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Vertical distribution of microphytobenthos in Cochin estuary

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Abstract

Microphytobenthos or benthic microalgae are recognised as the dominant flora in the littoral zone, estuaries and other shallow aquatic ecosystems. A preliminary study on the vertical distribution of microphytobenthos was carried out for a period of 13 months (2005-2006) at a selected station in Cochin estuary. Their distribution, biomass as chlorophyll *a* and community structure were investigated. Microphytobenthos included 27 species of diatoms and 5 species of blue-green algae. Pennate diatom, *Amphora turgida* Gregory, is a new distributional record from India. Both qualitative and quantitative distributions of flora along the vertical zone upto a depth of 5 cm were studied. During monsoon months, benthic chlorophyll *a* was lower than that of the planktonic counter part and was higher during late postmonsoon and premonsoon. The planktonic and benthic chlorophyll *a* showed lowest concentrations during June-July. The ratio of phaeopigment to chlorophyll *a* was found to be < 0.3 indicating an actively growing microflora with very low grazing pressure.

Keywords: Microphytobenthos, vertical distribution, Cochin estuary

Introduction

Planktonic microalgae form the major primary producers in any aquatic ecosystem and serve as the major synthesizers of primary organic matter in the pelagic zone. However, microphytobenthos are the dominant flora in the littoral zone and estuaries and are recognized as important primary producers in all the shallow aquatic ecosystems. Their biomass and production can equal or exceed those of phytoplankton in shallow waters (McIntyre *et al.*, 1996; Miller *et al.*, 1996; Cahoon, 1999; Underwood and Kromkamp, 1999). They have a significant role in the carbon budget of coastal ecosystems and can account for a substantial part (>30%) of the total primary productivity (Pinckney and Zingmark, 1993). Re-suspension of microphytobenthos serves as transient constituents of phytoplankton and contributes upto 60% to the total water column biomass (Lucas *et al.*, 2000).

Light penetrates upto a depth of 2-3 mm into the substrate and hence most benthic microalgae are found in top few millimeters of sediment (McIntyre *et al*., 1996). However, viable microalgal cells are observed in substantial numbers down to a depth of 10 cm or more in the sediment (Cahoon, 1999; Mitbavkar and Anil, 2002). The occurrence of photosynthetic microflora deep in the sediment has been attributed mainly to bioturbation by deposit feeders, migration, or physical hydrodynamic conditions (Pinckney and Zingmark, 1993; Barranguet *et al*., 1997). These microalgae have the capacity to survive at low irradiance and even in darkness without any damage to their photosynthetic potential. Diatoms can retain photosynthetic capacity in the dark, deeper sediments and can resume photosynthesis if resurfaced (Fielding *et al*., 1988).

Benthic microalgae of the marine and brackishwater habitats belong to various taxonomic classes such as Chlorophyceae, Bacillariophyceae, Haptophyceae, Dinophyceae and Cyanophyceae. Among the taxonomically diversified group of benthic microalgae, the dominant species are of diatoms (McIntyre *et al*., 1996). Though benthic environment exhibits substratal diversity, the present study was confined to a silt/mud substratum.

The biomass and productivity of benthic microalgae have been studied in several intertidal and estuarine habitats worldwide. Benthic microalgal biomass is always measured and expressed in terms of chlorophyll *a* concentration (Cahoon, 1999). Community structure of microphytobenthos of upper few millimeters in Cochin backwaters has been studied earlier (Sivadasan and Joseph, 1998). Information on the vertical distribution of microphytobenthos in the sediments is scarce. This study elucidates the temporal patterns in the vertical distribution of benthic microalgal distribution and biomass upto a sediment depth of 5 cm at a fixed station in Cochin estuary.

Material and methods

The study was undertaken in an intertidal zone of Cochin estuary (09^{0}56'51" N lat., 76^{0}17'24" E long.). Monthly samples were collected in triplicates during the lowest low tide from February 2005 to February 2006. Microalgal biomass was estimated as chlorophyll *a* concentration. Samples of the upper 5 cm sediment were collected by using a glass hand-corer, with an inner diameter of 25 mm. These 5 cm corer samples were cut at every 1cm length with the help of a very thin stainless steel knife and each layer was put into screw-capped glass bottles containing 10 ml of 90% acetone and kept at -4^{0}C in dark. Pigments were extracted over a period of 24 hours and measured spectrophotometrically ('Hitachi' U-2001 UV visible spectrophotometer). Sediment/water chlorophyll and phaeopigment to chlorophyll *a* concentration were measured by standard methodologies (Lorenzen, 1967, Strickland and Parsons, 1972).

For qualitative and quantitative analysis, the samples were preserved in 3% formaldehyde solution. For qualitative estimation, each layer of sample was diluted (Delgado, 1989) with a known volume of filtered estuarine water and sub-samples analyzed with the help of Nikon Eclipse E 200 Light microscope. Lens-tissue (Eaton and Moss, 1966) separation technique was also employed for harvesting microphytobenthos. The quantification of microalgae was done using a Sedgewick-Rafter counting cell at 200X magnification.

Shannon Wiener diversity (*H'*), richness (H'_{max}) and evenness (H'/H'_{max}) indices were calculated to understand the community structure of microphytobenthos. Statistical software '*Kyplot'* was used to find out 2-way ANOVA and linear correlation.

Temperature, salinity and pH were measured *in situ*. Salinity of interstitial water was measured with the help of salinometer. 'Takemura' DM-13 pH meter was used to determine the soil pH. The microalgae were identified based on the standard keys (Desikachary, 1959; Hendey, 1964; Gopinathan, 1984; Jin Dexiang *et al*., 1985).

Results and Discussion

Sediment surface temperature ranged from 22^{0}C in June to 31^{0}C in February and August; salinity from 7 psu in June to 29 psu in January; and pH from 6.4 in July to 8 in March, August and January (Fig.1). In all, 31 species were identified of which Class Bacillariophyceae was represented by 27 species and Cyanophyceae with 4 species. The diatoms included 21 pennate and 6 centric species.

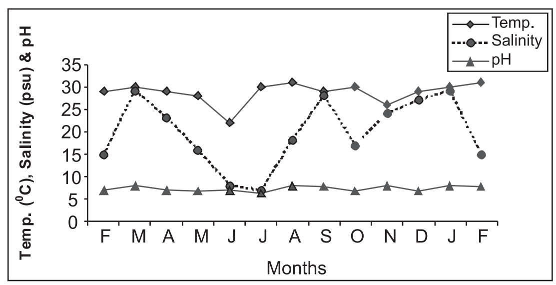

Fig. 1. Monthly variations of salinity, temperature and pH in the sediment during 2005-2006

The flora exhibited conspicuous monthly variation and no single species was found distributed in all the seasons in the entire strata. Pennate diatoms like *Amphora coffeaeformis*, *Cymbella marina, Achnanthes brevipes* and *A. coarctata* var. *parallela* were the most abundant species found in all the strata of sediment. The centric diatoms such as *Thalassiosira coramandeliana, Aulacodiscus orbiculatus, Biddulphia mobiliensis, B. pulchella* and *Coscinodiscus marginatus* were also represented in lower concentrations. Cyanophycean members such as *Merismopedia elegans, Chroococcus turgidus, Oscillatoria chalybea* and *Lyngbya* *aestuarii* were detected from isolated samples in small numbers. The stratum-wise analysis of diatom cell abundance has revealed that pennate diatoms invariably dominated the entire benthic strata and the dominant among these were pennate 'bi-raphid' species (Table 1).

The number of diatoms ranged from 35 to 169 cells/cm^3 on the upper 1cm^3 sediment in the first stratum, 23 to 102 cells/cm^3 in the second stratum, 11 to 56cells/cm^3, 8 to 41cells/cm^3 and 5 to 23cell/cm^3 respectively in the third, fourth and fifth strata of sediment (Table 1). During the premonsoon and

Table 1. Concentration of diatoms (class: Bacillariophyceae) (cells/cm3) in five vertical strata in the intertidal sediments in Cochin estuary during February 2005 - February 2006

Species	Feb-05					March					April					May					June					July				
	A*	B*	C*	D*	E*	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Achnanthes brevipes	5	2			3						5	3	3	1		12	4	4	1											
Achnanthes coarctata var. *parallela*						9	3	2	3		5		2													8	3	4	2	1
Amphora coffeaeformis	8	2	3	2		19	8	2			12	4	2	3	3	22	9	2	1		5	7	2							
Amphora turgida	18	6																								3		2	4	2
Aulacodiscus orbiculatus						12	3									6	3				3	2	1							
Biddulphia mobiliensis						3															6	13	5	2	2					
Biddulphia pulchella	2															6														
Caloneis permagna	6	9				12	9	3	2	3																				
Coscinodiscus marginatus																														
Cymbella marina	9	3	2			12	10	3	3		9	6				12	8	6	2	2						4	3	2	3	
Diploneis notabilis	12	7				5										30	13	3			2	8	3	2						
Diploneis weissflogii						8	2				19	12	10	3												8	6	6	10	5
Gyrosigma nodiferum	5	1		3							15	10	2			11	10	5	2	2										
Licmophora flabellata																5														
Navicula cincta			2	2																										
Navicula directa var. *remota*	6	2														13	5													
Navicula forcipata		2									12	10	1	2	2						5									
Navicula hennedyei	8	3	2		2	9	3	3	2	2																				
Navicula monilifera	9					7	3																							
Nitzschia closterium						10	7	3			23	15	4	2	2						6	2	2	2	3	2	3	3	3	
Nitzschia frustulum																15	10	2	3			7				8	6	7	9	3
Nitzschia longissima						5	2				12	7	3																	
Nitzschia panduriformis	2					9	2	1	2	5						12	6	4	3	2	5	3	2	1						
Pleurosigma aestuarii											8	7	5	2	2	8	3	4	1		8	14	3	2	2					
Podosira montagnei																					8	6				3	2	3	8	1
Surirella fastuosa	4	2	2	1		3		2	3																					
Thalassiosira coramandeliana	3																				8	2			1					
TOTAL (cells/cm^3)	**97**	**39**	**11**	**8**	**5**	**123**	**52**	**19**	**15**	**10**	**120**	**74**	**32**	**13**	**9**	**152**	**71**	**30**	**13**	**6**	**56**	**64**	**18**	**9**	**8**	**36**	**23**	**27**	**39**	**12**

Species	August					September					October					November					Decembe					January					Feb-06				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Achnanthes brevipes	3	2	2		2	5	4		5	2																12	3	6	6	3					
Achnanthes coarctata var. *parallela*																9	5									2		3							
Amphora coffeaeformis											19	13	6	5		18	16	5	3										23	18	12		4		
Amphora turgida	3	4	2	3		6	2	3	2												16	9	3	6	5						3				
Aulacodiscus orbiculatus																					3		2		2										
Biddulphia mobiliensis											10	4		5	5																				
Biddulphia pulchella																					7	3		6	2						4	5		6	4

Caloneis permagna																10		6	2	3	19	11	6	2											
Coscinodiscus marginatus						4	5	3	3	3																					12	9		9	3
Cymbella marina											13	11	12	4	2																				
Diploneis notabilis											31	17	12																						
Diploneis weissflogii	7	5	3	1																	22	15		3	4	#	21	5	8	3					
Gyrosigma nodiferum						6	2	3			6																								
Licmophora flabellata																																			
Navicula cincta						3			2																										
Navicula directa var. *remota*	4		2	1	2																														
Navicula forcipata											16	4		8		16	12	3	4																
Navicula hennedyei											13	6	4			11		4		6	19		15	4		8		5		3	15			5	1
Navicula monilifera						4	4	6	2	3	2		2	6																					
Nitzschia closterium																																			
Nitzschia frustulum	9	4	6	5																	18	3			5										
Nitzschia longissima											16	18	9	10	6																				
Nitzschia panduriformis							3	4		3	12	10	2			19	11	12	8	3	13	4	2		2	16	6	3	4		8	3	5	5	2
Pleurosigma aestuarii	6	5	4	3	4						13	10	6		3	10	10	6	6		20	11					2	3		3	17	11		5	
Podosira montagnei																16	12	6																	
Surirella fastuosa											18	9	3	3		12		7		4	10	2	8	3	3						9	15			2
Thalassiosira coramandeliana	3	3				8	3		2	2																					8	16			
TOTAL (cells/cm^3)	**35**	**23**	**19**	**13**	**8**	**36**	**23**	**19**	**16**	**13**	**169**	**102**	**56**	**41**	**16**	**121**	**66**	**49**	**23**	**16**	**147**	**58**	**36**	**24**	**23**	**76**	**32**	**25**	**18**	**12**	**99**	**77**	**17**	**30**	**16**

A*, B*, C* D* and E* denote the respective vertical stratum as 1, 2, 3, 4 and 5 cm

postmonsoon seasons, the microphytobenthos did not exhibit significant quantitative and qualitative variations, whereas during the monsoon, they were less in abundance. It may either be due to the surface washing out by land run-off to the intertidal zone or due to the unfavourable environmental condition caused by changes in salinity (Pillai *et al*., 1975). A two-way ANOVA has showed a significant interaction between abundance of cells in the sampling strata and months ($F_{4.64}$ = 3.24. $p < 0.05$).

Chlorophyll *a* concentration in the upper 1 cm sediment ranged from 2.6 to 86.3 µg/cm^3. In the second, third, fourth and fifth strata of sediment, it ranged from 1 to 30.8 µg/cm^3, 1.1 to 19.3 µg/cm^3, 0.76 to 16.9 µg/cm^3 and 0.26 to17.04 µg/cm^3, respectively (Fig. 2). Chlorophyll *b* concentration was lower while the concentration of chlorophyll *c* was comparatively higher and varied from 0 to 14.29 µg/cm^3 in the topmost layer, 0 to 10.2 µg/cm^3 in the second stratum, 0 to 10.3 µg/cm^3 in the third stratum, 0 to 8.7µg/cm^3 in the fourth and 0 to 10.3 µg/cm^3 in the fifth and last stratum of sediment (Fig. 2). The vertical distribution of benthic chlorophyll *a* in the sedimentary microhabitat was found to be influenced by the environmental variables and sedimentary characteristics besides the vertical migration (Lucas and Holligan, 1999). Southwest monsoon period was characterized by low chlorophyll *a* values indicating less standing crop of microalgae.

The depth at which the plants survive is termed as 'phytozone', which varies within the aquatic environment. A study along the Goa coast of India revealed that viable diatoms were present upto 15 cm depth (Mitbavkar and Anil, 2002). However, the depth of 'phytozone' observed in the study site was 5 cm. As no viable cells could be traced beyond this depth, the samples were taken only within this depth. The concentration of microphytobenthos was observed to be inversely proportional to the depth of the 'phytozone'. Vertical distribution of microphytobenthos, with the maximum values of chlorophyll *a* and cell abundance were observed in the topmost stratum, as also observed by several authors (Mitbavkar and Anil, 2002; Mundree *et al*., 2003). A two-way ANOVA showed a significant interaction between the chlorophyll *a* in the sampling strata and months of the samples collected ($F_{4.59}$ = 6.89; $p < 0.05$).

The surface water and surface sediment stratum chlorophyll *a* were analyzed to study the benthic-

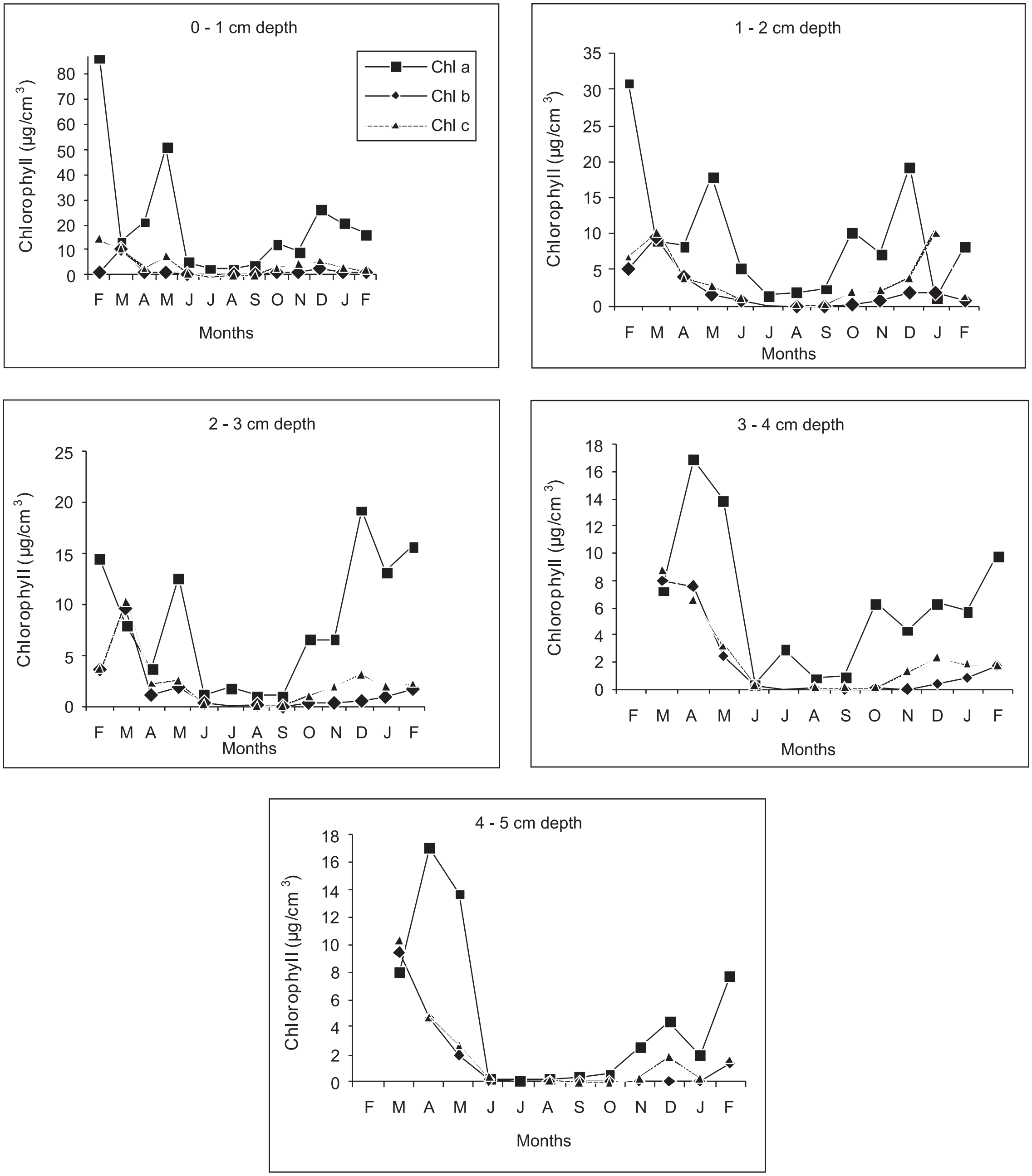

Fig. 2. Variations in chlorophyll *a*, *b* and *c* concentrations at different depth strata in the sediment

pelagic dynamics of microalgae. During the monsoon months, benthic chlorophyll *a* was lower than the planktonic counterpart, but was higher during the late postmonsoon and premonsoon. Overall, chlorophyll *a* from the two sources showed lower concentrations during June-July (Fig. 3). This is in agreement with the findings of Mitbavkar and Anil (2002, 2006), who found that diatom abundance

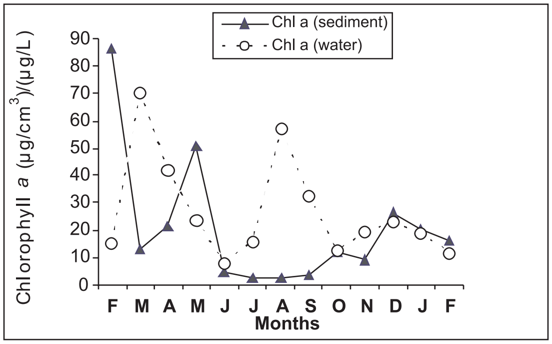

Fig. 3. Monthly variations of chlorophyll *a* concentration in sediment and surface water in Cochin estuary during February 2005 – February 2006

was the lowest during monsoon and higher during postmonsoon and early premonsoon seasons in the upper stratum of the sediment. However, most often, irrespective of seasons in coastal areas and estuaries benthic chlorophylls have had higher concentrations than the pelagic zone (Perissinotto *et al*., 2002, 2006).

The average chlorophyll *a* concentration in the upper 5 cm sediment ranged from 1.2 ($\pm$ 0.91) to 21.8 ($\pm$16.3) µg/cm^3. The average phaeopigment ranged from 0.29 ($\pm$0.11) to 3.59 ($\pm$ 3.4) µg/cm^3 (Fig. 4). Phaeopigment to chlorophyll *a* ratios were of low magnitude irrespective of strata during the entire period of analysis. The ratio of phaeopigment to chlorophyll *a* concentration gives a general indication of physiological or grazing state of microalgal communities (Shuman and Lorenzen, 1975). The low ratio (< 0.5) indicates an actively growing community of microalgae with relatively free of grazing pressure (Bidigare *et al*., 1986). The

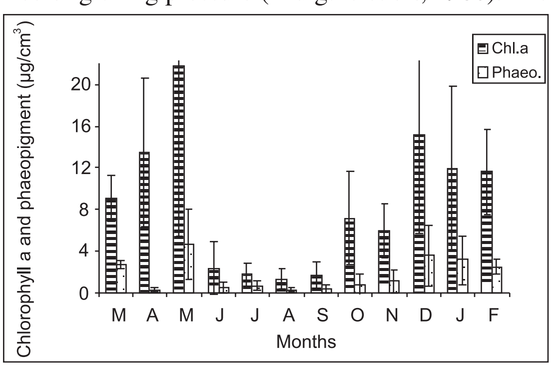

Fig. 4. Chlorophyll *a* and phaeopigment concentration (Mean + SD) in the upper 5 cm sediment column

ratio of < 0.3, derived from the present study is indicative of an actively growing community of microphytobenthos.

Fig. 5 shows the graphical representation of Diversity (*H'*), richness and evenness indices. The highest richness index (H'_{max}) of 2.84 was observed in the topmost stratum during February 2005 with 14 species and the lowest index of 0.92 in January, with only 5 species. The lowest richness of the entire season, 0.35, was observed in the third stratum during February 2006 with only two species. Generally it was observed that the maximum *H'* and maximum number of species coincided (Sivadasan and Joseph, 1998). The highest values were noticed in the upper stratum in all months. Evenness index (H'/H'_{max}) was detected without much variation in all the months and strata. The evenness index was 1 in the fifth stratum in May and January, when only 3 and 4 species were represented respectively and the cell numbers were the same. The lowest value observed was 0.71 in the second stratum in January, when 4 species were found and the cell numbers varied unevenly.

Most of the microphytobenthos remain buried in the sediments at a depth where no light is available for photosynthesis (Delgado, 1989). Survival of these species may be by mixotrophic/heterotrophic adaptations (Hellebust and Lewin, 1977). In shallow water ecosystems, the distinction between phytoplankton and phytobenthos is artificial, due to the exchange of living and non-living matters between sediment and water column. Under calm conditions, phytoplankton can move to the bottom and incorporate as microphytobenthos and *vice versa* (MacIntyre *et al*., 1996). However, Cahoon (1999) does not agree with this view. The study in Onslow Bay states that, on visual observation, concentration of microalgal biomass was only found at the top of sand ridges and not in the troughs, suggesting that these microalgae are firmly attached to the sediment. Pennate diatoms in sediment samples indicate that the benthic microalgae are distinct from planktonic microalgae, in which the centric diatoms, dinoflagellates and coccolithophores dominate. In this study, the pennate diatoms contributed about 67% to the total microalgae giving a strong support

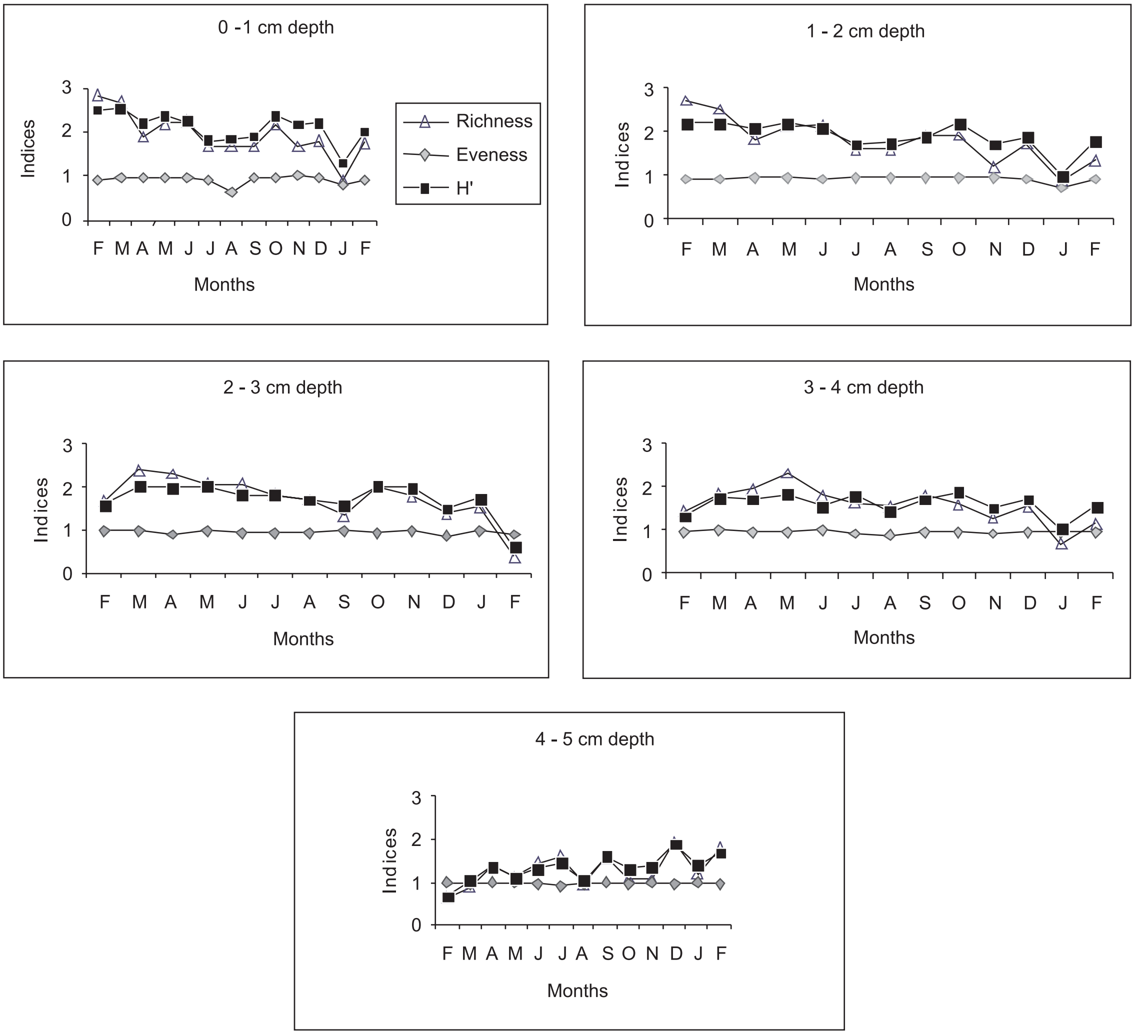

Fig. 5. Variations in Richness (s), Evenness (u) and Diversity (n) indices at different depth strata in the sediment

to the earlier findings and further clarifies that the benthic and planktonic microalgae are somewhat dissimilar. However, according to Pinckney and Lee (2008), the strict separation of microalgae into benthic or pelagic species is misleading and problematic at least for estuaries and coastal habitats as re-suspension and deposition processes effectively mix microalgae between sediment and water column. The vertical distribution of microphytobenthos showed a significant temporal variation. Heterotrophic species such as *Amphora coffeaeformis* and *Nitzschia frustulum* were found distributed in Cochin estuary. The pennate biraphid diatom species, *Amphora turgida* Gregory, recorded from Cochin estuary is a new distributional record for India.

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