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Biomarker pigment signatures in Cochin back water system – A tropical estuary south west coast of India

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ABSTRACT

Sedimentary biomarker pigments around Cochin estuary situated in the southwest coast of India were determined by HPLC. Fucoxanthin, an indicator of diatom was observed to be the most abundant carotenoid pigment in the estuary. Dinoflagellate derived carotenoid pigment peridinin was confined in the southern part of estuary and zeaxanthin pigment indicative of cyanobacteria were more found in sites influenced by anthropogenic activities. One compound having close similarity to fucoxanthin was also detected. Alloxanthin (cryptophyceae), chl b (green algae), canthaxanthin, neoxanthin, lutein and peridinin isomer were also detected by spectra and corresponding algal class were identified. The highest concentration of chl a (11.01 μg g^{-1}) found near to the anthropogenic affected area while the lowest chl a (0.65 μg g^{-1}) was recorded in industrial area. Degradation products of chl a, such as pheophorbide and pheophytin were observed and principal mode of mechanism of degradation were derived. Higher pheopigments content than chl a, reflects a density trapping of dead cells and early degradation of phytopigments from grazing activities.

1. Introduction

Estuaries are dynamic water bodies characterized by temporal changes occur over a spectrum of scales, ranging from short-term (hourly) driven primarily by tidal currents to long-term (seasonal or intra annual) caused by meteorological forcing or river discharge. In most tidal estuaries, the difficulty encountered in field observation is mainly due to the very variable stratification of water according to river flow and tidal coefficient. Therefore to distinguish and better understand the long-term development of estuarine ecosystems it is necessary to perform observations at different temporal scales. Some of the most direct indicators of estuarine development are the kinetics of carbon turnover and net primary production i.e. measurement of the preserved organic matter in sediments. (Wetzel, 1983; Dean, 1999). Earlier workers also reported (Bloesch et al., 1988) that about 10% of the estimated net primary production was deposited as organic carbon. Therefore, several studies in the marine system have looked for qualitative and quantitative relationships between the biomass abundances of the different phytoplankton class. Many methods of varying accuracy are available for phytoplankton analysis ranging from microscope to remote sensing.

Identification and enumeration of phytoplankton is usually done through microscopic examination which are mostly confined to micro (>2–200 μm) and nano (2–20 μm) plankton size. This procedure is time-consuming and also requires a high level expertise and taxonomic skill. Moreover, smaller organisms such as picoplankton (<2 μm) cannot be identified or counted with this approach. Conversely diagnostic biomarker pigment signatures can easily be studied to know the phytoplankton composition and their physiological status starting from old Paper Chromatography to spectrophotometric or fluorometric then recently by HPLC methods.

Recently these photosynthetic pigments markers have been used to a greater extent in oceanography for the quantification of the major taxonomic groups of phytoplankton and their degradation mechanisms (Barlow et al., 1997; Vidussi et al., 2001; Roy et al., 2006; Uitz et al., 2006) and individual carotenoids can be used as indicators of specific algae classes (Hodgson et al., 1997; Jeffrey et al., 1997). Indicator carotenoids include fucoxanthin (diatoms), diatoxanthin and diadinoxanthin (diatoms, dinoflagellates), alloxanthin (chryptophytes), lutein (green algae and higher plants), zeaxanthin (cyanobacteria) and peridinin synthesized by dinoflagellates (Johansen et al., 1974). Chlorophyll b (chl b) commonly ascribed to green algae while the β carotene and chlorophyll a (chl a) are more general indicators of total algal abundance.

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However, selective loss of pigments with different stabilities during deposition can affect the relative abundance of specific carotenoid pigments (Sanger, 1988; Hurley and Armstrong, 1990; Leavitt, 1993; Cuddington and Leavitt, 1999; Bianchi et al., 2000). Distribution of major taxonomically significant pigments across micro algal Divisions/Classes were given in the Table 1.

The pigment-derived classes defined here do not strictly refer to the true size of phytoplankton as can be the case for studies based on chlorophyll size fraction. HPLC based pigment analysis may be insufficient for understanding the finer scales of phytoplankton dynamics and cannot generally be used to make taxonomic distinction with in the class. Certainly no single methodology or technique is ideal for resolving all the information relevant to the structure and dynamics of a phytoplankton community. Appraisal of present literature reveals that very little information is available on these aspects in the back waters of south west coast of India. Considering these in view, the current article focused to study the distribution of fossil pigments and their taxonomy in surface sediments situated in the Cochin back water systems.

2. Materials and method

2.1. Study area

The study area located in the Cochin backwaters, along 9° 58′ N to 10° 10′ N and 76° 10′ E to 76°30′ E and forma multitudinal hydrographic system along the Kerala coast on the south west coast of India (Fig. 1).

The back water system covers an area of approximately 300 km^2 with one permanent bar mouth maintained at 12 m depth at Cochin and two seasonal openings during the peak monsoon period. The estuary is 16 km wide in the Vembanad lake area and there are several narrow canals along with those emptying municipal waste and other particulate organic matter into the estuary. Several major rivers Periyar, Muvattupuzha and Pampa discharge freshwater into the estuarine system. This estuary was classified as a tropical positive estuary, prone to strong tidal currents. The character of the estuary is also influenced by the adjoining rivers, altogether giving rise to seasonal and tidal fluctuations of hydrological conditions.

Station one (S1), (9′′ 58′ 084 N 76′′15′ 498 E) is near to Cochin port; Station two (S2), (9′′ 58′ 34 N 76′′ 16′ E) is Bolgatty Island in the middle of estuary 500 m away from the above site a popular tourist haunt. The station three (S3), (9′′ 57′ 387 N 76′′ 19′ 579 E) is in the Champakara canal in close proximity to a fish market which is highly polluted. The waste from the fish market is habitually drained into the canal. Moreover waste from Cochin Corporation sites and urban and domestic wastes also considerably affect the pollution status of the canal. Fourth station (S4), Cheranellur ferry (10′′ 04′ 350 N 76′′14′968 E) is also in the Periyar River. The ferry connects Cheranellur to Varapuzha and to Eloor. One of the largest industrial manufacturing centers, the Udyogmandal Industrial Estate, is located on the branch of the river periyar which passes to the north of Eloor, an island in the upper tidal reaches of the river. Industrial chemicals, leather and other goods are manufactured here. Many of the factories are located on the mainland, but several others are clustered on the north of the island, including FACT

Table 1
Distribution of major and taxonomically significant pigments across micro algal Divisions/Classes.

Pigments*		Cyanobacterial radiation		Green lineage			Red Lineage								
		Cyanophyta (Cyanobacteria)	Prochlorophyta	Chlorophyta	Prasinophyta	Euglenophyta	Rhodophyta	Cryptophyta	Bacillariophyta	Chrysophyta	Raphidophyta	Eustigmatophyta	Haptophyta	Dinophyta	Prymnesiophyceae
Chlorophylls															
Chlorophyll a	Chl a	■					■	■	■	■	■	■		■	
Chlorophyll b	Chl b			■	■	■									
	Chl c1								■		■				■
	Chl c2							■	■	■	■			■	■
	Chl c3									■					■
	MgDVP		T		■										
Carotene	[Caro]														
Old terminology	IUPAC														
α	β, ε		□	T	□			□				□			T
β	β, β	□	□	□	□	□			T	T	■	■		T	T
γ	β, ψ			T											
Xanthophylls															
Alloxanthin	[Allo]							■							
Antheraxanthin	[Anth]			T	T	T									
Astaxanthin	[Ast]			T											
But-fucoxanthin	[But-fuco]									■					■
Canthaxanthin	[Cantha]	T		T								T			
Crocoxanthin	[Cro]								T						
Diadinoxanthin	[Diadino]					■			■	■	■			■	■
Diatoxanthin	[Diato]					T			T	T	T			T	T
Dinoxanthin	[Dino]													□	
Fucoxanthin	[Fuco]						■		■	■			■		■
19′ Hex-fucoxanthin	[Hex-fuco]														■
Lutein	[Lut]			■	□										
9′-cis neoxanthin	[Neo]			■	■	T									
Peridinin	[Perid]													■	
Prasinoxanthin	[Pra]				■										
Violaxanthin	[Viola]			■	■							■			
Zeaxanthin	[Zea]	■	■	□			■					T			

* Code: ■ = major pigment (>10%); □ = minor pigment (1–10%); T = trace pigments (<1%) of the total Chlorophyll and Carotenoids, Jeffrey et al. (1997), Wright (2005).

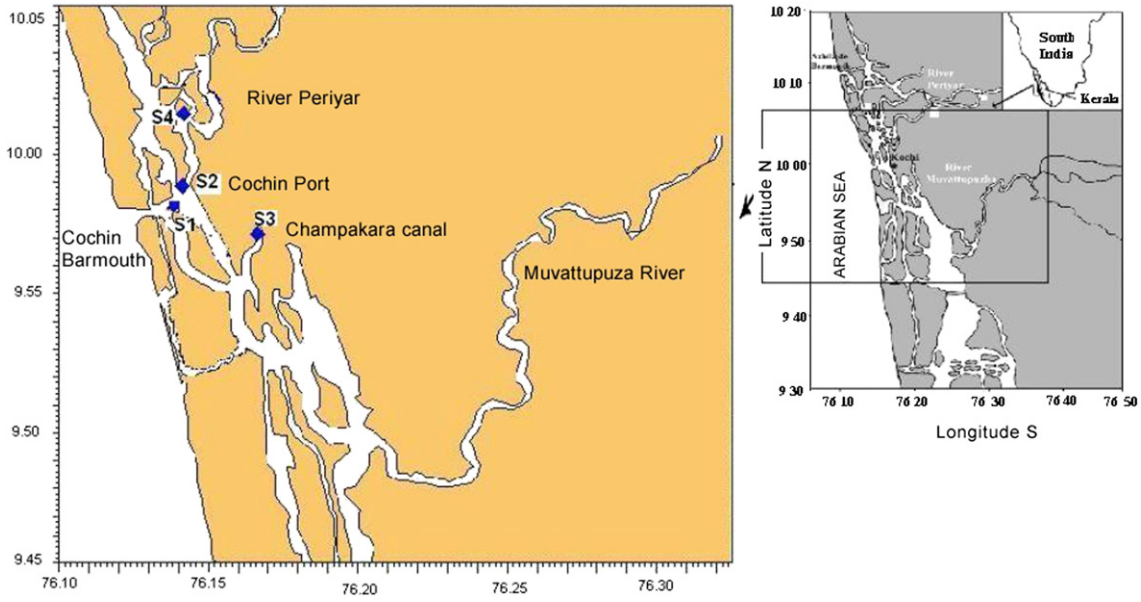

Fig. 1. Map of Cochin estuary showing sampling Sites.

(Fertilizers and Chemicals Travancore), IRE (India Rare Earths), and Merchem and HIL (Hindustan Insecticides Limited). Consequently this site is enormously putting up with the upshots of pollution.

2.2. Sample collection and preservation

Surface sediment samples were collected in peak summer (April 2007), using a stainless steel corer and stored in pre cleaned polyethylene bags for processing and transferred to the laboratory and preserved at 4 °C. All the sediment samples (one sample and a duplicate from each site) for pigment analysis were immediately transferred to 15 ml vials kept in ice bags in the dark on return to the laboratory, stored in a −70 °C freezer to render them more stable (Yacobi et al., 1990). These were finally transferred for preservation under −80 °C (SANIYO Ultra low MDFU-3086 maintained at −80 °C) and then freeze-dried (Viotis BENCHTOP-2K SI 213489 Lyophilizer) at −40 °C, 6–8 h at subdued light. All field and laboratory work was carried out in subdued light to minimize pigment degradation.

2.3. Analysis

HPLC analysis was carried out by DHI group Denmark as the HPL method (American Horn Point Laboratory) as reported by Hooker et al. (2005); NASA Technical Memorandum not separating α–β carotene as described below. The freeze-dried samples were homogenized prior to sub sampling. After weighing, (approx. 0.1 mg) each sub sample was extracted in 95% acetone with internal standard (vitamin E) sonicated in an ice cold sonication bath for 10 min, mixed on a vortex mixer allowed to extract at 4 °C for 20 h and vortexed again. Extracts were then filtered through 0.2 μm teflon syringe filters to remove cell and filter debris, transferred to HPLC vials and placed in the cooling rack of the HPLC. 357 μl buffer and 143 μl extract were injected on the HPLC (Shimadzu LC-10A HPLC System with LC solution software) using a pre treatment program. The detection wavelength was 420 and 450 nm and the flow rate was 12.5 μl min^{-1}.

2.4. Microscopic analysis of phytoplankton

For analyzing phytoplankton cell counts and composition, water samples were filtered through a phytoplankton net (20 μ mesh size) made of bolting silk. The filtrate was preserved in Lugol's iodine solution. A setting and siphoning procedure was followed to concentrate samples from 250 ml to 20 ml. For counting phytoplankton cells and identification of genera and species, the concentrated samples were thoroughly shaken and from each 1 ml replicates were transferred into a Sedgewick-Rafter plankton counting chamber and examined by using biological microscope (OLYMPUS; MLX) at 200× magnification. The whole slide (1000 fields) was counted for diatoms and dinoflagellates. Generic and species identification was done according to keys provided by earlier researchers. The planktonic micro algae filtered from 100 L of water was made up to a fixed volume concentrate. 1 ml of this sample was transferred to the sedge wick-Rafter counting cell (the volume of this chamber is 1 ml). The number of micro algae present in the cell 1000 grids was calculated. Repeated the counting for three times and took the average. The total number of planktonic algal species present in water sample was calculated using the formula,

$$N = m \times v/V$$

N = total number of phytoplankton cell per liter of water filtered; m = average number of phytoplankton cells in 1 ml of plankton sample; v = volume of plankton concentrate (ml); V = volume of total water filtered (L).

3. Results and discussion

3.1. Hydrological background

Kerala State is a strip of land with a coastline 560 km long and width varying from 11 to 124 km. About 16.40% and 54.17% of landforms are within 0–10 m and 10–300 m. Cochin Estuarine System (Fig. 1) is about 100 km long and 3–4 km wide and is a part of Vembanadu Lake, the largest estuary along the west coast of India. Two rivers, Periyar and Muvattupuzha discharge into the backwaters, whereas Thannermukkom bund (was constructed in 1974) regulates the flow from four rivers namely Meenachil, Manimala, Achankovil and Pamba and these total six rivers discharge about 20000 × 10^6 m^3 of freshwater into the estuary annually (Srinivas et al., 2003), which includes 104 million liters of

partially treated and untreated industrial effluents generated everyday by a large number of industries (Menon et al., 2000).

The Cochin harbor and its neighboring environment are natural and have a permanent connection (Cochin gut–tidal inlet) with the sea; houses the second largest port along the west coast of India. It has three dredged channels. All the dredged channels are maintained at a depth of 10–15 m. The climate is typical of tropical features yielding 60–65% of the total rainfall (≈300 cm) in monsoon (June to September), winter in post-monsoon (October–January) and summer at pre-monsoon (February–May). During the monsoon period, heavy rainfall results high river discharge which eventually reach the estuary through waterways of Cochin port. Stratification often develops and results in conditions with less dense river water at surface and high dense seawater at the bottom layers. The estuary is well-mixed and homogeneity exists in the water column and development of turbidity maxima during high tide within the estuary is very noticeable. The typical weather from March to May is hot (30–36 °C) and pleasant in December (22–25 °C). The seasonal wind direction is South West during monsoon and North East at post-monsoon and speed attain 45–55 km h^{-1} during such squally weather; also humidity is on the higher side (70–80%) due to naval influence. Such hydrographic features and circulation pattern complicate the sedimentation characteristics of these estuarine channels. The sedimentation features at the Cochin port vary according to season(s). The sediments are a mixture of clay and silt (70–85%) and sandy in the estuary. During pre and post-monsoon, sedimentation in the inner channels is minimum and maximum at monsoon and bed of approach channel rises to 1 m per annum (ICMAM PD, 2002). The tropical estuarine environment shows multitude features which characterize freshwater and seawater mixing and provides a breeding ground for marine organisms. The stations S1 and S2 are seaward end of the study area the estuary, salinity ranges between 0.1 and 34.64 (avg 16.8) while stations S3 and S4 are in back water where the salinity ranges from 0.02 to 13.97 (avg.2.13). The highest salinity was recorded at pre and post-monsoon and the salinity decreases with the onset of monsoon and became poorly freshwater in character. Nitrate varied between 0.44 and 2.96 at backwaters and 0.17–3.41 mg/L at estuary. Higher concentration were recorded during monsoon season while vertical mixing due to solar heating during post and pre-monsoon causes proliferation of phytoplankton leading rapid removal of nutrients from euphotic zone. Phosphate exhibits similar pattern as that of nitrate. Phosphate concentration varied between 0.05 and 4.41(backwaters) and 0.02–0.66(Estuary) mg/L. In the present investigation the high value of phosphate (1–4 mg/L) and Nitrate (0.4–2 mg/L) were recorded at Champakara canal (S3). Nitrite showed a different pattern compared to phosphate and nitrate, highest concentration were observed in pre-monsoon and lowest in peak monsoon. Spatially nitrite ranges from 0.01 to 0.43 (avg 0.16) at back water and ND – 0.60 (avg 0.18) mg/L estuarine zone. It is observed that region of high concentration of chlorophyll are characterized by the pockets of high anthropogenic discharge where high concentration of nutrient were observed.

Examination of existing literature on this area reveals that totally more than 700 species of flora and fauna comprising 65–194 species of phytoplankton, 135 species of zooplankton 199 species of benthos, 150 species of fishes and 7 species of mangroves were recorded between 1958 and 2002(ICMAM PD, 2002). Present and previous (Sreekumar and Joseph, 1995; Selveraj et al., 2003; Sanilkumar, 2009) phytoplankton analysis in the study area appended in the Table 2. The Present study reveals the presence of 43 species diatoms (Bacillariophyceae), 2 species chlorophyceae and 40 species of dinoflagellates at station S1. 42 species Diatoms (bacillariophyceae), 2 species chlorophyceae and 4 species of dinoflagellates at station S2 whereas 66 species of bacillariophyceae, 1 species of chlorophyceae, 4 species of dinofllagellates, 1 species of chrysophyceae and 1 stroptophyceae species were detected at champakara (S3) site. Station S4 Cheranellur, 52 species of Diatoms (bacillariophyceae) 2 species chlorophyceae and 6 species dinoflagellates were detected. The dominant species are *Cheatoceros*, *Amphiphora alata*, *Cosinodiscus marginatus*, *Biddulphia* sp, *Cyclotella striata*, *Skeletonema costatum*, *Thalassionema nitzschiodes*, *Thalassiosira* sp, *Heterocapsia*, *Pediastrum duplex*, *Nitzschia* sp. During pre-monsoon, the phytoplankton production in the estuary was high and fairly stable, with the dominant diatoms being *Chaetoceros*, *Coscinodiscus*, *S. costatum*, *Pleurosigma* and *Nitzchia* sp, and dinoflagellates of the genera Peridinium, Gymnodinium and Ceratium. During monsoon, the flora was mostly freshwater species of the genera Pledorina, Volvox, Pediastrum and Desmids. The abundance of species diversity of phytoplankton is varied in the estuarine and back water regions. Madhu et al. (2007) has inferred that the proliferation of diatoms or the 'Biological spring' falls during the monsoon months, when the diatom peaks coincide with low salinity and temperature, associated with high concentration of nutrients. From this data it is clear that the pico phytoplanktons and nano phytoplanktons are rarely detected by common microscopic methodology. This study attempted to find out the nano and pico phytoplankton in the surface sediment of these areas which are rarely detected by common microscopic method.

For more than thirty years oceanographers have been scrutinizing biomarker pigments in the sediments starting from Vallentyne (1954) to Uitz et al. (2006). These studies noted the universal presence of chlorophyll derivative and carotenoids in surface and older sediment material from corer. Chl a is a ubiquitous pigment and can be used as a global algal biomass indicator. Different degradation products (pheophorbide-a and pheophytin-a) from chl a also were characterized and are the derived degradation products by the demetallation of chl a associated to grazing activities were found in high concentrations essentially in the nutrient enriched areas (S3). The examination of possible pathways of degradation of

Table 2
The phytoplankton species determined form the study area by using the microscopic technique.

	Bacillariophyta	Chlorophyta	Chrysophyta	Dinophyta	Straptophyta	Year of study
S1	43	2	–	40	–	Present
S2	42	2	–	4	–	Present
S3	66	1	1	4	1	Present
S4	52	2	–	6	–	Present
	66	8		2	–	1995*
	58	–	–	2	–	2003†
	28	2		9		2009‡

* Sreekumar and Joseph (1995).
† Selveraj et al. (2003).
‡ Sanilkumar (2009).

chl a is usually limited by the success of these degradation pigment identification. Chl b was the next important pigment detected at low concentration. With the protocol followed, complete resolution and separation of α & β carotene could not be achieved and these pigments were therefore grouped together and treated as total carotenoids. Xanthophylls – alloxanthin, canthaxanthin, diatoxanthin, fucoxanthin, fuco like, neoxanthin, lutein, peridinin, peridinin isomer and zeaxanthin were detected (Fig. 2a and b).

3.2. Degradation products from chlorophyll pigments

Although most of the chlorophyll in healthy plant tissue is chl a, its degradation products may be relatively more abundant in sediments. Speculative pathways of degradation based on all the available portion of the plant detritus from both the plankton and benthos which are ultimately deposited on the bottom. They are observed to become a potential food source for bacteria, protozoa, and other benthic organisms whose feeding activities could possibly convert the chlorophyll to pheopigments (Currie, 1962). In the present scheme of research the degradation of chl a were evidenced by the detection of pheophytin a and pheophorbide a and the concentration of former were three times higher than later in all the studied stations, but pheophorbide a, was found below detectable level at station S4. Pheophorbide a and pheophytin a shows a remarkable increase in concentration of about three times to chl a. Pheophytin a is significantly (35.33 μg g^{-1}) present at station S3. S1 (2.33 μg g^{-1}), S2 (5.27 μg g^{-1}) and S4 (0.9 μg g^{-1}) shows about nearly 27% of chl a (Fig. 3). These results suggest that there was a shift in the dominant mode of chlorophyll degradation and earlier works (Kowalewska et al., 2004) support this scenario. One mechanism of degradation involving the loss of Mg^{2+} ions and $COOCH_3$, that is the formation of pyropheophytin a, was considered to be, but there is no evidence for the detection of pyropheophytin. In this contest a two step slow mechanism was proven, first the loss of Mg^{2+} ions resulting the formation of Pheophytin a and then phytol side chain resulting the formation of Pheophorbide a (Fig. 4).

This two step mechanism of chl a degradation is well established in the literatures (Scheer, 1991) and it involves the loss of Mg^{2+} ion

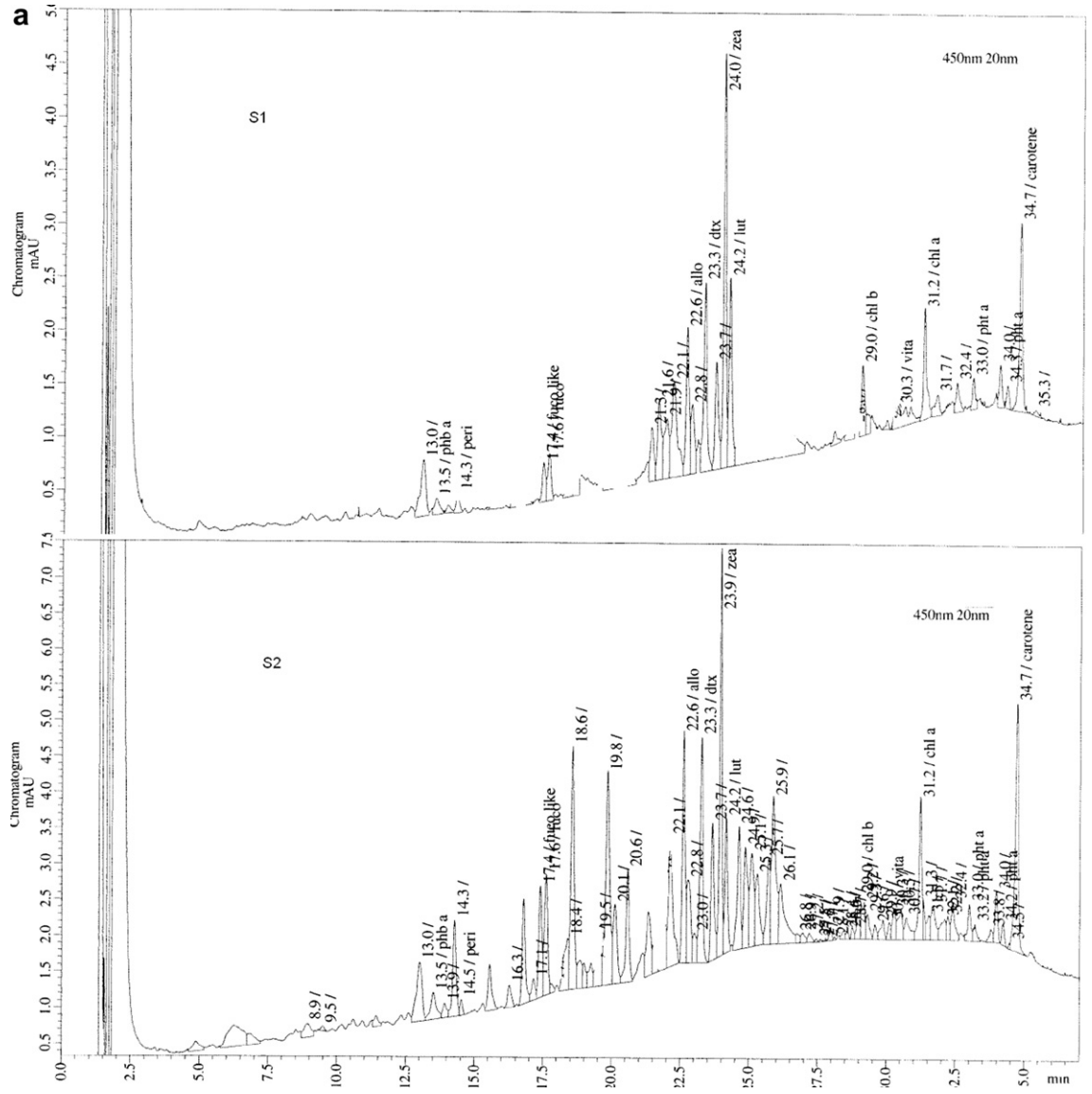

Fig. 2. a) Reverse phase HPLC spectra Absorbance (420 and 450 nm at stations S1, S2 from top to bottom). b) Reverse phase HPLC spectra Absorbance (420 and 450 nm at stations S3 and S4 from top to bottom).

from the tetrapyrrole ring (type 1 degradation, Brown et al., 1981) followed by the loss of phytol chain and various side groups. This could be further confirmed by the pheophytin a and pheophorbide a concentration i.e former is higher than later which indicates a more recent and advanced degradation state of the sediment. Since the pheophytin a will form first followed by the formation of pheophorbide a (Fig. 4). Very low concentration of pheophytin and below detectable level of pheophorbide at the station S4 indicates the low production and grazing activities in this station due to low light penetration owing to various anthropogenic activities.

3.3. Taxonomic pigments

Chromatographic analysis revealed the presence of a wide range of pigments exhibiting clear spatial variability. Identification was based on the retention time and peak shape, i.e. through fingerprint matching with known peak shape from the diode array spectral library which were created by running pure standard of individual pigments. The concentrations of the pigments were computed from the peak areas. Summary of sedimentary pigments recovered data; their taxonomic affinities and relative stability are given in the Table 3.

Distribution of chl a and its degradation products in the study area were given in the Fig. 3. chl a is maximum at Champakara station, S3 (11.01 μg g^{-1}); the next at estuarine site, S1 and S2 (0.95 and 1.69 μg g^{-1}) respectively. Low concentration was found at Cheranellur canal (S4) 0.65 μg g^{-1}. The high chl a concentration at S3 is the result of accumulation of fresh phytoplankton cells in the sediment, and could be explained by the proximity of the fish market

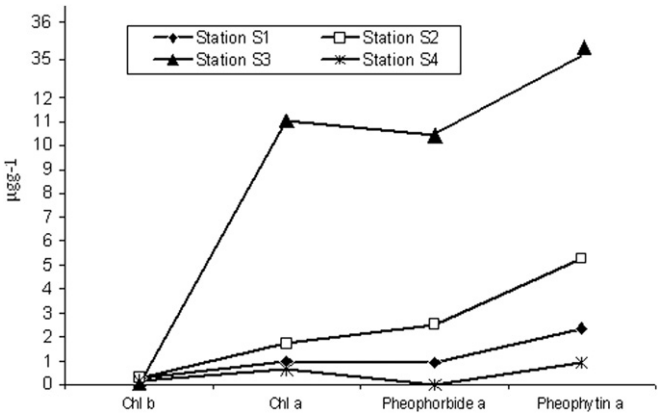

Fig. 3. Distribution of Chlorophylls and its degradation pigments.

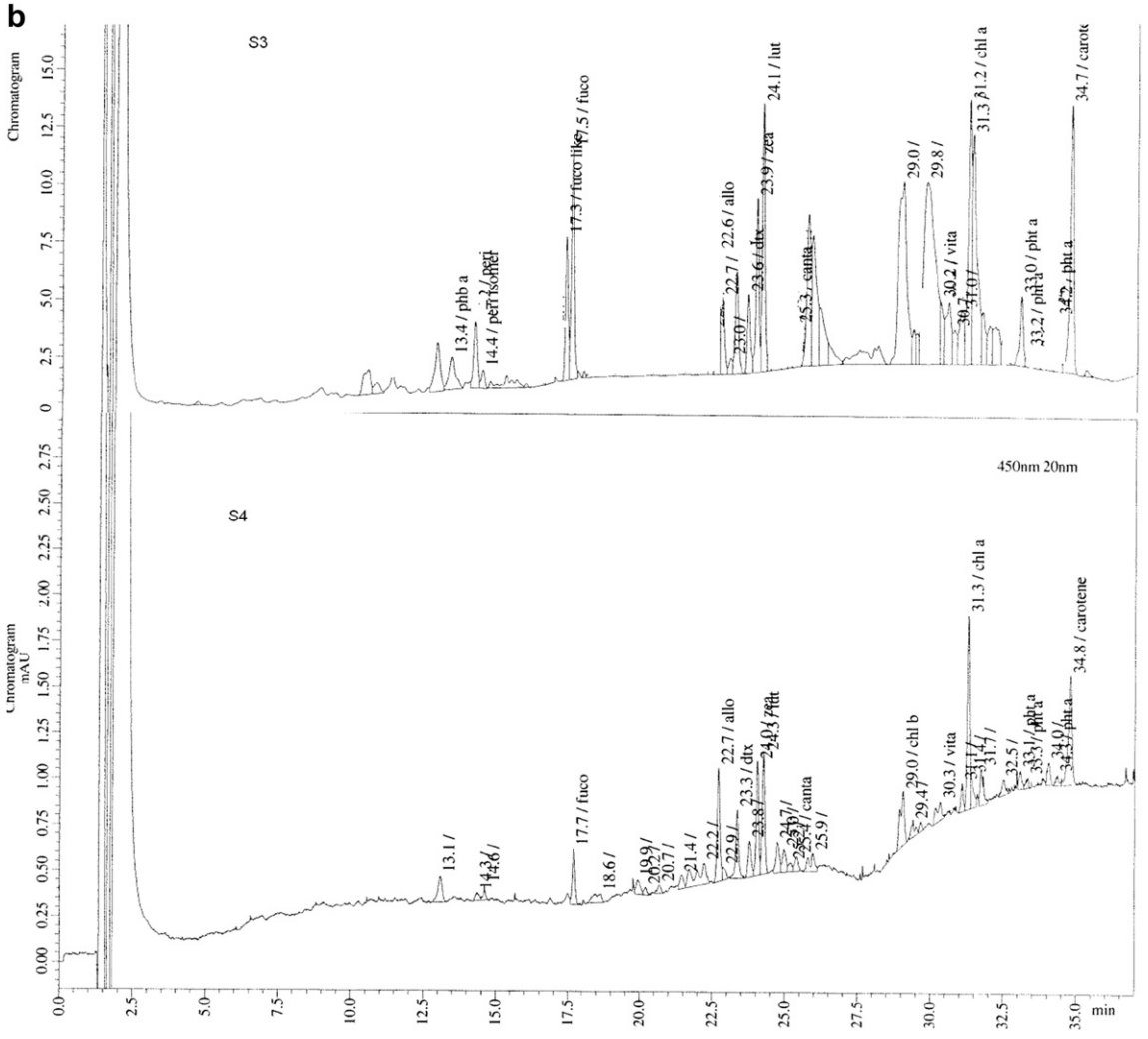

Fig. 2. (*continued*).

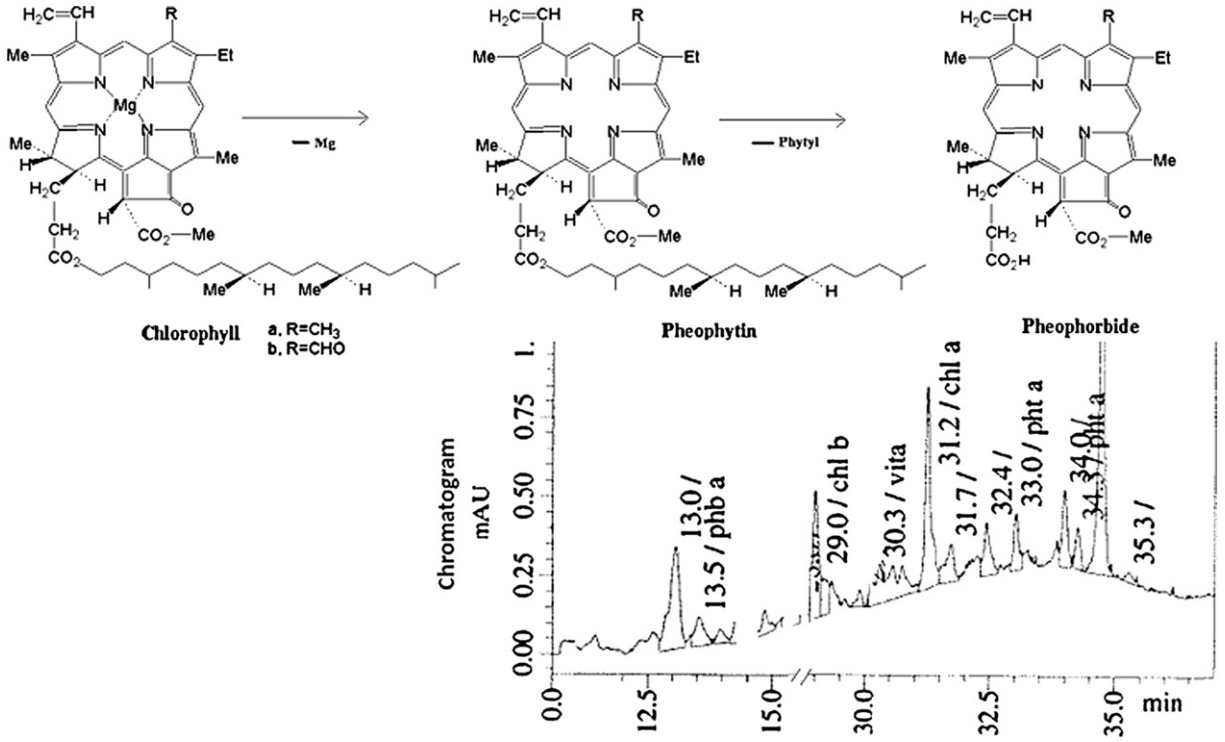

Fig. 4. Scheme of Chlorophyll a and b Transformation pathways.

where large nutrient introduction into the canal through the effluents from the harbor and sewage activities. Variations in evenness index are due to the influence of fluctuation in the hydrological characteristics of the estuarine environment and similar reports were acknowledged previously (Sreekumar and Joseph, 1998). The abundance in similarity of microflora between this area, S4 and rest of the estuary was low due to the effect of pollution.

Fucoxanthin or Fuco (Fig. 5), a tracer of diatoms, were found in high amounts at S3 (2.09 μg g^{-1}) similar to chl a. Insignificant concentrations were found in the station S4 (0.05 μg g^{-1}) while in the estuarine zone, 0.10 μg g^{-1} and 0.38 μg g^{-1} were reported at stations S1 and S2 respectively reflecting the wide distribution of diatoms. The photoprotecting zeaxanthin or Zea (Fig. 5), generally found in cyanobacteria and prochlorophytes was remarkably present in S1 (0.5 μg g^{-1}) and S2 (0.72 μg g^{-1}), and S3 (1.06 μg g^{-1}) stations. This zones are usually characterized by high and constant nutrient input but a reduction of cyanobacterial concentration were reported in the station S4 (0.07 μg g^{-1}) a nutrient deficient area.

Diatoxanthin typical pigments of diatoms and prymnesiophytes also showed same pattern as Fuco. Dinoflagellates derived marker pigment peridinin was 0.95 μg g^{-1} at S3 but very low concentration (0.06 μg g^{-1}) in estuarine station S1 & S2 and it was not detected at

Table 3
Summary of pigments recovered in the sediments during the study and their taxonomic affinities.

Sl	Pigments	Algal divisions	Stability*
1	Chl a	All photosynthetic Algae, Excluding Prochlorophyts	3
2	Chl b	Greenalgae, Euglenophyta, plants	2
3	Pheophorbide a	Grazing, Senescent diatoms	3
4	Pheophytin a	Chl a derivative (all)	1
	Carotenes		
5	α & β	Plants, Algae	2 & 1
	Xanthopylls		
6	Alloxanthin	Cryptophyta	1
7	Canthaxanthin	Cyanobacteria, Chlorophyta, Eustigmatophyta	1
8	Diatoxanthin	Bacillariophyta, Dinophyta, Chrysophyta	2
9	Fucoxanthin	Bacillariophyta, prymnesiophytes, Chrysophyta, Raphidophytes, several dinoflagellats	2
11	Fucoxanthin like	Chromophytes and nanoflagellates	
12	Peridinin isomer	–	
13	Lutein	Chlorophyta, Euglenophyta, Plantae	1
14	9′-cis neoxanthin	Prasinophyta, Chlorophyta, Euglenophyta	4
15	Peridinin	Dinoflagellates, Dinophyta	4
16	Zeaxanthin	Cyanobacteria (Cyanophyta)	1

* The relative degree of chemical stability is ranked from most (1) to least (4) stable, from Leavitt and Hodgson (2001). Pigments with least stability are rarely found in the sediment.

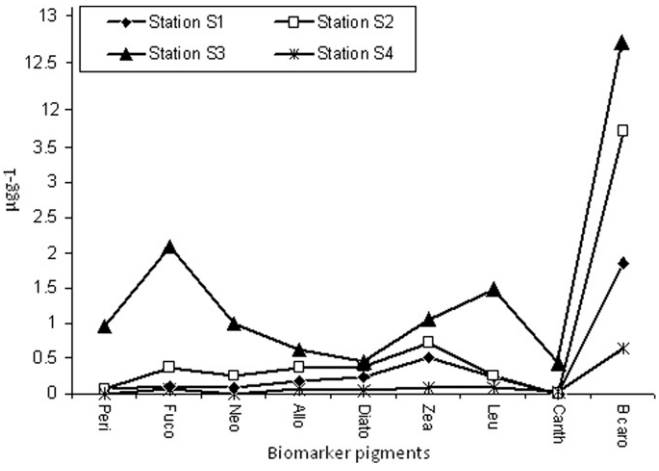

Fig. 5. Distribution of Marker pigments in the study area.

S4. This type of restricted distribution of dinoflagellates in estuarine environment was well described by Incze and Yentsch in 1981. The reason was observed to be due to prevailing salinity gradient. In addition to peridinin, peridinin isomer was also detected at station S3 only (Fig. 2b).

One compound that has a structure close to fucoxanthin (Fuco like) was detected and has a distinct distribution and was found only in the samples where peridinin was present (S1, S2 & S3) (Fig. 2a and b). Previous studies (Tangen and Bjornland, 1981; Wright and Jeffry, 1987) elucidates that dinoflagellates do not synthesize simultaneously peridinin and fucoxanthin like compounds. Similar observation was recorded by Denant et al. (1991) and assumed fuco like compound originates from species that are not dinoflagellates but those live in close association with them.

Associated with chl b, neoxanthin and lutein (typical indicators of chlorophytes and prasinophytes), were found in significant concentrations in the estuarine sediments. The presence of chl b, as an indicator of green algae is notable (Fig. 3) which is absent in the station S3 and very low concentration was reported at other stations. Lutein typical indicators of chlorophytes a green algae were present at all stations 0.5 $\mu g\ g^{-1}$ (S1), 0.72 $\mu g\ g^{-1}$ (S2) and 0.07 $\mu g\ g^{-1}$ (S4) $\mu g\ g^{-1}$ and 1.48 $\mu g\ g^{-1}$ at station S3 (Fig. 5) indicated that chl b is liable and undergoes rapid degradation. Lower or absence of chl b content along with some marker pigments at station S4 noted during the study supports the low contribution of green algae inferring an important localized contribution of green pico phytoplankton in coastal upwelling waters (S1, S2 and S3) (Fig. 5). Although diatoms (chl a) have been identified throughout the sediment core, chl c, which occur in all diatoms, were not observed in any of the extracts. Previous workers have also failed to detect chl c in sediments where diatom exist and have explained to a high chlorophyll a:c ratio in the diatoms (Vincent et al., 1993).

The ratio lutein to chl b, an indicator of chlorophytes and Type 1 prasinophytes (Leut: Chl b = 0.30–1.7 and 0–0.18; Wright, 2005) respectively were calculated and found at station S1, S2 and S4 with ratio 0.4, 0.8 and 0.84 respectively explains the presence of chlorophytes. Neoxanthin, an indicator of euglenophyta were not detected at S4 but it is distributed significantly in all other stations. Presence of alloxanthin, a carotenoid characteristic of strict planktonic cryptophytes appears to develop at all sites but is rare in the station S4. Over all a reduction of minor pigment was observed in the Cheranellur ferry (S4) indicating that decreased total primary production of phytoplankton (Lami et al., 2000). Moderate proliferations of phytoplankton production in the estuary and a eutrophic trend in Champakara site were observed due to high input of domestic and sewage wastes.

4. Conclusions

The Cochin estuary could be considered as a unique model for studying the chemotaxonomic compositions of sedimentary biomarker pigment signatures. Pigment concentration varied over a great range along all these stations studied. chl a distribution was found maximum at anthropogenic effected area and then at the estuary and then comes the industrial polluted area. These results suggest a marked influence of environment variability in the productivity and possible difference in exchange mechanism of nutrients across the different featured stations. The degradation product of chl a are found to be pheophytin a > pheophorbide a, indicating a recent and advanced situation of degradation of the sediment. The taxonomic pigments, fucoxanthin a marker of diatoms; zeaxanthin of cyanobacteria; alloxanthin of cryptomonads; peridinin of dinoflagellates and chl b of green algae, reflects specific distributions along the explored different sites which are having distinct aquatic character. Compounds with a fucoxanthin like structure showed good ally with peridinin evidences the potential presence of phytoplankton species which has close association with dinoflagellates. The association between the microscopically determined species diversity and HPLC determined species diversity was reasonably good. Some pigments that may exist in the estuary were not successfully isolated or identified including chl c, fucoxanthin derivatives and peridinin isomer. Further research is initiated to strengthen and evaluate the degree of pigment degradation and destruction in situ as well as to quantify how much breakdown occurs during core extrusion and handling analytical methodologies.

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