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The Trophic Status of the Continental Shelf Sediments of Bay of Bengal: Analysis Based on Sediment Biochemical and Microbial Variables

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Abstract: The trophic status of the shelf sediments of Bay of Bengal was assessed based on the biochemical and microbial variables. Total protein, total carbohydrate and total organic matter concentrations in the inner (< 100 m) and outer shelf (> 100 m) varied significantly ($p < 0.05$). Bacterial abundance and biomass were greater in the outer shelf regions. Sediment ATP concentration used as a proxy for living microbial biomass varied regionally. Accordingly, the measured biochemical and microbial variables clustered the shelf sediments 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 organically enriched sediments with high biopolymeric carbon and microbial biomass contributed to the eutrophic nature of the outer shelf.

Keywords: Continental shelves, Sediments, Organic matter, Bacteria, ATP assay, Trophic status

1. Introduction

Marine sediments are intricate systems affected by geological, hydrological, physicochemical, and biological factors (Koster and Reil, 2001). The energy content in these ecosystems defining its trophic status (Dell Anno et al., 2002) is a subject of great interest amidst the scientific community. The trophic status of marine ecosystem is generally assessed through inorganic nutrient concentrations (mainly N and P), phytoplankton biomass or alteration of chemical and physical parameters such as turbidity and oxygen concentration (Zurlini, 1996). Nixon (1995) proposed an alternative approach for evaluating the trophic state of benthic systems based on the supply of organic carbon (in terms of g $C/m^2/y$) and categorized the marine ecosystem as oligotrophic, mesotrophic, eutrophic and hypertrophic. But, due to the conservative nature of total sedimentary organic carbon, changes in the trophic state could be more evident in terms of the shifts in the biochemical composition of organic matter rather than

its quantity (Tselepides et al., 2000).Therefore, extending Nixon's concept, Dell'Anno et al., (2002) proposed a new approach to assess the trophic state of coastal marine ecosystems based on the biochemical composition of the sedimentary organic matter.

Organic matter in the marine environment is composed of labile and refractory compounds. Its quantity and quality reaching the seabed dependent upon several factors like origin, composition and biochemical transformations that occur on organic particles during their descent to the ocean floor (Danovaro et al., 1999). It is generally assumed that the lability or bioavailability of the organic matter is an important factor in regulating benthic community distribution in marine sediments (Tselepides et al., 2000).Information, therefore, on the quantity, quality and spatial distribution oforganic matter is significant since they represent the principal factors regulating benthic biomass (Grebmeier et al., 1988).

Microbes comprise the largest pool of total benthic biomass and play a vital role in benthic ecosystems by affecting the degradability of organic matter (Polymenakou et al., 2009). Marine sediments are documented as important domicile for microbial activity (Danovaro et al., 2000), and are colonized intensively by microorganisms including bacteria, cyanobacteria, viruses, fungi, algae and protozoans. Oceanographic researches depicted the biomass present in the marine ecosystem especially that of microorganisms at the base of the food chain (Koster and Reil, 2001; Steward et al., 2007). Reliable estimation of benthic biomass is essential to determine the quantitative importance of these groups in marine ecosystem. In sediments they play a key role in the disintegration, production as well as the consumption of organic matter and the release of inorganic nutrients to the environment (Meyer-Reil, 1993).In addition, because of their high turnover rate and metabolic activity the structure of microbial assemblages are assumed to respond rapidly to any environmental change and are sensitive to changes in trophic conditions (Hansen and Blackburn, 1992). Accordingly, the study of microbial community may give an indication on the extent to which the energy content is employed within the ecosystem and it symbolizes a functional measure for the analysis of the trophic state of the coastal marine environment (Vezzulli and Fabiano, 2006).

It is expected that changes in organic matter quality or quantity in marine systems will alter the microbial community and that the in-situ microbial community will in turn alter the composition of the organic matter pool (Dyda et al., 2009).During the past two decades correlation between the labile sediment fractions and benthic bacteria and their role in determining the sediment trophic status has repeatedly been documented (Danovaro, et al., 1999, 2000; Dell'Anno et al., 2002; Vezzulli and Fabiano, 2006; Pusceddu et al., 2007, 2009). But no work has been done along the Indian coasts. Hence following the concept by Dell'Anno et al., (2002) and Vezzulli and Fabiano (2006), our aim was to evaluate the trophic status of continental shelf sediments of south east coast of India (Bay of Bengal) using sediment biochemical and bacterial variables and to find out the bacterial

response to the sediment organic matter along the shelf. We also surveyed the ATP concentrations to characterize the biomass and analysed the spatial distribution of ATP as an indicator of the living microbial biomass in the shelf sediment.

2. Materials and Methods

2.1. Study Area

The study area was the continental shelf region of south east coast of India (Bay of Bengal), extending between latitude 10° 36' 00" N to 15° 14' 82" N and longitude 80° 07' 06" E to 81° 35' 09" E (Fig. 1), covering 18 stations over 6 transects. Across the transects, 3 stations each at a depth of 50 m, 100 m and 200 m were sampled.

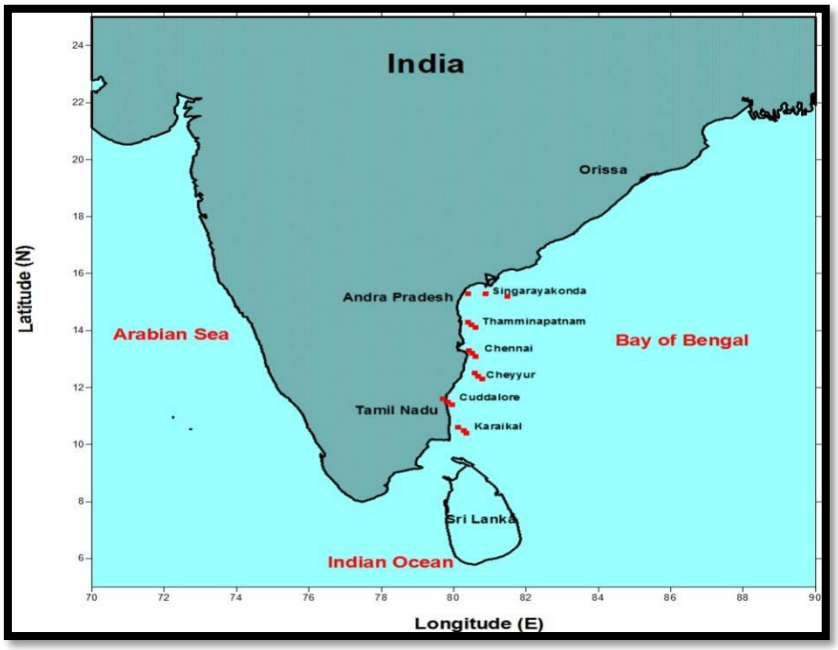

Fig. 1: Location of sampling stations in the study area (Bay of Bengal)

2.2. Sample collection

Sediment samples for the present study were collected onboard Fisheries and Oceanographic Research Vessel (FORV) Sagar Sampada (Cruise No.266), Ministry of Earth Sciences, Govt. of India, during May 2009.Hydrographical data for temperature, salinity and dissolved oxygen were collected from each station using onboard CTD(Sea bird, USA). Sediment samples were collected using Smith McIntyre Grab (0.2 m^2) from desired depths. Sediment from the surface layer (top 1-2 cm) was aseptically transferred into sterile polythene bags and immediately subjected to microbiological analysis. Sediment samples were preserved at -20°C in a deep freezer for organic matter and texture analysis.

2.3. Sediment analysis

2.3.1. Grain size analysis

The sediment samples were driedto constant weight 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. Grain size of the sediment was performed using a Laser Diffraction Particle Size Analyzer (SYMPA TECH, Germany).

2.3.2. Biochemical analysis

Sediment samples from each station were subjected to chemical analysis to determine the total organic carbon (TOC) and organic matter (OM) content. Samples were powdered well after drying in hot air oven at 60^0C for 48 hrs. The organic carbon was estimated by wet oxidation method with chromic acid (El Wakeel and Riley, 1957) and organic matter content was determined by multiplying the organic carbon concentration by the factor 1.724 (Nelson and Sommers, 1982). Three replicate analyses were made for each sample. Total carbon (TC) and total nitrogen (TN) analyses were performed using a Vario EL III CHNS Analyser.

Total protein (PRT) analysis was done following Lowry et al., (1951)as modified by Rice, (1982)after extraction with NaOH and using bovine serum albumin (BSA) as standard. The amount of protein nitrogen was obtained by multiplying protein with 0.16 (Mayer et al., 1986). Total carbohydrates (CHO) were analyzed by phenol sulphuric acid method (Kochert, 1978) with glucose as standard. Total lipids were extracted from dried sediment samples by direct elution with chloroform and methanol. Analyses were carried out using the methods of Bligh & Dyer (1959) and of Barnes and Black stock,(1973) rd. Blanks for each analysis were performed with pre-combusted sediments at 450 - 480°C for 4 h. PRT, CHO and LIP concentrations were expressed as BSA, glucose and cholesterol equivalents, respectively. All analyses were carried out in triplicate. Protein, carbohydrate and lipid concentrations were converted to carbon equivalents assuming a conversion factor of 0.49, 0.40 and 0.75 mg of C/mg respectively (Fabiano and Danovaro, 1994) and normalised to sediment dry weight. The sum of protein, lipid and carbohydrate carbon equivalents was reported as the biopolymeric carbon (BPC) and used as a reliable estimate of the labile fraction of organic matter (Fabiano et al., 1995).The protein to carbohydrate ratio (PRT:CHO) was calculated and used as indicator of the status of biochemical degradation processes (Galois et al., 2000). Lipid to carbohydrate ratio (LPD: CHO) which can be used as a good index to describe the energetic (food) quality of the organic contents in the sediments was also calculated.

2.4. Total bacterial count

Total counts (TC) were determined by acridine orange direct count method (AODC; Hobbie et al., 1977). Approximately 1 g of sediment was diluted immediately in filtered seawater supplemented with 0.2µ pore size filtered buffered formalin (5% final concentration) as fixative and stored refrigerated onboard until analyses. The samples were ultrasonicated for 5 sec at 10 Hz to separate bacteria from sediment. To 1 ml of sample taken in vials 100µl acridine

orange (1 mg/ml) stain was added and kept in dark for 5 minutes. After incubation, the stained samples were filtered through 0.2µ polycarbonate filter paper and the filter paper was placed over a drop of non epifluorescent immersion oil on a clean glass slide. Up to 25-30 microscopic fields per slide were counted were counted at 1000 x magnification via epifluorescence microscopy (Leica DFC 310 FX, coupled with an image analysis system) using UV excitation. The abundance was expressed as cells per gram dry weight sediment. Bacterial biomass was estimated using a mean bacterial volume of 0.2 μm^3 per cell (Kuwae and Hosokawa, 1999)and a conversion factor of 220 fg C μm^3 (Bratbak and Dundas, 1984). Data were normalized to dry weight after desiccation (60°C, 24 h).

2.5. ATP measurement for estimating active microbial biomass

ATP was extracted from the sediments on board after passing through a 1-mm stainless steel sieve. Extraction was done using sterile, boiling Tris- HCl (0.02M) buffer (pH 7.8; Parsons et al., 1984). 5 ml of buffer was brought to boiling point in a test tube, covered by a watch glass, on a recessed, heated aluminium block. To this 1 g of sediment was added. The temperature of the buffer should not fall to $<96^0$C after sediment was added. After 5 min the beaker was removed to a bath of crushed ice and centrifuged at 1,500 x g for 5 min. The supernatant thus obtained was then decanted into a graduated test tube and held at -20^0C until analysis. The ATP concentration was determined by the luciferin luciferase reaction, using an ATP bioluminescent assay kit (Sigma chemicals, USA). Bioluminescence ATP assays were performed using a Turner luminometer. The generated light signal was measured after a 3-s delay time and a 14-s integration time. ATP concentration is then converted to total microbial biomass carbon or dry mass.ATP (Sigma Chemicals, USA) at a concentration of 10-50 ng/ml was used as the standard. The factor used to convert measured ATP values to total biomass carbon was 250 (Holm-Hansen and Karl, 1978).

2.6. Statistical Analysis

Statistical analyses were carried out using statistical software XLSTAT v.2012.6.01 (Addinsoft), ORIGIN v.6.0,SPSS v.19.0 and PRIMER v6. Differences among stations, transects were investigated by means of one- way analysis of variance (ANOVA). In order to interpret statistically significant differences between respective depths and transects, Tukey's post-hoc test was carried out. A Spearman-Rank correlation analysis was performed to test for possible relationships among all of the investigated variables. Principal Components Analysis (PCA) was employed to assess differences among stations using sediment logical and biochemical data and also to ordinate them in a two-dimensional space after suitable transformations. To check the similarity between stations the protein to carbohydrate ratio at different depths were analysed by

hierarchical agglomerative cluster analysis based on Bray-Curtis similarities and the results were plotted into ordination graphs.

3. Results

3. 1. Sediment characteristics

The hydrographical parameters did not show significant ($p < 0.05$) spatial variation though it varied with depth. Sediment was fine sand at 50 -100 m depth and clayey silt at 200 m depth (data not shown). One way ANOVA elucidated a significant difference in total carbon, total nitrogen, total organic carbon and total organic matter between the inner (< 100 m) and outer shelf (> 100 m) regions (concentration shown in Table 1). Mean Total Carbon (TC) and Total Nitrogen (TN) in the shelf sediments were 48.6 ± 28.3 mg/g and 0.86± 0.4 mg/g respectively. Total organic matter along the shelf sediments ranged from 9.51to 37.66 mg/g (mean ± SD: 18.54 ± 6.7 mg/g) and was found to be maximum at 200m depth (mean ± SD: 24.91 ± 6.52 mg/g). Organic matter did not show any significant ($p < 0.05$) spatial variation between transects, though it was slightly greater towards northern latitudes of the study area. Organic matter quality as measured by molar Carbon/Nitrogen (C/N) ratio was found to be 14.28 ± 5.47 (range: 8.44 to 28.38). Significantly low values ($p<0.05$) were sited at 200m depth regions (9.64 ± 0.92).

TRANSECTS	TC (mg/g)	TN (mg/g)	TOC (mg/g)	TCHO (mg/g)	TPRT (mg/g)	TLIP (mg/g)	C-BPC (mg/g)
KRKL-A	47.38	0.51	5.52	0.82	0.30	5.04	4.26
KRKL-B	110.54	0.29	8.28	0.62	0.09	5.51	4.43
KRKL-C	65.19	1.15	13.11	1.17	1.87	4.79	4.98
CDLR-A	11.72	0.26	5.75	0.94	0.65	4.65	4.19
CDLR-B	87.46	0.60	5.98	0.79	0.60	4.64	4.10
CDLR-C	68.80	0.93	10.12	1.17	0.95	5.00	4.69
CHYR-A	35.85	0.43	9.43	0.81	0.80	8.77	7.30
CHYR-B	75.51	0.43	10.12	0.81	0.22	6.78	5.52
CHYR-C	22.61	1.84	21.85	2.17	2.19	9.83	9.31
CHNI-A	17.52	0.65	9.2	1.03	0.72	8.18	6.90
CHNI-B	64.14	0.78	9.66	0.98	0.70	8.32	6.98
CHNI-C	23.38	1.68	14.26	2.55	3.08	9.48	9.64
TPTM-A	51.60	0.65	9.43	0.87	0.92	7.51	6.43
TPTM-B	69.52	0.97	11.27	1.51	1.05	8.77	7.70
TPTM-C	16.78	1.33	11.5	1.81	1.85	7.75	7.44
SKDA-A	20.43	1.03	14.26	1.14	1.02	7.75	6.77
SKDA-B	69.25	0.69	8.05	0.66	0.40	7.40	6.02
SKDA-C	17.06	1.28	15.87	2.51	3.01	8.84	9.11

Table 1. Total Carbon (TC), Total Nitrogen (TN), Total organic carbon (TOC), Total Carbohydrate (TCHO), Total protein (TPRT), Total Lipid (TLIP) and Biopolymeric carbon (BPC) present in the shelf sediments of south east coast of India
(KRKL- Karaikal, CDLR- Cuddalore, CHYR- Cheyyur, CHNI- Chennai, TPTM- Thamminapatnam, SKDA- Singarayakonda)

3.2. Biochemical composition of sedimentary organic matter

A significant ($p < 0.05$) difference in total protein and total carbohydrate between the inner and outer shelf was evident from the results of ANOVA. However, total lipid did not show any significant ($p < 0.05$) bathymetric variation though they varied significantly ($p < 0.05$) between the northern and southern latitudes.

Total protein concentration ranged from 0.09 to 3.08 mg/g (mean ± SD: 1.14 ± 0.87 mg/g) and did not show any significant ($p>0.05$) latitudinal variation (Fig. 2A). Highest protein concentration was recorded from the 200 m depth of Chennai and lowest from 100 m depth of Karaikal. The protein nitrogen concentration ranged from 0.014 to 0.49 mg/g. Concentration of total carbohydrate in the shelf ranged from 0.62 to 2.55 mg/g (mean ± SD: 1.24 ± 0.59 mg/g) (Fig. 2B). As that of protein, carbohydrate concentration did not show any significant ($p>0.05$) latitudinal variation and highest and lowest concentration was recorded from Chennai and Kariakal respectively. Moreover, protein and carbohydrate concentrations were found to be higher at 200 m depth regions along the shelf sediment.

No significant ($p>0.05$) depth wise variation in the concentration of lipid was noticed in the shelf. Compared to total protein and total carbohydrate, total lipid exhibited higher values ranging from 4.64 to 9.83 mg/g (mean ± SD: 7.17 ± 1.73 mg/g) (Fig. 2C). Concentration of lipid was lowest along the Karaikal and Cuddalore transects and there after it increased towards the northern latitudes.

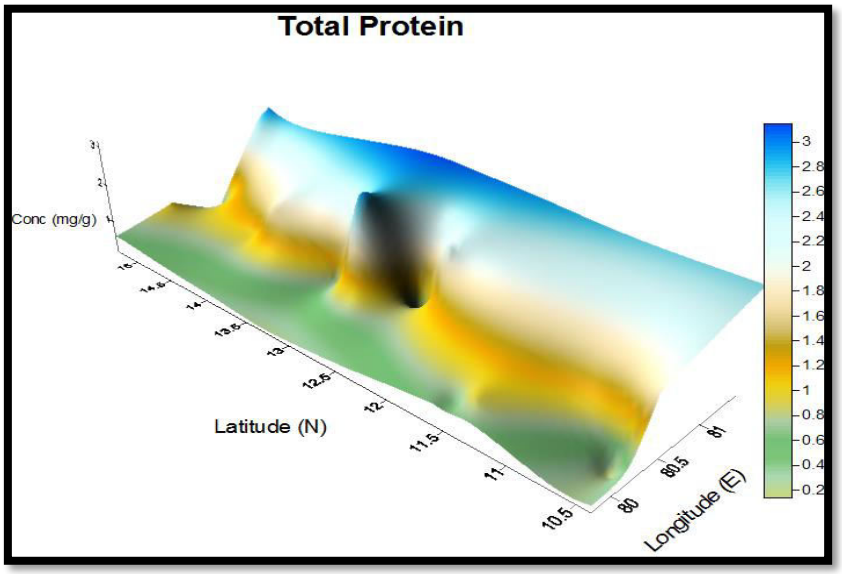

Fig. 2A

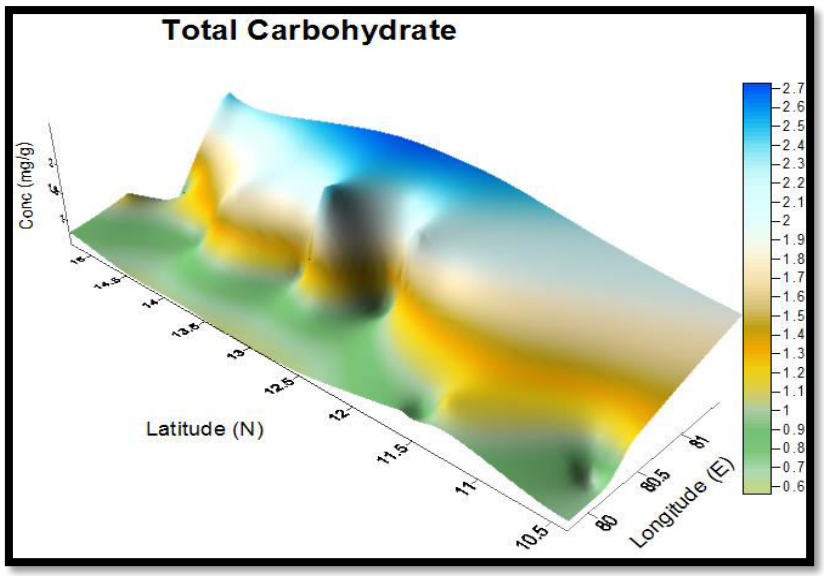

Fig. 2B

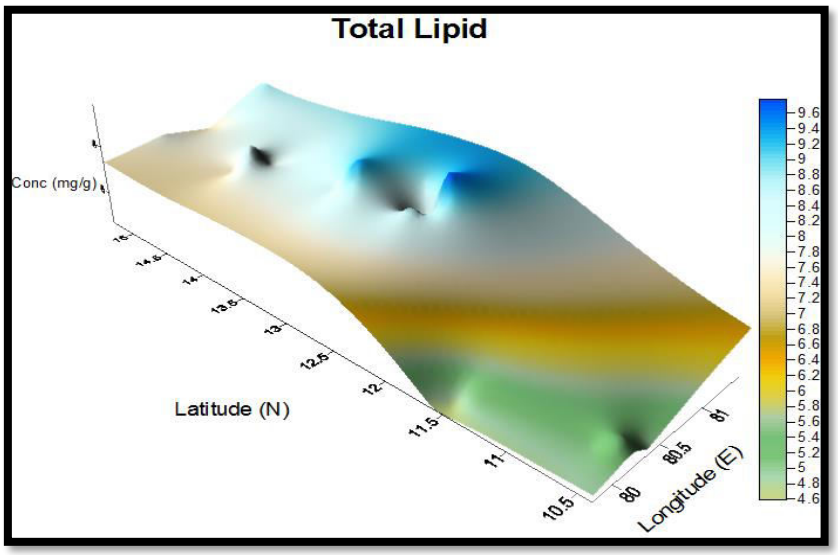

Fig. 2C

Fig 2: Concentration of total protein (2A), total carbohydrate (2B) and total lipid (2C) present in the shelf sediments of south east coast of India

The sum of carbohydrate, protein and lipid carbon (Biopolymeric carbon, BPC) was determined as a relative measure of the amount of food potentially available for heterotrophic metabolism.BPC ranged from 4.10 to 9.64 mg/g (mean ± SD: 6.43 ± 1.75 mg/g). Highest BPC was observed off Chennai and lowest off Cuddalore. No significant depth wise variation was noticed. But the results of ANOVA demarcated the northern latitude from the southern based on its concentration.

Total organic carbon constituted a mean of 36.22 ± 29.34 % of total carbon. Protein to carbohydrate ratio (PRT: CHO) was found to be increasing when moving from 50 m to 200 m depth. LIP: CHO ratio ranged from 3.52 to 11.11.

3.3. Bacterial density and Biomass

Total benthic bacterial density and biomass did not display any significant ($p < 0.05$) latitudinal variations though they exhibited significant ($p < 0.05$) depth wise variation. Bacterial density and biomass were higher in 200m depth when compared to other depth regions. Total bacterial count in the shelf sediment ranged from 7.07 x 10^8 to 1.44 x 10^9 cells/ g dry wt. with its maximum off Thamminapatnam and minimum off Karaikal (Fig. 3). Total benthic bacterial biomass (BBM) ranged from 31.11 to 63.26 µg C/g (mean ± SD: 44.30 ± 9.31 µg C/g). In the study area bacterial contribution to organic carbon pool (BBM/BPC) was generally very low ranging from 0.45 to 1.26%.

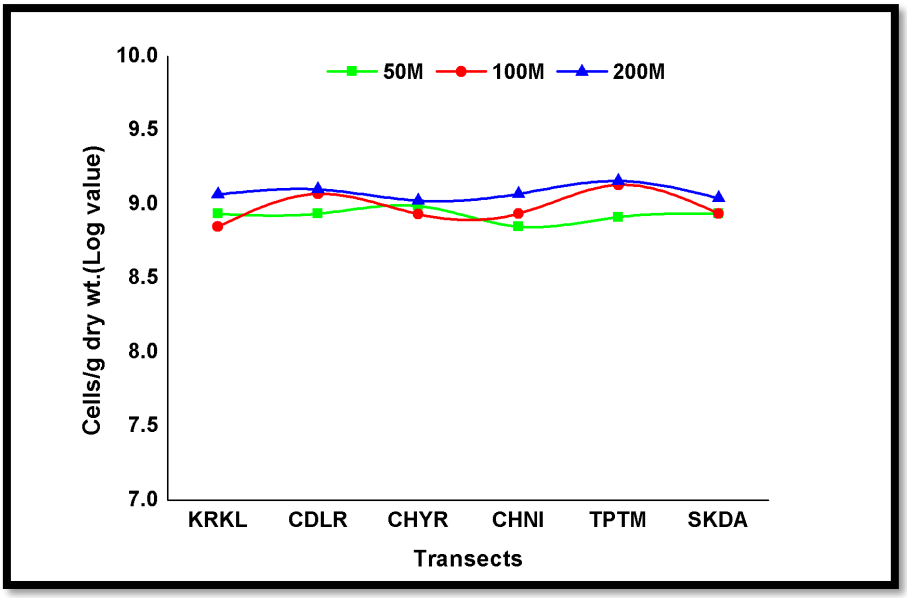

Fig. 3: Bacterial density (total count) in the shelf sediments of south east coast of India

3.4. Living microbial biomass and spatial distribution of ATP

ATP concentration was used as an indicator of living microbial biomass. Concentrations were very low and ranges from 196.80 to 840.27 ng/g (mean ± SD: 445.26 ± 216.74 ng/g) (Fig. 4). Significant ($p < 0.05$) differences in ATP concentrations between latitudes were observed. ATP was found to be significantly higher towards the northern latitudes nonetheless Singarayakonda strike down. Though no significant bathymetric variations in concentration were noticed, mean ATP concentration peaked towards the outer shelf.

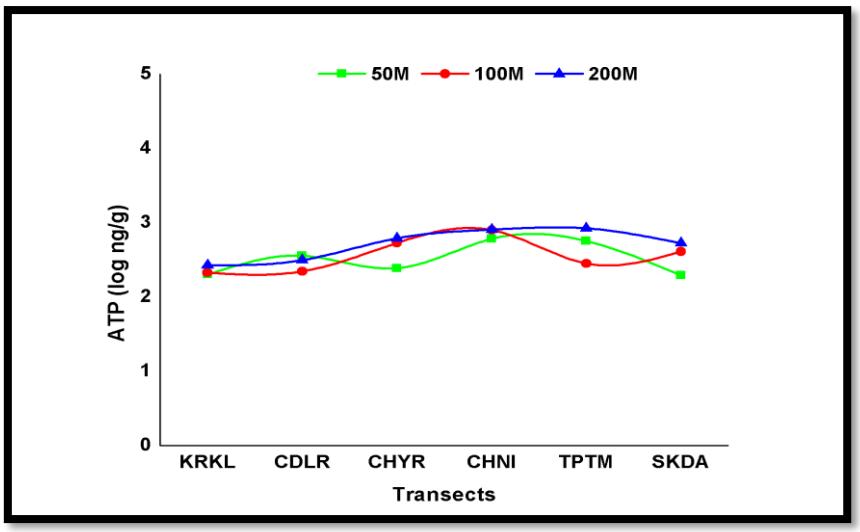

Fig. 4: ATP (microbial biomass proxy) in the shelf sediments of south east coast of India

Total living microbial biomass (MBB) estimated from the concentration of ATP ranges from 49.20 to 199.46 µg C/g (mean ± SD: 111.31 ± 54.18 µg C/g) and followed the same distribution trend for ATP. Total bacterial biomass determined from the bacterial bio volume contributed greatly to the total microbial biomass. Total bacterial biomass contribution to total microbial biomass (BBM/MBB) ranged from 20.51 to 93.39%. The difference between microbial biomass and bacterial biomass gives the non - bacterial biomass and it ranged from 3.66 to 161.43 µg C/g (mean ± SD: 67.01 ± 53.59 µg C/g). Total microbial contribution to organic carbon pool (MBM/BPC) in the shelf sediment was very low ranging from 0.72 to 2.85%.

3.5. Trophic classification of sampling area using biochemical and microbial variables

The biochemical variables selected to determine the sediment trophic status was the concentration of protein and carbohydrate. Based on the classification made by Dell Anno et al. (2002), we grouped the shelf sediments into oligotrophic inner shelf (protein < 1.5 mg/g; PRT: CHO < 1) and eutrophic outer shelf (protein >1.5 mg/g; PRT: CHO > 1). Protein to carbohydrate ratio in the shelf sediments ranged from 0.14 to 1.59 and was significantly higher towards the outer shelf. Though not statistically significant, the BPC values also showed slight increase towards 200 m depth.

Bacterial density was positively correlated with protein concentrations. When compared to oligotrophic inner shelf, bacterial density was higher in eutrophic outer shelf. Mean bacterial density which support the oligotrophic inner shelf is 9.09 x 10^8 cells/g dry wt. and 1.20 x 10^9 cells/g dry wt. support the eutrophic outer shelf. This shows that the bacterial cells displayed considerable increase in cell density in eutrophic sites. A similar trend was observed for microbial biomass.

3.6. Statistical analysis

Spearman rank correlation map (Fig. 5) shows a significant positive correlation of bacterial density and biomass with total nitrogen ($r = 0.601$, $p < 0.01$), total protein ($r = 0.581$, $p < 0.01$), total carbohydrate ($r = 0.542$, $p < 0.05$), depth ($r = 0.817$, $p < 0.001$) and a significant negative correlation with temperature ($r = -0.708$, $p < 0.001$).

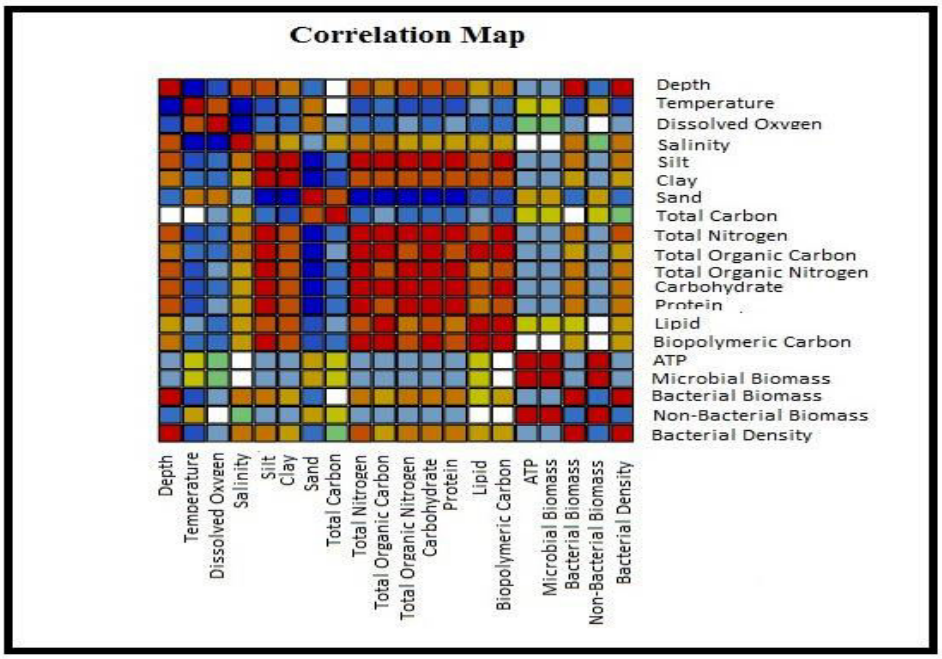

Fig. 5: Spearman rank correlation map showing possible relationships among all of the investigated variables(The blue colour corresponds to a correlation close to -1 and the red colour corresponds to a correlation close to 1. Green corresponds to a correlation close to 0 and white shows no correlation)

Principal component analysis (PCA) was carried out to identify clusters of sites with similar surface sediment (Fig. 6). The two-dimensional PCA ordination accounts for 92.1% of the total variance in the data indicating that the sampling sites can be satisfactorily grouped. The PCA results showed that the first component of the ordination (PC1) explained 82.7% of the total variance and presented a strong positive correlation with percentage of silt, clay, total protein, total carbohydrate, total lipid and biopolymeric carbon. The second component (PC2) explained 9.5% of the variance and was positively correlated with the percentage of clay, bottom water temperature and dissolved oxygen but negatively correlated with silt and sand. A clear cluster of sampling sites was observed with high organically enriched sediments in the outer shelf of Bay of Bengal, characterized by elevated concentrations of PRT, CHO, LIP and BPC. In contrast, the stations located in the inner shelf of the Bay showed lower organic content as indicated by PRT, CHO, LIP and BPC. The PCA analysis demonstrated that the sediment characteristics differ between the three regions in the study area.

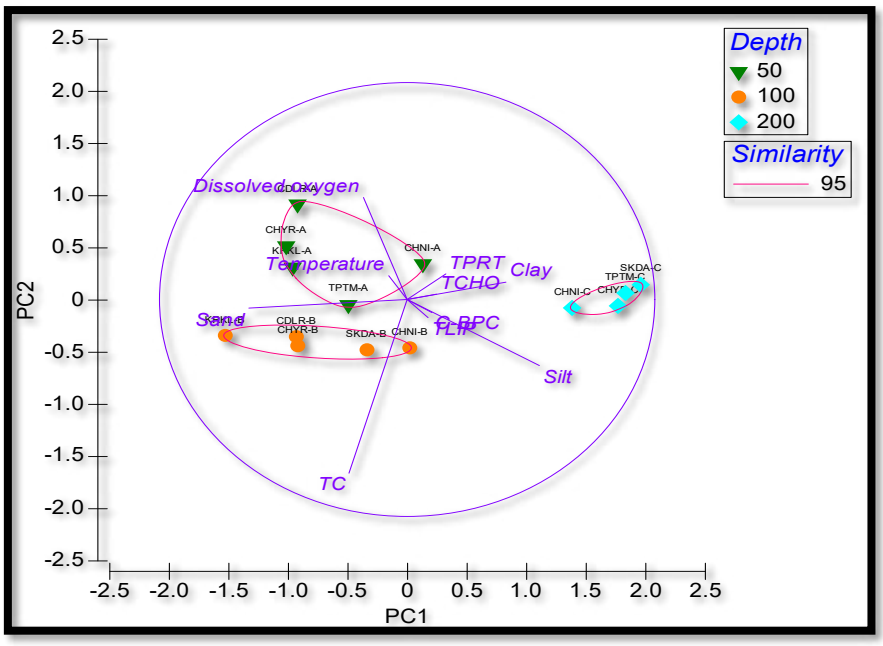

Fig. 6: Principal components analysis plot considering sediment characteristics and biochemical composition of the sampling stations

All sediment organic variables underwent a multivariate analysis for identifying the differences of these variables between transects. The result of MDS analysis clearly demarcates the northern latitude from the southern (Fig. 7). To demonstrate the trophic status of the continental shelf sediments of Bay of Bengal, cluster analysis was performed considering only protein and carbohydrate concentrations. The cluster identified the inner shelf as oligotrophic and outer shelf as eutrophic regions (Fig. 8).

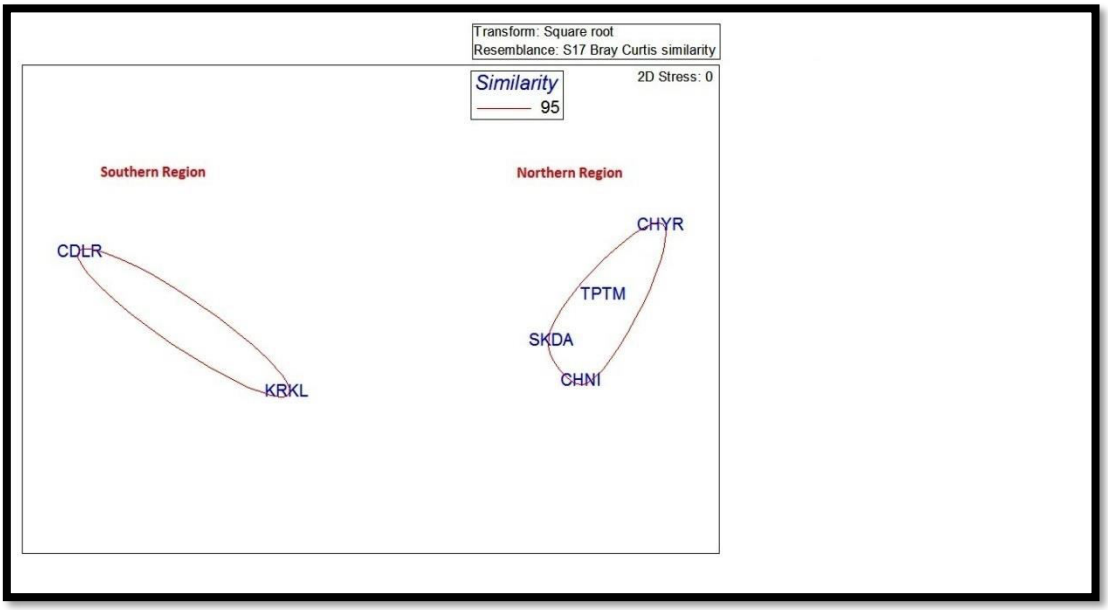

Fig. 7: MDS based on protein, carbohydrate, lipid, biopolymeric carbon and total organic carbon in the shelf sediments establishing the regional demarcation

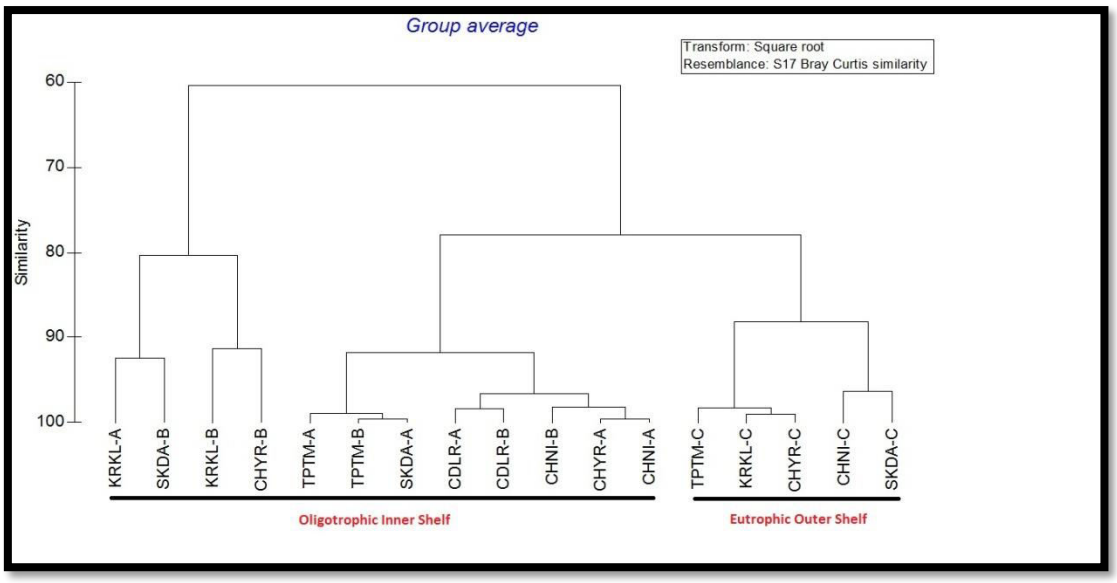

Fig. 8: Cluster analysis based on sediment protein and carbohydrate concentration classifying the shelf sediments of south east coast of India into oligotrophic inner shelf and eutrophic outer shelf

4. Discussion

Marine sediments function as a ''recorder'' of water column processes (Graf, 1992). Organic matter produced in the euphotic zone sinks through the water column and eventually reaches the ocean floor and forms an essential ecological link between the surface waters and the benthic ecosystem (Tselepides et al., 2000). Investigations on the quantity and quality of organic matter supplied to the bottom can be used as an alternative approach to assess the trophic status of benthic ecosystems (Dell Anno et al., 2002).

In our study the sedimentary organic matter concentration and its biochemical composition varied greatly between stations. This variation depends mainly on the grain size of the sediment. Organic matter was found to be maximum at 200 m depth where the sediment nature was clayey silt and minimum at 50 and 100 m where it was sandy. There is a general assumption that the organic contents in the sandy sediments were 1–2 orders of magnitude lower than those of muddy sediments (Rusch et al., 2003). Concentration of organic matter (18.54 ± 6.7 mg/g) present in the shelf sediment was comparable to that obtained from its earlier workers (Jacob et al., 2008). Estimation of quantity of organic matter alone cannot determine the nutritional status of the shelf. Due to the conservative composition of the sedimentary organic matter, variations in the trophic state of the sediments are more evident in terms of organic matter composition (proteins, carbohydrates and lipids) rather than quantity (Tselepides et al., 2000).

Sedimentary variables such as protein, carbohydrate and lipid displayed increasing values when moving from the inner to the outer shelf. Such variances indicate that different depth regionsare characterised by different organic loads which point to an assumption that the composition and concentration of

sedimentary organic matter are important indicators of the trophic state of the marine environments (Fabiano et al., 1995). Due to the high terrigenous input, the organic carbon flux to Bay of Bengal is much reduced (Ittekkot et al., 1991) which leads to the lower concentration of organic matter along the shelf sediments. Bay of Bengal is characterised by a narrow shelf, thereby permitting the nutrients that drains from the top to get lost to the deep. This leads to the increase of organic matter, particularly labile fractions in the outer shelf.

The concentration of sedimentary proteins is a key parameter to define the trophic characteristics of the system (Danovaro et al., 1999). Our study agrees this concept identifying that protein concentration alone demonstrates a clear difference in the trophic state of the benthic system. Protein concentration in the inner shelf was < 1.5 mg/g, which displays it as oligotrophic, whereas, outer shelf contains a protein concentration $\geq$ 1.5 mg/g identifying it as eutrophic. As stated by Dell Anno et al., (2002) oligotrophic system is characterized by a rapid exploitation of organic nitrogen and thus result in low PRT: CHO ratio (< 1) together with low BPC values and eutrophic systems are characterized by high nitrogen accumulation rates, high PRT:CHO ratio (> 1) and high BPC in the sediment. In the present study, PRT: CHO ratio in the inner shelf was < 1and outer shelf it was > 1. These results support the hypothesis of Fabiano et al., (1995) of a decrease in the PRT: CHO ratio moving from eutrophic to oligotrophic environments. PRT: CHO can be used as an index to determine the origin of material present in sediments and to determine the age of sedimentary organic matter (Danovaro et al., 1993). PRT: CHO ratio > 1 indicates living organic matter or 'newly generated' organic matter formed after the deposition of freshly produced phytoplankton (Pusceddu et al., 2003) or microphytobenthic bloom (Fabiano et al., 1995). On the other hand, low PRT: CHO ratios suggest the presence of aged or more degraded organic matter (Danovaro et al., 1993). Moreover, sediments with PRT: CHO ratios <1 are considered to be representative of nitrogen deficiency (Mayzaud et al., 1989), as witnessed from the low protein nitrogen values along the inner shelf.

Concentration of carbohydrate present in the inner shelf sediments was comparatively higher than protein suggesting that carbohydrate accumulation in oligotrophic environments is not unusual (Danovaro et al., 1993).This component may be largely composed of refractory compounds which are characterised by low degradation rates. Therefore, carbohydrate seems to behave as a reservoir of non-utilised organic carbon in oligotrophic sediments (Danovaro et al., 1999).

Lipids formed the main biochemical component in the shelf sediments. This is in contrast with most of the marine ecosystems where protein or carbohydrate dominate. The higher lipid content in this region may be ascribed by the flux of phytoplankton (Neira et al., 2001). Diatoms and faecal pellets of zooplankton are assumed to be important carriers of lipids to marine sediments (Baldi et al., 2010).The lipid content and lipid to carbohydrate ratio (LPD:CHO) have been used as good indices to describe the energetic (food) quality of the organic

contents in the sediments (Gremare et al., 2002). LIP: CHO ratio ranged from 3.52 to 11.11and these higher values are characterized by organic matter with a high energy value. The energy content per unit carbon of LPD is 1.4 times higher than that of CHO and 1.2 times higher than that of PRT (Salonen et al., 1976).

Biopolymeric carbon (BPC) another relative measure of potentially available food (Dell'Anno et al., 2000), displayed higher values along the outer shelf sediments. As specified by Vezzulli and Fabiano (2006) this was also evidenced in terms of biopolymeric composition of sedimentary organic matter as an increase in protein and a decrease in the carbohydrate contributions to total BPC. Higher lipid concentration in the shelf sediments results in larger amounts of BPC and, consequently, increased its contribution to TOC. The contribution of BPC to TOC was an average of 62.64 ± 12.32 %, confirming that a less fraction of TOC is represented by refractory material. The high contribution of BPC to TOC further suggests that the origin of TOC is autochthonous and that almost the entire organic carbon pool was represented by food material (Danovaro et al., 2000).

Stoichiometric ratios of carbon and nitrogen (C/N) can be utilised to determine the origin and transformation of organic matter based on the generalisation that organic matter derived from fresh living phytoplankton and bacteria is 7 and 4, respectively (Bale and Morris, 1998; Bates et al., 2005), while for the terrestrial organic matter, it varied from20 to 200 (Hedges et al 1986). Low values of C/N (< 10) ratio indicate the presence of relatively fresh and easily degradable organic matter of high nutritional quality mainly derived from phytoplankton whilst high C/N ratio (> 10) indicates the presence of more refractory organic matter of continental origin (Koster and Meyer-Reil, 2001). In the present study average C/N ratio at 200m depth regions were 9.64 ± 0.92 denoting the presence of high nutritional quality organic matter in the outer shelf sediments. Higher C/N ratios in the inner shelf suggest the presence of either the degraded organic matter or the influence of terrestrial organic matter. If, it was the latter then we would have expected higher concentrations of carbohydrates since terrestrial material is rich in cellulose. However, we did not observe high yields of carbohydrates at these stations indicating that the high C/N ratios were due to the presence of degraded organic matter (Khodse et al., 2007).

The concentration and relative importance of the main biochemical classes of organic compounds in the sediments vary in relation to the productivity of the system (Danovaro et al., 2000). The primary productivity in Bay of Bengal is relatively low due to narrow continental shelf and heavy cloud cover combined with high quantity of terrigenous organic matter which affects light penetration (Madhupratap et al., 2001). Conventional classification of the trophic status of water bodies based on the availability of inorganic nutrients, oxygen, and phytoplankton biomass the Bay of Bengal can be considered relatively oligotrophic (Prasanna Kumar et al., 2007). The comparison of the results obtained during the present study with those based on water column variables might disagree. Though the water column was relatively oligotrophic, the study

based on benthic biochemical parameters revealed that sediments vary from oligotrophic inner shelf to eutrophic outer shelf. Contrasting trophic conditions were also found in the Apulian coast (Mediterranean Sea, Dell Anno et al., 2002) and Marsala lagoon (Pusceddu et al., 2003). This difference in trophic status alters the functioning of benthic compartments and in turn the whole ecosystem (Venturini et al., 2011).

To provide an improved approach for the conceptual definition of trophic state in marine coastal ecosystems, Vezzulli and Fabiano (2006) demonstrated how density of microbial assemblages altered with changing nutritional status. This study confirms their findings asthe number of microorganisms in the shelf sediments varies with its trophic status. We used ATP as a global measure for calculating living microbial biomass (Karl, 1995) as exocellular ATP has a half-life of less than 1 hr (Contin et al., 2001). ATP obtained from the eutrophic sites was higher than that from oligotrophic sites. It has been demonstrated that the availability of organic material to microbial assemblages is one of the key parameters for the distribution of microbial biomass in the sediments (Boetius et al., 1996). The different organic matter concentrations at the two sites were reflected by ATP concentrations which displayed significant differences between sites.

The total microbial carbon biomass incorporated in the shelf sediment of Bay of Bengal was an average of 111.31 ± 54.18 μg C/g. Bacteria alone contributed about 50.05% of the total micro benthic biomass and was analogous to that found in the Yellow Sea (Meng et al., 2011). Previous studies have indicated that marine sediments support a remarkably constant bacterial density (approximately 10^9 cells/g), regardless of ocean depth (Schmidt et al., 1998).Total bacterial count in the shelf sediment ranged from 7.07 x 10^8 to 1.44 x 10^9 cells/g dry weight. As benthic biomass of the shelf sediments was dominated more by bacteria than other micro benthos, it is reasonable to assume that benthic microbial loop which controls the biological dynamics of Bay of Bengal shelf sediments, mostly hinge on the sediment bacteria.

It is the quantity and quality of the organic matter that reaching the seabed (Dugan et al., 2003) determines the distribution, composition and abundance of benthic biomass (Pusceddu et al., 2011). Vezzulii & Fabiano (2006) opined that heterotrophic bacterial population displays peculiar biological organization of marine ecosystems within distinct levels of its nutritional status. Dynamic sediment characteristics prevalent in the shelf region profoundly influenced the resident bacterial population. Recent studies have found a significant relationship between bacterial distribution and the concentrations of labile organic compounds, which are used as a measure of the bioavailable organic fraction (Danovaro et al., 1993; 2000).Benthic community abundance and diversity are positively correlated with protein (PRT), carbohydrate (CHO) and lipid (LPD) concentrations in the sediment (Fabiano and Danovaro, 1999; Albertelli et al., 1999; Dell'Anno et al., 2000; Medernach et al., 2001; Neira et al., 2001; Gremare

et al., 2002). Proteins can be rapidly utilized by benthic organisms (Burdige and Martens, 1988) due to the high nitrogen conversion efficiency of bacteria (Newell and Field, 1983). Bacterial density was positively correlated with protein concentrations, indicating a clear response to the accumulation of labile organic compounds in the sediments. When compared to oligotrophic inner shelf bacterial density was higher in eutrophic outer shelf. In our study low protein concentration observed in oligotrophic inner shelf may be due to the consumption of protein by benthic organisms and low protein concentration in turn reflect a still low organic matter input to the seafloor (Sane et al., 2012). It can be thus summarized that oligotrophic sites are assumed to be characterised by low food supply, compared to eutrophic sites where food supplied to the benthos is regarded as abundant. More over very low dissolved oxygen (data not shown) present in the outer shelf cause reduction in predation pressure and increases the availability of food to benthic bacteria (Neira et al., 2001). Bacterial contribution to organic carbon pool (BBM/BPC) was generally very low ranging from 0.45 to 1.26 %. Comparable observation was made by Danovaro et al. (1999) and they concluded that organic matter accumulations in such areas arenot the result of a bacterial 'protein enrichment' and that the entire pool may perhaps composed of detrital proteins.

From the study of sediment biochemical and microbial variables it can be concluded that Bay of Bengal which is considered as strictly oligotrophic can vary from oligotrophic to eutrophic conditions. Inner most shelf sediment shows its oligotrophic nature but as we move deeper it changes to more eutrophic environment. Increasing organic matter availability, greater ATP concentration and significantly higher microbial biomass further adds on to this perception.

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