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Advances in Applied Science Research, 2013, 4(4):490-500

ISSN: 0976-8610
CODEN (USA): AASRFC

Heterotrophic bacterial and fungal diversity in the inner shelf sediments of central west coast of India

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ABSTRACT

Vertical and horizontal distribution of culturable heterotrophic bacteria and fungi in the inner shelf sediments of central west coast of India (Arabian Sea) were examined. Physico-chemical parameters of the sediment and its influence on microbial diversity and abundance were evaluated. Microbial abundance displayed a significant positive correlation with sediment texture and organic matter. Bacterial population in the inner shelf regions increased towards northern transects and showed a decreasing pattern as core depth increased. A total of 221 bacterial cultures were isolated, out of which 63% gram negative and 37% were gram positive. Alteromonas (29%) was found to be the dominant genus in the sediment core samples followed by Micrococcus (18%) and Bacillus (10%). Other identified groups include Psychrobacter (10%), Moraxella (6%), Acinetobacter (5%), Flexibacter (5%), Corynebacterium (5%), Pseudomonas (3%), Alcaligenes (2%), Staphylococcus (2%), Enterobacteriaceae (1%) and Vibrio (1%). Out of the 47 fungal isolates, 34% were belonging to genus Aspergillus and 25% to Scopulariopsis.

Keywords: Arabian Sea, Inner shelf sediments, Heterotrophic bacteria, Fungi, Organic matter.

INTRODUCTION

Microorganisms inhabiting aquatic ecosystems play an important role in biogeochemical cycling by way of decomposing a wide spectrum of organic compounds ranging in molecular size from monomers to polymers [1]. Many of these microbes necessarily credit the oceans ability to sustain life on earth. Though a cosmopolitan distribution of free-living bacteria has been proposed to be the governing rule behind prokaryotic life, the knowledge on the distribution of marine sediment bacteria is still in its infancy [2]. The characteristics of the sediment greatly influence the microbial community [3].Within sediments, there is often a heterogeneous mixture of particles of different size, origin and surface features. These alterations influence the number and composition of the microbial community [4]. Nevertheless, benthic microbial community alters vividly over the course of a year [5]. At different redox depths microbial community structure is found to be significantly different. The structure of the microbial communities depends strongly on the sediment depth, organic carbon content, oxygen and salinity levels [6].

Sedimentary organic matter distribution across the Arabian Sea fuels an on-going debate over the controlling environmental factors [7]. The quantity and quality of organic matter in surface sediments are documented as major factors affecting benthic faunal dynamics [8]. Sediment microbial communities with bacteria as the primary mediators play a significant role in the decomposition of sinking organic matter from the water column and transport of dissolved inorganic matter to phytobenthos and phytoplankton [9].

Several studies have been undertaken to identify the microbial communities and dominant bacterial classes in diverse marine sediment environments. Studies conducted by Cavallo et al. [10] along the coastal sediments of Ionian Sea found gram positive bacilli as abundant genus together with *Aeromonas*, *Photobacterium* and *Pseudomonas*. The most abundant marine fungi encountered in various regions of the Sea of Japan belong to the

genera *Penicillium*, *Aspergillus*, *Wardomyces*, *Trichoderma*, *Chrysosporium*, and *Chaetomium* [11]. Recent studies by Jacob et al. [12] on the distribution and diversity of bacterial diversity along the shelf sediments of Bay of Bengal determined *Bacillus*, *Vibrio* and *Alteromonas* as the dominant genera. Studies conducted by Ramya et al. [13] along the Arabian Sea shelf sediments determined *Bacillus*, *Alteromonas*, *Vibrio*, Coryneforms and *Micrococcus*, as the dominant heterotrophic bacterial groups. Investigating the distribution and diversity of microbial communities is of great importance for gaining a better understanding of aquatic ecosystem. With this objective we determined the vertical and horizontal distribution of bacterial and fungal communities along the inner shelf sediments of central west coast of India. Sediment characteristics that influence the distribution and diversity pattern were also identified.

MATERIALS AND METHODS

Study Area

Sediment samples were collected from central west coast of India (Arabian Sea), which includes 5 stations off Cochin, Kannur, Mangalore, Goa, and Ratnagiri (Fig. 1). These study area lies between the latitude 09°55'99.5''N and longitude 75° 51'21''E to latitude 17° 00'40''N and longitude 73° 00'32''E. (Table1 and Fig 1).

Table 1: Details of the sampling stations in the inner shelf regions of central west coast of India (Cruise No.258 in Arabian Sea)

TRANSECTS	STATIONS DETAILS	DATE	LATITUDE (N)	LONGITUDE (E)	DEPTH (M)
COCHIN (CHN)	17 A	13/10/08	09^0 55' 99.5''	75^0 51' 21''	51
	17 B	13/10/08	09^0 55' 99.5''	75^0 51' 21''	51
	17 C	13/10/08	09^0 55' 99.5''	75^0 51' 21''	51
KANNUR (KNR)	27 A	11/10/08	11^0 59' 53''	74^0 55' 71''	54
	27 B	11/10/08	11^0 59' 53''	74^0 55' 71''	54
	27 C	11/10/08	11^0 59' 53''	74^0 55' 71''	54
MANGALORE (MGLRE)	32 A	8/10/08	12^0 51' 91''	74^0 29' 86''	51
	32 B	8/10/08	12^0 51' 91''	74^0 29' 86''	51
	32 C	8/10/08	12^0 51' 91''	74^0 29' 86''	51
GOA (GOA)	42 A	6/10/08	15^0 30' 06''	73^0 27' 04''	54
	42 B	6/10/08	15^0 30' 06''	73^0 27' 04''	54
	42 C	6/10/08	15^0 30' 06''	73^0 27' 04''	54
RATNAGIRI (RTNGRI)	47 A	4/10/08	17^0 00' 40''	73^0 00' 32''	52
	47 B	4/10/08	17^0 00' 40''	73^0 00' 32''	52
	47 C	4/10/08	17^0 00' 40''	73^0 00' 32''	52

A: 0-4 cm; B: 4-8 cm; C: 8-12 cm

Sample Collection

Samples for the present study were collected onboard Fisheries and Oceanographic Research Vessel (FORV) *Sagar Sampada*, Ministry of Earth Sciences (MoES), Govt. of India, during Cruise No. 258. Sediment samples were collected from 50m depth using Piston Corer and these sediments were sliced into 3 cores of 4cm each (0-4, 4-8, 8-12 cm). Bottom water was collected separately for analysis of abiotic factors such as temperature, salinity and dissolved oxygen from each station. Sediment samples were aseptically transferred into sterile polythene bags and were immediately preserved at -20°C in glycerol for further studies.

Grain size analysis

The sediment samples were dried overnight in a hot air oven at 60^0 C. 10g each of dried sample was accurately weighed and dispersed using sodium hexametaphosphate (10%) and kept overnight. The fine fractions of the sediment were separated by wet sieving. Grain size analysis was performed using a Laser Diffraction Particle Size Analyzer (SYMPA TECH, Germany).

Analysis of organic matter and microbial biomass

The sediment samples were homogenized and powdered well after drying in hot air oven at 60^0C overnight. 1g each of powdered sediment was ignited at 500^0C for 3 hours in a muffle furnace. The organic carbon content of the sample was determined by Loss on Ignition method (LOI) and was expressed as percentage of organic matter in sediment. ATP was extracted from the sediment sample to estimate the microbial biomass [14].

Microbiological Analysis

Standard plate count method was adopted. Sediment samples were subjected to serial dilution in sterile seawater and spread plated on to ZoBell's 2216e agar medium and Rose Bengal agar medium for the isolation of heterotrophic bacteria and fungi respectively. The plates were incubated at 28 ± 2°C for 5-7 days, the colonies were counted and expressed as colony forming units (CFU) per gram dry weight sediment. Morphologically different bacterial colonies were isolated, purified and identified by gram staining, spore staining and biochemical tests. The isolates

were identified up to generic level following Bergey's Manual of Systematic Bacteriology [15] and taxonomic scheme of Oliver [16].

Statistical Analysis

To study the variables that best explain the distribution of heterotrophic bacteria in sediments and their relation with environmental parameters, the spearman rank correlation was carried out using XLSTAT v.2012.6.01 (Addinsoft). Similarity between stations with respect to the generic composition and diversity indices were analyzed using PRIMER v6 [17].

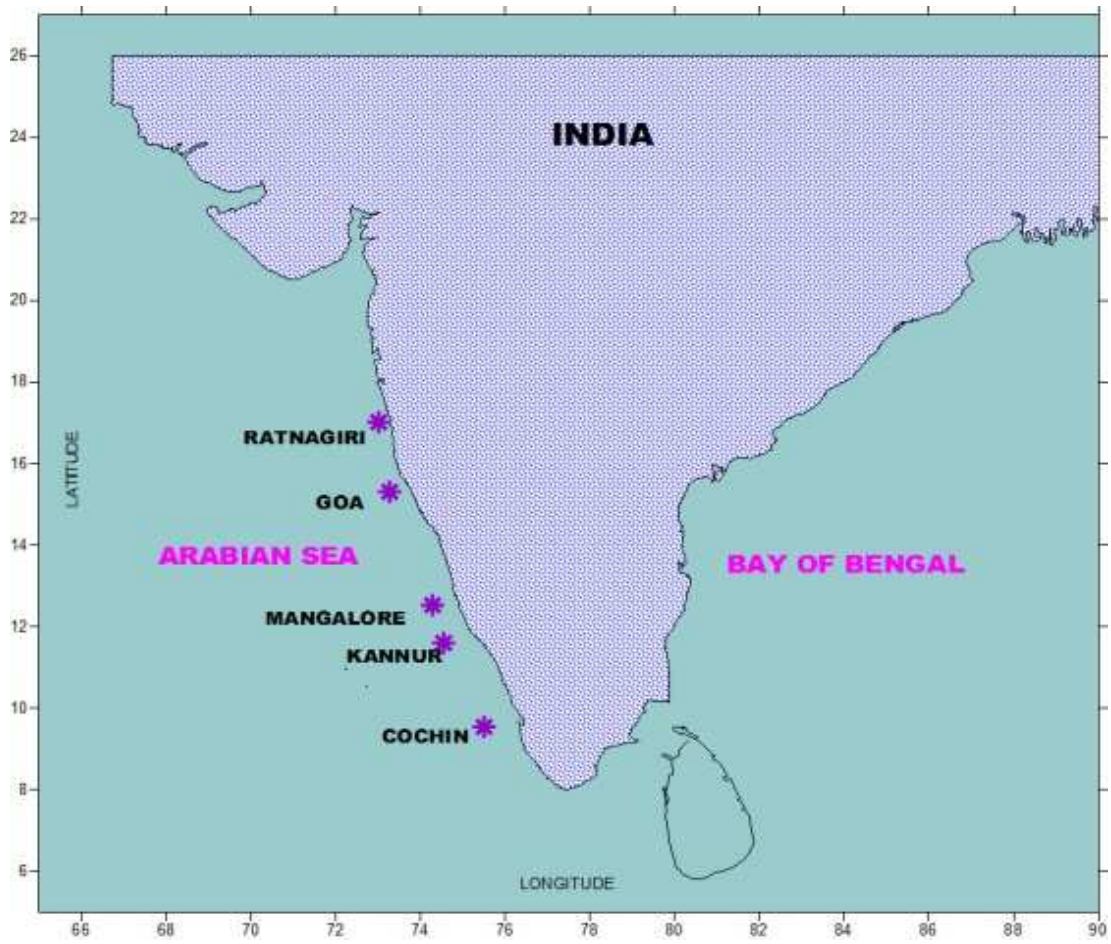

Fig1: Study area showing sampling stations

RESULTS AND DISCUSSION

Physico-chemical Parameters

The horizontal and vertical distribution of microbial population in sediment is influenced by various factors, such as the physico-chemical nature of sediment and the presence of organic matter [18-19]. In the present study temperature and dissolved oxygen were found to fall from the southern to northern latitude (Fig. 2). The study revealed no significant effect of temperature on THB. Similar findings were reported by Velankar [20] from Gulf of Mannar and Palk Bay near Mandapam (India).Variation in salinity was not so prominent in stations from north to south during the sampling. Dissolved oxygen was negligible towards the northern transects, similar to the previous report from Arabian Sea [21].

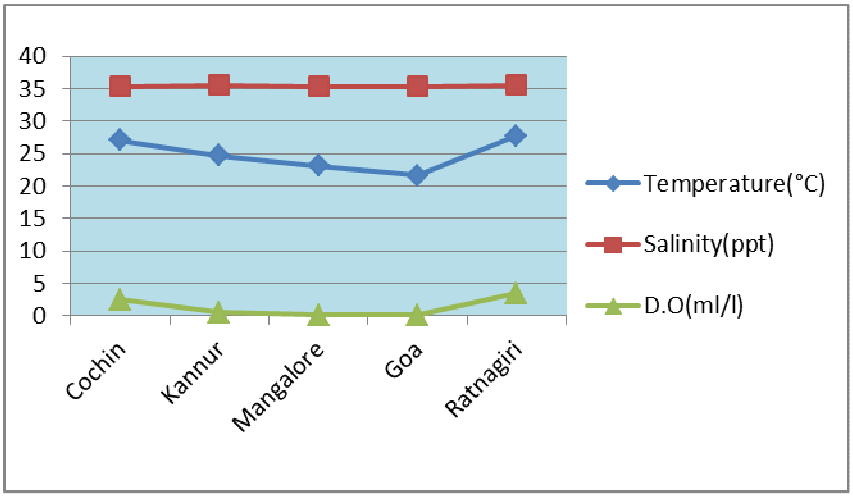

Fig 2: Temperature, Salinity and Dissolved oxygen profile of bottom water in the inner shelf regions of central west coast of India

Particle Size Characteristics of the Sediment

Texture

The textural types of sediments recorded were silty sand, sandy silt and clayey silt (Fig. 3). The inner shelf of southern latitude has more percentage of coarse fractions than northern region. Studies along the west coast of India by Murthy et al. [22] also have revealed that there existed a distinct zonation with regard to their distribution.

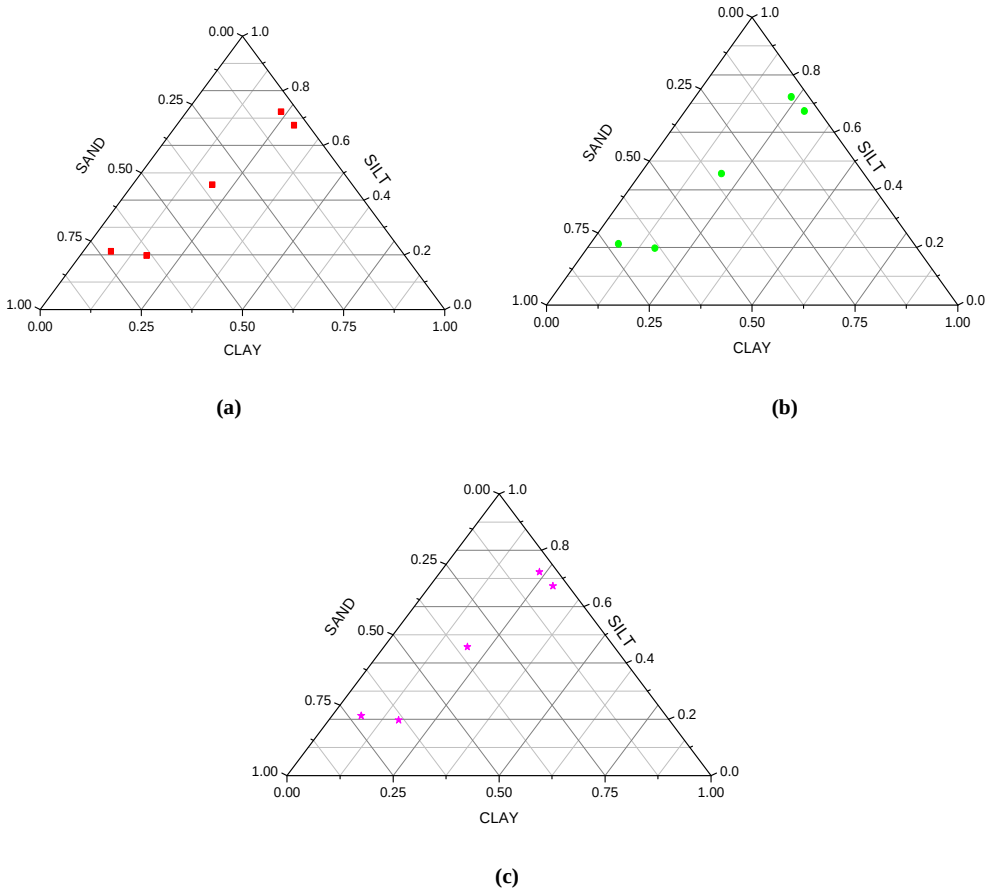

Fig 3: Ternary plot showing Sediment texture at (a) 0-4, (b) 4-8, (c) 8-12 cm cores in the inner shelf regions of central west coast of India

Organic Matter

Organic production was very high along the west coast of India [23]. Organic matter along the inner shelf sediments ranged between 6-10% in 0-4 cm of the cores; 5-7% in 4-8 cm of the cores and 4-7% in 8-12 cm of the cores of

various stations. The organic matter of sediment showed an increasing trend towards the north. Studies revealed that organic matter depends on the texture of the sediment and higher organic matter is associated with finer fractions than coarser ones [24]. Pearson correlation matrix showed that organic matter had significant positive correlation with clay (0.804, $p<0.05$); THB (0.892, $p<0.05$); and ATP (0.895, $p<0.05$). Along the west coast of India, sediment varied in their organic matter composition due to the existence of variable environmental conditions [25]. Organic matter in the sediment core decreased as the core depth increased.

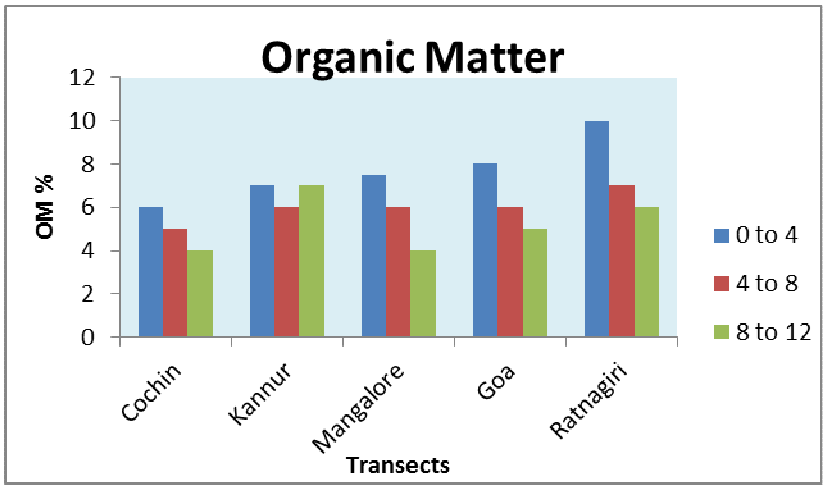

Fig 4: Organic matter (%) in the shelf sediments at various stations

Microbial Biomass Estimation by ATP Analysis

A marked depth wise variation in the microbial biomass was noticed in the sediment core. It is assumed that ATP content generally decreased with depth, but detectable amounts were always present even in the deepest sediments of Halifax Harbor (10 cm core) [26]. The present study illustrated that organic matter and bacterial biomass is positively correlated as reported by Dale [27] and Rublee [28]; a significant linear correlation between the organic matter of muddy sediments and the size of the microbial biomass. Microbial biomass showed a decreasing pattern as the core depth increased. Towards the north, the microbial biomass increased and Ratnagiri showed the highest concentration of ATP (Fig 5).

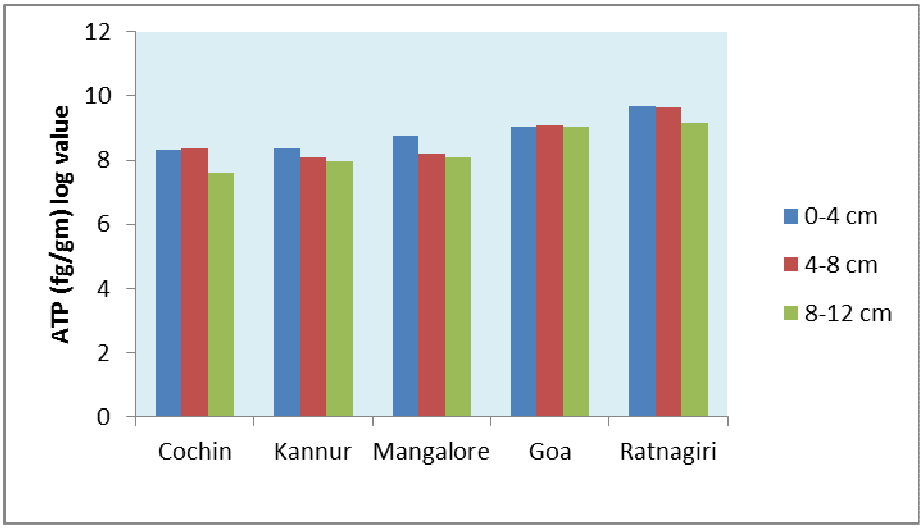

Fig 5: Concentration of ATP in the shelf sediments of central west coast of India

Culturable Heterotrophic Microbial Population

Total heterotrophic bacterial population

Cultivation dependent approach was adopted in the present study to describe the heterotrophic bacterial population of the sediment cores. The result showed that total heterotrophic bacteria had a significant positive correlation with organic matter (0.892, $p<0.05$); clay (0.629, $p<0.05$); silt (0.544, $p<0.05$); and ATP (0.890, $p<0.05$); whereas it showed negative correlation with sand (-0.596, $p<0.05$). Nair et al. [29] reported that bacterial population had a

direct relationship with organic matter. Report by Raghukumar et al. [30] found that clayey sediment of the deep sea harbour higher bacterial numbers.

Total heterotrophic culturable bacteria showed a progressive increase from southern to northern region and were found to be highest at Ratnagiri followed by Goa. Culturable bacterial population showed a significant depth wise variation (Fig. 6). Present investigation on microbial abundance in the cores of sediments from 50m depth along the west coast of Arabian sea illustrated that the THB values were in the range 10^4-10^5 cfu g^{-1} dry weight of the sediment. These results were similar to the observation of other studies from different areas which range between10^2-10^9cfug^{-1} [31-34].

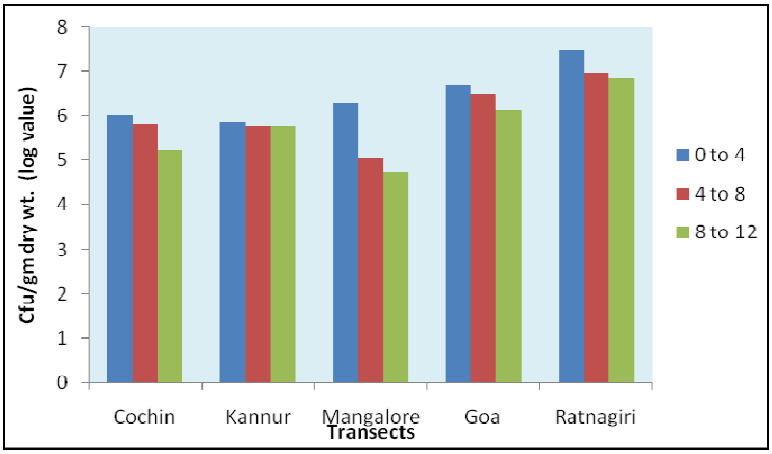

Fig 6: Total Heterotrophic Bacteria (culturable) in the inner shelf sediments of central west coast of India

Fungal Population

The present study showed that there was no significant pattern of fungal distribution within the sediment cores with respect to the 5 transects. Fungus population did not show any significant correlation with OM, clay, sand or silt (Fig 7). The present study showed that fungal population ranged between 10^2-10^3cfu g^{-1}dry weight of the sediment.

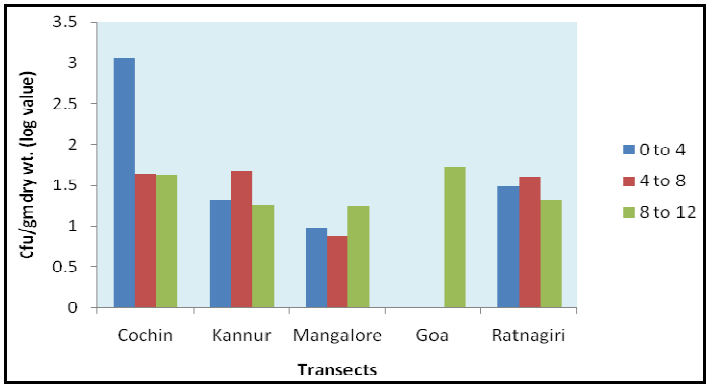

Fig 7: Fungal population in the inner shelf sediments of central west coast of India

Generic Composition of Heterotrophic Bacteria

The study revealed gram negative bacteria as the predominant group in different core depth at various transects compared to gram positive bacteria as was reported by Vasantha and co-workers [35]. Of the 221 isolates, 63% were gram negative and 37% gram positive (Fig.8). Among gram negative bacteria *Alteromonas* (29%) was the predominant group. Another important group identified during the study was *Micrococcus* (18%) and its occurrence in the coastal waters has been earlier reported by other researchers [36-37]. The present study illustrated the presence of heterotrophic bacteria such as *Bacillus* (10%), *Psychrobacter* (10%), *Moraxella* (6%), *Acinetobacter* (5%), *Flexibacter* (5%), Coryneforms (5%), *Pseudomonas* (3%), *Alcaligenes* (2%), *Staphylococcus* (2%), Enterobacteriaceae (1%) and *Vibrio* (1%) (Fig.9). Generic diversity of bacteria was found to be decreasing from south to north and was found to be higher off Cochin and minimum off Ratnagiri. Studies showed that significant changes in the bacterial species composition occur at various temporal and spatial scales [38].

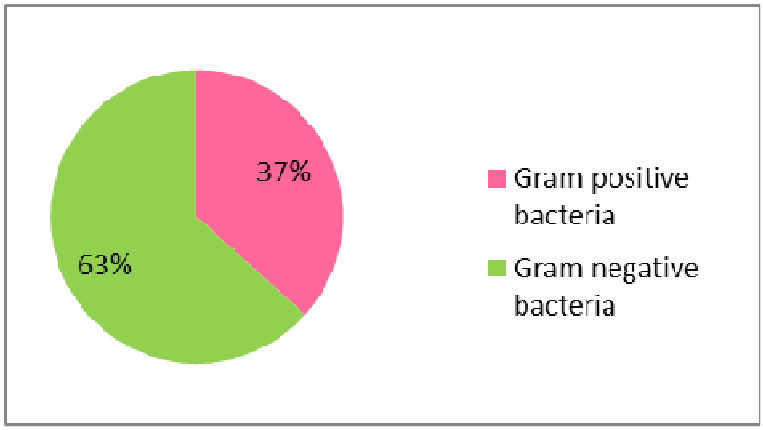

Fig 8: Percentage occurrence of gram positive and gram negative bacteria in the inner shelf sediments of central west coast of India

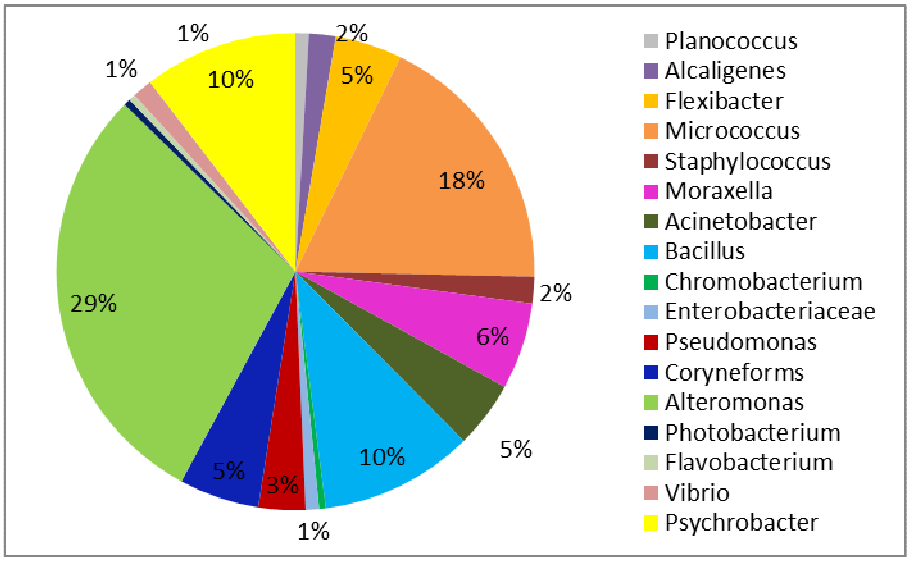

Fig 9: Percentage contribution of different genera isolated from the sediment cores of central west coast of India

Core Wise Distribution

1) 0-4 cm: The 0-4 cm depth core, *Alteromonas* (30%) was found to be the dominating genus followed by *Micrococcus* (18%). Genus *Psychrobacter* formed 14% of the bacterial composition in this core (Fig 10).

2) 4-8 cm: Core sediments of 4-8cm depth regions of all transects showed high abundance of *Alteromonas* (27%) and *Micrococcus* (21%) (Fig 10).

3) 8-12 cm: In general *Alteromonas* (31%) formed the major class of bacteria in this core followed by *Micrococcus* (15%) and *Bacillus* (12%). Coryneforms (9%), *Moraxella*, *Psychrobacter* and *Acinetobacter* (6% each) were the other dominant genera found in this core depth (Fig 10).

Generic Composition of Fungi

Isolates belonging to 5 different genera of fungi were obtained from the core sediments of inner continental shelf regions of the central west coast of India. Out of the 47 isolates, 34% belonged to genus *Aspergillus* and 25% to *Scopulariopsis*, *Penicillium* and *Cladosporium* (13% each), *Fusarium* (4%) and unidentified strains (11%) (Fig.11). Representatives of *Penicillium* and *Aspergillus* are reported to be versatile, ubiquitously distributed species capable of anaerobic denitrification [39]. The composition varied with different core depth. The fungal communities thriving under oxygenated conditions are distinctively different from fungal communities living under anoxic conditions [40].

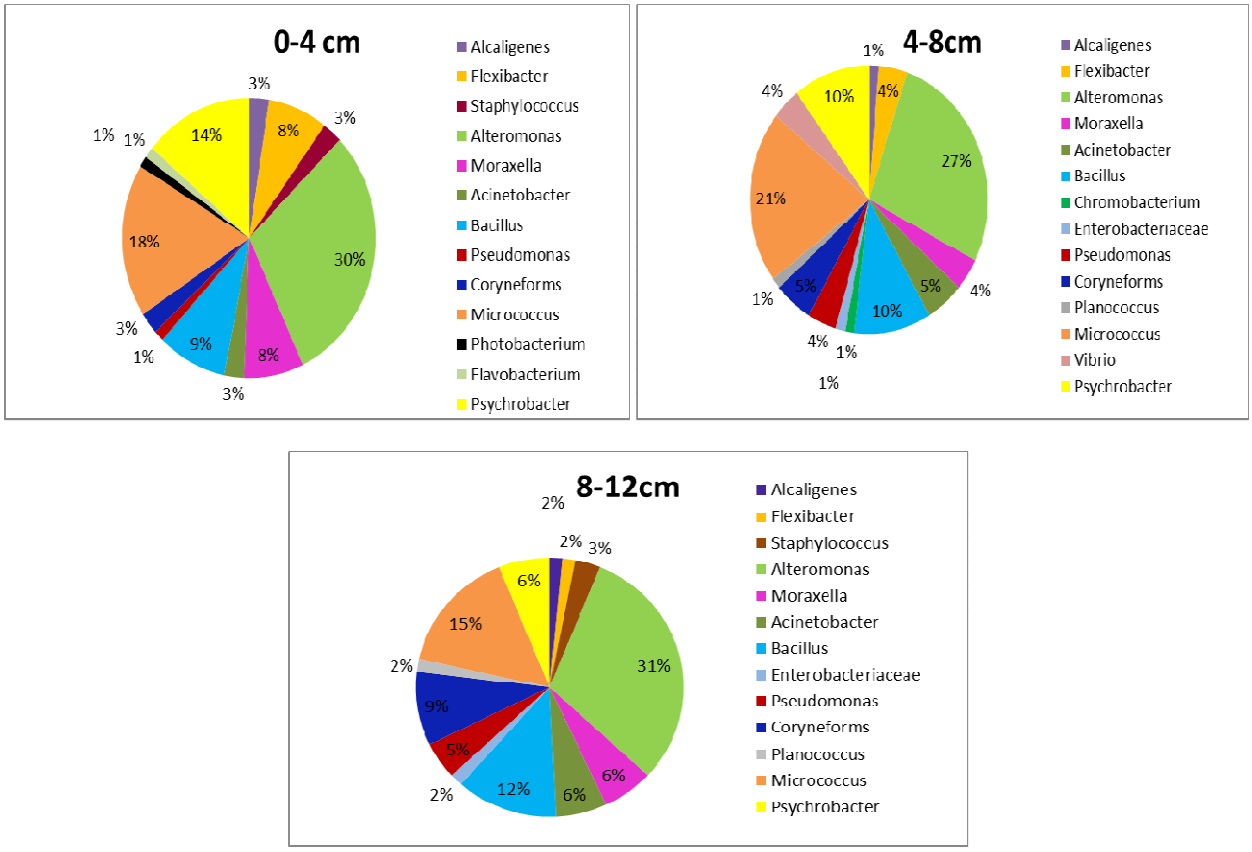

Fig 10 : Percentage distribution of different genera in sediment of core of (0-4),(4-8), (8-12) cm of various stations

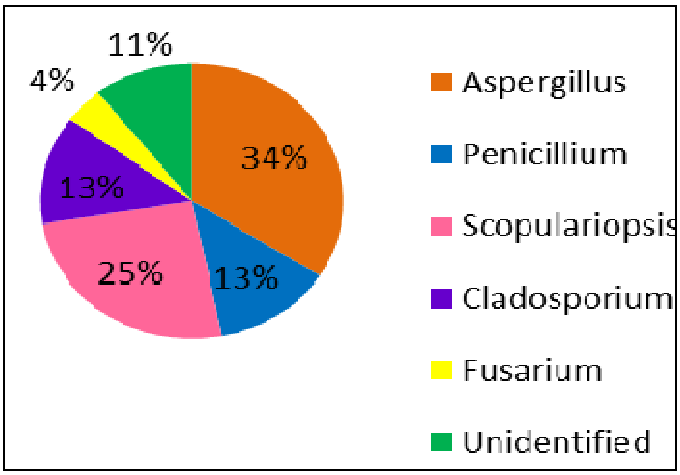

Fig 11: Percentage contribution of different genera of fungi isolated from the sediment cores of central west coast of India

Core Wise Distribution

1)0-4 cm: The genus *Aspergillus* (31%) formed the major fungal group in all the 0-4 cm core depth samples followed by *Scopulariopsis* (23%), *Fusarium* (15%), *Penicillium* (15%) and *Cladosporium* (8%) (Fig.12).

2)4-8 cm: *Scopulariopsis* and *Cladosporium* (31% each) were the two predominant genera observed in this core depth followed by *Aspergillus* (23%) and *Penicillium* (7%) (Fig.12).

3)8-12cm: The genus *Aspergillus* (40%) was found to be the dominant group followed by *Scopulariopsis* (25%), *Penicillium* (15%) and *Cladosporium* (5%) (Fig.12).

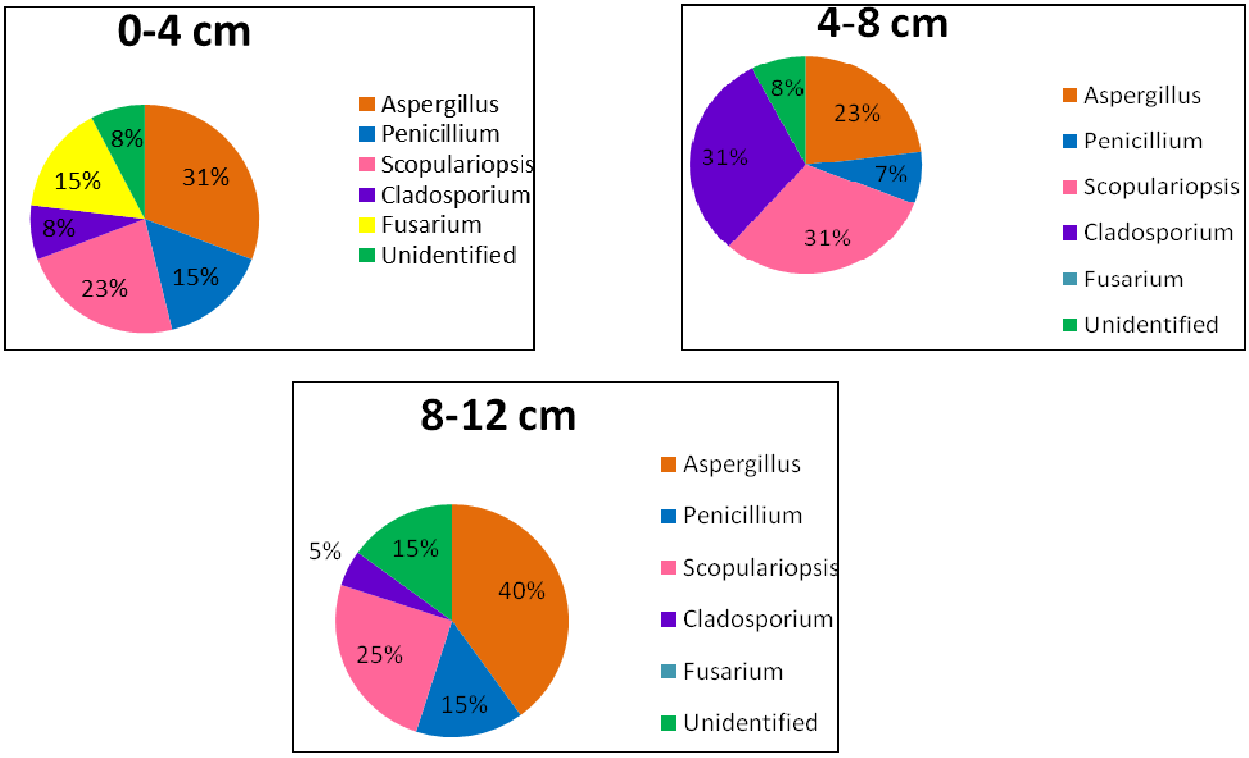

Fig12: Percentage contribution of fungi isolated from the sediment (0-4), (4-8), (8-12) cm core of central west coast of India

Table 2: Diversity indices of bacterial genera present in the inner shelf sediments of Central west coast of India (S- number of genera, N- total number, d- species richness, J'- species evenness, H'(log2)-species diversity, 1-λ'- species dominance)

Stations	S	N	d	J'	H'(log 2)	1- λ'
17A	10	14.43879	3.370889	0.987755	3.281251	0.961033
17B	11	13.79655	3.810368	0.981687	3.396078	0.970527
17C	11	14.47418	3.742002	0.97211	3.362947	0.961412
27A	8	11.38891	2.877533	0.988359	2.965076	0.952576
27B	10	13.38891	3.468975	0.987324	3.279818	0.966173
27C	8	9.650282	3.087799	0.976097	2.928291	0.959873
32A	7	11.23607	2.480232	0.946744	2.657845	0.906411
32B	6	8.146264	2.383723	0.976931	2.525329	0.933802
32C	6	9.388905	2.232613	0.994402	2.570492	0.928759
42A	2	3.645751	0.773059	0.847556	0.847556	0.548584
42B	7	10.24264	2.578915	0.953924	2.678005	0.918058
42C	6	7.464102	2.487432	0.97958	2.532177	0.947441
47A	5	6.732051	2.097668	0.969051	2.250067	0.915313
47B	4	5.828427	1.701889	0.97866	1.95732	0.887302
47C	3	3.828427	1.489809	0.98889	1.567354	0.891806

17- Cochin; 27- Kannur; 32- Goa; 42- Mangalore; 47- Ratnagiri; A:0-4cm, B:4-8cm, C:8-12cm.

Statistical Analysis

The combined use of species richness and diversity estimates provide information that enables deeper understanding of microbial diversity. Diversity indices showed a decreasing pattern towards the northern transects. The species richness was higher off Cochin and Kannur where as it decreased towards northern transects off Mangalore, Goa and Ratnagiri. There was no significant pattern of variation in species richness as core depth increased. Off Goa, the core depth of 0-4 cm had the least species richness but a prominent increase could be noted in deeper cores. Off Cochin and Kannur, the species evenness showed a decreasing pattern as core depth increased. In the case of Mangalore, Goa and Ratnagiri, it was found that the Pielou's Species evenness decreased as core depth increased (Table 2). The diversity was found to be high in southern region than in northern region. Shannon diversity does not showed a significant pattern with respect to core depth. Highest degree of diversity was found in southern region (Table 2). Species dominance showed similar pattern throughout the cores. Hierarchical clustering analysis delineates the bacterial communities of the study area into two main groups and these 2 major clusters showed a similarity below 60%. Within each cluster the sediment cores of different transects showed 70-80% similarities. First major cluster mainly showed the similarity between station 17 and 27. This major cluster was further grouped

into sub clusters based on their similarity. Each cluster showed a similarity range between 60 and 80%. Second major cluster showed the similarity pattern between the different cores of transects 42, 47 and 32. Similarity pattern ranged between 60-85%. The transects 17 and 27 belonged to the southern region and stations 32, 42 and 47 are northern region of the study area.

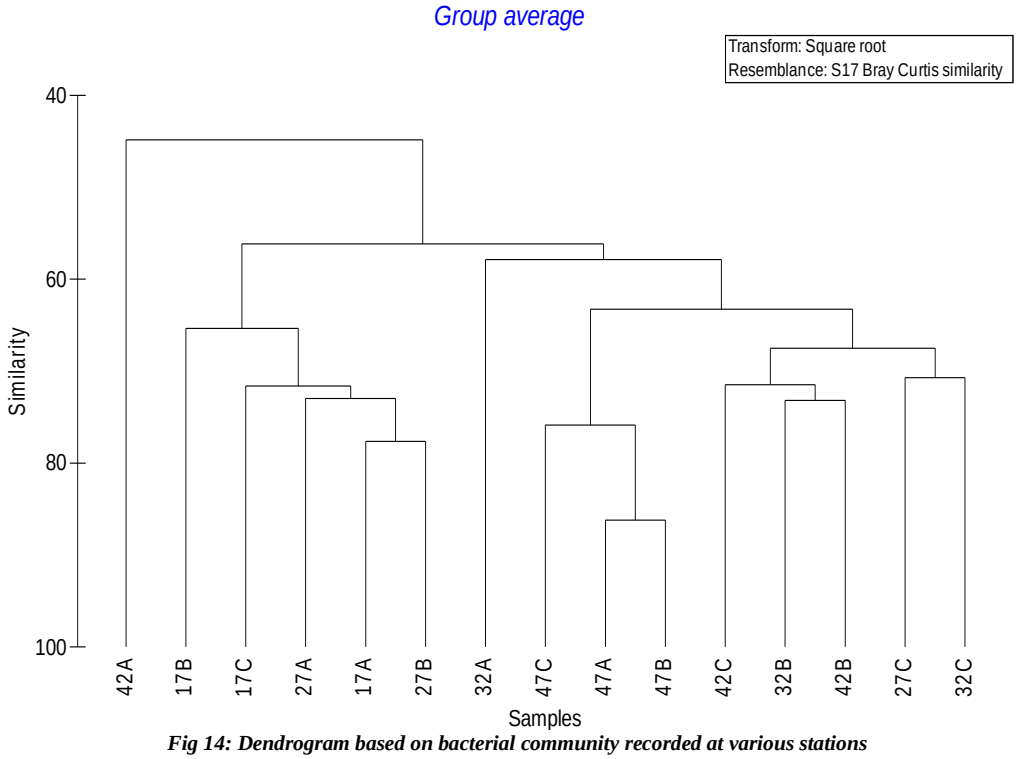

Fig 14: Dendrogram based on bacterial community recorded at various stations
17-COCHIN 27-KANNUR 32-MANGALORE 42-GOA 47-RATNAGIRI
A- (0-4 cm) B- (4-8 cm) C- (8-12 cm)

CONCLUSION

The study imparts knowledge on the microbial ecology of the inner shelf sediments of eastern Arabian Sea. Total heterotrophic (culturable) bacteria showed a progressive increase from southern to northern region and the study area could be demarcated into two ecosystems (southern and northern regions) in terms of the microbial diversity in the benthic realm of the region. The study revealed that the physico-chemical parameters and sediment texture greatly affected the total microbial population in the study area.

Acknowledgements
The authors are grateful to the Head, Department of Marine Biology, Microbiology and Biochemistry, Cochin University of Science and Technology for providing necessary facilities to carry out the work. We thank Centre for Marine Living Resources and Ecology, Ministry of Earth Sciences, Govt. of India for financial and technical support. The authors thank the captain and crew of FORV *Sagar Sampada* for their support in sample collection.

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