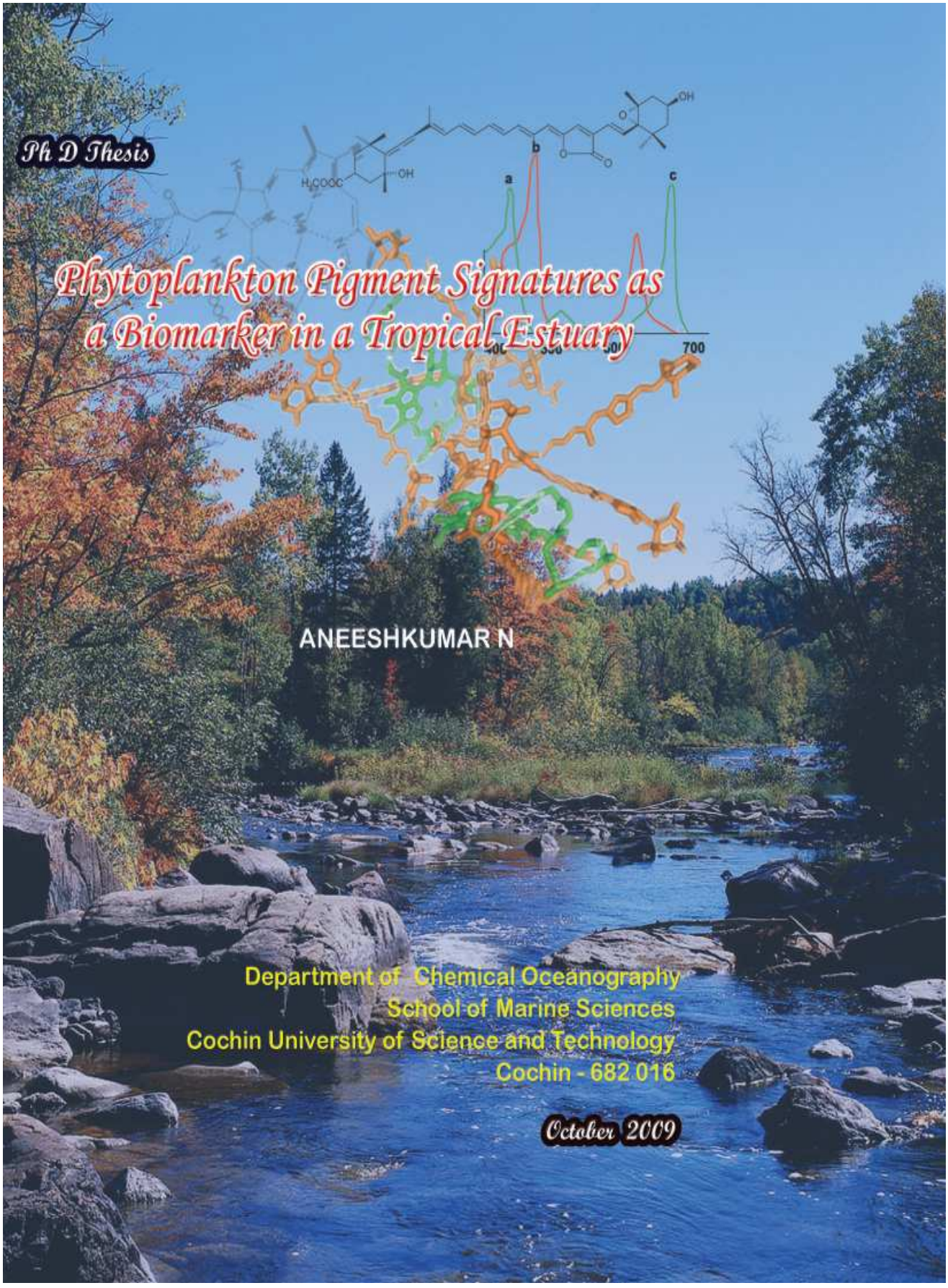
Ph D Thesis
Phytoplankton Pigment Signatures as a Biomarker in a Tropical Estuary
ANEESHKUMAR N
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Cochin - 682 016
October 2009

Dedicated to My Parents & Teachers

Dr. Sujatha C.H
Sr. Lecturer
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Certificate

This is to certify that this thesis titled, **"Phytoplankton Pigment Signatures as a Biomarker in a Tropical Estuary"** is an authentic record of the research work carried out by Mr. Aneeshkumar. N. under my supervision and guidance in the Department of Chemical Oceanography, Cochin University of Science and Technology, in partial fulfillment of the requirements for the Ph.D. Degree of Cochin University of Science and Technology under the Faculty of Marine Sciences and no part thereof has been presented for the award of any degree in any university/Institute.

Cochin–16
October 2009

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DECLARATION

I hereby declare that the thesis entitled, **"Phytoplankton Pigment Signatures as a Biomarker in a Tropical Estuary"** is an authentic record of research work carried out by me under the supervision and guidance of **Dr. Sujatha C.H**, Department of Chemical Oceanography, Cochin University of Science and Technology, in partial fulfillment of the requirements for the award of the Ph.D. Degree in the Faculty of Marine Sciences and no part thereof has been presented for the award of any other degree in any University/ Institute.

Kochi – 16 **Aneeshkumar. N.**

October 2009

There is a way that nature speaks that land speaks.
Most of the time we are simply not patient enough,
quiet enough, to pay attention to the story……

Linda A Hogan

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ANEESH KUMAR .N

PREFACE

We are in the cutting edge of a new era of development without leaving any promises to next generation. But the scale and size of the problem are only partially blamed. The juggernaut of Globalisation has trampled upon whatever little hope we might have had making a quick transition to a less energy – intensive world. "Environment friendliness begins at home". Our quest for productivity and profitability should progress simultaneous with our cooperative responsibility of leaving behind a clean and green earth for the generation to come. Climate change is the most pressing global environmental challenge being faced by humanity, with the quest for better productivity for our fragile ecosystem. It is too late to rely solely on reduction in Green house gas emissions to mitigate climate change although this is undoubtedly crucial. Coastal belts are more prone to these devastating impacts and its protection is an intensive filed of research. The present study describes how the colourful Carotenoids and Chlorophylls can be used in rapid hand on tool in conjunction with molecular biology to open sources and it also explores the fate of organic matter in the aquatic system and underlying sediments.

CONTENTS

Chapter 1

Introduction

The aquatic system comprises a great biodiversity including a variety of genes and biochemistries, hence a diverse array of organic compounds ranging from colourful carotenoids and chlorophylls with simple to complex structural molecules. Much of the chemical products and waste products of modern society are released in to the environment either during the production, storage, transport, use or ultimate disposal. These released materials participate in natural cycle and the reactions frequently lead to influence the disturbance of natural systems. Environmental chemistry is relatively young science which presents a reasonably uniform view of various aspects of chemistry of the environment and their processes. Characteristic feature of

today's environmental science is the need of skill and knowledge of the chemist, biologist and physical oceanographers to unravel the interactions between organisms in marine food webs and cycling of the major elements. A Multi- Marker approach is also necessary that make use of biomarkers, isotopes and DNA which are considered as ultimate biomarkers. Advancement in techniques involving Gas and Liquid Chromatograph linked to Mass spectra (GC-MS and HPLC-MS) and Continuous flow GC-irm-MS allowed to the identification of stable isotopes, which allow the measurements of more refractory compounds in the sea floor that may have been synthesized many (hundred to thousand) years previously.

The present biological productivity is of central importance to a world concerned about climate change. Phytoplanktons are recognized as the basis of all animal production in the open sea, supporting food webs upon which the world's fisheries are based. Nutrient enrichment due to natural or man made can result in damaging phytoplankton bloom which if toxic, can decimate not only wild fisheries but also inshore biota which causes illness or death of people who eat them. It is important to have an easy and rapid method to monitor these phytoplankton populations since the failure in the abundance and timing of algal blooms can lead to the collapse of whole year classes of fisheries.

The Phytoplankton derived from the words *phytons* (means plants) and *planktos* (means wandering) are the microscopic algae that makes up the floating pastures of the world's oceans. They

provide the food base which supports either directly or indirectly, the entire animal populations of the sea and they contribute significantly in climatic process. Their diversity is immense and ten thousands of species present at variety of forms. The best known are the exquisite silica walled diatoms (Bacillariopyta) which accounts for up to 1000 living species (Hostetter and Storemer., 1971; Margulis, 1990) and many more if fossil species are included. They are differentiated into three size classes:

- Microplankton 2-200µm
- Nanoplankton 2-20 µm
- Picoplankton 0.2-2 µm

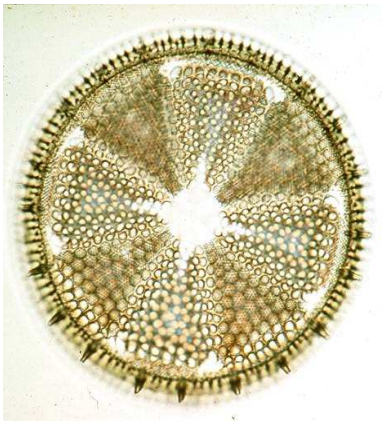
Phytoplankton identification and enumeration is usually done through microscopic examination. This procedure is time-consuming and also requires a high level of taxonomic skill. Moreover, smaller organisms such as picoplankton cannot be identified or counted with this approach. Alternatively, photosynthetic pigments can easily be studied to know the phytoplankton composition and their physiological status. Most of these pigments have chemotaxonomic association (Table 1.1). For example, fucoxanthin is considered to be a marker of diatoms; zeaxanthin of cyanobacteria; 19-hexanoyloxyfucoxanthin of Prymnesiophyceae; alloxanthin and crocoxanthin of Cryptomonads; prasinoxanthin of prasinophytes; peridinin and chlorophyll c_2 of dinoflagellates (Jeffrey and Mantoura., 1997). Identification of species composition in the field samples requires time and expertise, so routine analyses of phytoplankton are

usually limited to measurements of Chlorophyll biomass using a variety of techniques. Advances in pigment separation techniques now allow a great range of chlorophylls and carotenoids to be measured rapidly by chromatographic techniques to give chemotaxonomic signatures of algal type.

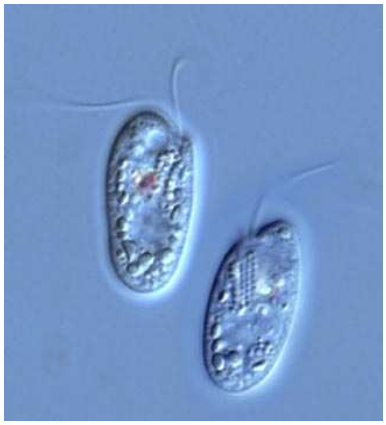
Taxonomic hierarchiel rankings used in phycology, in this monograph listed in as follows.

Division (-phyta)
Class (-phyceae)
Order (-ales)
Family (-aceae)
Genus usually a Greek name
Species usually a Latin name.

To assist the chemical oceanographer, we provide a simple introduction to the characteristics of each algal Division (or class), grouped with figure.1.1 to1.12, according to classical concepts of algal colour (Table .1.1) with taxonomically significant pigment in each algae divisions. Revisions are constantly appearing in the taxonomic literature as functions make it unique indicator of oceanic plant biomass and productivity. Phytoplankton biomass is typically approximated by quantifying Chlorophyll a concentration (Chl a), for which many methods ranging from the single cell to the synoptic (remote sensing) scale have been developed.



1.1 Bacillariophyta (Fucoxanthin)



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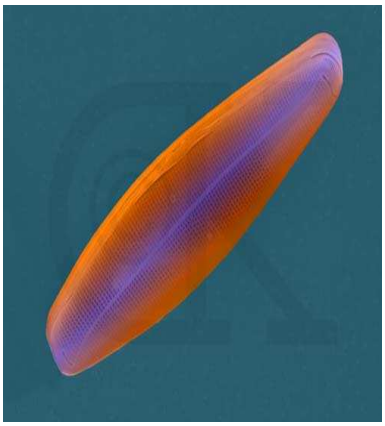
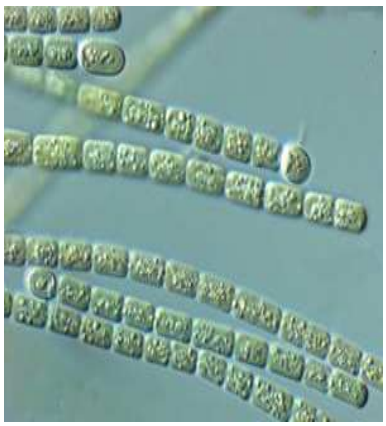
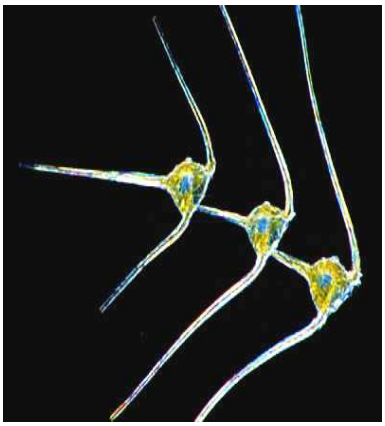


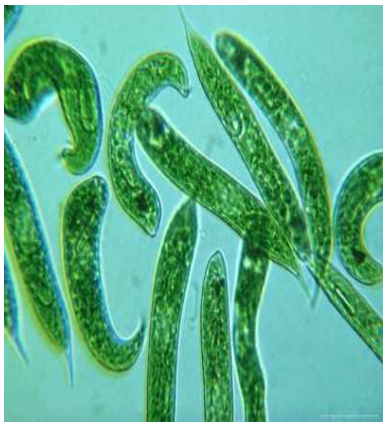
1.3 Chrysophyte (Diatoxanthin)



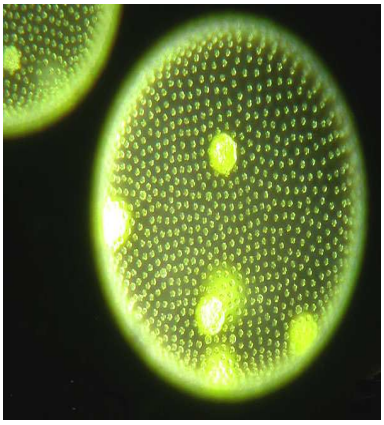
1.4 Cyanobacteria (Zeaxanthin)



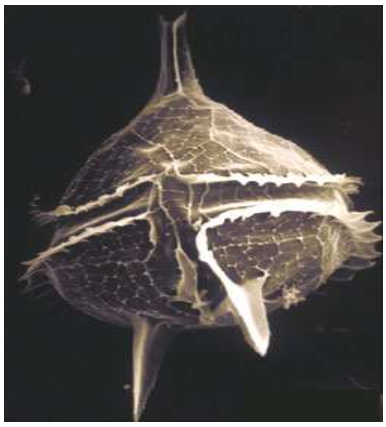
1.5 Dinoflagellates (Peridinin)



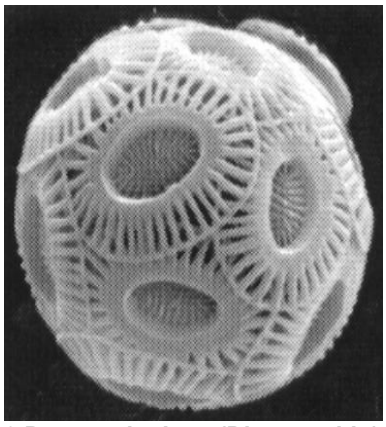
1.6 Euglenophyta (Lutein)



1.7 Chlorophyta (Lutein)

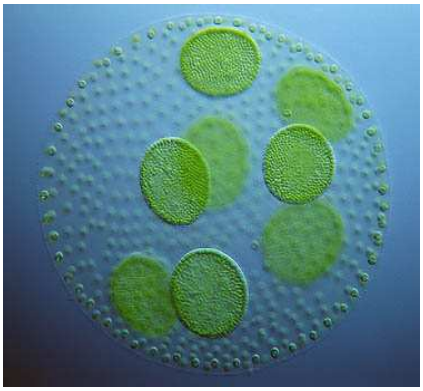


1.8 Dinophyta (Diatoxanthin & Peridinin



1.9 Prymnesiophyte (Diatoxanthin)

1.10 Raphidophyte (Diadinoxanthin)



1.11Green algae (Chl b)

1.12 Prochlorophyta (Zeaxanthin)

Table 1.1 Distribution of major and taxonomically significant pigments across microalgal 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		■										
Carotenes	**[Caro]**														
Old Terminology IUPAC															
α β,ε			□	T	□			□			□				T
β β,β		□	□	□	□	□			T	T	■	■		T	T
γ β,ψ				T											
Xanthophylls															
Alloxanthin	**[Allo]**							■							
Antheraxanthin	**[Anthr]**			T	T	T									
Astaxanthin	**[Ast]**			T											
But-fucoxanthin	**[But]**									■					■
Canthaxanthin	**[Can]**	T		T								T			
Crocoxanthin	**[Cro]**							T							
Diadinoxanthin	**[Diad]**					■			■	■	■			■	■
Diatoxanthin	**[Diato]**					T			T	T	T			T	T
Dinoxanthin	**[Dino]**													□	
Fucoxanthin	**[Fuco]**						■		■	■			■		■
19' Hex-fucoxanthin	**[Hex]**														■
Lutein	**[Lut]**			■	□										
9'-cis neoxanthin	**[Neo]**			■	■	T									
Peridinin	**[Peri]**													■	
Prasinoxanthin	**[Pra]**				■										
Violaxanthin	**[Viola]**			■	■							■			
Zeaxanthin	**[Zea]**	■	■	□			■					T			

Code ■ = major pigment (> 10%) □ = minor pigment (1-10%) T = trace pigments (< 1%) of the total Chloropyll and Carotinoids

1.1 Pigment Oceanography

Pigments are present in all photosynthetic algae but not in most bacteria and protozoa. Many pigments are limited to particular classes or even genera. They are strongly colored, fluorescent at visible wavelengths, allowing them to be sensitively detected. Most of them are labile and rapidly degraded after the death of the cell, thus distinguishing living from dead cells. Actual history of Pigment chemistry embark on late 1930's after purifying and crystallizing Chlorophyll a and b from higher plants by Mackinny (1941), Zscheile (1941) and Zscheile et al (1942). The key developments in pigment oceanography since purification was derived with its detection limit are:

- 1930 :-Purification and Crystallization of Chl a and b from higher plant.[1]
- 1949 :- First equation for the Spectroscopic determination.[2,3]
- 1952 :- Recognitions of Chlorophyll a as a selective biomarker of Phytoplankton.[3]
- 1963s :- Crystallization of Chl c and c1 and c2[4].
- 1966:- Fluorometric determination of Chl a. (μgl^{-1}). [5]
- 1965-87:- Thin layer Chromatography TLC. (μgl^{-1}). [6]
- 1980-90:- HPLC enabled onboard ship determination (ngl^{-1}).[7], Epi- Fluorescence microscopy[8] and Flow cytometry ($fg\ cell^{-1}$).[8]
- Early 90s:- Satellite remote sensing (2 km2= 1pixel).[9], Air Craft Color sensors (m^2).

(Reference: 1 (Mackinny (1941), Zscheile (1941) and Zscheile et al (1942)); 2. Arnon(1949) and 3. Richards and Thompson (1952); 4. Jeffrey (1963; 1969; 1972); 5. Holm-Hansen et al (1965); 6. Jeffrey (1968; 1974; 1976); 7. (Mantoura and Llewellyn (1983); Wright and Shearer (1984); Zapata et al (1987); Waterbury et al (1979)). 8. Yentsch (1983); 9. (Hovis (1980); Robinson (1983)).

Pigments are bound in pigment-protein complexes as part of the light harvesting complexes of reaction centres in photosynthetic organisms (Porra et al., 1997). The free pigment has no role in photosynthesis indeed they are toxic to cells not found in the photosynthetic cells. The initial process of photosynthesis is the absorption of light energy by the chlorophylls and carotenoids of the antenna of the pigment–protein complexes within the LHC's of PS I and PS II respectively. The energy of photon is rapidly and efficiently transferred by electromagnetic interactions which then form an electron which is then passed along electron transport chain to generate chemical energy (ATP) and reducing power (NADPH) (Jeffrey and Mantoura, 1997).

Phytoplankton contains three types of pigments involved in light harvesting and photo protection: chlorophylls, carotenoids and biliproteins. Their chemical structures and properties have been extensively reviewed (Rowan, 1989; Young & Britton, 1993; Scheer, 1991; 2003), as have their metabolism (Porra et al., 1997) and applications in oceanography (Jeffrey and Mantoura 1997). Comprehensive data and graphics sheets were compiled for 47 of the most important chlorophylls and carotenoids found in marine algae (Jeffrey and Vesk, 1997).

Chlorophylls are magnesium coordination complexes of cyclic tetrapyrroles containing a fifth isocyclic ring, often referred to as the Porphyrin, with a long-chain isoprenoid alcohol ester group, except in most of the chlorophyll c pigments. The carotenoids are hydrocarbons (carotenes) and oxygenated derivatives of carotenes (xanthophylls) (Figure.1.13). The chromophore, the part of the molecule that absorbs light of carotenoids consists of a system of regularly alternating single and double bonds. In comparison to other organic material, pigments are labile compounds and the individual stability of the pigments varies (Table 1.2). In a study of marine organic matter from the central equatorial Pacific, Wakeham et al (1997) assigned overall reactivity of different biochemical classes and found pigments (chlorophylls) to be the most labile, followed by lipids, amino acids and finally carbohydrates as the most refractory group. Pigments are degraded in the aquatic environment by chemical, photochemical, and biological processes.

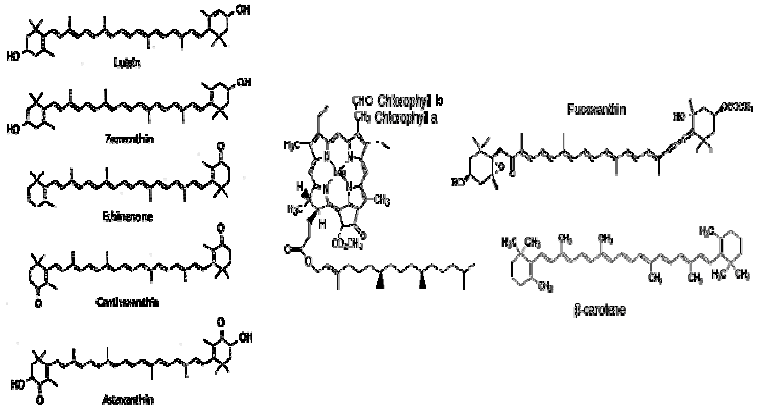


Figure 1.13 Schematic representations of some naturally occurring Carotenoids and Chlorophylls.

Chlorophylls contain nitrogen and are therefore more prone to being salvaged during senescence and biological breakdown than the carotenoids. Chlorophyll degradation pathways include allomerization (oxidation), demetallation (loss of the Mg), and dephytylation (loss of phytyl chain), with the five-ring phorbin being relatively stable (Porra et al.,1997) are explained by many workers (Currie.,1962, Brown et al., 1981, Scheer.,1991 and Kowalewska et al., 2004). Most of these breakdown products are detectable by regular pigment analysis. Carotenoids are found mostly in transform and are inherently more stabile than chlorophylls. However, unlike chlorophylls they are often broken down to colourless compounds, by destruction of the long chain of alternating double bonds that can not be detected by regular pigment analysis methods (Leavitt, 1993; Leavitt & Hodgson., 2001).

Chlorophyll, and its function of converting light energy to chemical energy via the process of photosynthesis, possibly began evolving in the ocean about 2,000 million years ago (Callot, 1991; Scheer, 1991). Photosynthesis had a dramatic impact on the biogeochemistry of the earth, changing its environment from the oxygen-poor, UV-rich and chemically- reducing to the oxygen-rich, geo-chemically more corrosive environment of the present day (Lovelock, 1979; Walker et al., 1983). More than 1,000 million tones of chlorophyll may be produced and destroyed each year both on land and in the sea, with chlorophyll turning over 40 times a year in the ocean because of the short generation times of phytoplankton (Hendry et al., 1987). Indeed the chlorophyll that sediments to the

ocean floor in the senescent or dead phytoplankton accumulates as a complex family of "geoporphyrins" in oil and shales (Treibs, 1936) enabling geochemists to link the organic geochemical record to the past (approx 10^6 years) biological productivity of the ocean (Baker and Palmer, 1978; Eckardt et al., 1991). Space and Time scale involved in aquatic realm was displayed in Figure: 1.14.

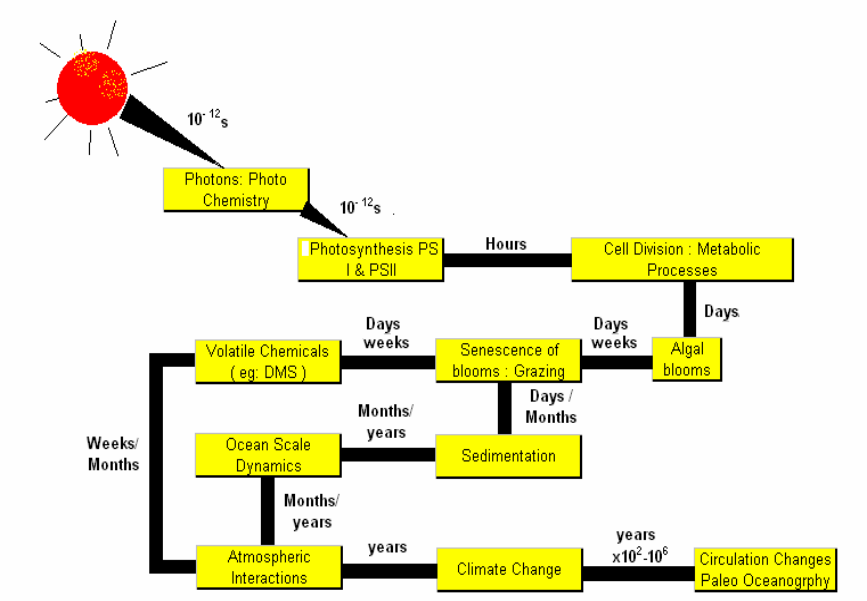


Figure 1.14 Space and Time scale involved in phytoplankton from molecular to Global (Modified from Jeffrey et al., 1997)

Table 1.2 Summary of common pigments recovered in the water column and in sediments and their Taxonomic affinities. The relative degree of chemical stability is ranked from most (1) to least (4) stable, from Leavitt & Hodgson (2001). Pigments with least stability are rarely found in the sediment.

Sl	Pigments	Algal divisions	Stability
	Phytopigments		
1	Chl a	All photosynthetic Algae, excluding Prochlorophyts	3
2	Chl b	Green algae,Euglenophyta, plants	2
3	Pheophorbide a	Grazing, Senescent diatoms	3
4	Pheophytin a	Chl a derivative (all)	1
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 dinoflagellates	2
10	4-keto-fucoxanthin	Haptophyta	
11	Hex-fucoxanthin	Chromophytes and nanoflagellates	
12	But-fucoxanthin	Chromophytes and nanoflagellates	
13	Lutein	Chlorophyta, Euglenophyta, Plantae	1
14	9'-cis neoxanthin	Prasinophyta, Chlorophyta, Euglenophyta	4
15	Peridinin	Dinoflagellates, Dinophyta	4
16	Zeaxanthin	Cyanobacteria (Cyanophyta)	1

1.2 Paleoecology

Estuaries are dynamic water bodies characterized by temporal changes that occur over a spectrum of scales, ranging from short-term (hourly) variations driven primarily by tidal currents to long-term (seasonal or interannual) variations caused by changes in meteorological forcing or river discharge. Estuaries are also spatially heterogeneous and often have large horizontal (or

vertical) gradients in water properties (e.g. salinity, suspended sediments, phytoplankton biomass) that result from local variations in bathymetry, circulation and mixing, or sources/sinks of dissolved and suspended constituents. Hydrodynamic processes are of crucial importance to estuarine ecosystem dynamics. First, all estuaries are to a certain extent influenced by advection of freshwater. Freshwater sources have been shown to enrich estuaries with nutrients (Mallin et al., 1993) as well as organic matter (Findlay et al., 1992; Soetaert & Herman, 1995) and, as a result net residual flow of nutrients in most estuaries directed towards the sea. In combination with tidal mixing, river discharge also induces an extensive exchange with coastal waters, referred to as estuarine circulation. Except for highly stratified estuaries (where mixing of freshwater and sea-water occurs in the sea) this estuarine circulation is a common phenomenon in all estuaries (Day et al., 1989). This circulation has been shown to be responsible for the transport of nutrients, organic matter, phytoplankton and zooplankton from coastal waters into estuaries by many investigators. The importance of this exchange with coastal water for estuarine phytoplankton dynamics is variable. In estuaries with a long residence time, local production often exceeds than import of coastal phytoplankton populations (Soetaert et al., 1994). In small estuaries, however, residence time often restricts the development of autochothonous phytoplankton populations despite high nutrient levels.

Aquatic organisms are extremely sensitive to physical and chemical conditions of marine water, with narrow tolerance ranges: water temperature between 25°C and 28 °C, high salinity, preferably 35 parts per thousand, water clarity, and high content of dissolved oxygen and an ample supply of sunlight. Any departure from these conditions results in stressful conditions hence their inability to survive near river mouths (low salinity and high turbidity), and at depths in excess of 50 m (lack of sunlight penetration). Many urban and industrial developments pose a threat to the eco-systems through: discharges of domestic and industrial effluents; agricultural/ aquaculture runoff contaminated with fertilizers and pesticides; dredging and reclamation activities with their high suspended sediment loads; and other stresses, which arise from high concentrations of population. It is also expected that sea temperature rise due to global warming, will also exacerbate this condition. As the pollution intensifies, the destabilized coastal systems undergo wild gyrations in population densities—some species dying off while others bloom in huge numbers the least desirable creatures, small, tough, and often poisonous, bloom in polluted environments while the most important creatures die. Biodiversity plummets and the ability of the ecosystem to maintain its high value for the production of food, recreation and tourism drops with it. In the century, the coastal ecosystems in the Asian region were seriously damaged by pollution or destruction. People in just four Asian countries—Malaysia, the Philippines, Thailand and Vietnam—cleared 7,50,000

hectares of mangroves, an estimated 10% of all remaining mangrove forests in South and South East Asia. The Philippines alone lost 70% of its mangroves (ESCAP, 2000). The total mangrove area in India is around 6,000 – 4,871 km^2 of which ≈ 57% are along the east coast, 23% are along the west coast and remaining 10% in the Andaman and Nicobar Islands. The wetlands of Kerala included a large mangroves swamp centuries ago among them a large portion are found in isolated patches along the banks of the Cochin estuary, total area amounts to 500km^2 south west of India. Like many other mangrove ecosystem all over the world, these are also subjected to increasing human influences and are being reclaimed for the land requirement subsequent to the industrial development.

As one of the most productive ecosystem in the tropical environment, Cochin back waters houses varieties of Zooplanktons, Phytoplankton and Benthos rendering these waters rich in biodiversity. Recent study conducted by Thomson (2003), clearly indicates an increasing trend in the intensity of industrial pollution in the Periyar river for the past 30 years. Kurian (1972)and Ansari (1977) reported considerable reduction in the density of bivalves and gastropods and isopods. Quasim et al (1972), Madhupratap (1977) and Joseph (2004) also pointed out the deterioration of Fauna and Flora of this region. The sequence of water quality issues arising in industrialized countries as shown below (Figure 1.15 and 1.16) (Modified from Meybeck and Helmer 1989).

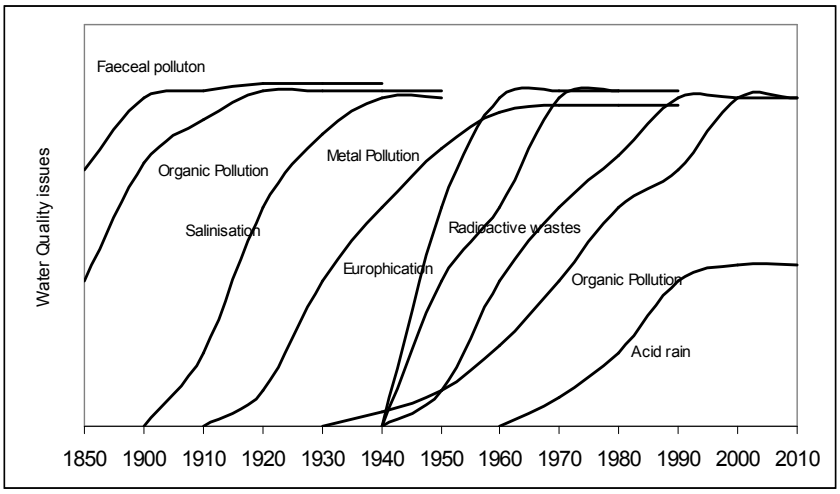


Figure 1.15 Sequence of water quality issues

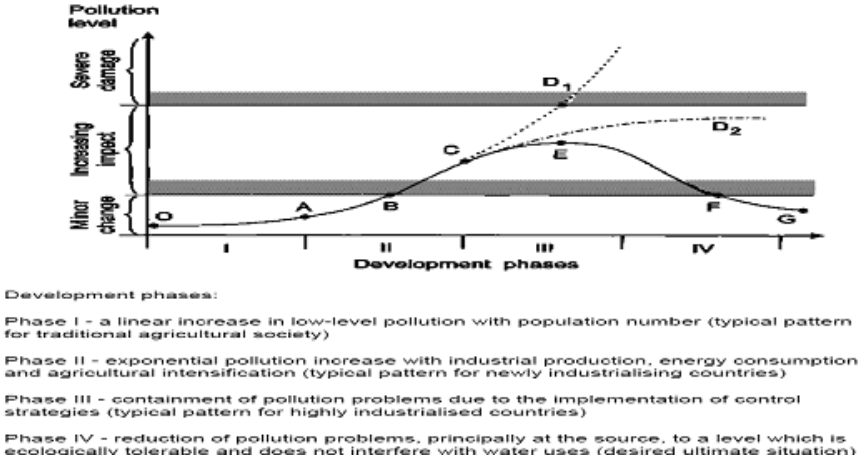


Figure 1.16 Long term impact and control of pollution of the aquatic environment (modified from Meybeck et al 1989)

Two major external factors are influencing the aquatic environment today: climate change and anthropogenic eutrophication. Global climate models predict that we are facing pronounced climate warming, which will have consequences for both terrestrial and aquatic ecosystems (Overpeck et al.,1997; IPCC., 2001). Globally, the coastal

zone has experienced increased anthropogenic impacts through the last centuries (Conley,1999; Rabalais & Nixon, 2002). The algal content of the water column is progressively incorporated to bed sediments through zooplankton grazing or by number of physical biological process which vary in time and space, algal growth also changes as a function of environmental forcing as mentioned above, nutrient regeneration, deoxygenating and reaeration, ultimately deposited in both freshwater and marine sediments (Brown, 1969; Sanger & Gorham, 1970). The fossil pigment records reflect the sedimentation history and also give indirect information about the dynamics aquatic system. Paleoecological assessments of phytopigment content can provide the long-term records of ecological status and natural variability that are needed to interpret changes observed in the environment today and predict future changes.

Analysis of fossil sediment records has become a widely used method for estimating changes in nutrient status and climate change, in both freshwater and marine systems (Cornwell et al., 1996; Battarbee 2000; Hodgson et al., 2003).A great diversity of pigments produced by aquatic algae, bacteria and higher plants are deposited in marine sediments. Often carotenoids and chlorophylls are the only fossil remains of nonsiliceous algae and bacteria and are therefore of considerable value to paleoecology and paleolimnology (Vallentyne, 1954; Brown, 1969; Sanger & Crowl, 1979; Sanger, 1988; Steenbergen et al, 1994 , Vaalgamaa, 2004; Weckstrom et al., 2004). Fossil pigments have been used as indicators of physical environment at the time of sedimentation as well as information on climate change through

changes imposed on the aquatic environment and past production (Smol & Cumming, 2000). Some of the most widely used biological indicators in paleoecological studies are diatoms, chironomids, foraminifera and pollen. In addition, statistical methods combining modern distributions of a biomarker in terms of their optima and tolerances to hydrochemical gradients have been developed, which can in turn be used to infer historical changes from fossil assemblages. This so called transfer function methodology has primarily been applied to diatoms and chironomids for lakes and coastal areas, and has been used for quantitative reconstruction of changes in nutrient status, temperature and salinity (Hall et al., 1997; Walker et al., 1997; McGowan et al., 2003; Weckstrom et al., 2004). The abundance and composition of phytoplankton in aquatic system are largely determined by the availability of light, temperature, inorganic nutrients, and rates of loss from grazing, sedimentation, respiration, and hydraulic flushing (continuous removal by downstream flow and mixing within the water column), (Skidmore et al., 1998; Bledsoe and Philips., 2000). Inputs of dissolved and particulate materials to rivers with small drainage basins should be better linked to local and regional changes in land use and weather patterns (e.g., rainfall events). Hydrodynamic processes and biological changes occur over different spatial and temporal scales. Consequently, any study of the former requires consideration of the latter as well as of the sampling scale and the interaction between the physical and biological scales (Legendre and Demers, 1984; Pinel-Alloul, 1995). Hence, the water chemistry of a system at any point reflects several major influences including the lithology of the

catchments, atmospheric, and anthropogenic inputs. Identification and quantification of these influences should form an important part of managing land and water resources within a particular river catchments zone. In addition, the transport of continental material to the oceans and their state during land–sea interaction can be best understood by the study of water quality parameters from rivers and estuaries.

1.3 Sedimentary pigments

Sedimentary plant pigments have been studied for years and have been proved valuable indicators of paleoecology. They are especially useful as indices of present and past production. The pigment data particularly Chlorophyll derivative and Carotenoids reflect the sedimentation history dynamics of the abundance of algae in lakes and changes in the balance between allochothonous and autochothonous organic contributions to the sediment (Sanger and Crowl, 1979). Calibration of fossil pigments against long-term phytoplankton records from lakes conform that pigment content reflects the trends in algal biomass (Leavitt et al., 1999). The main problem in the interpretation of the data on the distribution of fossil carotenoids and chlorophyll derivatives is related to the understanding of the degradation rates of living and moribund organisms in aquatic sediments (Sanger, 1988; Leavitt et al., 1989). In most systems, more than 90% of the pigments are degraded to colourless compounds during sinking, and the degradation of pigments also continues in surface layers of the sediment (Leavitt, 1993). In principle, fossil pigment records reflect the changes in decay factors, including chemical

oxidation, photo-oxidation and microbial activity. The quantification of pigment degradation rate needs a very comprehensive study due to the multiple factors that influence pigment deposition and fossil abundance in different aquatic systems.

1.4 Methodology for Pigment analysis

Chlorophylls, carotenoids and phycobiliproteins have many favorable characteristics as chemotaxonomic markers. Pigment also suffers several disadvantages as markers because they are labile. Their lability means that special conditions must be employed to preserve them, as they are sensitive to light, heat, oxygen, acid and alkali's as well as spontaneously forming families of isomers in solution. Their distribution is complex, with few unambiguous markers. Pigment analysis, however offers the best technique for mapping phytoplankton populations and monitoring their abundance and composition. Chromatographic analysis of algal pigments is a powerful tool for characterization of phytoplankton in field populations were developed recently makes it feasible to analyze several hundred phytoplankton samples from single oceanographic cruise, a task that would be completely impractical by microscopy. Pigment analysis is a powerful means of recognizing Nano- and Pico- planktonic organisms, which are normally unrecognizable by light microscopy. Many methods of varying accuracy are available for chlorophyll analysis ranging from microscope to remote sensing. If total phytoplankton biomass is the only estimate required, then spectrophotometric or flurometric analysis of Chl a may be appropriate.

1.4.1 Collection and Storage of Field Samples

Sediment samples collected from surface by corer. From the moment of collection, pigment ratios will begin to change due to the altered light so it better to analyse immediately or transport by preserving at 4^0C in stumpy light condition . It could be possible to store several days at -20^0C; or several weeks at -90^0C and -190 ^{0}C at least for one year. While storing samples should not be freeze dried since this cause degradation and reduces extractability of the pigments. It is better to store the crude sediment; freeze drying can be done immediate before the analysis for better results.

1.4.2 Extraction of Pigments

Accurate analysis of phytoplankton pigments depends on the effectiveness of the extraction technique. Several extraction techniques of varying effectiveness remain in current use. There is disagreement over the best physical techniques of sample disruption (grinding, bath sonication, high powered sonication, or soaking) and the most suitable solvents acetone, methanol, dimethyl formamide (DMF). Three criteria are important- the ability to completely extract all pigments from field samples irrespective of the phytoplankton species composition, compatibility with the chromatographic technique (the ability to produce sharp peaks), and stability of the pigments in the extraction solvent (since samples must often wait many hours in an auto sampler before injection). A disadvantage is that methanol promotes allomerization of Chl a and recent data suggests that holding methanol- extracted samples in a auto sampler at 4^0C

produces significant pigment degradation over 24 hours, more than that occurs in samples which are extracted in acetone. So 90-95 % acetone is recommended by many researches (present study also followed the same).

1.4.3 Choice of HPLC Methods

HPLC analysis of algal pigments presents a major challenge due to the diversity of large molecules spanning a wide range of polarities, but also including many that have closely similar chemical structures, some differing only by the position of a double bond.

Routine pigment analysis of field samples become feasible with the development of HPLC methods in the 1980's with automated analysis and quantification of pigments, and the possibility of on-line identification using diode -array detection. All current methods use reverse phase chemistry in which compounds are resolved primarily on their polarity, with C_8 – C_{30} stationary phase and gradient elution. Resolution of acidic chlorophylls, for which buffering and ion-pairing or ion suppression reagents are required, has until recently been achieved using ammonium acetate, sometimes coupled with tetrabutylammonium acetate (TBAA).

Two new methods using monomeric C_8 columns have improved resolution by replacing ammonium acetate with either pyridine or TBAA modifier. Zapata et al (2000) uses a gradient from aqueous methanol/acetonitrile to methanol/acetonitrile/acetone, with pyridine modifier (as the acetate, pH 5.0), achieving resolution of

seven polar Chl c derivatives, the Chl a /DV Chl a pair and partial resolution of Chl b /DV Chl b. Van Heukelem and Thomas (2001) method uses an aqueous methanol to methanol gradient at 60^0C with TBAA modifier (pH 6.5).

1.4.4 Peak identification and quantization

For samples containing a variety of pigments, a diode -array detector is essential to allow identification of peaks from their spectra collected during elution. The wavelengths 420-430 are useful for routine detection and integration of pheophytin a, pheophorbide a and their derivatives and 450-470 nm- detects all carotenoids, Chl b, and Chl c, without interference from Chl a derivatives.

A standard mixture of pigments is injected (after a blank column conditioning cycle) before each batch of samples. This is prepared by mixing extracts of (typically) *Pavlova lutheri, Palagococcus subviridis, Micromonas pusilla, Dunaliella tertiolecta, Amphidinium carterae and Chroomonas salina*. Individual algae are analyzed first, and then mixed, so that the peak heights of major pigments are approximately equal. Aliquots (0.5 ml) of the mixture are dispensed into cryotubes that are immediately frozen in liquid nitrogen. A freshly thawed sample is injected each day, providing an invaluable monitor of system performance as well as the basis of a retention time table. Peaks can be quantified using either the internal standard (IS) or external standard methods. Using an IS gives increased accuracy and precision, since it accounts for any volume change due to evaporation and dilution, and it also

provides a check on the injection status. The most commonly used commercially available internal standards are ethyl 8' – apo -β- carotenoate, 8' – apo - β- carotenoal , Vitamin E (present study) and canthaxanthin, the first of which is most suitable due to its stability and non- occurrence in natural systems.

1.5 Objectives

It is recognized that physical process affect the structure of plankton communities and the availability of "new nutrients" due to vertical mixing enhances phytoplankton biomass and production (Lohrenz et al., 1993; Claustre et al., 1994). The influence of these hydrodynamic forcing on phytoplankton community structure may occur at different partial scale that ranges from a few meters (mixed layer depth) to thousands of kilometers (basin scale). Given our present observation capabilities, this makes the observation of such an influence challenge for most oceanic areas. Remote sensing can resolve large and mesoscale patterns but not phytoplankton community structure. On the other hand, discrete sampling allowed phytoplanktonic structure to be characterize but not simultaneously over a large spatial scale.

The HPLC pigment analysis allowed a simultaneous and rapid technique of choice for achieving an objective survey of particulate organic matter of photosynthetic origin. Here, the study programmed pigment data to characterize the phytoplanktonic assemblages present during samplings in terms of the major taxonomic groups and addresses the importance of degradation processes and terrestrial inputs. In addition, pigment data have been combined with several basic physicochemical and geographical variables measured during the

study to develop a topology for the estuaries. It also enabled us to speculate on the pathways of pigment degradation in the lake and assess individual pigments as biomarkers of past algal and bacterial communities and the potential of pigment degradation products to signal changes in the historical depositional environment.

The main aim of the current research focuses:

- Hydrogeochemistry of **Cochin Back Water System (CBWS)**
  a) Controlling and interactive factors of regulating the phytoplankton production.
  b) Interrelationship between hydrographical parameters (seasonal and spatial periodicity).
  c) Adoption of Multivariate statistical approach and correlation techniques was employed to compile the data.
- Spatio – Temporal variation of pigment and its association in relation with macronutrient, micronutrient trace metals and Phytopigments.
- Isolation and identification of fossil pigments to study the distribution pattern of marker pigments at four prominent hot spots in **(CBWS).**
- Taxonomic composition of the phytoplankton was determined to Class level (micro-, nano-, and picophytoplankton) by high-performance liquid Chromatography.

- As a secondary part, the study attempted to derive Phytoplankton community structure and Tropical status of the system from Chlorophyll derivative to Total Carotenoid (CD/TC) ratios.
- Higher order associations of the individual pigments (sums and ratios) were calculated. An overview has been made, to account Sediment Pigment as a tool for the Preservation and degradation pattern for recent Ecological Reconstructions.

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Chapter 2

Materials and Methods

2.1 DESCRIPTION OF STUDY AREA

COCHIN ESTUARY:

The Cochin backwaters from the northern extension of the Vembanad Lake along the Kerala coast on the south west coast of India, located along $9^0$40'N to 10^0 10' and 76^0 l3' E to 76^0 50' E on the south-west coast of India, they form a multitudinal hydrographic system (Fig.2.1).

The backwater system covers an area of approximately 300 km^2 with one permanent bar mouth maintained at 12m depth at Cochin and two seasonal openings during the peak monsoon period. The estuary is wide (16 km) in the Vembanad lake area and several narrow canals along with municipal waste and other particulate organic matter empty into it. Several major rivers Periyar, the Muvattupuzha and Pampa discharge fresh water into the estuarine system. This estuary is classified as a tropical positive estuary. The characteristic of the estuary is influenced by the rivers flowing into it, while the estuary itself is prone to strong tidal currents. Both these phenomenon combine to give rise to seasonal and tidal fluctuations of hydrological conditions. Vallarpadam is situated next to Bolgatty Island on the west, and linked to the Ernakulam mainland via the Goshree bridges. It is about 3.5 km in length in north-south direction and hosts a population of 10,000 people. Vallarpadam is 1 km away from Ernakulam mainland. Cochin harbor, that is a major natural harbor, located on the southern side of Vallarpadam Island, is one of the major ports in the country. Most of the places in the Vallarpadam region of estuary are open lands. Population in this part is almost nil. Landmasses adjacent to the estuary are protected by rock pieces.

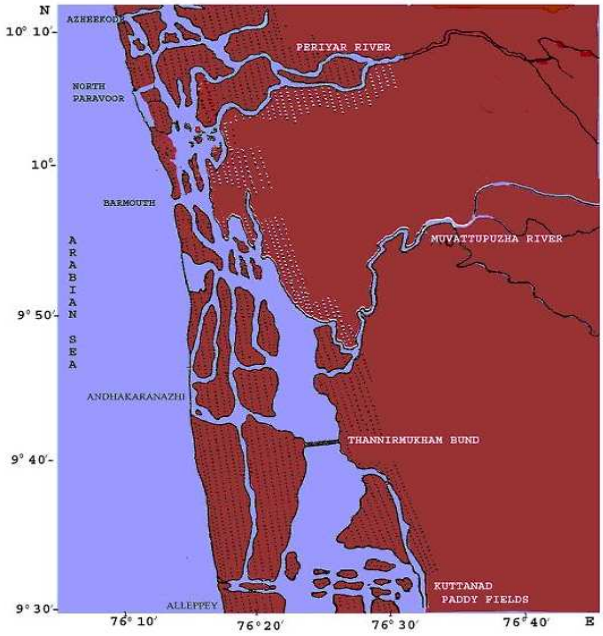

Figure 2.1 Map showing the Cochin estuary

2.1.1 AN OVER VIEW OF SAMPLING SITES

Based on the salinity characteristics of the surveyed area, stations 1 to 7 are grouped into three zones (Table 2.1). Zone 1 (Stations 1) is the riverine zone, where the salinity remains less than 1ppt in the monsoon, pre-monsoon, and post-monsoon seasons. Thus, this site remains as freshwater zone throughout the year. Zone 2 (stations 2, 3 & 4) is partially estuarine in character and shows saline only in the pre-monsoon season. The stations in this zone are canals and are closer to the industry and markets. Stations 5, 6 & 7 grouped together as zone 3, which becomes saline in the pre-monsoon and post-monsoon seasons and practically Cochin estuary - acts as a sink for the discharges from the Pamba, Vambanad Rivers and above mentioned canals along with ffluents from the municipal and industrial wastes from the Cochin City.

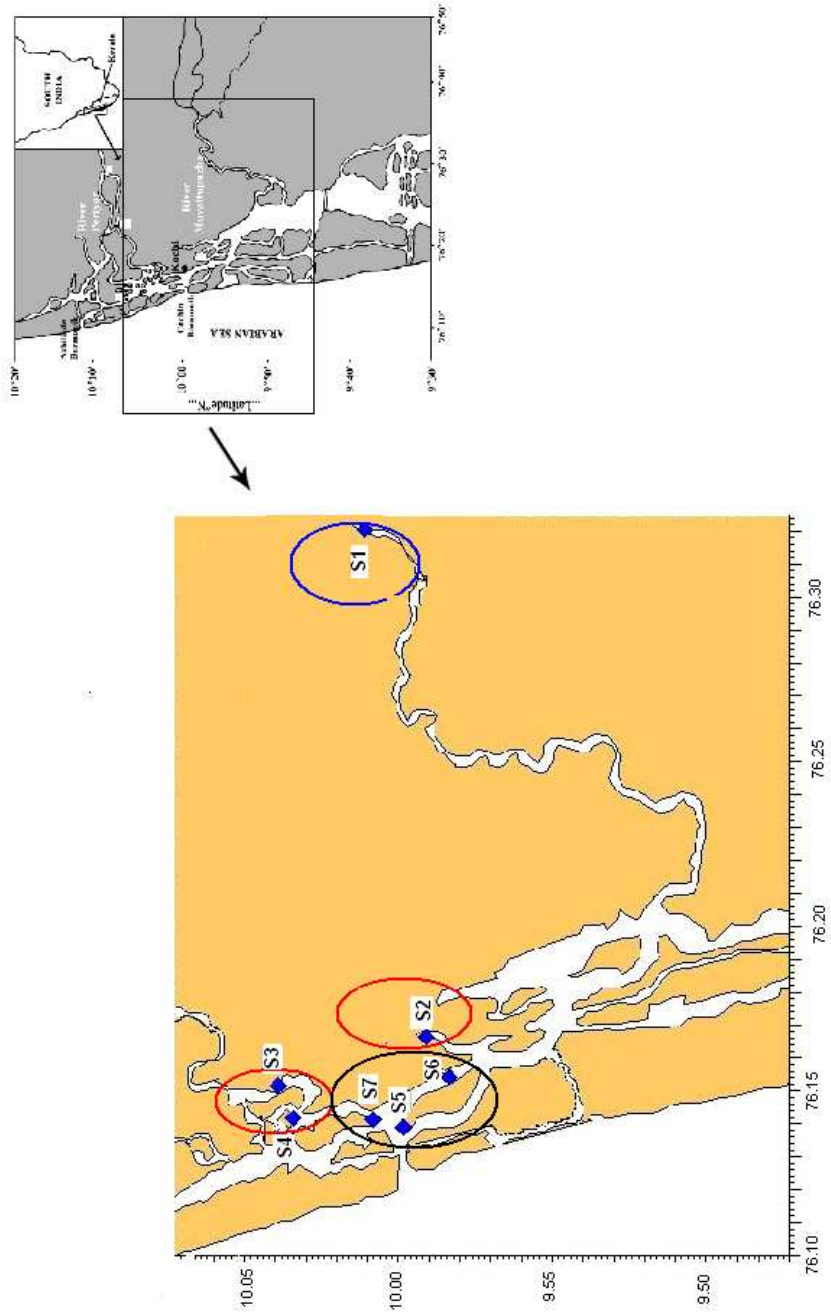

Figure 2.2 Map of Cochin estuary showing location of sampling sites.

Table 2.1 Description of sampling site

Zones		Station ID	Sample ID	Latitude and Longitude	Name of Sampling Site
A	Riverine	S1	1S	9" 59'134 76" 35' 006	Muvattupuzha (Surface)
			1B		Muvattupuzha (Bottom)
B	Backwater	S2	2S	9" 57'387 76"9'579	Champakkara canal (Surface)
			2B		Champakkara canal (Bottom)
		S3	3S	10"05'350 76"14' 968	Cheranelloor (Surface)
		S4	4S	10"04'350 76"14"968	Cheranelloor ferry(Surface)
			4B		Cheranelloor ferry (Bottom)
C	Estuarine	S5	5S	9"58' 084 76" 15" 498	Port-Taj (Surface)
		S6	6S	9"58' 209 76"50'668	Port-Jetty (Surface)
			6B		Port-Jetty (Bottom)
		S7	7S	9"58' 34 76"16'00	Bolghatty (Surface)
			7B		Bolghatty (Bottom)

Station 1:

This station is in the Muvattupuzha River at the Sangamam point. The name 'Muvattupuzha' is made up of three Malayalam words: 'Moo', which stands for 'three', 'aaru' - Small River, and 'puzha', which also means a river. The sampling site is the joining point of the three rivers- Kothamangalam River or Kothayaar, Kaliyar and Thodupuzhayaar, which merge to form a single river. Thus it is called Thriveni Sangamam or Centre Point in Malayalam. Muvattupuzha is comparatively less polluted.

Station 2:

This is in the Champakkara canal nearer to the Champakkara fish market and is found to be highly polluted. The wastes from the fish market drained to the canal. More over

wastes from Cochin Corporation, urban and domestic wastes are also considered to affect the pollution status of the canal.

Station 3:

This site is in the Periyar River opposite to the location of FACT Caprolactum plant.

Station 4:

This is also in the Periyar River. The ferry connects cheranellur to varapuzha and also to Eloor. One of the largest industrial manufacturing centers, the Udyogmandal Industrial Estate, is located around the branch of the Periyar which passes to the north of Eloor, an Island in the upper tidal reaches of the Periyar. Here industrial chemicals, leather and other goods are manufactured. Many of the factories are located on the mainland, but several others are clustered on the north of the island, including FACT (Fertilizers and Chemicals Travancore), IRE (India Rare Earths), Merchem and HIL (Hindustan Insecticides Limited). Hence this site is highly polluted.

Station 5:

This site is in the jetty nearer to the station S 6and is also polluted.

Station 6:

This site is subjected to pollution from oil tankers and also the remains from the fishing industry. Wastes from the Fishing Harbor and fish processing units enter the estuary.

Station 7:

Bolghatty Island also known as Ponjikara is one of the islands that make up the city of Kochi, Kerala, India. The island is a popular tourist haunt, and houses the Bolghatty palace. Bolghatty Island has a local name Mulavukad. This island is on the western side of Ernakulam. The site is near the Bolghatty palace.

2.1.2 Sample Collection and Preservation.

The result of any testing method can be no better than the sample on which it is performed. Sample collection and preservation were done as per Standard methods (1995) and Grasshoff et al (1999). The objective of the sampling is to collect a portion of the material, small enough in volume to be transported conveniently handled in the laboratory, while still accurately representing materials being sampled.

Sample collection were made bimonthly from November 2005 to September 2007, surface sample were collected approximately 5 cm below the water surface. Bottom samples were collected approximately 25 cm above the sediments. The depth at each sampling site varied throughout the year depending on the season and tide, the intervals observed were (1) 5-10 m, (2) 3-4 m (3) only surface sample (4) 1to 2m, (5) only surface sample (6) 3- 7m (7) 1- 7 m. The highest water level was found in the monsoon season and lowest in the pre-monsoon. Water samples grabbed from the sites using plastic Niskin sampler which can accommodate 1.5L sample at a time with out any significant alteration and transferred to 2.5L clean pre-washed plastic cans

with tight fitting plug, which are rinsed initially with a portion of sample. Color, odor, taste were noted first and pH was noted using pH meter. Water for determination of DO was collected first. Samples were siphoned off in a DO bottle, and then are fixed by adding Winkler A and B on the spot for DO. Samples of 1L each were taken for trace metal and pigment analysis and quickly transferred in to an insulated container filled with ice. The whole samples are transported to the laboratory without any alteration. For the pigment analysis the water sample is immediately filtered through GF/C of pore size 0.7µm and the filter were extracted in 10 ml 90% acetone. The samples for trace metal analysis was acidified to pH <2 and preserved at about 4°C until extraction.

Sediment samples were collected using a Van veen Grab and Sampling of the upper 10 centimeters of sediments at four distinct locations at studied estuary was performed in April 2007 using improvised gravity corer. The samples were immediately packed into special plastic cooling boxes and transported to the laboratory then the sample divided into two sub-samples one for the spectrophotometrical analysis of chlorophyll derivates (CD) and total carotenoids (TC), other portion for HPLC analysis of fossil pigments. First part is again divided into four at the interval of 2 cm from the surface of the sediment. This sediment samples for the pigment analysis is immediately collected in a 15cm plastic vials for preservation in -80^0C and/or directly freeze dried according to the availability of the lyophilizer instrument in the laboratory. A portion of the sample that collected in the April 2007

was preserved at 4^0C in order to study the stability of Chlorophyll and their degradation products. Form that sample a portion was taken out at April 2008. Portion of the sample is kept for air drying in a pre cleaned glass petty dish for the analysis of trace metal, nutrients and organic matters.

All chemicals used in the study were obtained from Merck, India/Germany and were of analytical grade. Deionised water was used throughout the study. All glassware and other containers were thoroughly pre cleaned with acid and rinsed with de ionized water several times prior to use.

2.2 ANALYTICAL METHODOLOGY

Brief descriptions of the analytical method adopted in this work are appended in the Table 2.2.

2.2.1 Physical parameters

Temperature were noted at site for surface and bottom samples using thermometer of precision 0.01^0C and conductivity measured in the lab using conductivity meter . The pH values were determined using a digital direct readout pH meter, which is a combination of glass and AgCl electrodes. The instrument should be calibrated using a standard buffer solutions having pH 4, 7 and 9. After calibration the electrodes are washed with distilled water, blot dried and immersed in the sample for recording pH.

Salinity sample was collected and preserved in the ice bags and measured Argetometrically in the lab. Analysis of trace metals, organic compounds like carbohydrates, protein and nutrients are

carried out according to Grasshoff et al (1999) & Standard method (1995), Dissolved oxygen was estimated by Winkler's methods. Chlorophylls and its degradation products were determined by filtering the sample through GF/C filter paper and extracting with 90% Acetone (Parsons et al., 1984; Standard method 1995).

2.2.2 Geo Chemistry

Textural analysis of the sediment was done based on stocks law using the methods of Mudorch et al (1997). Samples were analyzed for concentration of the trace metals, TOC, percentage carbon, hydrogen, nitrogen and sulpher (%C, H, N&S) and nutrients (total phosphate, orthophosphate and exchangeable nitrate). For the determination of trace metals, 1 g of the sediment was digested at 90°C with HNO_3 + $HClO_4$ + HCl mixture in the ratio 1: 1: 3 for about 10 hrs. The acidic solution was centrifuged at 5000 rpm and made up to 25 ml with dil HCl. Blanks were set up concurrently. All the samples and blanks were then analyzed by Atomic Absorption Spectrometer (Perkin Elmer model 3110) using air-acetylene flame and the nutrients were estimated spectrophotometrically (Grasshoff et al., 1999) after extraction of the sediment as described by Mudorch et al (1997). TOC was determined using wet digestion followed by back titration with ferrous ammonium sulphate and %C, H, N&S analyses were carried out using Vario EL III CHN Analyzer.

Table 2.2 A brief description of the analytical method adopted in this work

Water analysis					
Parameter	Method	Calibration	Preservation	Detection Limit	Reference
Temperature	On site	Calibrated	NA	0.01oC	APHA 1995; Grassoff et al 1999
pH	Digital read out pH meter	Buffer 4.7 and 8.2	NA	0.1	APHA 1995; Grassoff et al 1999
DO	Winkler's		immediate		APHA 1995; Grassoff et al 1999
Salinity	Argentometric	Standard NaCl Solution	Ice Cold Condition < 6hrs	0.01mg/L	APHA 1995; Grassoff et al 1999
Nutrients	Spectophotometric	With Concerned Standard solution	Ice Cold Condition < 6hrs	0.01mg/L	APHA 1995; Grassoff et al 1999
Sulphate	Spectophotometric	With Concerned Standard solution	Ice Cold Condition < 6hrs	0.01mg/L	APHA 1995; Grassoff et al 1999
Carbohydrate	Spectophotometric	With Concerned Standard solution	Ice Cold Condition < 6hrs	0.01mg/L	Parson et al 1984
Protein	Spectophotometric	With Concerned Standard solution	Ice Cold Condition < 6hrs	0.01mg/L	
Chlorophyll a	Spectophotometric	N/A	Ice Cold Condition < 6hrs	0.01mg/L	
Chlorophyll b	Spectophotometric	N/A	Ice Cold Condition < 6hrs	0.01mg/L	
Chlorophyll c	Spectophotometric	N/A	Ice Cold Condition < 6hrs	0.01mg/L	
Pheophorbide a	Spectophotometric	N/A	Ice Cold Condition < 6hrs	0.01mg/L	
Pheophytin a	Spectophotometric	N/A	Ice Cold Condition < 6hrs	0.01mg/L	
Total Carotenoid	Spectophotometric	N/A	Ice Cold Condition < 6hrs	0.01mg/L	
Geochemistry					
Texture	Pipette method		N/A	0.01mg/L	
TOC	wet digestion		N/A	0.01mg/L	
Exchangeable Nitrate	Extraction ,Spectophotometric	With Concerned Standard solution	N/A	0.01mg/L	Mudroch et al 1997, Grasoff et al 1999
Ortho Phosphate	Extraction ,Spectophotometric	With Concerned Standard solution	N/A	0.01mg/L	
Total phosphate	Extraction ,Spectophotometric	With Concerned Standard solution	N/A	0.01mg/L	
Organic Phosphate	Extraction ,Spectophotometric	With Concerned Standard solution	N/A	0.01mg/L	
C H NS				0.01mg/L	
Pigment analysis	HPLC	Authentic Standard spectra with Vitamin E	-80oC	5 ng/g	Hooker et al 2005

2.2.3 Statistical Analysis

Statistical Parameters (Correlation and Multiple Regression) were calculated employing the statistical software SPSS v10 for windows.

2.2.4 Pigment analysis

Chlorophyll a, b, c and its degradation products in water samples were determined by filtering the sample through GF/C filter paper and extracting with 90% Acetone (Parsons et al., 1984; Standard method 1995). The mixture sonicated at 5 atm for 30 sec to disrupt the cells and kept in dark at 4^0C about 5 hr in order to ensure the complete extraction of Pigment. The mixture was then centrifuged at 3000 rpm to separate the pigment solvent complex. This process was repeated until the color of the extract was almost clear. The supernatant was then transferred to UV spectrometer GENESYS 10 UV Thermospectra.

Sediment Samples for pigment analysis were collected in the field, immediately transferred to 15 ml vials kept in ice bags in the dark on return to the laboratory, stored in a -70 ^{0}C freezer to render them more stable (Yacobi et al., 1990). The sediment for the pigment analysis is immediately sub sampled in a 15cm plastic Vials for preservation under -80^0C (SANYO Ultra low MDFU-3086 maintained at -80^0C) and/or directly freeze-dried (Buffan- Dubau and Carman, 2000), in Viotis BENCHTOP-2K SI 213489 Lyophilizer) at -40^0C, 6-8 hrs at subdued light according to availability of the instrument in the laboratory. All field and

laboratory work was carried out in subdued light to minimize pigment degradation.

HPLC analysis carried out by DHI group Denmark as described below. The Freeze dried sample was stored at -80^{0}C until analysis. The freeze dried samples were homogenized prior to sub sampling and after weighing (Table 2.3)

Table 2.3 Weight in g used for HPLC Extraction

Zone	Stations	Weight in g used for HPLC Extraction
B	S2	0.1100
	S4	0.1210
C	S5	0.1123
	S7	0.1153

each sub sample was extracted in 95% acetone with internal standard (vitamin E) sonicated in an ice cold sonication bath for 10min, mixed on a vortex mixer allowed to extract at 4^{0}C for 20hrs and vortexed again. Extracts were then filtered through 0.2µm 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 HPLC method is the HPL (American Horn Point Laboratory of the University of Maryland Center for Environmental Science method) (Van Heukelem & Thomas, 2005 *in* Hooker et al., (2005); NASA Technical Memorandum) not separating α-β carotene. Internal method No. SF. No 30/ 852:01).

The detection wavelength was 420 & 450 nm and the flow rate was 12.5 µl min^{-1}. Pigments were identified by comparing their retention times and spectra against standards.

For the analysis of the sedimentary chlorophyll and their degradation products a known wet weight of sediment was added in a plastic vials and vacuum dried. Approx 0.5g of vacuum dried sample is added to conical glass centrifuge tube with 90% acetone and the mixture sonicated at 5 atm for 30 sec to disrupt the cells and kept in dark at 4^0C about 5 hrs in order to ensure the complete extraction of Pigment. The mixture was then centrifuged at 3000 rpm to separate the pigment solvent complex from the remaining sediment. This process was repeated until the color of the extract was almost clear. The supernatant was then transferred to UV spectrometer GENESYS 10 UV Thermospectra.

2.2.5. Calculations

The Concentrations of chlorophyll a, b and c and its degradation pigment (Pheophytin) and Carotenoid in the water and Sediment were Calculated the in the extract by inserting the corrected optical densities in the following equations (Parsons et al., 1984; Standard method, 1995). Use the optical density reading at 664,647, 630 and 665a nm to determine chlorophyll a, b, c and Pheophytin respectively. The carotenoids were measured at 480 and 510 nm. The OD reading at 750 nm is correction of turbidity. Subtract this reading from each of the pigment OD values of other wavelengths before using them in the equation below. Because the OD of the extract at 750 nm is very sensitive to changes in the

acetone to water proportions, adhere closely to the 90 parts acetone: 10 parts water (v/v) formula for pigment extraction. The sediment pigment concentrations were calculated by converting µg/l to corresponding µg /g by multiplying density factor.

$$\text{Chlorophyll a, mg/m}^3 = \frac{26.7\left(664_b - 665_a\right) \text{x V1}}{\text{V2 x L}}$$

$$\text{Pheophytin a, mg/m}^3 = \frac{26.7\left[1.7\left(664_b\right) - 665_a\right] \text{x V1}}{\text{V2 x L}}$$

Where:

V1 = volume of extract, L

V2 = volume of sample, m3 (Water)

L = light path length or width of cuvette = 1cm and

664_b, 665_a = optical densities of 90%acetone extract before and after acidification respectively.

Carotenoid mg/ l = $(C_p \text{ x } v) / (\gamma \text{ x } l)$.

where: C_p = 7.6 (E480 -E750) - 1.49(E510-E750)

v = volume of acetone, ml

γ = weight in g of sediment, path length of cuvette = 1 cm of spectrometer

Calculate the concentrations of chlorophyll a, b and c in the extract by inserting the corrected optical densities in the following equations:

a) C_a = 11.85(OD664) – 1.54(OD647) - 0.08(OD630)

b) C_b = 21.03(OD647) – 5.43(OD664) - 2.66(OD630)

c) C_c = 24.52(OD630) – 7.60(OD647) – 1.67(OD664)

where: C_a, C_b, and C_c = concentrations of chlorophyll a, b and c respectively mg/ L, and OD664, OD 647 and OD630 = corrected optical densities (with a 1-cm light path) at the respective wavelengths. After determining the concentration of pigment in the extract, calculate the amount of pigment per unit volume as follows:

Chlorophyll a mg /m^3 = (C_a x extract volume, L) / Volume of sample, m^3 (water).

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Chapter 3

Hydro geochemistry of Cochin Back Water System (CBWS)

3.1 Introduction

Awareness about environment has given tremendously all around the world in the recent years. The current generation has a huge responsibility to ensure that the environment where we all live and harmony to live behind a better world for the possibility of

the present as well as the future generation with the growing population and the consequent demand on natural resources. The environment in general is imperative to adopt a comprehensive and integrated view in space and time for the development process. Being an integral part of the global sustainable development process oceans, coasts and islands support a diverse array of activities yielding enormous oceanic and social benefits. In this context a research insight into the aquatic realm that would strengthen to deal with the essence and profiles related to the above said scientific scenario.

Estuaries are among the most productive, diverse, economically important, and hydrologically variable ecosystems on Earth (Neilson and Cronin, 1981; Hobbie, 2000). A bulk of the world's commercial and recreational fish stocks depend on estuaries as nurseries, refuges and feeding grounds. Estuarine and coastal watersheds support approximately 75% of the world's human population and the number of coastal residents continues to rise (Vitousek et al., 1997). The productive nature and resourcefulness of estuaries depends on nutrient inputs. Current nutrient loading rates often exceed those needed to sustain desirable production leads to excessive production of organic matter. Many estuaries are now facing nutrient-over-enrichment, or "too much of a good thing" in the form of nutrient-enhanced primary production (D'Elia, 1987; NRC, 2000). This condition prevail excessive production of organic matter in the form of algal blooms and earlier literature also supports (Nixon, 1995).

The abundance of chlorophylls and nutrients in the water column are of paramount importance for determining the biological productivity and potential resources. Light penetration decides the depth of the euphotic zone while the nutrient (especially the nitrate, nitrite and phosphate) indicate the fertility of the water to promote productivity. Availability of photosynthetic pigments that have been modified biogeochemically in estuaries and coastal waters are transported to the open ocean (Ackroyd et al., 1986; Saager et al., 1997). Hence, the water chemistry at any point reflects several major influences including the lithology of the catchments, atmospheric, and anthropogenic inputs. The physical, chemical, biological, sedimentological and more recently anthropogenic factors control the behavior and biogeochemical cycles of nutrients including carbon in estuaries. The biogeochemical cycles of these nutrients maintain concentrations that are adequate for the production of the phytoplankton communities. However, increase in human activities in the form of urbanization, industrialization and agriculture in the last two decades has resulted in an increase in nitrogen and phosphorus compounds, most of which bound to sediments are immobilized (Fisher et al., 1982; Kennish, 1986; Valiela, 1995; Balachandran et al., 2003). In addition to this numerous estuarine studies throughout the world clearly demonstrate that interactions between the sediment and the water column play an important role in regulating phytoplankton production and the extent of bottom water hypoxia/anoxia

(Matson et al., 1983; Nixon and Pilson, 1983; Kemp and Boynton, 1992; Madhu et al., 2007).The imbalance between relatively high rates of O_2 consumption and low rates of O_2 re-supply causes dissolved oxygen (DO) content to drop to levels that are low enough to adversely affect oxygen-requiring animal and plant life. DO concentrations of less than 4mg O_2 L^{-1} are commonly referred to as hypoxic and are frequently stressful to higher life forms, while no detectable O_2 concentrations are termed anoxic and potentially fatal to finfish, shellfish and invertebrate species (Renaud, 1986; Pihl et al., 1991; Diaz and Rosenberg, 1995).

Up to 1000 times higher phosphorus and 300 times higher nitrogen concentrations have been reported in nutrient immobilized sediments in comparison to the overlying water column (Rivas et al., 2000). The mechanism of immobilization of nutrients and contaminants by sediments involves absorption on ion exchange sites, binding to organic matter, incorporation into lattice structures, and precipitation into insoluble compounds (Chan et al., 1982; Dunbabin and Bowmer, 1992). Contaminants and organic matter show an affinity for the finer fraction of aquatic sediments due to the exponential increase in surface area with decreasing grain size and an increase in surface charge (Forstner et al., 1982; Sakai et al., 1986; Bubb et al., 1990; Birch and Taylor, 2000; Narayanan, 2006; Sujatha et al., 2008, Niffy, 2009).

Estuarine sediments are rich in organic matter (3–10% organic carbon) and represent vast storage reservoirs for nutrients and oxygen demand which delivers nutrients to the coastal zone

and determines hydrologic properties (flushing or residence time), vertical stratification, turbidity and color of the water column, all of which mediate productivity, nutrient cycling, DO, and habitability in an interactive manner. The fluxes of trace elements that have been modified biogeochemically in estuaries and coastal waters are transported to the open ocean and the original composition of seawater is altered (Lalu raj et al., 2002; Renjith and Chandramohankumar, 2007). The transport of continental material to the oceans and their state during land–sea interaction can be best understood by the geochemical studies of sediment and water in the rivers, estuaries and marine basins. It is estimated that about 2.73×10^9 t/yr dissolved materials are brought into the oceans from the land mass by rivers and a further 9.3×10^9 t/yr of suspended solids before extensive disturbance of the land surface by man (Whittaker, 1975). Current estimates of particle erosion rates are about 24×10^9 t/yr. When biomass levels are considered to be too high, nutrient reduction is a proven method to reduce levels in both marine and freshwater systems. However, it is not always obvious which nutrient should be reduced and sometimes results are disappointing. The effectiveness of nutrient reductions can be addressed by statistical data analysis; i.e. empirical relations between total P loadings and algae concentrations. Algal blooms usually consist of various species of phytoplankton belonging to different taxonomic or functional groups such as diatoms, flagellates, green algae and cyanobacteria, commonly referred to as blue green algae and some species are harmful which would effect

the ecosystem. Their resource requirements vary (nutrients, light) and they have different ecological properties and it is important to find an easy way to characterize these species more accurate and reliable way.

3.2 Estuarine setting

Geologically the study area is of South Indian Precambrian terrain covered by Archaean age consist of granulites, granites, Grasitoid gneisses and green stones. 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. These elevations are termed coastal plains, lagoons and lowlands respectively. This estuary 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 20,000 x $10^6 m^3$ of fresh water 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, 2000). The estuary recognized as one of the largest brackish water bodies in India, stretches to over 24000 ha in area and contributes to ≈50 percent of the total area of estuaries in the Kerala state. The bathymetry of the water body indicates that depth variation occurs between 1.5 m and 6.0 m in

most parts except the dredged channels, which are 10–13 m deep. The variation in depth ranges in the estuary for the last seventy years has given in the table 3.1. The annual maintenance dredging volume of 10×10^6 m^3 from the Cochin harbor region indicates the intensity of sedimentation (Rasheed, 1997; CPT, 2000) and the present study also have given importance to the behavior of the CBWS.

Table 3.1 Variation of depth ranges in the locations in Cochin Estuary 1930-2001. Modified from Gopalan et al.,(1983) .

Stations	Depth (m) ranges		
	1930	1980	2001
Between Thaneermukkam bund & Vaikom	8--9	3--4	3.5--4
Between Vaikom & South Paravoor	7--9	4--5	3.5--4
Between South Parvoor and Aroor	5--6	3--4	3--4.5
Between Aroor and South of Wilington	7--8	7--8	7--8
Between Cochin Habour Region	7--8	7--8	7--8
Between Bolgatty and Cherai	3--4.5	2--2.5	1.5--2
Between Cherai to Munabam	3--6	2.5--4	2.5--4

The Cochin estuarine 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. Historically, this area is known for trade, commerce and cultural activities with countries like Arabia, Portugal and Holland. It has three dredged channels (Figure 2.1), one being the approach channel oriented along east–west direction of around 10 km length and 500 m width and the two inner channels located on either side of the Willington Island, i.e. Ernakulam channel of around 5 km length with a width of 250–500

m and Mattancherry channel of 3 km long with a width of around 170–250 m. All the dredged channels are maintained at a depth of 10–15 m. The tropical estuarine environment shows multitudinal features (Quasim, 2003) which characterize freshwater and seawater mixing (Joseph & Kurup, 1987; Menon et al., 2000) and provide breeding ground for marine organisms (Remani et al., 1983; Nair et al., 1988; Sarala Devi et al., 1991 Madhu et al., 2007). The extent of freshwater–seawater mixing in Cochin tidal inlet, with partially mixed conditions in the month of May and a salt wedge in August–October. From November to January, partially mixed conditions prevail whereas in June, moderately stratified to partially mixed waters were observed. The sedimentation features at the Cochin port vary according to season(s). There are three seasonal conditions prevailing in this estuary, i.e. monsoon (June–September), post-monsoon (October–January) and pre-monsoon (February–May). During the monsoon period, heavy rainfall results in high river discharge which eventually reach the estuary and 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. Such hydrographic features and circulation pattern complicate the sedimentation characteristics of these estuarine channels. In post-monsoon, river discharge gradually diminishes and tidal influence gains momentum and the estuarine conditions change to a partially mixed type, weakening stratification. This is mainly a transitional period. The river discharges minimum in pre-monsoon and seawater influence is

maximum at upstream. 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 sediments are a mixture of clay and silt (70–85%) and sandy in the estuary; but sand is prominent in the Muvattupuza river and surf zones (Zone B) clay and silt is found to be higher. The climate is typical of tropical features with monsoon (June–September) yielding 60–65% of the total rainfall (≈ 300 cm). The temperatures from March to May are hot (30–36 ^{0}C) and pleasant in December (22–25 ^{0}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. Humidity is on the higher side (70–80%) due to naval influence. During pre and post-monsoon, sedimentation in the inner channels 1m per annum, and average sedimentation rate is 0.51cm per annum. The circulation pattern helps to bring more silt and clay into the estuary and sedimentation is highest during the tide slack period. This sedimentation feature results from the heavy discharge of water and sediments brought out to the Cochin gut and deposited in the outer harbor. Simultaneous turbulence due to currents suffered in the inner harbor and leads to resuspension and reduction of sediment accumulation. The sedimentation in the port area creating a reduction of depth (up to 1–2 m per year) is clearly a problem for marine traffic. Previously heavy silting was observed in certain years at Cochin port and was influenced by the appearance and movements of mud banks in the close vicinity of

the spot. Anto et al (1977) observed that the longshore currents could also bring sediments into the channels.

The coastal region (2,500 km^2) of Cochin is studied seasonally to derive the salient features which characterize its biological productivity. The study aims to understand the (a) controlling and interactive factors of regulating the phytoplankton production and (b) interrelationship between hydrographical parameters both seasonal and spatial periodicity. In order to achieve this, multivariate statistical approach and correlation techniques were adopted.

3.3 Result and discussion

3.3.1 Temperature

Temperature in any study area is an important factor which determines the estuarine circulation and stratification. Aquatic organisms are extremely sensitive to physical and chemical conditions of marine water, with narrow tolerance ranges:

Water temperature between 25°C and 28 °C

High salinity, preferably 35 psu

Water clarity

High content of dissolved oxygen

Ample supply of sunlight

any deviation results stressful conditions .Temperature determines the fate of the inorganic materials and effect the physico chemical parameters like salinity, pH, nutrient distribution and indirectly to the adaptation of Flora and Fauna of the system. Temperature in

the study area shows highest in the premonsoon (31^{0}C) and lowest in the Monsoon season (29^{0}C) (Fig 3.1). Surface water temperature shows comparatively higher than bottom layer temperature (AppendixFigure3.14&3.15).

Distribution of Temperature and Salinity during the study period

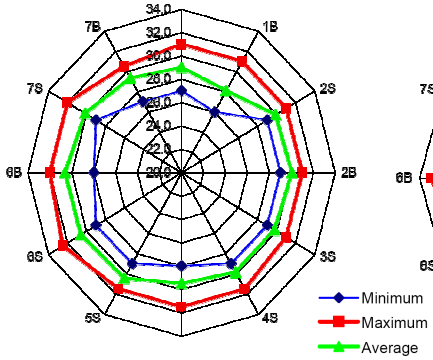

Figure 3.1 Temperature°C

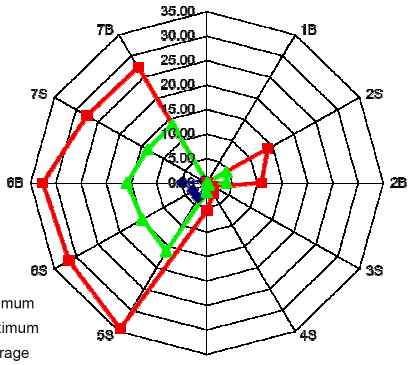

Figure 3.2 Salinity psu

3.3.2 Salinity

The Stratification is controlled by temperature and salinity which might play an important role in the setting of detritus and hence in the metal fluxes in the estuary. Generally in pre-monsoon (Feb-May) there was no salinity stratification a stable water table is observed in the estuary whereas with the onset of monsoon (June) a rapid build up of stratification occurred and upper water column separated from bottom layer due to high salt content in the lower layer.

During the monsoon the estuarine conditions were transformed to fresh water body salinity falls (avg 34.0 to 0.75 psu). Post monsoon period was a typical transition period. During low tide, the sewage water flushes and spreads into the estuary

resulting in localized low saline conditions, except for areas very near the bar mouth .

Like temperature salinity always show an inverse relation with precipitation as observed earlier researchers. During post monsoon months due to lower tidal amplitude and freshwater inflow did not allow salinity of this canal water to rise even after rainy season. This is confirmed by high dilution factor during these post monsoon months. The salinity was highest during pre monsoon season, which gradually declined to almost zero during monsoon. Spatially the total salt content (Salinity) varied from 0.01 to 34.0 from Zone A to C. Generally surface water column salt content was lower compared to bottom layer (Appendix Figure 3.16 &3.17). Salinity recorded relatively high variations between station to station and season to season. An increasing trend from riverine to estuarine regions noted. Zone 1 is upstream has the lowest recorded salinity in the study period and practically nil and fresh water in character (0.01- 0.12, (avg 0.07) psu) and no significant fluctuations observed in this site. Zone B, back water Zone where the salinity ranges; (0.02- 13.97 (avg 2.13) psu). Maximum salinity in all periods recorded in the estuarine region as expected and more prone to variations (Figure. 3.2). The highest salinity was recorded at Estuarine stations (Zone C) during pre monsoon (avg 34.0psu) and the salinity decreases with the onset of monsoon and it was become practically a fresh water Zone (Appendix Figure 3.16 &3.17). Compared to Estuarine zone fluctuation in the salinity was not observed in the riverine and back

waters zones. It could be due to the influx of fresh water from the upper reaches of river and intrusion of seawater via bar mouth to the estuarine zone (Salinity intrusion were very low at the Zone A and B).

3.3.3 Rainfall

The annual average rainfall in the Cochin estuary is about 3.2cm, which exhibits considerable variations from year to year. Approximately 75% of the total rainfall occurs during the monsoon period which starts from late May or early June to September. With the onset of monsoon and within the course of a few weeks, the hydrographic conditions in the estuary undergoes remarkable changes due to the influx of freshwater as the system rapidly changes from marine to brackish water condition and similar observation reported by earlier workers (Menon et al., 2000). The early morning drizzling in the Cochin estuarine area was noted by many meteorologists because of the proximity of industrial area and the influence of Arabian sea which results high humidity (>75%).

Monthly precipitation ranged between 2 mm (March to May) and 150-248 mm (July & August) (Figure 3.3). A seasonal pattern in precipitation became apparent; heavier precipitation occurred during south west monsoon (May–August) and north-east monsoon (September–December) there was little precipitation (2-15) during January–April. 90% of precipitation occurred during the monsoon and rest fall on the post monsoon and April is always characterized by a summer precipitation.

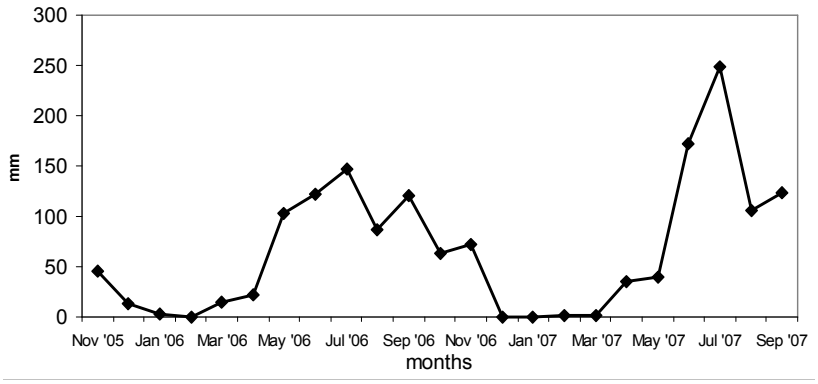

Figure 3.3 Rainfall during the study period (Monthly Average Kerala State)

3. 3. 4 pH

pH is an important factor that determines the suitability of water for various purposes, including toxicity to animals and plants. pH of natural waters is governed by the carbonate–bicarbonate–carbon dioxide equilibrium. Slightly alkaline pH is preferable in waters, as heavy metals are removed as carbonate or bicarbonate precipitates and is not as toxic to aquatic life as they are present mostly in the unavailable form. Significant temporal and spatial variations exhibited in the pH values. Highest value observed at January and June, lowest value recorded in the monsoon periods both in the south west and north east monsoon (Appendix Figure .3.18&3.19).

The pH varied slightly avg (6.61 ± 0.10) in riverine zone; (6.6 ± 0.2) in the back waters and (7.61 ± 0.7) in estuary. pH showed seasonal fluctuation. It drops significantly in the month of October 2006 up to 5.42 (river), 5.92 ± 0.3 (back waters) and 6.14 ± 0.5 (estuary) and increased to 8.16 ± 0.2 ,

river ; 7.20 ± 1.2 , back waters and 8.3 ± 0.2 for estuary (Figure 3.4). This trend creates an alkaline behavior at monsoon and pre monsoon. No significant fluctuation was observed in the Station 1, (zone A - acidic). Lowest pH 5.34 (October) and highest pH 8.5(August) immediate after the monsoon onset in Zone B and C. The highest and lowest values were noted during the precipitation time (October, June and Aug) this indicated that highly acidic or alkaline effluents were discharged during the rain fall time and then the pH indicated a recovery in pre monsoon (Appendix Figure 3.18A & 3.18B). Similar observation was reported by Martin et al, (2007). The changing acidic and alkaline pattern in this zones may be due to the discharge of effluent from the neighboring industries which are located in the proximity of the Zone B. They are flushed and discharged directly into the bays with out any treatment. During the rainy season the high water flows and washed well and dilution taking place. Zone B, back waters (site S2, S3 and S4) water samples were very turbid due to the mixing process occurred soon after the rain fall. They are light brown to yellowish color because of excessive effluents. Often acidic pH (less than 6.0) was reported in back waters Zones during the north east monsoon where there is no persist rain fall to clear the water.

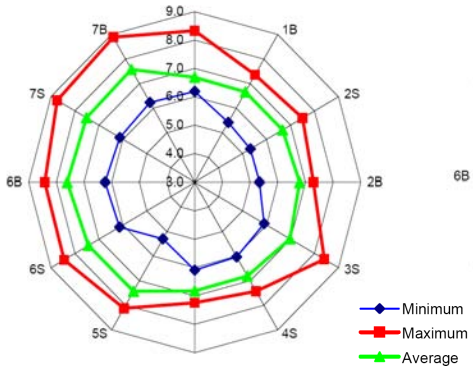

Figure 3.4 pH

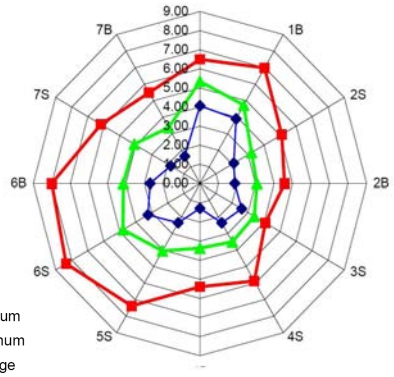

Figure 3.5 Dissolved Oxygen mg/l

Distribution of pH and Dissolved oxygen during the study period

The pH value in estuary (7.5 to 8.0) usually indicates the presence of carbonates of calcium and magnesium. These substantiate the dominance of bicarbonate, sodium and chloride towards downstream as compared to upstream.

3. 3. 5. Dissolved Oxygen

Generally, variation in Dissolved oxygen (DO) is basically governed by photosynthesis, respiration, mineralization and decomposition activities in water. The imbalance between relatively high rates of O_2 consumption and low rates of O_2 re-supply causes DO content to drop to levels that are low enough to adversely affect oxygen-requiring animal and plant life .Significant seasonal and tidal fluctuations were experienced in the surface and bottom waters. Lowest and highest values in DO content of both bottom and surface waters (sewage receiving areas- Zone B) was always fluctuating (1.28 to 8.0 mg/L) due to the availability of decomposing materials - detritus mixed with organic matters at the bottom and suspended decomposing material on the surface column. These areas were vertically stratified, slowly flushed,

and/or stagnant, the consumption of O_2 may exceed its re-supply from either atmospheric or in-stream photosynthetic (i.e., O_2 evolution) sources.

Concentration of dissolved oxygen measured bi monthly and it is observed that in overall study the dissolved oxygen is ranged from eutrophic (1.3 mg/L) to mesotrophic (8.4 mg/L). There is no significant variation of DO observed in riverine stations 4.0 to 6.5 (avg 5.3) in surface and 3.9 to 6.98 (avg 4.7) bottom water; where as in Back waters a significant fluctuation noticed (1.28 to 5.4) (Figure 3.5). Dissolved Oxygen is sensitive to season, it droppes to an average 4.0 ± 0.1, 2.1 ± 0.5 and 2.5 ±0.4 at river, back waters and estuarine respectively in pre monsoon. It increased up to 6.5 ± 0.2 in river; 5.5 ± 1.7 in back waters and 6.5 ± 1.4 in estuarine site during post monsoon. In the monsoon seasons the dissolved oxygen ranges from 2 to 5 mg/L. Compared to post monsoon DO reported lowest in monsoon but higher than that of pre monsoon season (Appendix Figure 3.19&3.20). Stations (S1 & S6), where there is no direct anthropogenic waste the oxygen content was always higher, in the surface water DO was higher than in the bottom. The intensification of algal community was grater in the surface compared to bottom waters while this trend is not strictly followed at Zone B a reverse trend was noted. High value was found in the bottom than in the surface samples in some pockets may be due to the fact that surface layer enriched with floating readily degraded or "reactive" organic matter and tend to consume large amounts of O_2. It is stated that oxygen consumed by

decomposition of organic matter is often reflected in the disappearance of oxygen from the deeper stratum of the water body. The study periods the oxygen content is always low at the bottom waters at Zone B and C. The estuarine Zone three stations were showed less oxygen content (< 3.0 mg/L) reported and having anoxic condition at pre monsoon and monsoon seasons.

Lowest oxygen contents were usually found in pre monsoon where the temperature reached in the highest peak. Highest value was recorded at the winter may be to low water temperature and considerable growth of algae, which may release appreciable amount of oxygen as a result of photosynthetic activities. The deficiency of DO content during summer might be due to active utilization of oxygen for bacterial decomposition of organic matter for the production. Owing to the high temperature during pre monsoon months (March-April) the process of decomposition is accelerated with the uptake of DO and low values were found in the month of April during the study (Appendix Figure .3.19&3.20). The brunt of pollution is usually experienced during pre monsoon periods, and the combined effect of high temperature and rapid decomposition of organic material occurs also favor the trend. No drastic recovery of DO was observed in monsoon from pre monsoon as estimated. During monsoon the sewage gets diluted and considerable amounts of organic material are deposited in the lower reaches of canal and estuarine openings resulting in the lowering of oxygen content in surface water and thereafter a recovery of DO was observed in the Post monsoon season.

3.4. Nutrients

3.4.1 Nitrate

Nitrate- nitrogen is one of the most important indicators of pollution of the water body. Nitrate represents the highest oxidized form of nitrogen. The most important source of nitrogen is the biological oxidation of organic nitrogenous substances, which arise in sewage and industrial waste or it can be produced indigenously in the water. The highest and lowest nitrate content was obtained in the lower (Zone B & C) and upper discharge areas (Zone A). The former is characterized by extensive anthropogenic pollution and later is upper area literally no anthropogenic discharge. The minimum value was reported at station I, Zone A (0.02 mg/l) and maximum value was at station 6, Zone C (3.41 mg/l) as shown in Figure. 3. 6.

Nitrate varied between 0.02 and 0.61 mg/L (avg 0.2) riverine site and 0.4 and 3.0 (avg 1.6) backwater zone and 0.17-3.41(avg 1.92) mg/L at the estuaraine zone. There was a net increase in nitrate concentration during monsoon and post monsoon and a decrease in pre-monsoon (April-may). A sharp increase in the nitrate concentration during monsoon months was observed. In General the bottom layer is more enriched compared to surface water particularly at estuarine regions. Little exemption in the riverine and back water stations were also noted.

The station 1 is an unpolluted riverine site, highest concentration 0.62 and 0.63 mg/L (surface and bottom) were found

in summer and 0.23 and 0.35(surface and bottom) reported in monsoon. While lowest concentration was found to be in winter both in surface and bottom. The Zone B (S2,S3 & S4) is typically canals collectively named as back waters showed a uniform distribution of nitrate ranges from 0.4 -3.0 mg/L(avg 1.71mg/L) . Among this S2 located near by a Fish market and always prone to higher nutrient enrichment with high anthropogenic discharges (> 1.2 mg/L) irrespective of season which indicates a constant load of nutrient. As reported by many researchers in Zone C, i.e. Cochin estuary, an increase in the nitrate concentration during monsoon season was detected with respect to pre-monsoon (Appendix Figure 3.21& 3.22). The onset of south west monsoon was always accompanied by a general rise in the nitrate level and the concentration of the nutrients was high during the monsoon months in Cochin estuary and previous study Lakshmanan et al (1987) also found similar results. There was a net increase in nitrate concentration during monsoon and post monsoon and a decrease in pre-monsoon period was recorded in this area. It is observed that during monsoon and post monsoon there was net addition of nutrients while in pre-monsoon there was net removal and earlier works supports this trend (De Sousa et al.,1981). An increasing trend of nitrate is observed from 2006 to 2007 (Appendix Figure 3.21&3.22) and bottom was always higher compared to surface in all season.

3.4.2 Nitrite

Spatially nitrite ranges from ND - 0.3(avg 0.06) in riverine, 0.01-0.43 (avg 0.16) at back water and ND- 0.60 (avg0.18) mg/L estuarine zone (Figure 3.7). Distinct relation was not observed between surface and bottom values in the estuarine and back waters but in riverine site the bottom layer always higher than surface layer. Seasonally Nitrite shows in the peak level in premonsoon than in the Post monsoon and lowest or even below detectable level were reported during the peak monsoon periods. High nitrite concentration was noted at zone B and C and low at zone A. Seasonal average of nitrite concentration was low during monsoon months, the highest value reported at summer and winter (Appendix Figure . 3.23&3.24). Spatially the nitrite shows an increasing order from river, back water and estuary. Highest value is reported at Champakara (S2) and cheranallur ferry (S4) where Low O_2 (some time <4.0 to 1.28 mg/L) in summer and winter season were noted with foul smell. Anthropogenic activities were prone and high biological productivity reported and excessive proliferation of flora leads to eutrophication.

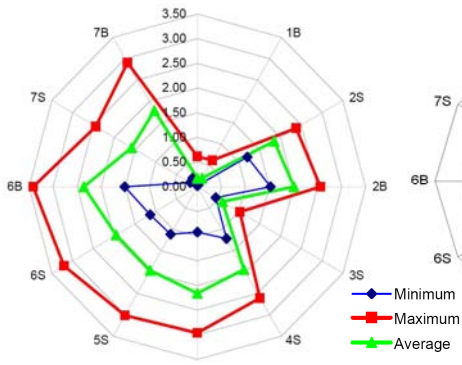

Figure 3.6 Nitrate mg/l

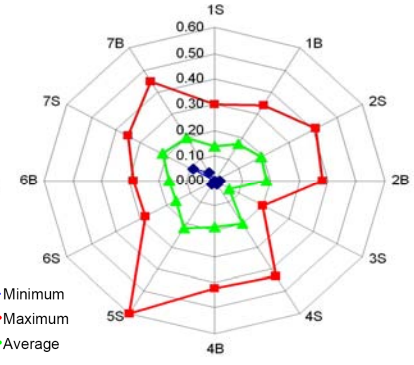

Figure 3.7 Nitrite mg/l

Distribution of Nitrate and Nitrite during the study period

Station S2 which was situated near the fish market and slaughtering unit - a perfect example of direct human intervene area and these animal waste were floating all the time which in turn decay from the surface of the water leads to excess nitrite compared to bottom layer. High NO_3 was observed in this station indicating that the upward mixing and displacement of this NO_2 to NO_3 complete. Unlike nitrate and Phosphate, nitrite least stable nutrient in the water and there is no distinct relation between surface and bottom layers observed in the estuary.

3.4.3 Phosphate

Phosphate shows spatial and temporal variations. In the upstream viz Movatupuza River varies between 0.02 and 0.27 (avg 0.075) while in the back waters it ranges from ND to 4.41 mg/L (avg 2.5 ± 1.5). High values reported in some pockets.

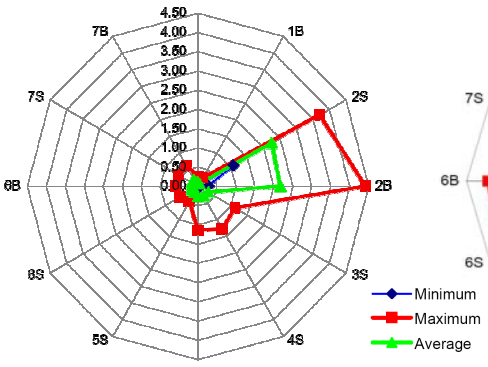

Figure 3.8 Phosphate mg/l

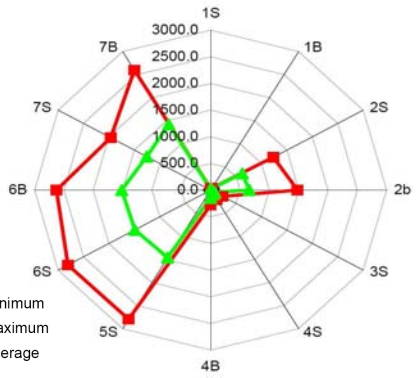

Figure 3.9 TDS mg/l

Distribution of Phosphate and TDS during the study period

Spatially phosphate content showed drastic seasonal zig-zag. In riverine zone the concentration of phosphate was reported a sharp increase in the peak winter season while estuarine sites always shows a consistent distributions 0.02 to 0.61 (avg 0.2) mg/L. It is observed that unlike nitrate, PO_4 is more enriched at the surface layer than at the bottom layer in all studied area (Figure 3.8). Monsoon and winter season which dropped in pre monsoon season while in the estuary phosphate showed maximum in the pre monsoon than in the monsoon and lowest was reported in the winter season. Seasonal distribution of Phosphate in the back waters varied differentially in all stations due to high anthropogenic discharges (Appendix Figure 3.25 &3.26). Spatially Phosphate showed highest in back waters than in estuary and least in the riverine samples. The lowest value is observed at Movattupuza (S1) and highest > 4.0 mg/L was at Champakkara - a back water site. Champakara as mentioned earlier is a direct anthropogenic effected area recorded highest PO_4 concentration through out the study, concentration up to 4.4 mg/L recorded in October 2006. Estuarine sites always showed a consistent distributions (0.02 - 0.66; avg 0.2 mg/L). It was observed that generally the nutrients are more enriched at the surface than in the bottom and often these nutrients available in the water column are not fully utilized by the phytoplankton and high concentration were detected in the surface column similar results was also reported by Gopinathan et al (2001). Nair et al (1988) reported higher productivity in bottom waters in tropical waters and well

low concentration of nutrients at bottom layer in the study area. World rivers have a nitrate: phosphate ratio of approx 15 Redfield ratio. This implies that the phosphate is biologically mobilized and always a limiting nutrient. The present study outweighs that phosphate is enriched relative to nitrogen by a factor of three. The increased phosphate is no doubt caused by inorganic phosphate.

Phosphate shows seasonally and spatially different pattern. In the riverine station the concentration of phosphate was reported a sharp increase in the monsoon and winter season which dropped in pre monsoon season while in the estuary phosphate showed maximum in the pre monsoon than in the monsoon and lowest was reported in the winter season. Seasonal distribution of phosphate in the back waters were varied differentially in different station due to high anthropogenic discharges, a high maximum peak was observed in summer and winter while low values reported during the monsoon due to dilution effect with fresh water.

3.5 Carbohydrate and Proteins

Carbohydrates (CHO) and Protein serve both as structural and storage components and comprise up to 40% of the organic matter (OM) in marine and 75% of the OM in terrestrial organisms (Aspinall, 1983; Parsons et al., 1984; Biersmith and Benner, 1998). Proteins are the most important nitrogen-bearing compound in most organisms. Both compound classes together quantitatively dominate the organic carbon in living organisms (Wakeham et al., 1997). Their analysis provides a useful tool to evaluate the reactivity of particulate organic matter (OM) (Kerherve et al., 2002;

Pantoja and Lee, 2003). Carbohydrate and Proteins are common structural and storage compounds in both marine and terrestrial organisms and represent the major form of photo chemically assimilated carbon in the biosphere and are potentially powerful tools in elucidating the sources, processes, and pathways of biologically important organic materials in nature. Carbohydrate varied from 1.9 to 6.39 (avg 3.45) river zone; 1.74 to 29.71 (avg 10.7), back water zone and 2.55 to 17.14 (avg 9.95) mg/L at estuarine zone (Figure 3.10). Seasonally carbohydrate showed highest in the post monsoon and lowest in the monsoon except at some pocket of back water where a sharp peak in carbohydrate was observed in the monsoon season. Generally at all stations the surface column was more enriched compared to bottom water column in Zone A and B but a inverse pattern was observed with Zone C. Spatially carbohydrates showed higher in back waters followed by estuary lowest in the river station as observed as nutrient concentration . Carbohydrates produced from photo assimilated carbon in benthic diatoms, but its production depends on nutrient availability and growth phase (Buzzelli et al., 1997; Goto et al., 1999; Staats et al., 2000) as well as tidal and photosynthetic rhythms (Smith and Underwood, 1998). As expected the concentration of carbohydrate was found to be lower at bottom column on the other hand protein was enriched in the bottom layer may be attributed that most of the production took place in bottom layer and carbohydrate were assimilated by predators and release nitrogenous compounds.

Protein ranges from 1.33 – 50.0 (avg 21.7) mg/L at river zone; 24.7- 107(avg 63.4) mg/L at back water stations and 13.08-58.1(avg36.82) mg/L at estuarine stations. In contrast to carbohydrate, distribution of protein was more concentrated at bottom layer than surface layer with few exceptions. Seasonally the protein showed spatially mixed distribution, highest concentration observed in monsoon season but at some pockets, observed in the back waters the sharp peak was observed at pre monsoon. (Figure 3.11).

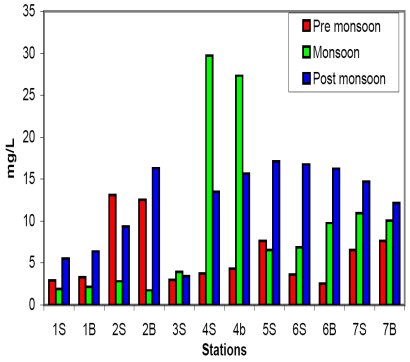

Figure 3.10 Carbohydrate

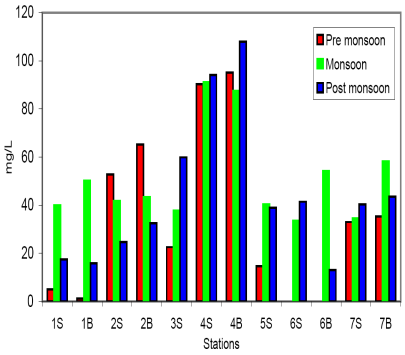

Figure 3.11 Protein

Distribution of Carbohydrate and Protein during the study period

3.6. Sulphate

Sulphate in aquatic system might be highly inflated by river discharges. Sulphate content from 12.55 – 100 mg/L, river; 34.42-2314 mg/L, backwater and 132-3422 mg/L at estuary (Figure 3.12). Maximum concentration of SO_4 was found during the dry season in April due to low dilution and lowest values recorded in monsoon, large quantities of rain water and flow dilute the

minerals. There is no significant distinct relation in sulphate content observed between surface and bottom column and varied spatially and seasonally. The high level of sulphate recorded in Zone B and C indicating an additional source of sulphate to the back water and estuarine sites. The occurrence of sulphate ions is in the form of dissolved sodium sulphate, calcium sulphate and magnesium sulphate practically at alkaline condition was common. The main source of sulphate ions was probably from industrial effluent, discharged unit and river discharges and the relatively high sulphate concentration accounts for the major difference between studied water and seawater. SO_4 concentration up to 3422mg/L found in pre monsoon along with high salinity ≈ 34 PSU at estuarine region this value is compared to 2750 mg/L present in seawater having salinity ≈ 35PSU suggesting additional SO_4 during the dry season . Seasonally Sulphate showed sharp peak value in pre monsoon and than in post monsoon least was recorded in the monsoon.

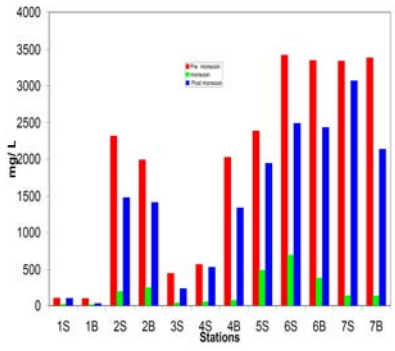

Figure 3.12 Sulphate

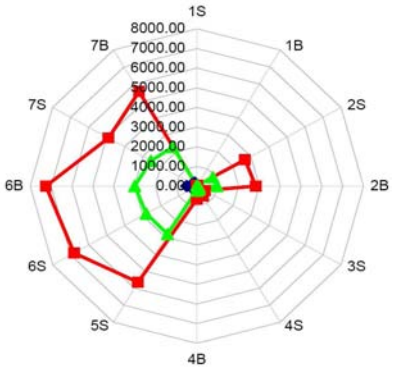

Figure 3.13 Hardness mg/L

Distribution of Sulphate and Hardness during the study period

3.7. Hardness

Natural hardness of water depends upon the geological nature of the drainage basin and mineral levels found in natural water. The hardness of water is not a pollution parameter but indicates water quality. In the present study it shows significant temporal and spatial fluctuation, total hardness as $CaCO_3$ ranged between 9.14 to 7250.0 mg/L from river to estuarine stations. Yearly averages show a decreasing trend. Highest value reported in estuary from 203- 7250 (avg 2691) mg/L, 22.33-2822 (avg 481.4) in back waters and 8.72 – 80.00 (18.8) mg/L for river (Figure 3.13). The total hardness fluctuating seasonally, highest values reported in the pre monsoon and lowest value reported at monsoon.

The maximum values for hardness were observed due to evaporation and reduced inflow in summer, and minimum values due to dilution in the rainy season. The principal source of calcium and magnesium in groundwater is the silicate mineral groups like Plagioclase, Pyroxene, and Amphibole among Igneous and Metamorphic rocks and limestone's and dolomite and gypsum among sedimentary rocks. It assumes that ion concentrations of Cl^-, SO_4^{2-}, HCO_3^-, CO_3^{2-} , Na^+, Ca^{2+} and Mg^{2+} are the most elevated ions in water with pH values ranging between 5.5 and 8.5 (Boaventura and De Freitas, 2006) . pH of the study area always found between 6.5- 8.3 which will bump up Cl^-, SO_4^{2-}, HCO_3^-, CO_3^{2-}, Na^+, Ca^{2+} and Mg^{2+} concentration. The low discharge areas zone B and C noted a high concentration of these ions. Such high concentration along with high alkaline water derives from waste

dumping of materials from the fertilizer factories and other chemical industries near the water body.

3.8. Total Dissolved Solids

The TDS contents of the estuarine waters ranged from 10.8 to 2805 (avg 1428) mg/L but riverine water shows a even distribution 14.4-44 avg (24.4) and 32-1480 (avg 319) mg/L at back waters, much variation was not observed between surface and bottom layers but later is generally higher than former (Figure 3.9). Highest concentration was recorded in the pre monsoon and post monsoon while lowest concentration was recorded in the monsoon. While sampling, it was visually clear that the estuarine waters looks muddy almost up to the bar mouth while Movattupuza river water was almost clear in all season except during the high precipitation during the south west and north east monsoon seasons. The highest particulate contents were encountered at estuarine and some pockets of backwaters where a lot of human discharges find its way. As expected the total dissolved solids are more concentrated in lower discharge areas of estuary than in back water zone; least was found in the riverine with the high discharge site. The TDS was more pronounced in summer due to evaporation, concentrate the ions and least was recorded at monsoon due to dilution and post monsoon stage is typically a transition stage. The common source of solids in natural waters are rain and wind erosion of soil surfaces along with municipal and industrial waste waters also originated from domestic wastes, road run off and industrial process all these contribute considerably at

high pH commonly observed in these area as indicated earlier. It assumes that ion concentrations of Cl^-, SO_4^{2-}, HCO_3^-, CO_3^{2-}, Na^+, Ca^{2+} and Mg^{2+} are the most elevated ions in water with pH values ranging between 5.5 and 8.5 (Boaventura and De Freitas, 2006)

3.9 Sedimentary analysis

The components and composition of the sediments have received much attention. Most of the work in India relates to the texture and organic content of marine and estuarine sediments. The nature and extent of fluctuation in the composition of sediments can indicate the balance between the erosion and depositional forces of the ecosystem. The supply and sources of these materials and the sites of the deposition mainly depends on the system, river discharge, currents, wave action and tidal regime. Organic carbon content in the sediment of the estuarine and riverine system is of paramount interest as a potential food for the benthic fauna. As many researchers have demonstrated, the organic matter in sediments is often determined by preservation rather than production (Wetzel, 1983; Dean, 1999), it is expected that about 10% of the estimated net primary production is deposited as organic carbon in eutrophic lakes (Bloesch et al., (1988). Generally the state of preservation depends partially on its texture as well as microbial and redox potential of the sediment.

This section presents sediment data collected over a period (2005 -2007) from 7 stations and divided into 3 Zones: river, estuary and backwaters , a hot spot with in the vicinity of industrial belts where a variety of effluents are discharged into these sits . Details regarding the sites were described under the chapter 2.

Sediment samples were collected with a Van veen grab sampler. Samples were shade dried in the laboratory. The samples were analyzed for grain size and percentage sand, slit and clay were determined by pipette method. Samples for texture analysis were washed repeatedly with distilled water to remove salts. Organic Carbon was estimated by wet digestion method and percentage organic matter values by using a factor of 1.724.

Colour and Grain size of the sediments varied temporally and spatially (Table 3.1). Station 1 sample had almost sandy in nature and no fine grained material was observed in monsoon. Monsoonal floods flushed away all accumulated material exposing the gravel underneath. Station 2, Champakara a effluent discharge area where the color of sediment was constant and the seasonal changes being less evident. The sample were a collection of organic debris and dark green to black in nature, this could be attributed to the settlement and decay of organic material. Station 3 is characterized by sediment having light black shade with variation in the grain size. Station 4 is mostly sandy in nature but in monsoons an occurrence of slit was noted: due to the settlement of sediment. The estuarine sediment collected from Stations 5, 6 and 7 are largely affected by dredging activities and leaching due to seasonal changes. They were dark brown to red in color. Dredging changed the sediment size character totally into fine sandy and slity in the pre monsoon. While monsoon and post monsoon was characterized by slit and clay with dark brown clay type sediment. The textural characteristic was studied by the

method based on Mudroch et al (1997) and the following observation was made.

Table 3.1 Seasonal Textural Characteristic of the sediment

Station	Monsoon			Post monsoon			Pre monsoon		
Monsoon	Sand %	Clay %	Silt %	Sand %	Clay %	Silt %	Sand %	Clay %	Silt %
S1	97.45	2.16	0.39	97.19	1.79	1.02	96.87	2.56	0.57
S2	.....	.....	.....	83.51	9.69	6.8	91.58	7.44	0.98
S3	84.85	6.39	8.76	.....	.....	.....	42.76	19.37	37.87
S4	15.58	27.03	57.39	60.15	38.34	1.5	74.72	12.67	12.61
S5	42.29	18.99	38.72	42.29	28.99	48.72	.....	.....	.....
S6	34.63	21.98	43.39	94.35	5.57	0.08	82.3	14.3	3.4
S7	19.1	27.31	53.59	4.89	34.88	39.77	91.15	7.59	1.26

3.9 Organic Matter and Carbon Content

The maximum, minimum and average values of Organic carbon and Organic matter are given in the Figure 3.14 & 3.15 respectively. Comparison of average organic matter values at station 1 situated in the fresh water regime through out the year, only showed a marginal enrichment (avg 0.56 and maxi and mini was 1.48 and 0.05 % respectively). High values were reported during the monsoon seasons. Organic matter also exhibited the same trend (avg 0.96 % and ranges from 0.1 to 2.56 %).

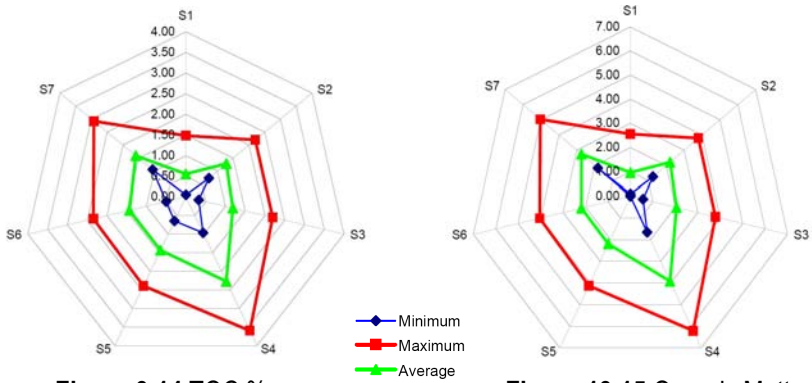

Figure 3.14 TOC % **Figure 13.15** Organic Matter %

Distribution of TOC and Organic matter (%) during the study period

TOC values at back water Stations 2, 3 and 4 ranges from 0.33 to 3.60 %. Station 2 characterized by anthropogenic organic waste; reported moderate TOC and organic matter content (0.73 to 2.29; avg 1.28 %). No significant seasonal variation was observed in this site and always showed same pattern. Organic carbon showed highest in the pre monsoon and lowest at monsoon. Organic matter ranges from 1.6 to 3.79 (avg 2.21 %). As expected the highest values were reported at station 3 (avg 1.19 %) and then 4 (avg 2.27 %). Organic matter in this site varied between 0.51-3.78 (avg 2.05) at station 3 and 0.56-6.21 (avg 3.92 %) at Station 4. These sites are nearer to canals which are connecting Cochin Harbor to industrial areas a water transport channel to transport organic chemical , oil etc for the near by industries through boat. Many researchers previously reported high organic load at these areas. The organic carbon value near the area of Cochin Harbor (stations 5, 6 and &7) 0.5-2.92(avg 1.45%) and absolutely agrees with the previous studies. Murthy et al (1969) reported a value of 3.8 and a value ranges from 0.74-3.48 % by Sankaranarayanan and Panampunnayil (1979) and recently 0.06-3.57% by Renjith (2006). They have reported the high organic content in the shallower area (station 7) and to the contribution of terrigenous sources and Sarala devi et al (1979) also reported high organic load based in the study related to water quality aspects.

3.11. Correlation Analysis

Statistical parameters (Correlation and Regression) were designed employing the statistical software SPSS v.10 for windows and the results were shown in the Table 3.2. Significant correlation was found between nutrient with the salinity, Dissolved Oxygen and temperature. Along with physicochemical parameter the statistical analysis were carried out for the phytopigments with their associates and the result discussed were discussed in the Chapter IV.

The processes influencing dynamics of dissolved substances within estuaries can be investigated by testing for conservative behavior. This is usually done by plotting concentration of each parameter and testing the behavior one another. The Dissolved Oxygen(DO)and nutrients (nitrate, nitrite and phosphate) were plotted against salinity for each sampling zone; if the concentration of a substance relates linearly to salinity, its dynamics are controlled by mixing processes and no significant uptake or release occurs within the estuarine boundaries. DO versus salinity showed a strong inverse correlation as expected in all the zones both in surface and bottom. The inverse relationship between salinity and DO in Cochin estuary has reported by Haridas et al (1973) and Kumaran et al. (1975).

Table 3.2 Correlation analysis Surface and Bottom water samples

Correlation analyis River Zone A (surface)						
	NO_3	NO_2	PO_4	Salinity	Temerature	DO
NO_3	1.00	0.10	-0.19	0.39	0.49	-0.24
NO_2		1.00	-0.01	-0.07	0.46	-0.28
PO_4			1.00	-0.27	-0.02	-0.06
Salinity				1.00	0.61	-0.81
Temerature					1.00	-0.33
DO						1.00

Correlation analyis River Zone A (Bottom)						
	NO_3	NO_2	PO_4	Salinity	Temerature	DO
NO_3	1	-0.035	-0.048	0.361	0.476	-0.405
NO_2		1	0.117	0.125	0.234	-0.348
PO_4			1	0.712	0.129	-0.314
Salinity				1	0.585	-0.498
Temerature					1	-0.629
DO						1

Correlation analyis Zone B (surface)						
	NO_3	NO_2	PO_4	Salinity	Temerature	DO
NO_3	1.00	-0.56	0.36	-0.05	-0.86	0.17
NO_2		1.00	0.36	-0.07	0.62	0.09
PO_4			1.00	-0.06	-0.24	0.22
Salinity				1.00	0.20	-0.40
Temerature					1.00	-0.03
DO						1.00

Correlation analyis Zone B (Bottom)						
	NO_3	NO_2	PO_4	Salinity	Temerature	DO
NO_3	1	-0.663	0.408	-0.056	-0.332	-0.031
NO_2		1	0.214	0.351	0.026	-0.193
PO_4			1	0.019	-0.151	0.033
Salinity				1	-0.098	-0.163
Temerature					1	0.335
DO						1

Correlation analyis Zone C (surface)						
	NO_3	NO_2	PO_4	Salinity	Temerature	DO
NO_3	1.00	-0.68	-0.05	-0.68	-0.26	0.06
NO_2		1.00	-0.17	0.23	-0.21	-0.16
PO_4			1.00	0.61	-0.24	-0.66
Salinity				1.00	0.29	-0.53
Temerature					1.00	0.61
DO						1.00

Correlation analyis Zone C (Bottom)						
	NO_3	NO_2	PO_4	Salinity	Temerature	DO
NO_3	1	-0.546	0.303	-0.744	-0.398	-0.157
NO_2		1	-0.283	0.397	0.224	-0.006
PO_4			1	0.055	-0.579	-0.472
Salinity				1	0.005	-0.363
Temerature					1	0.605
DO						1

Salinity play an important act in the nutrient distribution and the negative relation may be due to high uptake of oxygen by flora is characterized by large bloom. Salinity vs. Nutrients shows variable results Table 3.2 . Salinity is correlated linearly with nitrate and phosphate, a positive with former and negative with later. But there is no significant correlation was observed with nitrite in the surface water of riverine zone, while the nutrients show good positive relation with bottom water. A negative correlation for salinity and nutrients in the back waters indicates that salts and nutrients inputs are from common source and behave conservatively and are utilized by predators. The nitrates in the bottom column were related inversely but phosphate and nitrite showed a positive relation. In estuarine region surface and bottom nitrate concentration showed a clear inverse relationship with salinity while phosphate and nitrite correlated linearly with salinity.

The relationship of the nutrients to dissolved oxygen reveals deviation from a simple scenario of biological uptake in the surface waters and regeneration of nutrients from settling organic matter at increasing depth. It is observed that nutrients (phosphate, nitrate and nitrite) were inversely related to dissolve oxygen in riverine site while in the back waters variable relation was observed due to high release of nutrients. In the back waters phosphate and nitrate showed positive association but nitrite related inversely with DO in surface samples and in the bottom column the nitrite and nitrate shows negative and phosphate showed a positive correlation. In

the estuary nitrate, nitrite and Phosphate (-0.54) related inversely throughout the water column. In the bottom water column DO correlated inversely with nutrients in all the study Zones.

Very low relation with DO obtained with nitrite in back waters may be due to the dissolved nitrite is depleted relative to that of oxygen utilization even though these stations recorded higher nitrate which may be due to the fast conversion of NO_2 to NO_3. This is supported by low dissolved oxygen with positive correlation between nitrates. It can be suggested due to the nutrient starved sinking algae replenishing their nutrient pools or denitrification by chemoautotroph. High discharge of nutrients may produce oxygen starved areas with a positive correlation with nitrate and phosphate attributed to low consumption and eutropication trend in some pockets (Cheranellur Ferry and Champakara) which were worse due to anthropogenic activity and high discharges . These results conclude that in the back waters Nitrite acting as a limiting nutrients but Nitrate and Phosphate is excess and conversion of NO_2 to NO_3 is complete.

From correlation analysis it is evidenced that there is no depletion in any nutrient in the riverine zone. In the estuarine and riverine zones the phosphate were inversely related to dissolve oxygen. Moderate and constant enrichment of PO_4 and low oxygen content with strong linear relation ship with oxygen indicating a strong uptake of regenerated PO_4 in these sites. This uptake could be the result of chemical adsorption reaction (Callender,1982) or a non- photosynthetic biological uptake. This

high inverse correlation is concordant with the observation of Quasim et al, (1972) stated that salinity has apparently no influence on primary production but help mixing process. There is a close correlation (negative) between cycles of phosphorus and organic production in the back water but unlike his observation the nitrogen cycles is well connected to productivity. The negative correlation with DO suggested that back waters zone with moderate consumption and no eutropication trend is observed in estuarine zones and phosphate is acting a limiting nutrient.

3.12. Conclusion

Availability of nutrients has been recognized as one of the major factors controlling primary production. The concentrations of nitrite, nitrate, phosphate exhibited pronounced seasonal variation and also indicated large inputs from industrial units, sewage works and agricultural runoffs. On the estuary, a core of high values during monsoon suggests the presence of an external input source while in the Zone 1 remains low concentration. A comparison of the nutrient distribution with earlier reports suggests the resultant behavior linked to inter basin transfer of water and the construction of Thannirmukham bund which are affecting the nutrient load. The observed nutrient values were due to the control exercised by arrested oxidation of organic matter, soil leaching and seasonal depletion brought about by primary production. Nitrate, nitrite and phosphate concentration showed more complex distributions and increased dissolved nutrients concentrations at low salinity regions during high discharge areas

may be due to release of river borne suspended sediments followed by removal due to scavenging in the turbid zone. These leads to lower the concentrations at higher salinities. Dissolved and particulate organic matter supplied by rivers and canals to the estuary exhibited pronounced seasonal variation and also indicated large input from industrial unit's sewage works and agricultural runoff (B and C Zones). The riverine zone and Canal nutrient supply may cause enhanced surface-water productivity.

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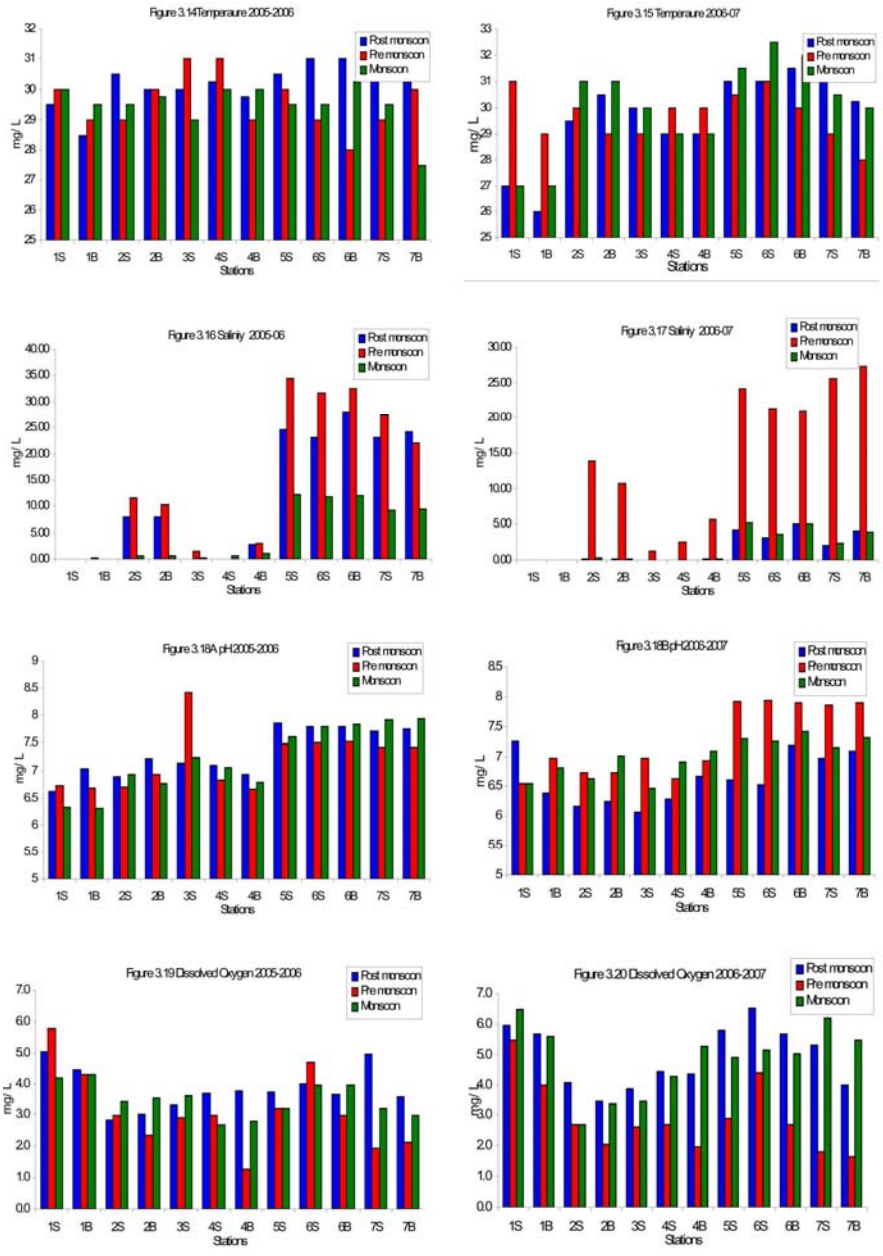
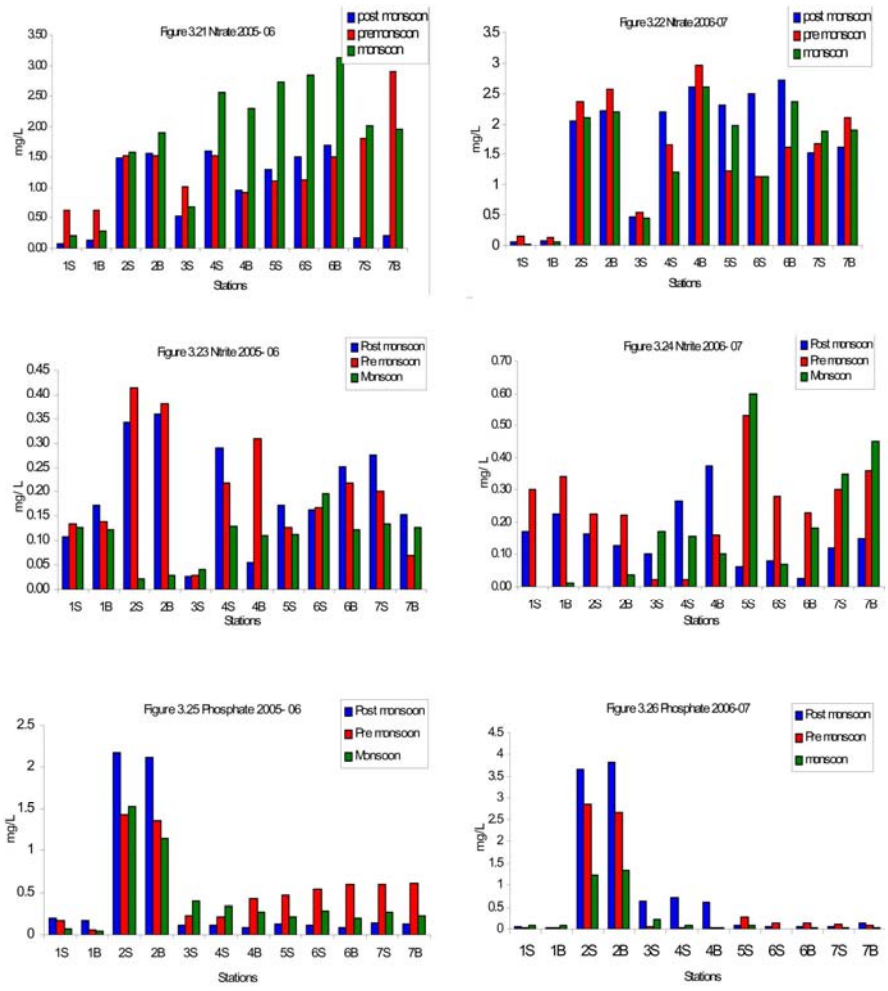
Appendix



Distribution of hydrochemical parameters during the study period



Distribution of hydrochemical parameters during the study period

Chapter 4

Spatio-Temporal variation of Pigment and it associates in Cochin back waters

4.1 INTRODUCTION

The pigment chemistry of the ocean is of central importance to a world concerned about climate change. Marine phytoplanktons comprise a quarter of the world's vegetation and call it by name "Green Gold" or "plant blood". Chlorophyll is proven pigment of life and its function of converting light energy

to chemical energy has recognized long back. The ***Chlorophyll - A biomass indicator of these plants*** is the most frequently measured biochemical parameter in aquatic chemistry. The Chemistry of Phytoplankton pigments in aquatic niche reveals a cutting edge for both quantitative and qualitative estimation of Chlorophylls and Carotenoids which would give a background to pigment status and pigment processes. Besides evaluates a classical and modern method for identification, compilation, interpretation and graphics it infers vivid conclusions on selective significant key pigment.

It is well known that photosynthetic pigments are the index of primary production and playing a significant role in the ecological characteristics of an ecosystem. The essence and intensity of the factors and processes influencing the ecosystem, and their consequences are extremely complicated, which causes high temporal and spatial variability in the chemical and biological characteristics of water masses in estuaries. Perusal of literature reveals that extensive works were carried out on the qualitative and quantitative aspects of chlorophylls and primary productivity in the South West Coast of India , mostly confined to the Cochin Estuary (Qasim et al.,1967, 1973; Gopinathan et al.,1972, 1974, 1975; Joseph and Pillai, 1975; Thangaraj 1984; Jayalakshmi et al. , 1986, Selveraj, 2000, 2002 & 2003; Madhu et al., 2007; Sanilkumar, 2009). On the other hand, in recent years tremendous change in the urbanization, industrial developments and the pertinent continuous fluctuations in the precipitations from normal to actual, have aggravated the physicochemical and biological characteristics

of coastal waters. Very little information is available on these aspects to their change in the characters of the Cochin Back waters and surf zones. Since in estuarine environments, phytoplankton is quantitatively the second source of particulate organic matter after terrestrial inputs from soil erosion which supply the highest quality food source and constitutes premier basis of the estuarine food web (Heip et al.,1995; Gasparini et al.,1999; Burdloff et al.,2000). Restoration of this Cochin back water system (CBWS) requires an understanding of the Phytoplankton biomass- A fundamental importance for the biology. Behavioral chemistry of Nutrients, Micro trace elements and other Organic Matters (OM) like Carbohydrate, lipids and Proteins and its contributing pattern would provide a pre requisite knowledge for the biogeochemical process occurring in the above said aquatic realm. The phytoplankton biomass comprises: ***Chlorophyll a, b and c*** and its degradations products (Pheophytin a). Chlorophyll a (Chl a) has been a subject of constant interest to researchers for many years not merely because of its abundance in the marine environment but also it has become a recognized marker of different processes taking place in seawater (Jeffrey et al., 1997). It is a marker of organic carbon (Bianchi et al., 1995) and is present in all phytoplankton species, it can serve, as a measure of changes in their biomass. The parent molecule- ***Chl a,*** its derivatives like *Chl b* and c, and its degradation products in seawater are good markers of different processes taking place in the water column, principally grazing by heterotrophic organisms. (Welschmeyer and Lorenzen,

1985). Large amounts of chlorophyll a derivatives, especially of pheophorbides a, are indicators of intensive activity of these organisms (Bianchi et al., 2002).Figure (4.00)

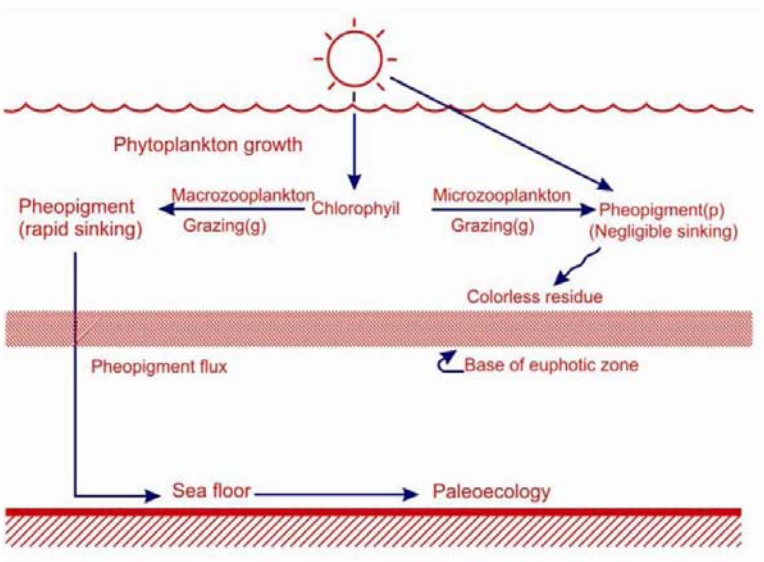


Figure 4.00 Dominant process affecting the concentrations of phytopigments in the euphotic zone

The spatial and temporal gradients in environmental parameters offered by the coastal waters Cochin make it suitable for examining the relationship between physico-chemical parameters and phytoplankton in terms of chlorophyll a which forms the main objective of the present study. In order to achieve this, multivariate statistical approach is adopted. Chlorophyll a can be related to the environmental parameters by means of linear regressions, though it provides only the prediction efficiency of a single factor at a time. This Chapter contributes to the seasonal

variation of Chlorophylls and productivity in the different segments in the Cochin Back Waters system (CBWS) situated along the Kerala coast on the South West Coast of India, (9^0 40'N to 10^0 10'N and 76^0l3' E to 76^0 50' E) (Detailed description are given in Chapter 2 : Materials and Methods). In order to explain the hydrography and the distribution pattern of various forms of Nutrients, Micro trace elements and other organic matters (OM) like carbohydrate and proteins and its correlation with phytopigments (chlorophyll a , b , c and pheophytin a) a set of observations were carried out bimonthly during 2005-2007. Seven prominent stations were identified and categorized into three zones (Table.2.1 and Fig. 1.2). In order to recognize the magnitude of the salinity influence and also to represent the socio economic importance of pollution loads. The study aims to understand

a. Controlling and interactive factors of phytoplankton production and
b. Interrelationship between macronutrients, micronutrient trace elements and Phytopigments in the tropical estuarine system – Cochin estuary with respect to spatial and seasonal change.

4.2 Results

4.2.1 Hydrographical Conditions

The details of the sampling handling, sample preservation procedure and analytical methodology were described in Chapter 2. The hydrographical conditions of the CBWS are greatly

influenced by seawater intrusion and influx of river water as indicated by the distribution of temperature and salinity explained in Chapter 3 (Figures 3.1 to 3.13) at surface and bottom (0.25 m above the estuary bed). There are three pronounced seasonal conditions prevailing in this estuary, i.e. monsoon (June–September), post-monsoon (October–January) and pre-monsoon (March–May). During the monsoon period the region receives about 290-320 cm rainfall annually, of which, nearly 60% occurs during the southwest monsoon season, rest would fall on north east monsoon. The estuary is connected to the Arabian Sea at two locations, Cochin (Latitude 9°58′ N) and Azhikode (Latitude 10°10′ N) (Figure. 2.1). During December to April, a salinity barrier at Thanneermukkom virtually cuts off the tidal propagation further south and modifies the circulation in the remaining part of the estuary. The onset date and duration of the southwest monsoon vary from year to year. Likewise, the associated quantity of rainfall contributes to the variability of the estuarine water levels, flow and chemistry. It is recognized that the water quality plays an important role in selecting the phytoplankton community (Chavez, 1996; Legendre and Le-Fevre, 1989). Temperature , salinity , substrate concentration , suspended particulate matter , DO, and pH have been shown to exercise an influence on nitrification which in turn proliferate phytoplankton production at one time or other , their relative importance as controlling factors may vary between different estuaries or even with in segment of an estuary.

Zone 1 is upstream has the lowest recorded salinity in the study and practically nil and a fresh water zone 0.01- 0.12 (avg 0.07psu). Zone II, Back water where the salinity ranges from 0.02 to 13.97 (avg.2.13) psu. While in the seaward end of the study area the estuary, salinity ranges between 0.1 and 34.64 (avg 16.8) psu (Figure 3.2, 3.16 &3.17Appendix). The highest salinity was recorded at pre and post monsoon and the Salinity decreases with the onset of monsoon and became poorly fresh water in character. The key water column parameters such as Secchi disc depth, nutrient concentration, abundance of Chlorophyll were monitored spatially and temporally. The water column were comparatively transparent and Secchi depth varied from 1.2 m at riverine site to 0.6 m at estuarine Zone. The availability of nutrients in the euphotic zone and its subsequent biochemical response is the basis of any biological properties of the aquatic systems.

The distribution of chlorophyll a, b c and pheophytin a, at the euphotic layer are presented in Figure 4.1. Concentration level of chlorophyll **a** as well as the composition of pigment is variable, which in turn depends strongly on the hydrological situation in the river watershed, distance of the given location from its mouth, seasonal circulation in the basin, water column stability and depth. Large variations in physico chemical and biological characteristics were observed in the CBWS system (3.1-3.26) therefore reliable evaluations of Phytoplankton composition and biomass require repeated sampling and analyses performed accordingly in the present study.

4.2.2 Chlorophyll *a*

Over all concentration in the entire sampling season (pre monsoon to post monsoon) ranges from 0.84 – 29.75 mg/m^3. Chl a value were high during the pre monsoon at all stations. Seasonal fluctuations in the chlorophyll content were recorded in all the stations (Figure. 4.1-4.7). The Chlorophyll **a** ranged from 1.18 to 14.06 (avg 5.07) mg/m^3 at river (station 1), 1.05 to 29.75 mg/m^3 (avg 5.87) at Zone B and 2.75 to17.97 mg/m^3 (6.5) at Zone C. The highest Chlorophyll **a** value (29.75 mg/m^3) was observed at Champakara in the month of April 2006. Moderately high and lower values were noticed in the back waters; where as a regular pattern was observed in the estuarine and riverine sites. In general, lower values observed in the monsoon and higher values recorded in the pre monsoon and post monsoon seasons.

4.2.3 Chlorophyll b

Chlorophyll **b**, an accessory pigment of Chlorophyll a showed the same trend, and followed in the entire sampling site (with slight variations in some pockets). The highest values were recorded in some pockets of Zone B and C at pre monsoons. The concentration ranged from 0.62 - 3.6; 0.59 – 9.98 and 1.32- 10.42 mg /m^3 at Zone A, B and C respectively. Moderately high and low values observed at Zone B (Champakara) and Zone C (Bolghatty) in monsoon and pre monsoon periods respectively (Figure 4.1).

4.2.4 Chlorophyll c

The concentration of chlorophyll **c** fluctuated between 1.17-11.1 mg/m^3 in Zone A, 0.16-14.13 mg/m^3 Zone B and 0.98- 10.67 mg/m^3 Zone C (Figure 4.1to 4.7).

4.3. Nutrients

Phytoplankton normally synthesis their proteins from nitrite, nitrate and NH_3 but bacteria usually use only these forms of nitrogen when organic nitrogen is not available. Bacterial oxidation ,commonly referred to as nitrification, is the only known biological mechanism of conversion of ammonium to nitrite and then to nitrate(Kaplan,1983) .This transformation of inorganic nitrogen(N) in the euphotic zone of the aquatic system and below influences the availability of the most oxidized form of nitrogren to primary producers which differ in their ability to utilize combined nitrogen nutrients. The rate at which nitrate is produced through nitrification also determines the magnitude of new production a key component in global biogeochemical models of N and C.

The present chapter focus to the surface seasonal distribution of nutrients owing to most of the photosynthetic activity took place at or near by euphotic Zone. The physical, chemical and biological characteristics of surf zone and the back waters sites are summarized in figures 3.1-3.13. During the period of peak biomass, temperatures recorded were 31^0C (April) and water temperature varied between 26.0 and 31.0^0C (Figure 3.1). The corresponding salinity 1 7.89 - 34.54 psu values were observed in

the peak summer at estuarine sites (S5,S6 &S7) and 3.75-6.87 psu in backwaters (S2,S3&S4) and very low 0.06 psu in river station (S1). Salinity values fluctuated between 0.05 and 34.64 psu from riverine to estuarine water samples.

Nitrate varied between 0.02 and 0.61 mg/L (avg 0.2) river, 0.44 and 2.96 backwaters and 0.17-3.41 mg/L at Estuary (Figure 3.6). Higher concentration were recorded during monsoon vertical mixing due to solar heating during post monsoon 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 was always lower than nitrate concentration and varied between 0.02-0.27 (River), 0.05-4.41(backwaters) and 0.02-0.66(Estuary) mg/L (Figure3.8). Higher concentration was recorded during monsoon and post monsoon periods when the regeneration of phosphate dominates over utilization in photosynthesis. Lower concentration coincided with the periods of peak production. Lower in summer and winter (Nov – Dec) coinciding with higher production of Phytoplankton. The increased light intensity, long duration of light and inhibition of nitrite shows a different pattern compared to phosphate and NO_3 highest concentration was observed in the Pre monsoon and lowest in the peak monsoon. In the present investigation the high value of phosphate (1- 4 mg/L)(Figure 3.8) and Nitrate (0.4 -2 mg/L) (Figure 3.6) were recorded at Champakara canal with the moderate Chlorophyll a was observed (Figure 4.2). 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. 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 ND - 0.3 (avg 0.06) in riverine, 0.01-0.43 (avg 0.16) at back water and ND- 0.60 (avg0.18) mg/L estuarine zone (Figure 3.7). It is generalized the region of high concentration of chlorophyll a are characterized by the pockets of high anthropogenic discharge where high concentration of nutrient observed.

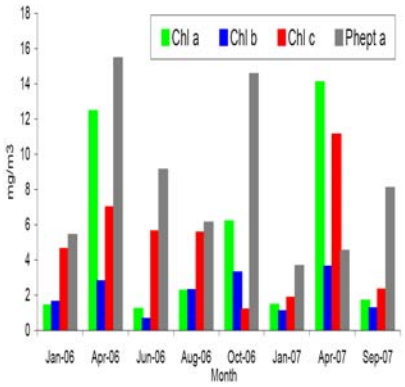


Figure 4.1 Station S1

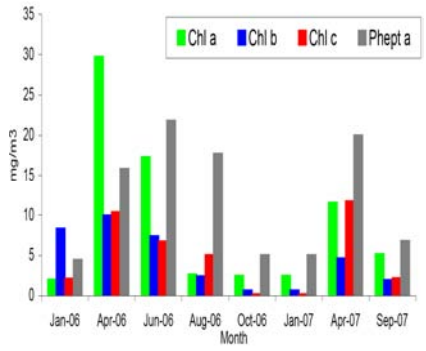


Figure 4.2 Station S2

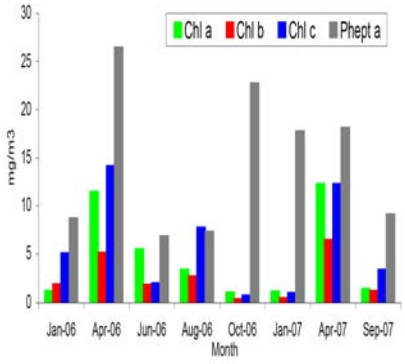


Figure 4.3 station S3

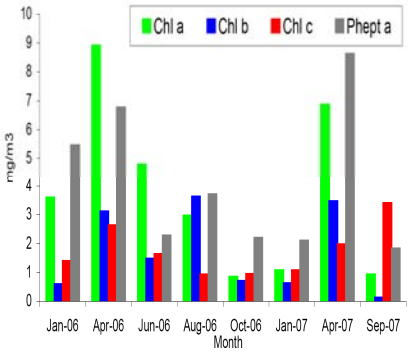


Figure 4.4 Station S4

Distribution of phytopigmnets

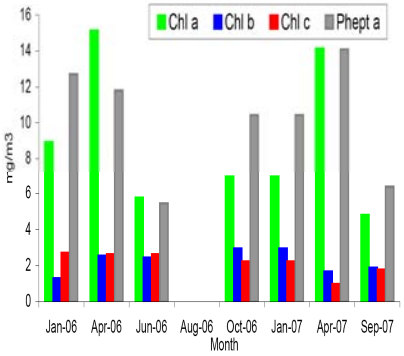


Figure 4.5 Station S5

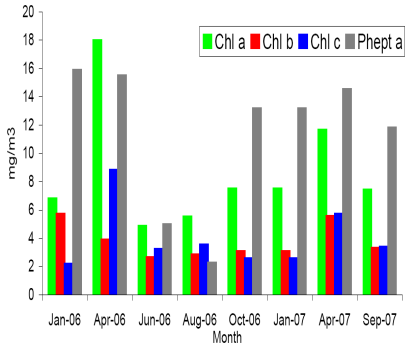


Figure 4.6 Station S6

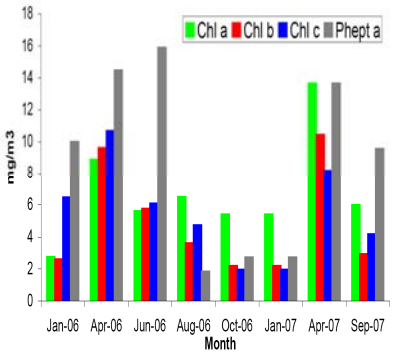


Figure 4.7 station S7

Distribution of phytopigmnets

4.4 Micronutrients trace elements (Fe, Mn & Zn)

The nutrients most frequently considered to limit the reproductive rates of phytoplankton in the aquatic setting are the macronutrients (nitrogen, Phosphorus and Silicon). The role of micronutrient trace metals in the ecology of the phytoplankton has been recognized recently. Phytoplankton affects trace metal chemistry in the natural waters not only by surface reaction but also by metal up take and by production of

extracellular organic matter with metal complexing process. The release of extracellualr organic matter - a labile substrate which in turn depends on environmental factors are a major sources of dissolved organic matter. Recent data indicate order of magnitude difference in the Fe, Mn & Zn and concentrations between oceanic and Nertic zones which leads to difference in the requirement of these micronutrient and could result in species shifts in phytoplankton communities and an excess of these metals may inhibit. Here a statically approach were conducted in order to find out the relation with Fe , Mn and Zn with Phytopigments along with other macronutrients.

Fe, which is the enriched element in the study (19.0-852.0 avg 176.6 µg/L) (Figure 4.8). Recent studies revealed that Fe to phytoplankton is controlled by ferric and ferrous ion activities which in turn controlled by redox reaction. During the present study high concentration of iron were recorded at the backwaters (Stations S2, S3 & S4) then at estuarine site and lower at riverine stations . Iron is positively correlated with in the back water and inversely with estuarine and riverine zone with phytoplankton. This indicated that even though the Fe is most available to phytoplankton, relative to cellular requirement iron seems to be less available in riverine and estuarine zones than in back water zone.

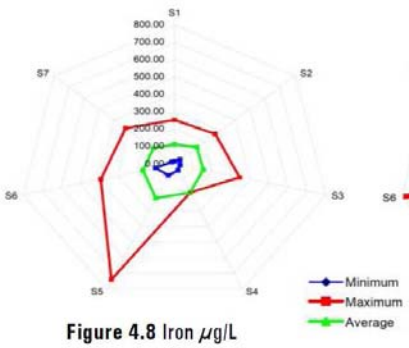


Figure 4.8 Iron µg/L

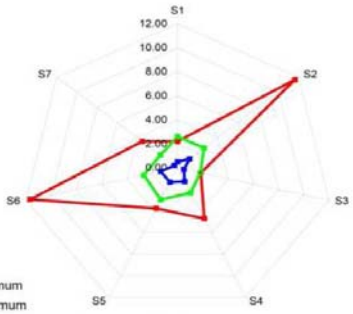


Figure 4.9 Manganese µg/L

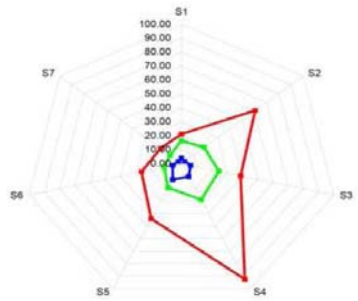


Figure 4.10 Zinc µg/L

Distributions of micronutrient trace metals during the study

An inverse relation in all the zones indicated that the uptake of Mn is maximum and limited due to high up take, only exception with Chl b which is correlated linearly with Mn. Manganese were found comparatively lower in concentration during the study (ND to 16.00µg/L; avg 2.43µg/L) (Figure 4.9).

Zinc is an important micronutrient for phytoplankton due to their involvement in enzyme systems and vitamins. Zn was found moderate enrichment (2.52-92.4; avg 18.8 µg/L) (Figure 4.10). A linear relation was found with phytoplankton pigment in all the zones. So unlike Fe and Mn , Zn was not a limiting factor and excessive zinc enrichment were found in this areas.

The present data suggested that Fe, Mn and Zn can be important environmental factors and that may act as a limiting factor as like macronutrients from proliferating or surviving the phytoplankton community. Independently, measurements of the combined elemental effects are rare. So future studies should concentrate on to have a better understanding and to know how the biogeochemistries of these elements interact and how they influence phytoplankton pigment variability.

4.5 Statistical analysis

4.5.1 Correlation analysis

Regression and correlation analysis has been carried out to find out the relationship between various physico chemical parameters by employing SPSS v 10.0 for Windows and the results are given in the Tables 4.1. to 4.3. A positive Correlation between Chlorophyll a, b and c was noticed in the selected light depth. Chl a indicated a linear relationship with temperature, and nutrients. In riverine system nitrate and nitrite is positively correlated with Chl a, b & c but an inverse trend is obtained with phosphate. In the back water system the phosphate and nitrate shows a negative trend while nitrite represented a positive correlation pattern. In case of estuarine waters an inverse relation observed with nitrate but chlorophylls correlated positively with phosphate and nitrite. Correlation analysis with micronutrient trace metals where also achieved to finding out the relation between one another.

Table 4.1 Correlations River (Surface)

	Chl a	Chl b	Chl c	Phe a	NO3	NO2	PO4	Salinity	Tem	DO	Fe	Mn	Zn	Hardness
Chl a	1.00	0.83	0.69	0.34	0.53	0.58	-0.48	-0.04	0.57	0.13	-0.14	-0.87	-0.14	-0.04
Chl b		1.00	0.45	0.33	0.23	0.46	-0.47	-0.07	0.38	0.13	-0.10	-0.79	0.21	0.13
Chl c			1.00	-0.17	0.45	0.53	-0.24	0.57	0.92	-0.37	0.07	-0.72	0.02	-0.18
Phe a				1.00	0.50	0.29	0.19	-0.43	-0.12	0.07	0.07	-0.24	0.18	0.06
NO3					1.00	0.10	-0.19	0.39	0.49	-0.24	0.20	-0.31	0.21	-0.34
NO2						1.00	-0.01	-0.07	0.46	-0.28	-0.28	-0.51	-0.41	0.09
PO4							1.00	-0.27	-0.02	-0.06	0.68	0.15	0.39	0.52
Salinity								1.00	0.61	-0.81	0.17	0.73	0.44	-0.60
Tem									1.00	-0.33	0.47	-0.79	0.33	0.14
DO										1.00	0.19	-0.56	-0.19	0.60
Fe											1.00	-0.28	0.98	0.77
Mn												1.00	0.01	-0.45
Zn													1.00	0.28
Hardness														1.00

Table 4.2 Correlations Back waters (Surface)

	Chl a	Chl b	Chl c	Phe a	NO3	NO2	PO4	Salinity	Tem	DO	Fe	Mn	Zn	Hardness
Chl a	1.00	0.87	0.86	0.86	-0.12	0.07	-0.50	0.30	0.04	-0.60	0.23	-0.28	0.06	0.20
Chl b		1.00	0.90	0.72	-0.19	0.06	-0.53	0.53	0.32	-0.60	0.27	0.12	0.39	0.49
Chl c			1.00	0.82	-0.06	-0.10	-0.49	0.56	0.08	-0.66	0.13	-0.14	0.03	0.34
Phe a				1.00	0.22	0.04	-0.07	0.44	-0.24	-0.61	0.15	-0.40	-0.24	0.17
NO3					1.00	-0.56	0.36	-0.05	-0.86	0.17	-0.59	-0.78	-0.90	-0.36
NO2						1.00	0.36	-0.07	0.62	0.09	0.40	0.60	0.83	0.39
PO4							1.00	-0.06	-0.24	0.22	0.04	0.10	-0.29	-0.08
Salinity								1.00	0.20	-0.40	-0.94	0.43	-0.07	0.78
Tem									1.00	-0.03	0.36	0.90	0.97	0.65
DO										1.00	-0.73	0.11	-0.03	0.10
Fe											1.00	0.24	0.57	-0.27
Mn												1.00	0.80	0.87
Zn													1.00	0.61
Hardness														1.00

Table -4.3 Correlations Estuary (Surface)

	Chl a	Chl b	Chl c	Phe a	NO3	NO2	PO4	Salinity	Tem	DO	Fe	Mn	Zn	Hardness
Chl a	1.00	0.52	0.65	0.48	-0.37	-0.04	0.64	0.51	0.41	-0.09	-0.60	-0.27	0.49	0.00
Chl b		1.00	0.75	0.79	-0.61	0.49	0.55	0.82	0.01	-0.60	-0.18	0.49	0.50	0.54
Chl c			1.00	0.45	-0.37	0.21	0.92	0.79	-0.23	-0.68	-0.19	-0.25	0.90	0.36
Phe a				1.00	-0.93	0.57	0.19	0.78	0.30	-0.18	-0.57	0.41	0.35	0.67
NO3					1.00	-0.68	-0.05	-0.68	-0.26	0.06	0.62	-0.38	-0.38	-0.65
NO2						1.00	-0.17	0.23	-0.21	-0.16	-0.32	0.90	0.22	0.27
PO4							1.00	0.61	-0.24	-0.66	0.01	-0.60	0.84	0.20
Salinity								1.00	0.29	-0.53	-0.42	0.15	0.65	0.84
Tem									1.00	0.61	-0.80	0.25	-0.46	0.10
DO										1.00	-0.45	-0.13	-0.75	-0.26
Fe											1.00	-0.16	0.04	0.02
Mn												1.00	-0.58	0.35
Zn													1.00	0.25
Hardness														1.00

4.5.2 Regression analysis

Multiple regression analysis was performed to discriminate the most significant properties involved in the phytoplankton growth. This analysis substantiate the interpretation of the depended variable phytopigment (chl a, b, c and pheopigment a) as a function of a number of independed variables (NO_3, NO_2, PO_4 and Dissolved Oxygen). In order to investigate the factors effecting the phytoplankton growth, a stepwise regression was employed, keeping the collinearity between the independent variables to the minimum. The analysis displays the R squared and the adjusted R squared, which attempt to correct R squared to closely reflect the goodness of fit of the model in the population (Table. 4.4). Two different multiple regression were done the former considering as independent variable the nutrients (NO_3, NO_2, PO_4 and Dissolved Oxygen) and latter as independent variable the micro nutrient trace elements (Fe, Mn & Zn).

Table 4.4 Regression analysis

Independent Variables	**NO3,NO2,PO4 and DO**			
Dependent Variables	R	R Square	Adjusted R Square	Std. Error of the Estimate
Chl a	0.931	0.866	0.687	2.974
Chl b	0.713	0.508	-0.147	1.164
Chlc	0.705	0.497	-0.174	3.519
Pheo a	0.699	0.489	-0.193	4.882
Independent Variables	**Fe,Mn & Zn**			
Dependent variables	R	R Square	Adjusted R Square	Std. Error of the Estimate
Chl a	0.89	0.79	0.15	5.88
Chl b	0.99	0.97	0.90	0.38
Chlc	0.74	0.55	-0.81	4.20
Pheo a	0.99	0.98	0.95	0.96

4.6 Discussion

Earlier workers reported (Nair et al., 1975; Selveraj et al., 2003; Renjith, 2006) mean Chlorophyll **a** concentration in estuarine and coastal waters with respect other scientific aspects . (Table 4.5). In Cochin estuary the Chl **a** content have been reported as 2-21 by (Nair et al., 1975), 4.93- 8.93 (Selveraj et al., 2003) and 1-34.61 mg/L Renjith, (2006). Chl **a** value and biomass recorded in the present study agrees with the earlier reported values. Observation of high surface chlorophylls during pre and post monsoon in the present study agree with the earlier observations by Gopinathan (1972), Devessy and Bhattathiri, (1974) in Cochin estuary , Edward et al, (1992). Estimation of photosynthetic pigments of flora of the Cochin backwaters has brought about contrasting results. Nair et al; (1975) have estimated an overall range of 1.5–18 mg m^{-3} for chl *a.* The findings of Joseph & Pillai (1975) indicate that maximum chl *a* concentration for pelagic flora occurs during monsoon, whereas for benthic microflora, the maximum were reported during pre-monsoon and monsoon (Sivadasan & Joseph, 1995). Maximum chl *b* concentration for pelagic as well as benthic micro flora was noted in the monsoon. Maximum chl *c* was

Area	Chlorophyll a mg/m^3	Reference
Cochin estuary	1.5 - 18	Nair et al 1975
Surf Zone Cochin	8.35- 15.64	Selveraj et al 2003
Cochin estuary	4.93- 8.85	Selveraj et al 2003
Cochin off	1.0-5.0	Gopinathan et al 2001
Cochin Back water	1.7- 47.0	Madhu et al 2007
Cochin Back water	1-34.6	Renjith 2006
Cochin Back water	9.6-13.7	Madhu et al 2009
Cochin Back water (Zone C)	2.75-17.97	Present study
Moovatupuza River(Zone A)	1.18-14.06	Present study
Back waters (Zone B)	0.84-29.75	Present study

Table 4.5 The Chlorophyll a content in the study area from 1975-Present study.

found during pre monsoon and monsoon from benthic area, whereas it was found more in monsoon from pelagic area. The present result findings agree with these results and maximum chl b is recorded in pre monsoon and post monsoon. Gupta (1976) recorded chlorophyll b from traces to 10.2 mg/L in surface water off Cochin and well coincided with present findings 10.42mg/L at Bolghatty an estuarine station during pre monsoon . The higher peak during pre and post monsoon is attributed to the stability of water, less turbidity and improved light conditions. A ten fold increase in the TDS (Total dissolved solids) was observed in the study area as compared to the earlier report (Renjith 2006). High density of chlorophyll a during pre and post monsoon correlated with the greater adaptability of phytoplankton to utilize the

Dissolved Oxygen, nutrients temperature and light conditions (Dehadrai (1970); Thangaraj (1984): De et al .,(1991); Tiwari and Nair (1998)). It is observed from previous reported findings and the present study that Chl a, content a indicator biomass increases from 1975 to 2007 indicated not because of the increase of productivity but eutrophication trend in this area due to prevailing industrial activities. These observations were supported by the productivity studies in the study area. Organic production of the Cochin backwaters was studied by Qasim et al., (1969) using various techniques and stated that the gross production (GP) ranged from 272 to 293 g C m^3 yr^{-1} with an average of 280 g C m^3 yr^{-1}, and the net production (N P) was 193 g C m^2 yr^{-1}; and Kunjukrishna pillai et al., 1975 reported GP 164-481 g C m3 yr^{-1} and Selveraj et al., (2003) approximated 225-803gm^3C/year while a reduction in NP reported by Renjith (2006) about 114-486 g C m3/year even though the nutrient showed an enrichment trend.

It is well known that wealth of chlorophylls and nutrients in the water column are of paramount importance for determining the biological productivity and potential resources. Light penetration decides the depth of the euphotic zone while the nutrients especially the nitrates and phosphate indicate the fertility of the water to promote productivity and the availability of the photosynthetic pigments help the production at the primary level. The peak production was coinciding with the periods of low nutrients concentration is observed by Qasim, (1980), Murugan and Ayyakkunu, (1993) and Jagadeeshan, (1986) in Cochin back

waters, Cuddalore, Uppanan back waters and Coleron estuary but this kind propensity is not strictly observed in the study, this is true in case of nitrate and phosphate but reverse is observed with nitrite in back waters and in estuary . This may be attributed that the availability of nutrients in the study area both in the back waters and estuary is largely due to tidal activities and discharge from anthropogenic (both point and nonpoint sources). Very often the nutrients available in the surface column are not fully utilized by the phytoplankton and high concentrations are detected in some region where the surface concentration is higher than the bottom Column. The nutrients do not have direct bearing on density of biomass as nutrients could not replaced due to regeneration and exchange from bottom waters to surface waters. In present study the level of nitrate and phosphate showed moderate they are not act as limiting factors.

It is quite interesting that there is a positive correlation between Chl **a** and **b** even though chl **b** is an accessory pigment of Chl **a**, its concentration in the regions are significant for assessing the fertility area. High values of Chlorophyll a observed at Champakara Canal station with high concentration of Chlorophyll **b**, next higher concentration recorded at Estuarine sites (Figure 4.1-4.7)than the river . Highest Chl **b** recorded at Bolagatty with Chl a. The availability of nitrates and phosphates in the Champakara is mostly from anthropogenic waste from the near by fish markets repeatedly recorded high values of nutrients (Figure 3.7) but in case of Estuary the nutrient input basically from tidal activates,

river runoff and also drainage from the land. Very often these nutrients available in the water column are not fully utilized by the phytoplankton and high concentrations are detected in the surface water column, the present investigation the highest values of phosphate, nitrate and nitrite 3.7, 1.2 and 0.40 respectively coincided with moderate to high (29.5 mg/L) Chlorophyll a and b observed from these station. Estuaries also indicated same trend. This indicated that the Pigment that Phytoplankton distribution, biomasses are closely associated with prevailing hydrographic parameters and nutrients.

Correlation and Regression

Phytopigments showed a strong correlation between salinity at Zone B and C where as an inverse relation obtained with Zone A. The linear relation with salinity and Pheopigment my due to the fact that the stratification which often develop in the system will control the nutrient distribution in the Zone B and C which in turn effect the Phytopigment biomass. This is confirmed by the inverse relation of nutrient with salinity during the study. A negative correlation between Chl a is observed with NO_3 and PO_4 but a positive with NO_2 at the back waters. These observations indicated that phosphates and nitrates were fully utilized by the phytoplankton for its growth while in case of NO2 is partially utilized by the plankton. A strong negative correlation especially with the PO_4 with Chlorophylls in the riverine stations indicated that phytoplankton is able to meet the requirement for growth and multiplication through inorganic PO_4. In case of Estuary a positive

correlation between Phosphate and Nitrite and inverse relation with nitrate is noted. A positive correlation between Chlorophyll a and Phosphates has been reported by Balachandran et al, (1989) from the inshore waters of Cochin while negative correlation was reported between these parameters from the Laccadive sea during the south west coast monsoon season (Balachandran et al.,1997).

The correlation analysis revealed that even though the Fe is most available to phytoplankton, but relative to cellular requirement iron seems to be less available in riverine and estuarine zones (negative correlation) but in back water zone (A positive correlation) it is available for their physiological activities. The back water zones are characterized by low oxygen content < 2 mg/L in some pockets. The Fe maximum is common in this type hypoxic condition due to decomposition of sinking organic matter, a process in which O_2 is consumed and CO_2, NO_3 and Fe regenerated. Iron is being released via desorption and or colloidal/oxide solubilization as the particles sink through the O_2 minimum environment (Martin et al., 1988).

The regression analysis Calculation model yielded data shown in Table 4.4 The variability of Chl **a** content can be predicted as a linear regression of the nutrient(r^2 =0.87) Nutrients can used to Chl b(r^2=0.51), Chl c (r^2=0.5) and Pheophytin a (0.5). with regards to micronutrient trace elements approximately 80% of the variability of the Chl **a** content can be predicted as a linear regression of the Chl **a** , Chl **b** , Chl **c** and Pheophytin **a** with (Fe, Mn and Zn) are r^2=0.79 , 0.97, 0.55 and 0.98 respectively, suggesting that this element shows the major influence on the phytopigment growth.

4.7 Conclusion

It can be concluded that the species diversity of phytopigment biomass was maximum during pre and post monsoon period and minimum during monsoon period. The phytoplankton distributions, species composition and dominance are closely associated with the prevailing hydrographic condition and nutrients. Consistent increase was observed along the season watercourse. Instead fluctuation occurs in response to the proximity of pollution sources (point and non point) of changes in the water flow which depends spatially and temporally. Significant correlation was observed between salinity and DO between nutrients. Phytopigments often controlled by salinity which in turn control the distribution of nutrient due to stratification and tidal activities. Strong correlation observed between phytopigment, nutrients and micronutrient trace metals. It seems plausible that the Trace metal such as Zinc, Manganese and Iron contributing for the reproduction of phytoplankton biomass as like macronutrients.

Measurements of the combined Micronutrients trace metal effects are rare. So future studies should concentrate on to have a better understanding and to know how the biogeochemistries of these elements interact and how they influence phytoplankton pigment variability.

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Chapter 5

Biomarker Pigment Characterization- As a "Hands on" Research

5.1 Background

Our global environment remains under threat from our changing lifestyles and our unreasonable demand on earth resources especially the oceans embracing coasts estuaries, lakes, river etc. The essence and intensity of factors and processes influencing the water realm- estuarine and lake ecosystems and

their impact are extremely complicated, the temporal and spatial variability in the biochemical characteristics and the monitoring data of water masses in lakes is great. As many researchers have demonstrated, the organic matter in sediments is often determined by preservation rather than production (Wetzel, 1983; Dean, 1999). Bloesch et al. (1988) showed that about 10% of the estimated net primary production is deposited as organic carbon in eutrophic lakes. The deposition rate of organic carbon in the sediment varies over-proportionally with changes in net primary production (Gruber et al., 2000). In the works of many researchers, the content of fossil pigments (Swain, 1985; Sanger, 1988; Lami et al., 1994; Reuss et al., 2005) characterizes mostly the level of degradation and preservation of organic matter. The biogeochemical and lithological information stored in accumulative deposits over certain time periods (Chambers, 1993; Leavitt, 1993 Hassan et al., 1997 Lami et al., 1997) are needed to establish the sensitivity of ecosystems, and, thus, create a scientific basis for the introduction of necessary mitigation measures (Eriksson, 1996). Studies by Hurley & Armstrong (1990) and Leavitt & Carpenter (1990) have shown that there are different rates of decay between pigments and that the actual amounts of pigment delivered to the sediments are dependent on the relative importance of decay factors. Chlorophylls and carotenoids have different decay rates and ratios of these pigments have been used as a paleo-limnological tool to reconstruct tropic status (Sanger & Gorham, 1972) and

hypolimnetic anoxia (Steenbergen et al., 1994). Paleoecological assessments can provide the long-term records of ecological status and natural variability that are needed to interpret changes observed in the environment today and predict future changes. Thus, it is very rarely possible to reconstruct the correct state of past ecosystems on the basis of short-term monitoring data. Paleo records in lake sediments can be used for monitoring long-term changes in land-use.

Sediments can be a sensitive indicator for both spatial and temporal trend and monitoring of contaminates in the marine environment. Consequently they have formed an essential component in many international monitoring programmes. Great diversity of pigments produced by aquatic algae, bacteria and higher plants are deposited in both freshwater and marine sediments (Brown, 1969; Sanger & Gorham, 1970). Often carotenoids and chlorophylls are the only fossil remains of non siliceous algae and bacteria and are therefore of considerable value to palaeoecology and palaeolimnology. (Vallentyne, 1954; Hutchinson & Vallentyne, 1955; Gorham, 1960; Brown, 1969; Sanger & Crowl, 1979; Swain, 1985; Sanger, 1988; Steenbergen et al., 1994).

Pigments represent the phototrophic community as they are produced by algae and other photosynthesizing organisms and are specific to particular groups (Jeffrey et al. 1997). The sedimentary pigment records can therefore be used to reconstruct past phototrophic communities and production, and

have been shown to reflect changes due to a wide variety of forcing factors such as increased nutrient load, changes in grazing pressure and acidification (Leavitt & Hodgson 2001). To date, the most extensive sediment pigment analyses have been conducted on lake systems and the focus has been on changes in the phytoplankton structure as a response to increased nutrient load.

The Phytoplankton's are the microscopic algae that make up the floating feed of the world's oceans. They provide the food base which supports either directly or indirectly, the entire animal populations of the sea and they contribute significantly in climatic process. Their diversity is immense and representative of most algal divisions may be found in these ocean populations. The Dinoflagellets (Dinophyta) encompass about 1200 species only about half of which are photosynthetic. Green algae (Chlorophyceae, Prasinophyceae) and the euglenophytes (Euglenophta) are common in coastal water and former occurs in the open ocean (Jeffry et al 1976; Jeffry and Hallegraeff 1980). The Polyphyletic golden brow flagellates of the Haptophyta and Chrysophyta Prymnesiophyceae, Chrysophyceae,Raphidophyceae) are very diverse and may dominate the phytoplankton in particular regions and at certain times of the year. Picoplanktonic blue green algae (Cynobacteria; Cynophyta) and free living prochlorophytes (Prochlorophyta) are ubiquities in the world's oceans, often preferring the dimly lit regions at the base of eutopic zone

(Waterbury et al., 1979; Chissholam et al 1988) Cryptomonads (cryptophyta) and eustigmatophtes (Eustigmatophta) are also widly distributed. The unicellular red algae (Rhodophyta) are common in benthic habitats of tropical reef waters and Norwegion coastal waters (Paasche and Throndsen 1970) are not generally detected in oceanic phytoplankton.

5.2 Development in Pigment Characterization.

Pigments are present in all photosynthetic organisms and function primarily as light harvesting agents for photosynthesis and photo protection. Moreover, it was shown that some phytoplankton pigments, often referred to as biomarker pigments, can be regarded as selective biomarkers of certain micro algal classes which led to an increasing use of chemotaxonomic approach in marine phytoplankton studies. An important prerequisite for the application of the biomarker approach in extensive field studies was the development of analytical methods for the determination of photosynthetic pigments using high performance liquid chromatography (HPLC) techniques which enabled a routine and rapid simultaneous determination of various Chlorophyll and carotenoid pigments along with their degradation products.

During the past decades, HPLC techniques have rapidly evolved, allowing for phytoplankton biomass and composition in the oceans to be described in detail using algal pigment biomarkers. Indeed, the [TChla] has been a widely used biomarker for the phytoplankton biomass in the oceans (Yentch

and Menzel, 1963; Parsons and Strickland, 1963; O'Reilly et al., 1998). The determination of chlorophyll and carotenoid pigment concentrations by high-performance liquid chromatography (HPLC) is a method which fulfills most of these requirements. Indeed, many carotenoids and chlorophylls are taxonomic markers of phytoplankton taxa, which means community composition, can be evaluated at the same time.

5.3 Methods of Identification and Enumeration

Phytoplankton identification and enumeration is usually done through microscopic examination. This procedure is time-consuming and also requires a high level of taxonomic skill. Moreover, smaller organisms such as picoplankton cannot be identified or counted with this approach. Alternatively, photosynthetic pigments can easily be studied to know the phytoplankton composition and their physiological status. Most of these pigments (Table 5.1) have chemotaxonomic association. For example, fucoxanthin is considered to be a marker of diatoms; zeaxanthin of cyanobacteria; 19-hexanoyloxyfucoxanthin of Prymnesiophyceae; alloxanthin and crocoxanthin of Cryptomonads; prasinoxanthin of prasinophytes; peridinin and chlorophyll c2 of dinoflagellates (Jeffrey et al., 1997 a &b).

Table 5.1 Chemotaxonomic associations of most frequently detected pigments and algal class

Sl	Pigments	Algal divisions/occurrence
	Chlorophylls	
1	Chl a	All photosynthetic Algae, excluding Prochlorophyts
2	Chl b	Green algae,Euglenophyta, plants
3	Pheophorbide a	Grazing, Senescent diatoms
4	Pheophytin a	Chl a derivative (all)
	Carotenes	
5	α & β	Plants, Algae
	Xanthophylls	
6	Alloxanthin	Cryptophyta
7	Canthaxanthin	Cyanobacteria, Chlorophyta,Eustigmatophyta
8	Diatoxanthin	Bacillariophyta, Dinophyta, Chrysophyta
9	Fucoxanthin	Bacillariophyta, prymnesiophytes, Chrysophyta, raphidophytes, several dinoflagellates
10	4-keto-fucoxanthin	Haptophyta
11	Hex-fucoxanthin	Chromophytes and nanoflagellates
12	But-fucoxanthin	Chromophytes and nanoflagellates
13	Lutein	Chlorophyta, Euglenophyta, Plantae
14	9'-cis neoxanthin	Prasinophyta, Chlorophyta, Euglenophyta
15	Peridinin	Dinoflagellates, Dinophyta
16	Zeaxanthin	Cyanobacteria (Cyanophyta)

The current research aims an integrated geochemical dealing with chemotaxonomic association incorporating riverine, estuarine and near shore shelf areas which has not been carried out so far. The study area located with in the stretches of Periyar and Muvattupuzha rivers situated in the "*Vempanadu kayal*".

The application of high-performance liquid chromatography (HPLC) was introduced in this study for the first time which has been

found to be more accurate and reliable for estimating not only Chl a but also for other pigments as well. This technique allows quantification of additional fifty phytoplankton pigments and carotenoids in marine phytoplankton (Wright et al., 1991; Jeffrey et al., 1997). Geochemical and phytoplankton taxonomic studies of sediment and water in the rivers, estuaries and marine basins are very helpful in understanding hydrodynamic factors, sources, distribution pattern and their complex interplay existing in an area . In seawater sample phytoplankton are always identifiable microscopically, the sample preserved by formalin or Lugol's fixatives. However, the fragile phytoflagellates, which are often equally abundant, are only recognized when gentle fixatives (e.g., buffered glutaraldehyde) are used for their preservation. Recognition of the importance of the delicate flagellates (5-10μm) (Tomas, 1993) and minute coccoid forms (0.2-2 μm) has come only in the past two decades when more appropriate techniques for studying the phytoplankton were developed recently. These included examination of species in the living state after concentrating them in a plankton centrifuge, gentler methods of fixation, examination by electron microscopy, size-fractionation of phytoplankton, use of enrichment cultures, onboard cell-flow cytometry and chromatographic pigment analysis (TLC, HPLC) for assessment of algal pigment signatures.

Phytoplankton can be differentiated into three size classes: the microplankton (20-200 μm), nanoplankton (2-20 μm) and picoplankton (0.2-2 μm). The nanoplankton can at times account for up to 90%of the total phytoplankton chlorophyll in coastal and

open ocean waters (Jeffrey and Hallegraeff, 1990). Picoplankton which is also contributes significantly but rarely detected by common methods.

The carotenoids (Table 5.1) were chosen based on the fact that they are the most common pigments used in chemotaxonomic or photophysiological studies in open Ocean or coastal waters (Gieskes et al., 1988; Bidigare and Ondrusek, 1996; Barlow et al., 1993; Claustre et al., 1994). Subsequent grouping of pigments (including chlorophyll sums) permits the formulation of variables useful to different perspectives. For example, the pool of photosynthetic and photoprotective carotenoids (PSC and PPC, respectively) are useful in photophysiological studies (Bidigare et al., 1987), and the total amount of accessory (non-chlorophyll a) pigments (TAcc) are useful in remote sensing investigations (Trees et al., 2000). Accessory pigments have either photosynthetic properties allowing the phytoplankton cells to increase their light harvesting spectrum, or a role of photo protection in dissipating the excess of light energy received and reducing the oxidation that takes place due to stress in conditions of strong irradiance. The ratios that can be derived from these pooled variables, e.g. [PSC]/ [TChl a], are dimensionless and have the advantage of automatically scaling the comparison of results from different areas and pigment concentrations.

The Diagnostic Pigment [DP] criteria were introduced by Claustre *et al.* (1994) developed by Vidussi et al., (2001) and recently extended by Uitz et al.,(2006), to derive size-equivalent pigment

indices which roughly correspond to the biomass proportions relevant to picophytoplankton (less than 2µm), nanophytoplankton (between 2 and 20µm) and microphytoplankton (greater than 20µm); [pPF] or pBP, [nPF] or nBP, and [mPF] or mBP, respectively. Macro variables are composed of pigment sums and ratios, so they should be particularly useful in reconciling inquiries applied to databases from different oceanic regimes. Note that these variables are equivalent to the Fp ratio (Claustre, 1994), defined as the biomass ratio of phytoplankton involved in new production over total phytoplankton and as such is equivalent to the f- ratio (new production/ total production by Eppley and Peterson, 1979. This means that together with size significance, some of the criteria defined, also have a functional/ biogeochemical significance (Vidussi et al., 2001). 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.

5.4 Phytoplankton abundance in the study area - Literature Review

Several studies have been conducted in the Cochin backwaters on various physico- chemical (Sankaranarayanan and Qasim, 1969) and biological characteristics (Rao et al., 1975). Being eutrophic, primary production in the estuary is always high and is mainly constituted by nanoplankton (<20 mm) community. Perusal of literature stand in 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- 2007.

Joseph & Pillai (1975) have divided the phytoplankton in the estuary into

(a) flora adapted to fluctuating estuarine conditions, i.e. typical estuarine forms which are permanent residents, and

(b) those which are not adapted or less adapted i.e. either freshwater or marine forms entering the estuary, and resident for short periods. Of the various categories, the concentration of nanoplankton (<20 mm) community largely composed of diatoms (Bacillariophyceae), is relatively high throughout the year, around 70% of the total phytoplankton was contributed by Skeletonema costatum (Qasim et al., 1974, Kumaran & Rao, 1975

Madhu etal., 2007). Gopinathan (1975) & Madhu etal 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. During pre-monsoon, the phytoplankton production in the estuary was high and fairly stable, with the dominant diatoms being Chaetoceros, Coscinodiscus, Skeletonema, Pleurosigma and Nitzchia, 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. During post-monsoon, gradually the freshwater species disappear coinciding with the predominance of marine forms. Kumaran & Rao (1975) are of the opinion that most of the species recorded in the Cochin backwaters were marine forms and the area near the barmouth was the most productive area; conditions immediately after or following a break in the monsoon are favorable for the sudden spurts in plankton abundance. Periphytic algae growing on stones, aquatic macrophytes and other submerged objects form a major group of autotrophs in shallow waters. Usually they attain high biomass and may contribute up to 80% of primary production. They are a major food resource for benthic invertebrates. Sreekumar& Joseph (1995) estimated that periphytic algae of the Cochin backwaters are comprised of 66 species of Bacillariophyceae, eight species of Chlorophyceae and two species of Cyanophyceae. Sivadasan & Joseph (1995) found that the estuary is rich in benthic micro flora which plays a dominant role in the total productivity of the ecosystem. Recently Sanilkumar (2009) characterized about 28 species Bacillariophyta , 9 species of dianoflagellets and two species chlorophyceae in 2006-07 and 54, 19 and 2 in 2007-2008 respectively (Table 5.2). Furthermore, the flora in the backwaters were similar upto 60% in composition, while at the end of the postmonsoon and pre-monsoon, flora composition varied considerably.

Table 5.2 A review of literature Phytoplankton and abundance in the study area

Phyto plankton Group/Species	No Species	Time of Sampling	Reference
Bacillariophyceae,Dinoflagellates,Cyanophyceae,Chlorophyceae & Filamemntous algae	194	1958-1975	George 1958 a & b , Devassy and Bhattathiri 1974 ; Gopinathan, 1972 & 1975; Joseph and Pillai, 1975; Kumaran and Rao, 1975
(Periphytic algae)			
Bacillariophyceae	66		Sreekumar& Joseph (1995)
Chlorophyceae	8		
Cyanophyceae	2		
Bacillariophyceae	89	1999	Project report, Department of Ocean Deveplopment 2002
Dinophyceae	31		
Chlorophyceae	2		
Cyanophyceae	1		
Bacillariophyceae	58	2001-02	Selveraj et al 2003
Dinoflagellates	2		
Bacillariophyceae,Dinoflagellates & others	89 (Pre monsoon) 65 (Monsoon)	2003	Madhu et al 2007
Bacillariophyceae	28	2006-07	Sanilkumar 2009
Dinoflagellates	9		
Chlorophyceae	2		
Bacillariophyceae	54	2007-08	
Dinoflagellates	19		
Chlorophyceae	2		
Algael Class identified during the study by pigment signature HPLC analysis		Size um	Present study
Green algae	Picoplankton	< 2	
Cynobacteria	Picoplankton	< 2	
Eustigmatophyta	Nanoplankton	2-20	
Prymnesiophytes	Nanoplankton	2-20	
Chrysophyta	Nanoplankton	2-20	
Dinophyta	Nanoplankton	2-20	
Bacillariophyta	Microplankton	> 20	
Dinoflagellates	Microplankton	> 20	
Cryptophyta	Microplankton	2-200	
Chlorophyta	Microplankton	2-200	
Euglenophyta	Microplankton	20-200	
Raphidophytes	Microplankton	20-200	
Prasinophyta	Microplankton	20-200	

A great diversity of pigments produced by aquatic algae, bacteria and higher plants are deposited in both freshwater and marine sediments (Brown, 1969; Sanger & Gorham, 1970). Often carotenoids and chlorophylls are the only fossil remains of nonsiliceous algae and bacteria and are therefore of considerable value to paleoecology and paleolimnology (Hodgson et al., 1997) Individual carotenoids can be used as indicators of specific algae classes (Jeffrey et al., 1997). Indicator carotenoids include fucoxanthin (diatoms), diatoxanthin and diadinoxanthin (diatoms, dinoflagellates), alloxanthin (chryptophytes), lutein (green algae and higher plants), and zeaxanthin (cyanobacteria), while the β-carotene and chlorophyll-a are more general indicators of total algal abundance. Perdinin synthesized by dinoflagellats chlorophyll b (Chl b) has been commonly ascribed to green algae. Chlorophytes and Type I Prasinophtes can be identified by their relative ratio of **leutin to Chl *b*** (Leut: Chl b = 0.30-1.77, 0-0.18 respectively) (Wright, 2005). 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., 2000b). Distribution of major and taxonomically significant pigments across micro algal Divisions/Classes were given in the Table 5.1.

Smaller organisms such as picoplankton cannot be identified or counted with microscopic examination alternatively; photosynthetic pigments can easily be studied to know the

phytoplankton composition and their physiological status. To study the evolution and the nature of organic matter in the estuary from phytoplankton the current study focused analysis of algal chlorophyll and carotenoids pigments using high performance liquid chromatography (HPLC). These photosynthetic pigments markers have been used increasingly in oceanography for the quantification of the major taxonomic groups of phytoplankton and their degradation mechanisms (Wright et al., 1991; Letelier et al., 1993; Barlow et al., 1997). Inspection of literature reveals that no information is available on these aspects in the Cochin back waters. Considering these in view, the main aim of the current research is to study the distribution of fossil pigments and their taxonomy in surface sediments in four different tropic statues around Cochin back water systems as follows.

However, it should be noted that marker pigments are not exclusive of any one group of algae. In natural environment pigment composition may well vary with prevailing light condition and photoadaptive state (Falkowski and LaRoche, 1991).

5.5 Factors affecting pigment content

Environmental factors strongly influence pigment composition of microalgae includes:

- Irradiance (Johnsen et al., 1994; Goericke & Montoya, 1998; Schlüter et al., 2000;Henriksen, 2002),
- Spectral distribution of light (Wood, 1985; Bidigare et al., 1989; Partensky et al., 1993),
- Day length (Sakshaug et al., 1986),

- Diurnal cycle (Tukaj et al., (2003),
- Nutrient status (Goericke & Montoya, 1998; Henriksen et al. 2002), notably iron concentration (Wilhem et al., 1996; van Leeuwe et al., 1998),
- Growth phase (Schlüter et al., 2000; Wilhem & Manns, 1996, and
- Strain differences (Stolte et al., 2000; Zapata et al., 2000).

This variability is usually limited to changes in the total pigment quantity per cell rather than the type of pigments present, although in senescent or nutrient-limited populations secondary pigments may be produced.

5.6 Study area

Stations 1 and 2 located in the Cochin backwaters, along 9^0 40'N to 10^0 10' and $76^0$13' E to 76^0 50' E on the south-west coast of India they form a multitudinal hydrographic system along the Kerala coast on the south west coast of India. The backwater system covers an area of approximately 300 km^2 with one permanent bar mouth maintained at 12m depth at Cochin and two seasonal openings during the peak monsoon period. The estuary is wide (16 km) in the Vembanad lake area and several narrow canals along with municipal waste and other particulate organic matter emptying into it. Several major rivers Periyar, the Muvattupuzha and Pampa discharge fresh water into the estuarine system. This estuary is classified as a tropical positive estuary; the characteristic of the estuary is influenced by the rivers flowing into it, while the estuary itself is prone to strong tidal currents. Both these

phenomenon combine to give rise to seasonal and tidal fluctuations of hydrological conditions supports earlier studies (Lakshmanan et al, 1982, 1987). Station 5 (90 58'' 084'N 760 15'' 498'E) is near to port Station 7 (9058'' 34'N 760 16'' 00'E) is Bolghatty Island in the middle of estuary 500 m away from the above site a popular tourist haunt, and houses the Bolghatty palace.

Third site (9° 57''387' N 76°19'' 579'E) is in the Champakkara canal S 2 nearer to the Champakkara fish market and is found to be highly polluted with the organic wastes. The wastes from the fish market are mainly drain to the canal. More over wastes from Cochin Corporation, urban and domestic wastes are also considered to affect the pollution status of the canal. This Canal is particularly suited to fossil pigment analysis because anoxia, aphotic conditions, and low biological activities also favor the sedimentary pigment preservation (Gorham & Sanger, 1972; Sanger & Crowl, 1979; Sanger, 1988). Fourth station (10°04''350' 76°14''968') is also lies in the Periyar River. The ferry connects Cheranallur to Varapuzha and also to Eloor industrial area, one of the largest manufacturing centers. The Udyogmandal Industrial Estate, is located around the branch of the Periyar which passes to the north of Eloor, an island in the upper tidal reaches of the Periyar. Industrial chemicals, leather and other goods are manufacturing in this industrial belts. Many of the factories are located on the mainland, but several others are clustered on the north of the island, including FACT (Fertilizers and Chemicals Travancore), IRE (India Rare Earths), Merchem and HIL

(Hindustan Insecticides Limited). Hence this site endangered with high loads of industry based waste.

5.7 Objectives

This research theme focused on the use of sedimentary pigments as a biomarker of long-term changes in phototrophic community structure. The aim was to obtain fossil pigment records from different environments and evaluate its use as an indicator for anthropogenic and climate induced changes. Pigment analysis was carried out by high-performance liquid chromatography (HPLC) and UV-Visible spectroscopy and the interpretation of the pigment data was conducted in a multiproxy perspective.

a. Signature Marker pigments were identified at different lake systems in the estuary and their surf zone situated along the southwest coast of India. Geography of the area was briefly appended Chapter 2 Materials and methods.
b. Grouping of pigments (including chlorophyll sums) which permit the formulation of variables useful to different perspectives.
c. An overview has been made to account how sediment pigment act as a tool for the Preservation and degradation pattern for recent Ecological Reconstructions

5.8 Sample Collection and Analysis

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 lab and

preserved at 4°C. The sediment for the pigment analysis is immediately sub sampled in a 15cm plastic vials for preservation at -20°C until Freeze drying (Details are presented in Chapter II material and methods).

Four specific samples (S2, S4, S5 and S7) were selected according to the variations in the environmental chemical differences. HPLC analysis carried out by DHI group Denmark as described below. The sample was stored at -80°C until analysis. The freeze dried samples were homogenized prior to sub sampling and after weighing (Table 5.3) each sub sample was extracted in 95% acetone with internal standard (vitamin E) sonicated in an ice cold sonication bath for 10min, mixed on a vortex mixer allowed to extract at 4°C for 20hr and vortexed again. Extracts were then filtered through 0.2µm 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 HPLC method is the HPL (American Horn Point Laboratory (HPL) of the University of Maryland Center for Environmental Science method) (Hooker et al 2005).

Table 5.3 Weight in gram of the sediment used for HPLC Analysis

	S2	S4	S5	S7
Stations	Champakara	Cheranellur ferry	Port Taj	Bolgatty
Weight of sediment (mg)	0.1100	0.1210	0.1123	0.1153

5.9 RESULTS AND DISCUSSION

5.9.1 Signature Pigments distribution and interpretation as Bio marker.

Chromatographic analysis revealed the presence of a wide range of pigments, exhibiting a 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 created by running pure standard of individual pigments. The concentrations of the pigments were computed from the peak areas. Figure5.1, 5.2, 5.3 and 5.4 represents the HPLC spectra for the samples S2, S3,S4 and S7 respectively.

Chloropyll a (Chl a) is a ubiquitous pigment and can be used as a global algal biomass indicator. Chlorophyll a is present in all groups of photosynthetic organisms except some bacteria (Moss, 1988). It is the most abundant form in sediment as pheophytin a, pheophorbide a, chlorophyllide a, or isomerized or allomerized form (Daley et al., 1977). Other chlorophylls occur similarly in derivative form. Undecomposed chlorophyll is less common and is usually reported from very recent sediments (Swain, 1985). Different degradation products from chl a will be considered. The degradation products derived from the demetallation of chlorophyll-a associated to grazing activities (phaeophorbide-a and pheophytin-a) were found in high concentrations essentially in the upwelling area. Chlorophyll b was the next important pigment detected in low concentration.

With the protocol followed, complete resolution and separation of α & β carotene could not be achieved; these pigments were therefore grouped together and treated as total carotenoids. Xanthophylls-alloxanthin, canthaxanthin, diatoxanthin, fucoxanthin, fucolike, Neoxanthin, Leutin peridinin, Zeaxanthin are detected and depicted Figure.5.1-5.4.

5.9.2 Taxonomic Pigments

Fig 5.5 shows the distribution of Chl a and its degradation products in the study area. Chl a is maximum at Champakara S2 (11.01 μgg^{-1}) than at estuarine site S5 and S7 0.95 and 1.69 μgg^{-1} respectively and it decreased at Cheranelloor canal (S4) to 0.65 μgg^{-1}. The high chl a concentration in the station S2 is a consequence of the accumulation of fresh phytoplankton cells and flora in the sediment, these high level can be easily explained by the proximity of the fish market and slaughter units which empties large nutrient inputs especially of phosphate was introduced into the canal and also the results of sewage effluents from the harbor activities. While the stations S5 and S7recorded a moderate Chl a content compared to that of the S2, the species diversity of benthic diatoms was low in Cochin backwaters. It is assumed that variations in evenness index are due to the influence of fluctuation in the hydrological characteristics of the estuarine environment (Sreekumar & Joseph, 1998). Similarity at station S4 due to the effect of pollution, the percentage of same type micro flora between this area and rest of the estuary was low.

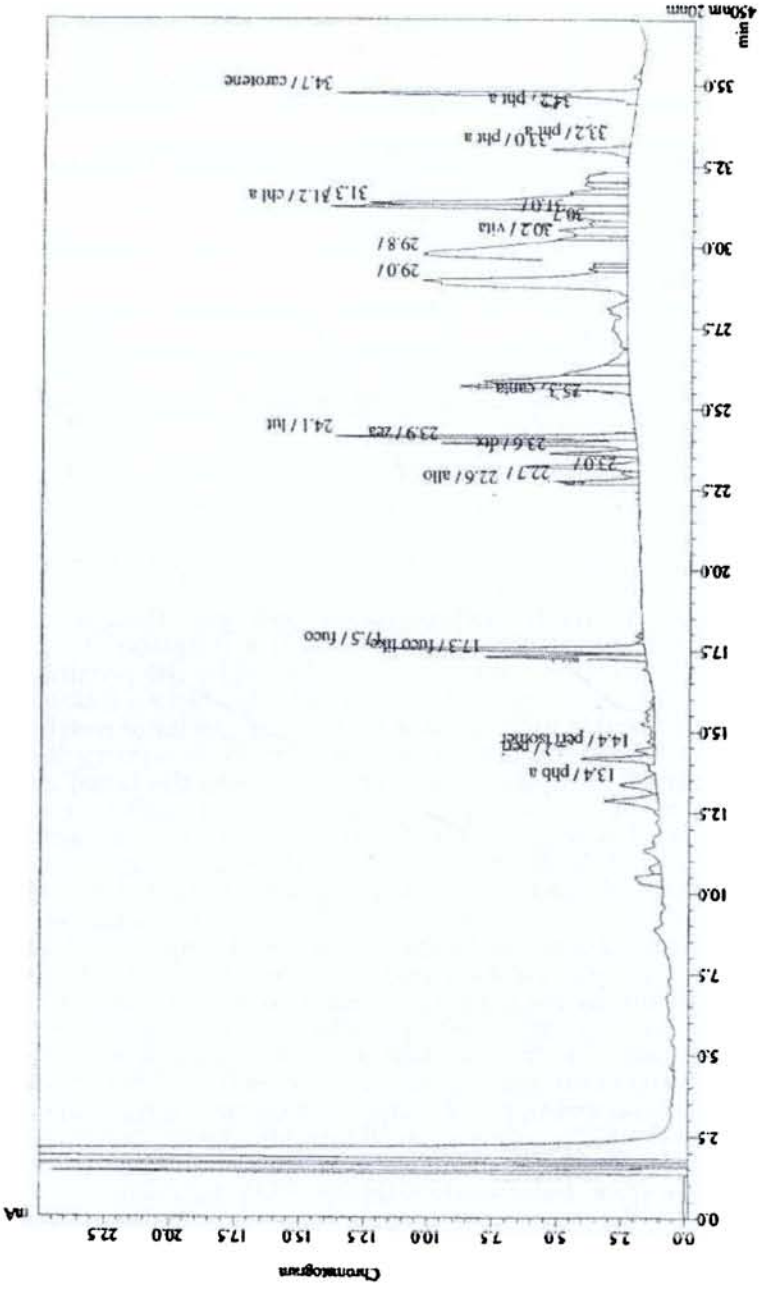

Figure : 5.1 Reverse phase HPLC Spectra Absorbance (λ = 420 & 450 nm) at station S2

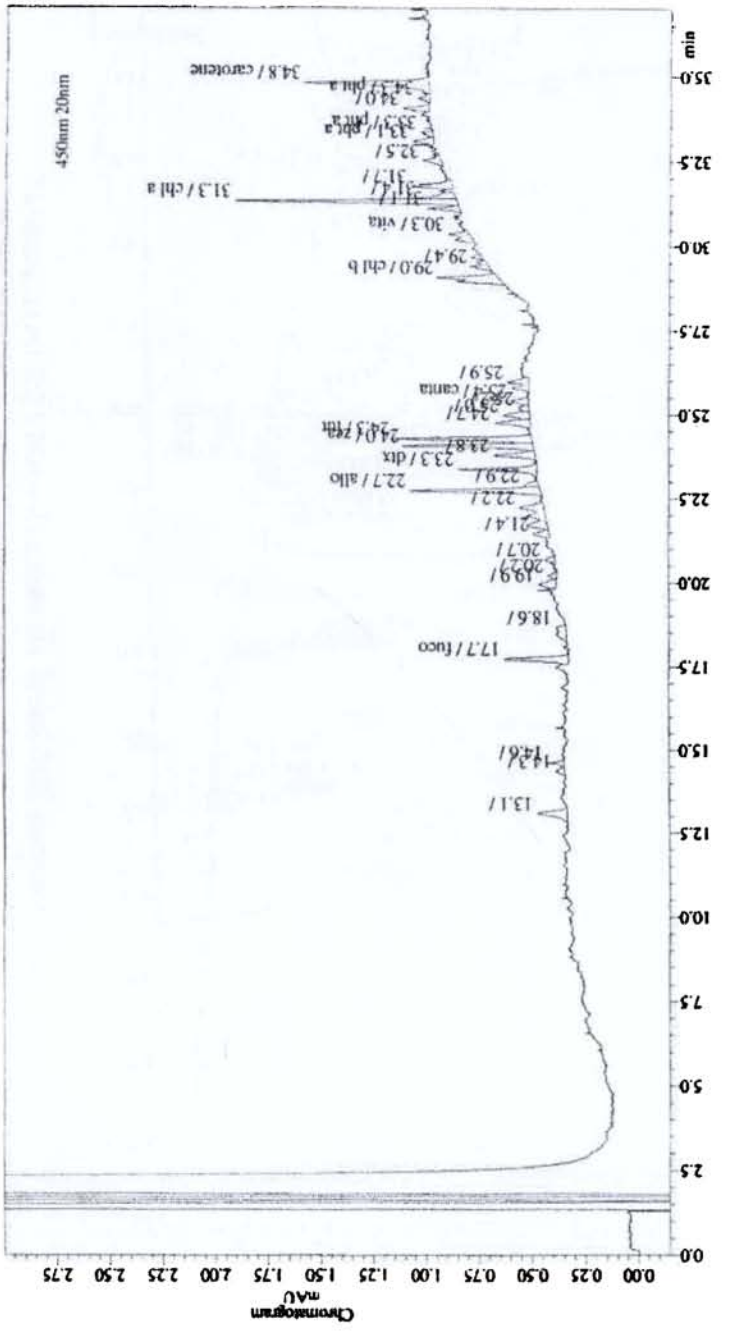

Figure: 5.2 Reverse phase HPLC Spectra Absorbance (λ = 420 & 450 nm) at station S4

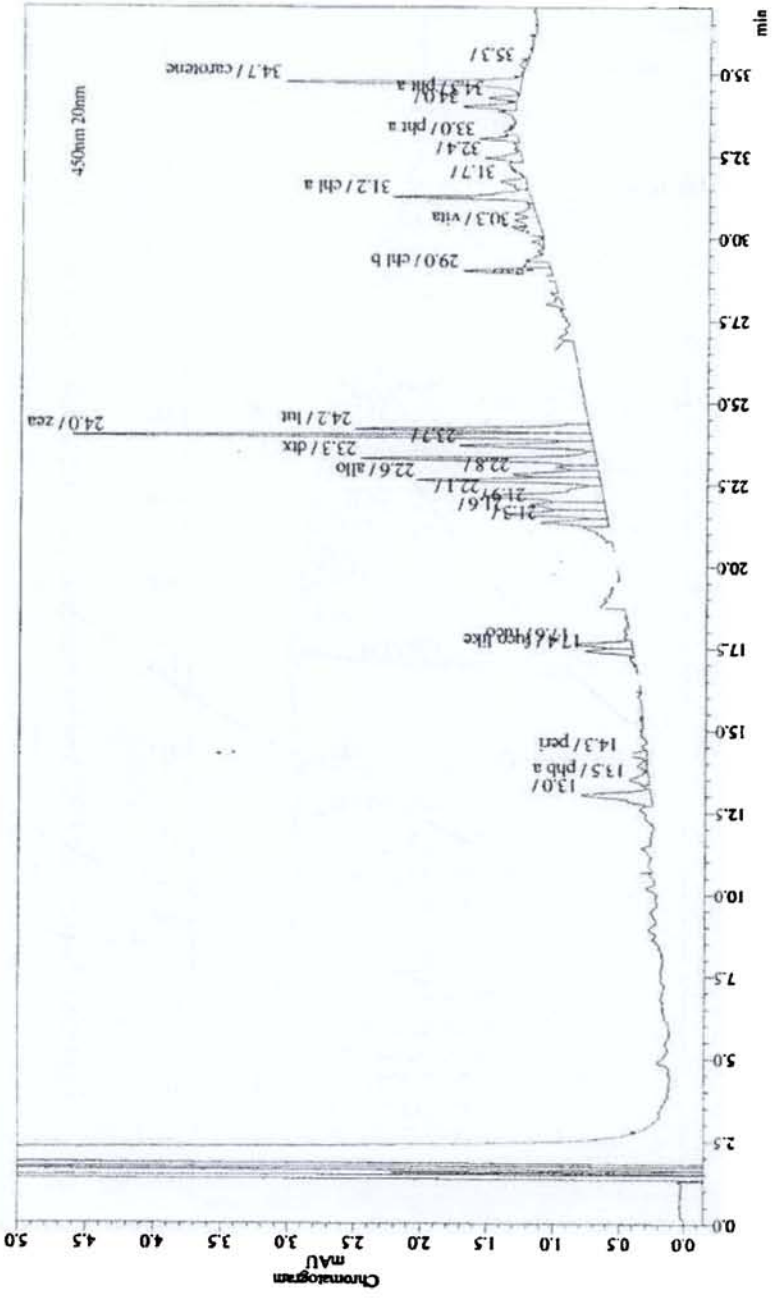

Figure 5.3 Reverse phase HPLC Spectra Absorbance (λ = 420 & 450 nm) at station S5

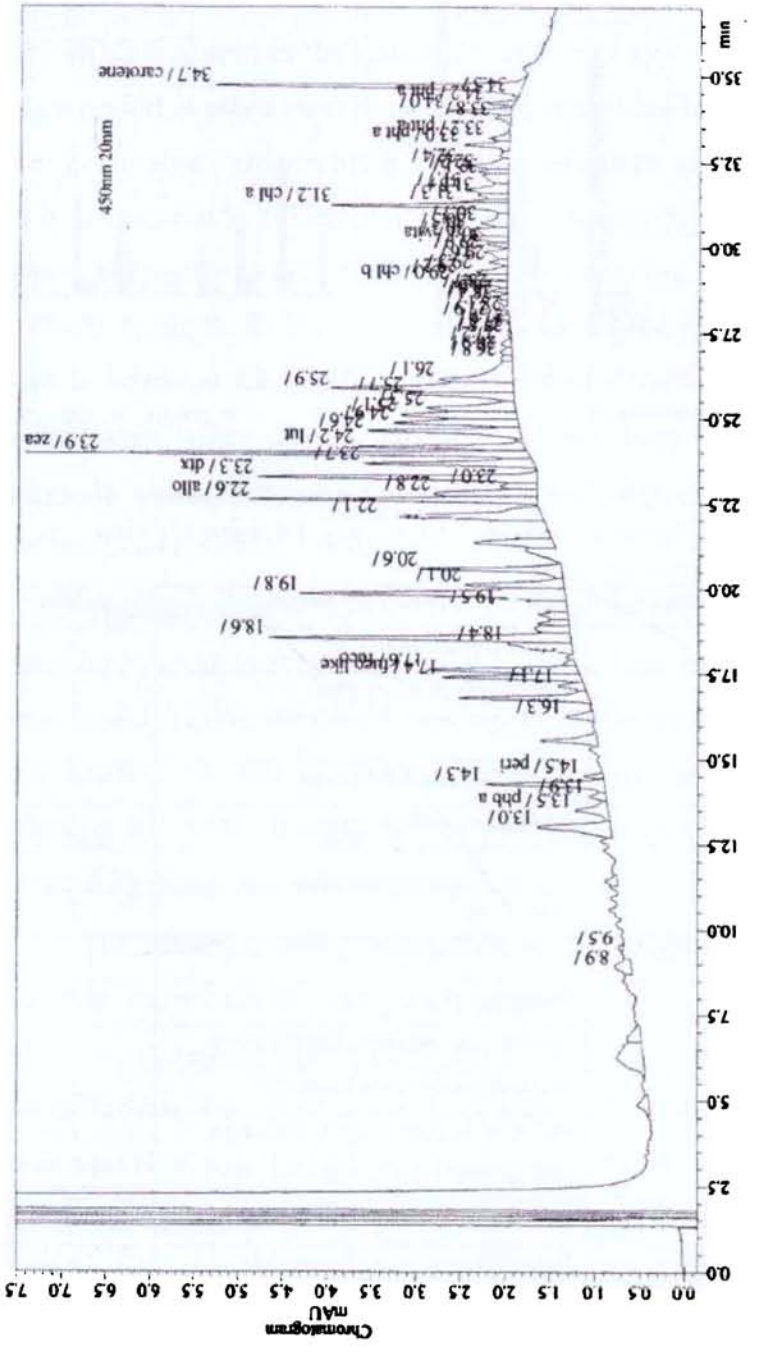

Figure: 5.4 Reverse phase HPLC Spectra Absorbance (λ = 420 & 450 nm) at station S7

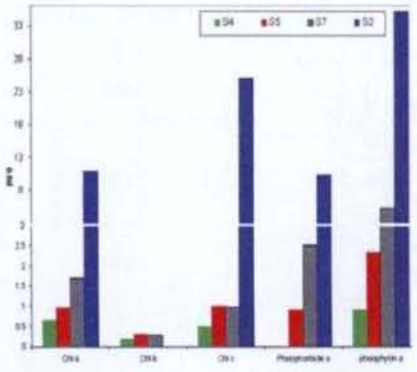

Figure 5.5 Distribution of Chlorophylls and its degradation pigments

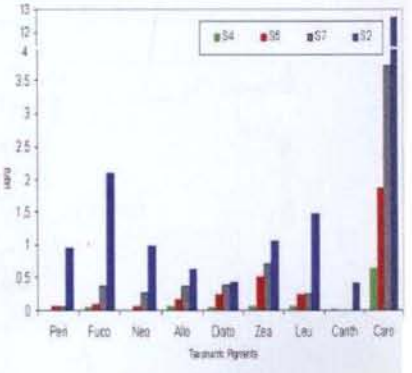

Figure 5.6 Distribution of marker pigments in the study area

Table:5.4 Distribution of major and taxonomically significant pigments across microalgal Divisions/Classe in the present study

Pigments	Taxonomic Significance	Stability
Chl a	All photosynthetic Algae, plants	3
Chl b	Green algae,Euglenophyta, plants	2
Biliproteins		
Phaeophorbide a	Grazing, Senescent diatoms	3
Phaeophytin a	Chl a derivative (all)	1
Carotenes		
α & β	Plants, Algae	1
Xanthophylls		
Alloxanthin	Cryptophyta	1
Canthaxanthin	Cyanobacteria, Chlorophyta,Eustigmatophyta	
Diatoxanthin	Bacillariophyta, Dinophyta, Chrysophyta	2
Fucoxanthin	Bacillariophyta, prymnesiophtes, Chrysophyta, raphidophytes, several dinoflagellates	2
Lutein	Chlorophyta, Euglenophyta, Plantae	1
9'-cis neoxanthin	Prasinophyta, Chlorophyta, Euglenophyta	4
Peridinin	Dinoflagellates, Dinophyta	4
Zeaxanthin	Cyanobacteria (Cyanophyta)	1

The presence of chlorophyll b derivatives is notable (Figure 5.5), which is absent in the station S2 and very low concentration was reported at other stations as it can be attributed to low inputs from green algae (chlorophyta), since higher plants are absent and chl b is susceptible for rapid degradation. Although diatoms (Chl a) have been identified throughout the sediment core, chlorophylls c, which occur in all diatoms, were not observed in any of the extracts. Previous workers have also failed to detect chlorophyll c in sediments where diatom remains exist and have corroborated this, with an explanation of a high chlorophyll a: c ratio in the diatoms (Vincent et al., 1993).

Fucoxanthin or Fuco (Figure, 5.6), a tracer of diatoms, found high amounts at S2 as reported as Chl a. Low concentrations were found in the station S4 (0.05ug/g) while in the estuarine zone, station S5 0.10 $\mu gg^{-1}$and at S7 0.38 μgg^{-1} were reported reflecting the wide distribution of diatoms in these stations as reported by many researchers (Table 5.2).

The photoprotecting zeaxanthin or Zea (Figure. 5.6) were found in cyanobacteria and prochlorophytes, showed very low values at station S4 (0.07 μgg^{-1}) and progressively increasing in concentrations from estuarine to Canal (S2) stations with a maximum (1.06 μgg^{-1}) which is a particular zone, characterized by high and constant nutrient input. Diatoxanthin, essential pigments of diatoms and prymnesiophytes also showed same pattern as Fuco. Peridinin was 0.95 μgg^{-1} at S3 but very low concentration 0.06 μgg^{-1} in estuarine zone (stations S5 & S7) where it was not

detected at S4. This type of type of distribution on dinoflagellats in estuarine environment was described by Incze and Yentsch in 1981 due to gradients in salinity. In addition to Peridinin, peridinin isomer also was detected at station S 2. One compound that have a structure close to fucoxanthin (Fuco like) detected and was present only in samples where peridinin (S5, S7 & S2) occurs. Previous studies elucidates that Dinoflagellats do not synthesis simultaneously peridinin and fucoxanthin like (derived) compounds. (Tangen and Bjornland 1981; Wright and Jeffrey, 1987). Same observation was recorded by Denant et al 1991 and it is inferred that the fucoxanthin like compound originates from species that are not dinoflagellats; but 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, thus suggesting an important localized contribution of green picophytoplankton in these coastal upwelling waters. But Chl b is absent in the station S2 indicates low contribution of green algae. Neoxanthin indicator of Euglenophyta was not detected at S4. The presence of compound alloxanthin- a carotenoid characteristic of strictly planktonic cryptophytes appears to develop all sites but rare at station four. The ratio Lutein to Chl b an indicator of Chlorophytes and Type 1 Prasinopytes were calculated and found that all Stations S4, S5 and S7 are having the ratio 0.4, 0.8 and 0.84 respectively indicate the presence of Chlorophytes, Over all

reduction of minor pigment was observed in the Cheranalloor ferry (stn.S4) indicating a declining in total primary production, results from phytoplankton blooming (Lami, et al 2000) where as a moderate production in the estuary and an eutophic trend occurred Champkara site due to the high input of anthropogenic effluents.

In this study about 12 algal classes were identified which belongs to Pico plankton (<2 µm), Nano plankton and (2-20 µm) micro plankton (20-200 µm)

Table 5.5 Algal Class identified during the study by marker signature pigment by HPLC.

Name of Algal Class	Classes	Size um
Green algae	Picoplankton	< 2
Cynobacteria	Picoplankton	< 2
Eustigmatophyta	Nanoplankton	2-20
Prymnesiophytes	Nanoplankton	2-20
Chrysophyta	Nanoplankton	2-20
Dinophyta	Nanoplankton	2-20
Bacillariophyta	Microplankton	>20
Dinoflagellates	Microplankton	>20
Cryptophyta	Microplankton	2-200
Chlorophyta	Microplankton	2-200
Euglenophyta	Microplankton	20-200
Raphidophytes	Microplankton	20-200
Prasinophyta	Microplankton	20-200

Previous studies conducted in this area mostly confined to microscopic and identified only very few algae classless. The microscopic analysis always fails to detect the pico plankton which contributes a higher percentage among total algae lineage. Algal

class identified in this work is given in the table (5.5) along with the previous studies in table (5.2). Among them two fall in the Pico plankton , four belongs from Nano plankton and six from micro algae .The micro algae proportion were found highest in the Anthropogenic effected area and pico plankton were houses in the productive areas. nano plankton distributes equally in all area contributes about < 20% of total composition of the algae class.

5.10 Taxonomic pigments and size structure indices

The taxonomic composition of phytoplankton influences many change in the biogeochemical processes, so it is essential to simultaneously determine phytoplankton biomass and its composition over the continuum of phytoplankton size. Generally seven pigments are used as biomarkers of several phytoplankton taxa: fucoxanthin, peridinin, alloxanthin, 19-butanoyloxyfucoxanthin, 19-hexanoyloxyfucoxanthin, zeaxanthin and total chlorophyll-b (for abbreviations see Table 1.1). In order to know the contribution of each community in the study area, data reduction was performed and size structure indices were made.. The diagnostic pigment (DP), mPF, nPF and pPF are defined below (Vidussi et al., (2001) ; Uitz et al.,(2006) and Roy etal., (2006). These taxa are then grouped into three size classes (micro-, nano-, and picophytoplankton), according to the average size of the cells.

[pPF]	Pico plankton proportion factor	([Zea]+[TChl b])/[DP]
[nPF]	Nano plankton proportion factor	([Hex]+[But]+[Allo])/[DP]
[mPF]	Micro plankton proportion factor	([Fuco]+[Peri])/[DP]
DP	Total diagnostic pigments (DP)	[PSC]+[Allo]+[Zea]+[TChl b]

Other marker pigments were categorized into photosynthetic pigments (PSP) and photo protective carotene or pigments (PPC), Photo synthetic Carotene (PSC), Total accessory pigments (TAcc), latter so-called macro variables are composed of pigment sums and are ratios, so they should be particularly useful in reconciling inquiries applied to databases from different regimes. Total pigments and Pigment ratio are calculated and are represented as in Table.5.7.

The higher order pigments shown with their symbols, names, and calculation formulae (Claustre et al., 2004)

Symbol	Pigment sum	Calculation
PPC	Photo protective carotenoids (PPC)	([Allo]+[Diad]+[Diato]+[Zea]+[Caro])
PSC	Photosynthetic carotenoids (PSC)	([But]+[Fuco]+[Hex]+[Peri])
PSP	Photosynthetic pigments (PSP)	([PSC]+[TChl a]+[TChl b]+[TChl c])
TAcc	Total accessory pigments (TAcc)	([PPC]+[PSC]+[TChl b]+[TChl c])
TPig	Total pigments (TPig)	([TAcc]+[TChl a])

Typically, large-sized phytoplankton have greater potential to export organic matter through a short classical food chain, whereas the small-sized phytoplankton are utilized by complex microbial food webs that favor the recycling of organic matter (Cermeno et al., 2006). Many studies have confirmed that small sized phytoplankton is an integral component in environmental monitoring assessment of the plankton community (Legendre and Le Fevre, 1988; Cermeno et al., 2006), although their relative contribution to the total community varies with the abundance of large-sized phytoplankton (Raimbault et al., 1988). Generally, nutrient enrichment favors the growth of large phytoplankton, while the production of small phytoplankton (nano-) is mainly

controlled by microzooplankton (cilites and flagellates) grazing (Riegman et al., 1993; Jyothibabu et al., 2006). The Composition with in each class is determined at each site from the pigment-derived criteria (Table 5.6 & Figure 5.7-5.10).

Table 5.6	Size indices			
Classes	Stations			
	S2	S4	S5	S7
DP	4.73	0.35	1.13	1.82
Bp pico	22.41	68.57	70.80	55.49
BP nano	13.32	17.14	15.04	20.33
BP micro	64.27	14.29	14.16	24.18

In estuarine site the pico phytoplankton was predominat. 70.8 %, pico , 15.0 % nano and 14.2 % micro and 55.5 % pico, 20.3 % nano and 24.2 % micro were observed at station S5 and S7 respectively, same trend 68.6% pico, 17.1 % nano and 14.3 % micro was observed at Cheranallor ferry (S4) (a water high way) (Cyanobacteria and prochlorophytes). In Contrast to other site a different proportion was measured at Champakara (S2) .Highest portion contributed by micro plankton and then pico and nano 64.3, 22.4 and 13.3 % respectively. The results confirm the predominance of micro plankton cells and Diatoms. The greater abundance of pico plankton at above stations as compared to the Champakra stations probably reflects the difference in micronutrient availability. Upwelling, tidal activities along with southwest, north east monsoon, and river discharges will often reflect nutrient enrichment in these stations. The period of study was close to the end of the summer period. This process was

therefore more developed at the former stations, supporting larger biomass of pico plankton. A significant contribution was also seen from the nanoplankton community. Picoplankton communities are generally dominates to prochlorophyte and cyanobacteria, which are common in tropical oceans and most likely, represent systems associated with regenerated production (Claustre, 1994).More over exogenous nitrates from anthropogenic inputs are principally used by large phytoplankton (microphyoplankon) and mainly contributes to new production (Goldman 1993). While regenerated forms of nitrogen (ammonia and urea) are the likely source for pico planktons and nano planktons) (Vidussi et al., 2001). The station S2 were one among the high discharge area of anthropogenic waste contain easily available exogenous nitrate.

Distribution algal class during the study period

Figure 5.7 S2 Charrapakara

Figure 5.8 S4 Cheranellor

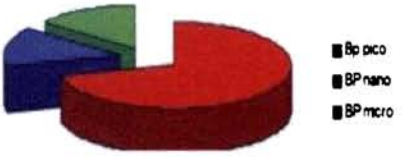

Figure 5.9 S5Port Taj

Figure 5.10 S7 Bolghatty

Accessory pigments have either photosynthetic properties allowing the phytoplankton cells to increase their light harvesting spectrum, or a role of photo protection in dissipating the excess of light energy received and reducing the oxidation that

takes place due to stress in conditions of strong irradiance. The major accessory pigments have also proven to be useful in chemotaxonomic indicators (Goericke and Repeta, 1992; Wright and Jeffrey, 1987; Moore et al., 1995; Guillard et al., 1985). Hence, the chlorophyll-a and their accessory pigment distributions have become important descriptors for the spatial and temporal variations of the autotrophic biomass and taxonomic composition. Subsequent grouping of pigments (including chlorophyll sums) permits the formulation of variables which are useful in different perspectives. The pool of photosynthetic and photoprotective carotenoids (PSC and PPC, respectively) are useful to photophysiological studies (Bidigare et al., 1987), and the total amount of accessory (nonchlorophyll) pigments (TAcc) are useful in remote sensing investigations (Trees et al., 2000) (Table 5.7). The ratios that could be derived from these pooled variables, e.g., [PSC]/[TChl a], are dimensionless and have the advantage of automatically scaling the comparison of results from different areas and pigment concentrations.

The maximum PPC, PSC and PSP observed at Champakara canal than at the estuarine site. The highest value of the photo protecting Chlorophylls and Carotinoids at these stations may be attributed to the contribution from large bloom of hydrophytes which contribute Chl a and zeaxanthin a indicator of picoplankton bio mass and largely by Fucoxanthin a indicator of Micro plankton and was found to be the most abundant of all photosynthetic carotenoids, making the largest contribution to the PSP budget.

While a lowest contribution of PPC, PSC and PSP at the station were examined at the station S4 (Cheranallur), as the result of low production flora . A moderate value was observed at estuarine sites indicated the mesotropic nature.

The pigment derived ratios are dimensionless and are used for scaling. It can be seen from our results that the ratios are almost the same indicating the elution and identification of each pigment are authentic (Table 5.7).

Table 5.7 Accessory pigments

Accessory pigments	Stations			
	S2	S4	S5	S7
PPC	14.81	0.82	2.77	5.20
PSC	3.04	0.05	0.16	0.44
PSP	39.25	1.34	2.39	3.39
TAcc	54.06	1.99	4.86	8.30
TPig	65.07	2.64	5.81	9.99
Ratios				
Tacc/TCha	4.91	3.06	5.12	4.91
PPC/TPig	0.23	0.31	0.48	0.52
PSP/TPig	0.60	0.51	0.41	0.34
PSP/Tcha	3.56	2.06	2.52	2.01

Significant linear correlations were found between Chl *a* and the photosynthetic carotenoid and photosynthetic pigment and photo protective carotenoids (Figure 5.11-5.13). A linear regression analysis performed between DP and Chl a and found significant correlation (r^2 =0.84,), indicating that DP can also act as a proxy of phytoplankton biomass. An existence of similar correlation between DP and Chl a has previously been reported by Vidussi et

al (2001) at eastern Mediterranean Sea; Barlow et al., 2005 at southern Benguela and Roy et al; 2006 at south west coat of India. However the DP/Chl a ratio may change with variations in nutrient dynamics and prevailing light condition, the DP can still be used as a surrogate of phytoplankton biomass and for identifying general trends (Roy et al 2006). A good correlation were observed between PPP and PSP &PSP and TChl a gives r^2=0.9 and 0.99 respectively.

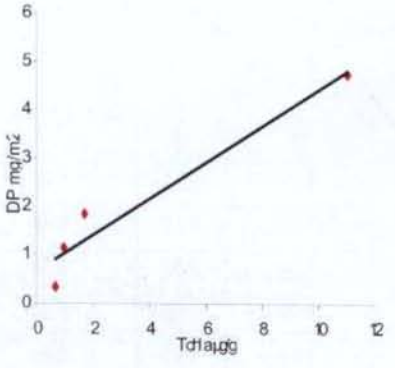

Figure 5.11 Relationship between DP and T Chl a

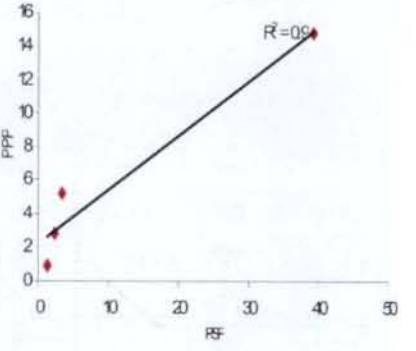

Figure 5.12 Relation between PPP and PSP

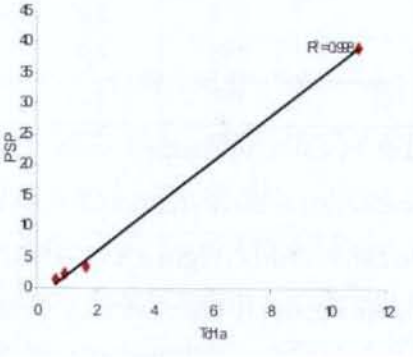

Figure5.13 Relation between PSP and T chl a

5.11 CD/TC ratio and classification of the aquatic system

Total carotenoids provide some indication of tropic status and also some evidence for the relative importance of allochthonous vs. autochthonous detritus in the sedimentary organic matter. In decaying leaves and soil organic layers, chlorophyll derivatives are ultimately better preserved than carotenoids, even though the initial breakdown of chlorophyll is faster (Gorham and Sanger 1967; Sanger et al. 1972), On top of the absolute values, information about the bio-production and degradation of organic matter in lakes can also be obtained from the Total Chlorophyll derivative to Total Carotene (CD/TC) ratios. With the protocol followed, complete resolution and separation of α- and β-carotene could not be achieved; these pigments were therefore grouped together and treated as total carotenoids (Roy et al., 2006). However, detailed studies in this field have not yet given a direct answer about which stage, production or destruction of the matter cycling in the lakes, which is more responsible for the forming of CD/TC ratios in sediments. Swain (1985) suggested that intensive production of pigments in eutrophic lakes may be the most important factor in the formation of the CD/TC ratio in sediments. The increase in CD/TC in lakes might be explained by faster degradation of carotenoids by Sanger (1988). Algal decay in lakes favors preservation of carotenoids and, hence, lowers the ratios. Because the bulk of the organic matter in eutrophic lakes is autochthonous, while in oligotrophic lakes allochthonous detritus from the drainage basin is the major source, ratios are higher in the

oligotrophic lakes. High ratios reflect a greater proportional input of allochthonous detritus and possibly a greater degree of aerobic decomposition of the autochthonous sedimentary organic matter. Both these phenomena are being compatible with shallowing and invasion of the aquatic system. The CD/TC values in the study area of the sediment cores differ sharply (Table 5.8).

Table 5.8 Carotenoids to Total Chlorophyll ratio

Stations	S2	S4	S5	S7
CD/TC	1.75	1.34	0.69	0.48
Classification	Allochothonous	Allochothonus	Autochthonous	Autochthonous

Overall a high value is reported at back water sites and lower values were found in the estuary. In samples from S2 this ratio is 1.75. 1.34 was estimated at S4. Same trend in the mesotropic system was observed by many researchers where the input of allochothonus detritus from the drainage basin and decaying of organic matter would cause better preservation of chlorophyll derivative along with the lower sedimentation rate. As pointed out by many authors (Swain, 1985; Sanger, 1988), these conditions influence more and the degradation pattern of carotenoids than that of chlorophyll derivatives. The CD/TC ratios in samples from S5 & S7 the estuarine stations have low 0.69 and 0.48 rather stable values. Low ratios are probably indicative of eutrophic trend with autochthonous plankton production prevailing. The former stations S2 and S4 are characterized by moderate carbon, and high Total, inorganic and Organic phosphate compared to latter estuarine station. The

phytopigments Chl a , b, c and Pheopigments shows different pattern. The degradation pigment found to be about two times higher than its parent pigment which indicate the excessive proliferation of flora and than it deposited rapidly after the faster degradation. The Pheopytin to carotenoids ratio were found out at three seasons. The ratio was almost constant in all stations indicating that the degradation is controlled by same mechanism; but the relative rate of degradation and preservation were different which were coupled with long residence time due to low flow conditions can result in an accumulation of organic matter in the surf zones compared estuarine. The percentage Nitrogen, Carbon, Sulphur and Hydrogen analyzed by CHN reveals that the percentage of nitrogen was very low in back water stations and but moderate Carbon and Suplhur detected (Table 5.9). This indicating that the grazing activities are predominates in this station. Nitrifying bacteria's and Sulphur bacteria will acting an important roll in this process.

Table 5.9 C,H,N&S analysis

Stations	N %	C%	S%	H%
S2	0.03	1.76	0.77	0.47
S4	0.2	1.96	0.18	0.67
S5	0.44	3.64	0.50	1.77
S7	0.34	2.60	0.63	1.44

5.12 Degradation products from Chlorophyll pigments

Chlorophylls are tetrapyrroles with a porphyrin head in the center of which is coordinately bound with magnesium atom (Aronoff . 1960). The porphyrin is attached to a long-chain terpene alcohol (phytol) which serves to bind the molecule to the intercellular membranes in prokaryotes and in the thylakoids of eukaryotic chloroplastids. It follows that chlorophyll a should be the most abundant, because it serves as the electron donor in pigment systems I and II to drive the electron transport mechanisms of the light reaction of photosynthesis. Sedimentary chlorophyll degradation products have been examined in many surface and sediment core studies, but their value in paleoecology and paleolimnology remains the subject of considerable uncertainty. One major difficulty occurs in distinguishing concentrations that are controlled by diagenesis and those controlled by primary productivity. The complexity of the molecules and the great array of possible degradation products, each with a different light-absorption spectrum and their chemical and specific absorption coefficient, obscure structures that have been reported earlier including paleoecological interpretation (Daley and Brown, 1973; Daley et al., 1973). Degradation pathways seem to be initiated by rather subtle differences in their sedimentary environment making it impossible to sort out reasons for the presence of a particular derivative. Various organisms, including bacteria, fungi, protozoans, crustaceans, oligochaetes, etc are present in various enzyme systems, operating over differing

pH ranges that act to promote variety in chlorophyll degradation. Additionally, chemical attack by action with various organic acids is different from oxidation that occurs in the chlorophyll-containing matrices which were absorbed on clay surfaces.

Pheophorbide a and Pheophytin shows a remarkable increase in concentration compared to Chl a where it accounted about three times to Chl a. Pheophytin is significantly present at station S3 (35.33 $\mu g g^{-1}$). S1, S2 and S4 show about 27% of Chl a and depicted in Figure. 5.6.

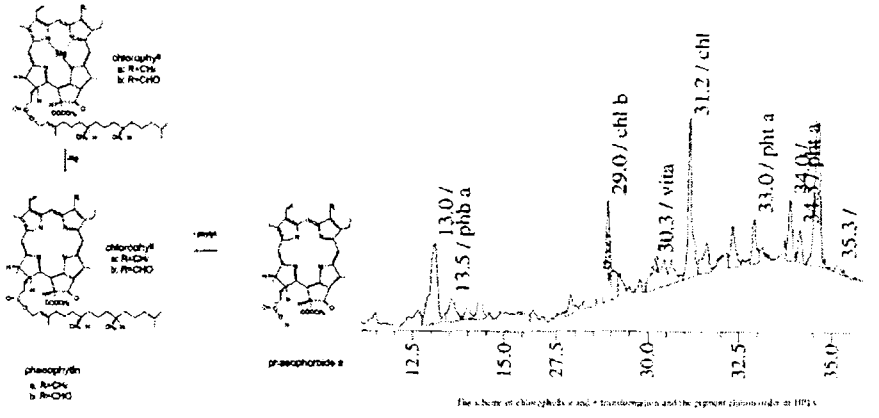

Figure 5.14 Scheme of Chlorophyll a and b transformation

Although most of the chlorophyll in healthy plant tissue is chlorophyll a, its degradation products may be relatively more abundant in sediments. Speculative pathways of degradation based on all the available large portion of the plant detritus from both the plankton and benthos is ultimately deposited on the bottom. Here it becomes a potential food source for bacteria, protozoa, and other benthic organisms, whose feeding activities

could possibly convert the chlorophyll to phaeo-pigments (Currie, 1962). The examination of possible pathways of degradation is limited by the success of pigment identification. From the above study proposed that an array of chlorophyll ***a*** degradation is the due to presence of pheophytin and phephorbide and the concentration of former is three times higher than later at all stations except S4 where later was found below detectable level (Figure 5.6) which suggests that there was a shift in the dominant mode of chlorophyll a degradation. Similar works also reported by Kowalewska et al (2004) from one involving the loss of Mg^{2+} ions and $COOCH_3$ (i.e. pyropheophytin a) (Figure 5.14) because no Pyro pheophytin detected, but involving a two step slow mechanism first the loss of Mg^{2+} ions resulting the formation of Pheophytin a and then phytol side chain ending the formation of pheophorbide a. These two step mechanism of chlorophyll a degradation was well established in the literature (Scheer, 1991) and involves the loss of Mg^{2+} atoms from the tetrapyrrole ring (type 1 degradation, Brown et al., 1981), and loss of the phytol chain and various side groups. This could be further confirmed by the observation that pheophytins and pheophorbide concentration that is former was higher than later which indicate that a more recent and advanced degradation state of the sediment.

5.13 Pigment as a tool for recent Ecological reconstructions

For more than 30 years Limnologists and Plaeoecologists have been scrutinizing fossil pigments in the sediments of fresh water starting from Vallatine 1954 to Utiz et al., 2006. These studies noted the universal presence of Chlorophyll derivative and Carotenoids in surface and older sediment material from corer. They suggested that the sedimentary Chlorophyll degradation products (SCDP) that have preserved in the sediment have been shown to be useful indicators of Recent/ Paleoecological changes in phytoplankton production and assemblages (Sanger, 1988; Millie et al., 1993; Leavitt and Hodgson, 2001), and can therefore potentially provide the long-term records needed for interpreting current changes in the environment caused by eutrophication. However, it is well known that the majority of pigments are degraded in the water column and in the uppermost sediment before being incorporated into the fossil record (Furlong and Carpenter, 1988; Hurley and Armstrong, 1990; Leavitt, 1993; Bianchi et al., 2002b). Factors influencing the sediment pigment record include photo- and chemical oxidation as well as herbivore digestive processes in the water column during deposition and post depositional degradation in the sediment. Changes in grazing pressure can affect both the digestive degradation and preservation through escape from oxidation in the water column by faster downward transport in faecal pellets (Leavitt, 1993; Cuddington and Leavitt, 1999), while the presence of anoxia at the sediment-

water interface and lack of benthic macrofauna has been shown to substantially increase the preservation of pigments in sediments (Sun et al., 1993; Bianchi et al., 2000b). The preservation of pigment biomarkers may improve over time with increased primary production reducing oxygen concentrations in the bottom waters. These phenomena can be difficult to distinguish from an increase in biomarker concentration from simple increases in primary production (Leavitt, 1993). Studies of pigments preserved in Baltic Sea sediments have demonstrated the occurrence of cyanobacterial blooms over thousands of years (Bianchi et al., 2000a) and changes in the concentration of chlorophyll derivatives have been linked to changes in primary production, preservation conditions and climatic phenomena (Kowalewska et al., 1999; Kowalewska, 2001). In addition, the sediment pigment record has recorded fluctuations in plankton community structure (Bianchi et al., 2002), and over a glacial cycle in Antarctica (Hodgson et al., 2003). In the Black Sea and Gulf of Mexico, sediment bacterial pigments have been used to investigate anoxygenic primary production and extent of anoxia, respectively (Repeta, 1993; Chen et al., 2001). In the present study the few samples were analyzed by UV-Visible spectrophotometer in order to analyze stability during the storage. For this; one portion of the sediment were analyzed immediately after freeze drying and another portion were kept for one year and took the spectra. The U V spectra reveal that

there is no significant effect on sample during the time if preserved at -80^{0}C Figure 5.15& 5.16.

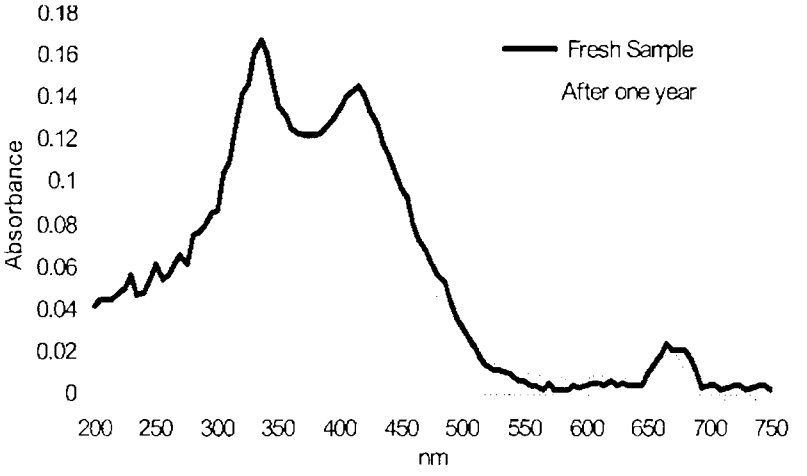

Figure 5.15 UV Visible spectra

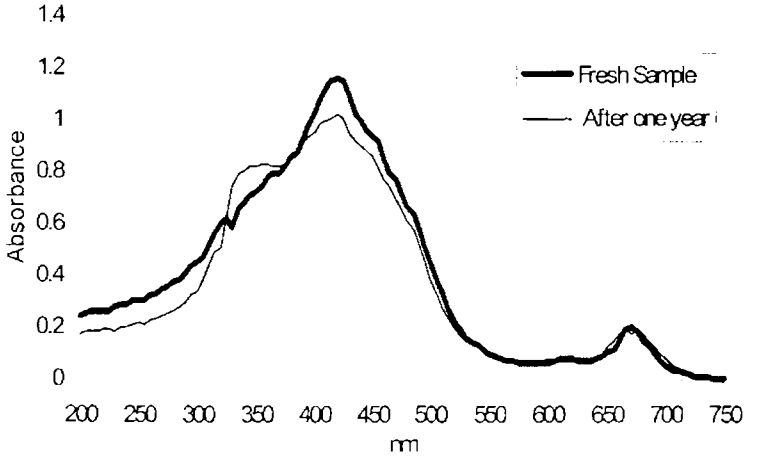

Figure 5.16 UV Visible Spectra

Sedimentary chlorophyll degradation products have been examined in many surface sediment core studies, but their value in paleoecology and paleolimnology remains still unraveled. The degradation and preservations pathways which are seem to be initiated by rather subtle differences in the sedimentary environment. Various organisms, including bacteria, fungi, protozoans crustaceans, oligochaetes, etc present most abundant in the sediment form as pheophytin a, pheophorbide a, chlorophyllide a, or in the various enzyme systems, operating over differing pH ranges, that could act to promote variety in chlorophyll degradation.

In order to study the preservation and degradation at different regimes, four specific characteristic sites where chosen in the CBWS and their surface zones. Sites were distinct in their physical and chemical characteristics (Table 2.1), which were reflected in the chlorophyll-a and total carotenoid data (Figure 5.5 & 5.6). The ratio of pheopigment- a to chlorophyll-a and carotenoid to total Chlorophyll-a was used to describe the pigment preservation at each site. This ratio is commonly used as the parent compound, chlorophyll- a, is readily degraded while the degradation products are much more stable. Good preservation of pigments, as indicated by a low ratio of pheopigment-a to chlorophyll-a. In the present study the ratio in the surface sediment was found to be almost constant ($\approx$ 1.32) (Table 5.10) which indicate that the process and influence

occurring in surface sediment for the control of the phytoplankton preservation are governed by similar pattern.

Table 5.10 Pheophytin toTotal Chlorophylls and Chlorophyllderivetives to Carotenoid derivatives

Seasons	Stations	phe a/ Total Chlorophyll a	CD/TC
Monsoon	2S	1.75	1.96
	4S	1.26	1.38
	5S	1.67	1.06
	7S	1.39	1.30
Post Monsoon	2S	1.50	1.42
	3S	1.37	1.22
	4S	1.46	1.52
	7S	0.71	1.93
Pre Monsoon	2S	1.21	1.22
	4S	0.80	1.30
	5S	0.77	1.03
	7S	2.00	0.51

The Vertical profile of the Chlorophyll a, its degradation pigments and carotene were found out after collecting the sediment core on April 2007 from zero depth to 6 cm deep. The core is immediately sub sampled in the interval of 2 cm (0, 2, 4 and 6 cm). Highest concentration was recorded at surface and the concentration of pigment decreases from top to bottom (Figure 5.17-5.19).

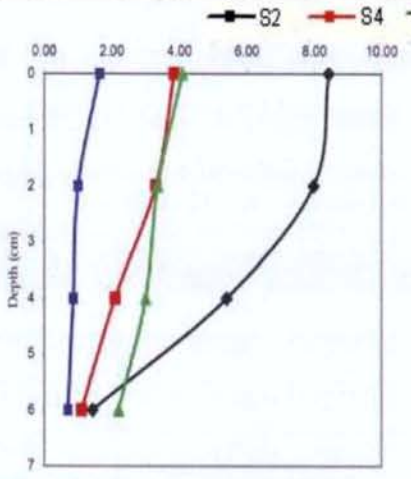

Figure 5.17 Vertical Chlorophyll a profile μg/g

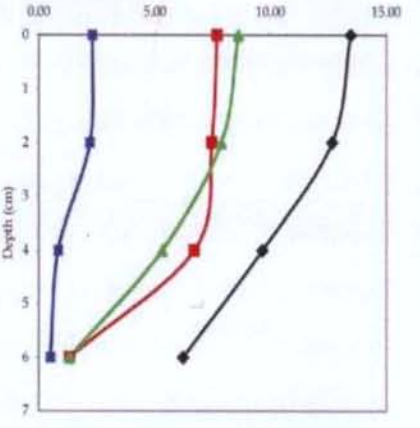

Figure 5.18 Vertical Total Carotenoids profile μg/g

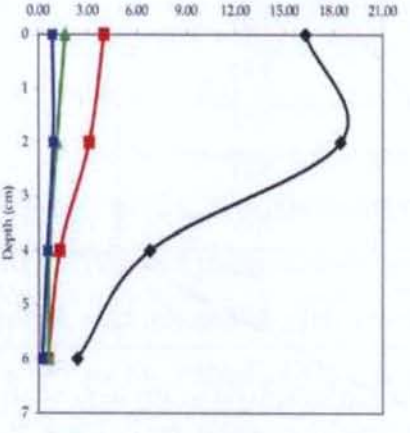

Figure 5.19 Verticlal Pheophytin a profile

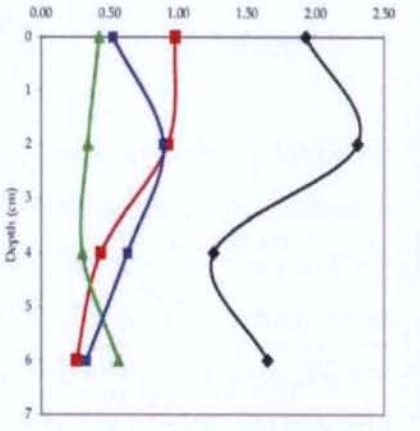

Figure 5.20 Vertical Pheophytin to Chl a ratio profile

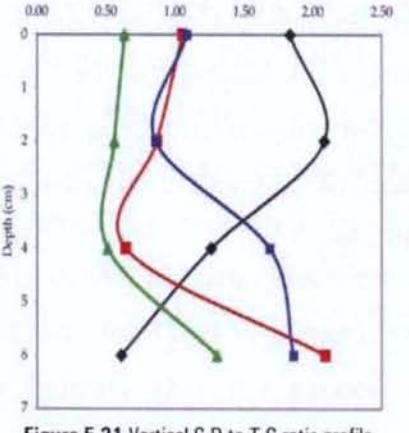

Figure 5.21 Vertical C D to T C ratio profile

Highest concentration of surface chlorophyll recorded at station 2 Champakra ranging 8.42-1.42 µg/g; 3.83-1.1 µg/g at S4; 4.09- 2.2 µg/g at S5 and lowest inventory pigment 1.64 -0.7 µg/g were recorded at station S7 from top to bottom. Pheophytin as expected showed almost double in concentration compared to Chl a concentration. Vertically (0 to 6 cm depth it ranges from 16.27-2.36; 3.99- 0.56, 1.62- 0.62, 0.86- 0.23 µg/g and at stations S2,S4 ,S5 and S7 respectively . Total Carotenoids varied 13.46- 6.22 µg/g at S2; 7.68 - 1.32 µg/g at S4 ; 8.61- 1.32 µg/g at S5 and 2.30 - 0.5 µg/g at S7.Similar observation were reported recently by Sanilkumar (2009).Concentration of pigments below 6 cm characterized by lowest inventory pigments, and often difficult to separation in ordinary means due to leached materials indicating substantial degradation caused by the daily tidal exposure of the sediment. A rapid decrease in concentration from top to bottom was observed at station 5 and 7. A interesting vertical distributions of Chlorophyll, Pheophytin and Carotenoids noticed in these productive sites and a rapid degradation were observed immediate after the surface layer up to 2cm than the degradation found to be slow down, where the anthropogenic input were higher and which would help rapid degradation of the pigment in surface and sub surface layers, prevailing anoxic conditions which retard the degradation of pigment at bottom layers. Pheophytin to Chloropyll ratios at these station showed very variable concentration (Figure 15.20). Pheopigment-a/chlorophyll-a ratio at station 2 and 7 indicating a net increase in ratio at 2 cm and

degradation is pronounced in surface. Rapid deposition of fresh sediment and oxic conditions may the prevailing reason. Beyond this layer the ratio was low, degradation slow down due to low availably of oxygen. While Station 4 and 5 shows a slow degradation pattern .The highest pigment concentrations and rapid degradation were found at Champakara canal, a productive estuary with anoxic bottom waters. Anoxic conditions in bottom waters results primarily from reduced water exchange due to the bathymetry and highly stratified waters. The CD (Chlorophyll derivative) to TC(Total Carotenoid) ratio(Figure 5.21) support the Pheophytin a to Chl a ratio. The increase in CD to TC ratio indicates the slow degradation of Chlorophyll or degradation of algae. The low rato indicates the preservation of carotenoids.

The pigment profiles at Champakara (S2) showed a distinct subsurface maximum, preservation of major productive changes in the estuary. Good oxygen conditions before this time could be explained the lower pigment rate in the bottom of the sediment core, however, no significant trend were observed in the pheopigment- a/chlorophyll-a ratio resulting the preservation regime has been fairly constant. Degradation products of chlorophyll origin, especially pheophytin- a, heavily dominated in the sediment pigment record. The low salinity at this site includes mostly large macrofauna, e.g. polychaetes, influencing much in the sediment. Resuspension and focusing of sediment are important processes at estuarine site and may be reasons for limited preservation of pigments. Resuspended material in this steep-sided

estuary experiences an extended period of exposure to high oxygen concentrations and light intensity in the water column in turn causing high degradation of pigments (Bianchi et al., 2000 ; Leavitt and Hodgson, 2001; Bianchi et al., 2002). The high sedimentation rate at this site was also reflected in the pigment supply.

5.14 Conclusion

The chapter introduces chemical oceanographer and related reaserches an insight to the Chemotaxonomic accounts of sediment associated pigments. The CBWS a tropical positive estuary and the back water system could be considered as a unique model for studying the chemotaxonomic composition of sedimentary pigment signature. Pigment concentration varied over a widely along the stations. Chl a distribution showed maximum at anthropogenic effected area and then the estuary and then polluted area, which suggest that marked influence of environment on productivity and possible difference in exchange mechanism of nutrients across the different stations. The degradation product of Chl a in this study found to be Pheophytin a greater than pheophorbide a indicates a recent and advanced degradation state of the sediment.

The other taxonomic pigments such as fucoxanthin, a marker of diatoms; zeaxanthin of cyanobacteria; alloxanthin of Cryptomonads; peridinin of dinoflagellates and Chl b of green algae reflects specific distribution along the different zones selected for the research work. In natural environment, pigment composition may well vary with prevailing light condition and

photoadaptive state. Compounds with a fucoxanthin like structure showed an interesting correlation with peridinin, suggesting the potential presence of phytoplankton species which has close association with dinoflagelates.

Some pigments that may exist in the estuary were not successfully isolated, including chlorophylls b, c, and the carotenoid fucoxanthin derivatives. Further research is required to determine the degree of pigment degradation and destruction in situ as well as how much breakdown occurs during core extrusion and in the performed analytical procedures.

The size structure provides useful insight into the ecosystem functioning as the larger phytoplankton are known to be generally dominant in nutrient- rich, productive waters whereas smaller phytoplankton are more abundant under mesotropic conditions. The biomass proportions derived from the marker pigments and DP (Diagnostic pigment) as defined above indicate that the picoplankton community contributed significantly to the biomass structure in the southernmost part of our study region. In contrast to other sites micro plankton appear to be most abundant in the Champakra canal whereas pico plankton contribute predominant species in riverine and estuarine stations . For the whole sampling area, the order of abundance was microplankton (38.6%), picoplankton (32.7%) and nanoplankton (24.6%).

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Summary

Climate change could cause irreversible damage to ecosystem and biodiversity rendering several species extinct. Temperature in an area is an important which determines the circulation and stratification. Aquatic organisms are extremely sensitive to physical and chemical conditions of marine water, with narrow tolerance ranges any deviation results stressful conditions. In addition to this numerous estuarine studies throughout the world clearly demonstrate that interactions between the sediment and the water column play an important role in regulating phytoplankton production and the extent of bottom water hypoxia/anoxia.

The present Doctoral Research work entitled "Phytoplankton Pigment Signatures as a Bio marker in a Tropical Estuary" focused an appraisal of particulate organic matter of photosynthetic origin, here; the study programmed pigment data to characterize the phytoplanktonic assemblages in terms of the major taxonomic groups and addresses the importance of degradation processes and terrestrial inputs. In addition, pigment data have been combined with several basic physicochemical and geographical variables measured during the study to develop a topology for the estuaries. It also enabled us to speculate on the pathways of pigment degradation in the lake and assess individual pigments as biomarkers of past algal and bacterial communities and the potential of pigment degradation products to signal changes in the historical depositional environment.

First chapter dealt with the opening of the subject which gave an insight to the coming chapters. Brief introduction to taxonomically significant pigment in each algae division along with its history of development, paleoecology and the use of sedimentary pigment as Bio Marker Signatures were included.

Second chapter spotlighted the sampling locations with its environment and analytical methodology adopted in this work.

Chapter three gave a portray about the Cochin estuarine hydrographical settings which are regulating the production and the interrelationship between hydrographical parameters both seasonal and spatial periodicity. A multivariate statistical approach and correlation techniques were implemented.

Spatio -Temporal variation of Pigment and it associates in Cochin back waters were illustrated in the chapter four. It was found that the phytoplankton distributions, species composition and dominance are closely associated with the prevailing hydrographic condition and nutrients and fluctuation occurs in response to the proximity of pollution sources. Phytopigments controlled by salinity which in turn control the distribution of nutrient due to stratification and tidal activities. A strong correlation observed between phytopigment , nutrients and micronutrient trace metals . It seems plausible that the trace metal such as Zinc, Manganese and Iron contributing for the reproduction of phytoplankton biomass as like macronutrients.

Final chapter gave an insight to the Chemotaxonomic accounts of sediment associated pigments. The **CBWS** a tropical positive estuary and the back water system could be considered as a unique model for studying the chemotaxonomic composition of sedimentary pigment signature. Pigment concentration varied over a widely along the stations. Chl a distribution showed maximum at anthropogenic effected area and then the estuary and then polluted area, which suggest that marked influence of environment on productivity and possible difference in exchange mechanism of nutrients across the different stations. The degradation product of Chl a in this study found to be Pheophytin a greater than pheophorbide a indicates a recent and advanced degradation state of the sediment.

The other taxonomic pigments such as fucoxanthin, a marker of diatoms; zeaxanthin of cyanobacteria; alloxanthin of Cryptomonads; peridinin of dinoflagellates and Chl b of green algae reflects specific distribution along the different zones selected for the research work. In natural environment, pigment composition may well vary with prevailing light condition and photoadaptive state. Compounds with a fucoxanthin like structure showed an interesting correlation with peridinin, suggesting the potential presence of phytoplankton species which has close association with dinoflagelates.

The size structure indices worked out which are useful into the ecosystem functioning as the larger phytoplankton are known to be generally dominant in nutrient- rich, productive waters

whereas smaller phytoplankton are more abundant under mesotropic conditions. The biomass proportions derived from the marker pigments and DP (Diagnostic pigment) as defined above indicate that the picoplankton community contributed significantly to the biomass structure in the southernmost part of our study region while other sites micro plankton appear to be most abundant. For the whole sampling area, the order of profusion was microplankton (38.6%), picoplankton (32.7%) and nanoplankton (24.6%).

Appendix

Temperature °C									
Stations	Nov-05	Jan-06	Apr-06	Jun-06	Aug-06	Oct-06	Jan-07	Apr-07	Sep-07
1S	29.0	30.0	30.0	31.0	29.0	27.0	27.0	31.0	27.0
1B	29.0	28.0	29.0	31.0	28.0	26.0	26.0	29.0	27.0
2S	30.0	31.0	29.0	29.0	30.0	30.0	29.0	30.0	31.0
2B	30.0	30.0	30.0	30.0	29.5	31.0	30.0	29.0	31.0
3S	29.0	31.0	31.0	29.0	29.0	30.0	30.0	29.0	30.0
4S	29.0	31.5	31.0	31.0	29.0	29.0	29.0	30.0	29.0
4B	28.0	31.5	29.0	31.0	29.0	29.0	29.0	30.0	29.0
5S	31.0	30.0	30.0	29.0	30.0	31.0	31.0	30.5	31.5
6S	31.0	31.0	29.0	29.0	30.0	31.0	31.0	31.0	32.5
6B	31.0	31.0	28.0	30.5	30.0	32.0	31.0	30.0	32.0
7S	31.0	31.0	29.0	30.0	29.0	30.0	32.0	29.0	30.5
7B	30.0	30.5	30.0	27.0	28.0	30.0	30.5	28.0	30.0

pH									
Stations	Nov-05	Jan-06	Apr-06	Jun-06	Aug-06	Oct-06	Jan-07	Apr-07	Sep-07
1S	6.5	6.7	6.7	6.2	6.4	6.2	8.3	6.5	6.5
1B	7.2	6.8	6.7	6.2	6.4	5.4	7.4	7.0	6.8
2S	6.9	6.8	6.7	7.5	6.4	5.3	7.0	6.7	6.6
2B	7.3	7.1	6.9	7.0	6.5	5.3	7.1	6.7	7.0
3S	7.2	7.1	8.4	8.0	6.5	5.9	6.2	7.0	6.5
4S	7.3	6.9	6.8	7.4	6.7	6.0	6.6	6.6	6.9
4B	6.8	7.1	6.7	6.7	6.9	6.1	7.2	6.9	7.1
5S	8.0	7.8	7.5	8.1	7.1	5.3	7.9	7.9	7.3
6S	7.9	7.7	7.5	8.5	7.2	6.1	6.9	7.9	7.3
6B	7.9	7.7	7.5	8.4	7.3	6.2	8.1	7.9	7.4
7S	7.7	7.7	7.4	8.7	7.1	6.1	7.8	7.9	7.2
7B	7.8	7.7	7.4	8.9	7.0	6.2	8.0	7.9	7.3

Dissolved Oxygen mg/L									
Stations	Nov-05	Jan-06	Apr-06	Jun-06	Aug-06	Oct-06	Jan-07	Apr-07	Sep-07
1S	4.12	5.92	5.77	4.06	4.27	5.87	6.04	5.47	6.50
1B	3.90	4.98	4.27	4.27	4.27	4.37	6.98	3.97	5.60
2S	2.11	3.55	2.99	3.14	3.71	3.00	5.10	2.69	2.69
2B	1.88	4.14	2.35	3.21	3.85	2.35	4.57	2.05	3.36
3S	2.71	3.91	2.90	3.78	3.42	3.63	4.08	2.60	3.47
4S	3.24	4.15	2.99	2.35	2.99	3.00	5.88	2.69	4.26
4B	3.00	4.50	1.28	2.56	2.99	3.29	5.39	1.98	5.26
5S	2.81	4.62	3.21	2.35	4.06	4.21	7.38	2.91	4.93
6S	3.24	4.74	4.70	3.85	4.06	4.70	8.35	4.40	5.15
6B	3.14	4.14	2.99	3.85	4.06	3.35	8.00	2.69	5.04
7S	3.90	5.98	1.92	2.56	3.85	4.92	5.72	1.82	6.18
7B	2.69	4.45	2.14	2.56	3.42	3.27	4.72	1.64	5.49

Nitrate mg/L									
Stations	Nov-05	Jan-06	Apr-06	Jun-06	Aug-06	Oct-06	Jan-07	Apr-07	Sep-07
1S	.....	0.07	0.61	0.20	0.23	0.04	0.09	0.14	0.02
1B	.....	0.12	0.62	0.22	0.35	0.04	0.09	0.13	0.05
2S	.....	1.49	1.53	1.20	1.98	2.03	2.06	2.37	2.10
2B	.....	1.56	1.52	1.80	2.01	2.43	1.99	2.56	2.20
3S	.....	0.52	1.02	0.62	0.75	0.44	0.49	0.53	0.45
4S	.....	1.60	1.53	2.60	2.50	1.98	2.40	1.66	1.21
4B	.....	0.96	0.92	2.20	2.41	2.83	2.39	2.96	2.60
5S	.....	1.30	1.11	2.43	3.01	1.75	2.87	1.22	1.97
6S	.....	1.50	1.13	2.50	3.20	2.10	2.90	1.13	1.13
6B	.....	1.70	1.51	2.83	3.41	2.15	3.27	1.62	2.37
7S	.....	0.17	1.80	1.60	2.44	2.40	0.65	1.68	1.88
7B	.....	0.20	2.90	1.90	2.02	2.50	0.73	2.10	1.90

Nitrite mg/L									
Stations	Nov-05	Jan-06	Apr-06	Jun-06	Aug-06	Oct-06	Jan-07	Apr-07	Sep-07
1S	0.14	0.08	0.14	0.25	0.00	0.22	0.12	0.30	0.00
1B	0.14	0.21	0.14	0.24	0.00	0.26	0.19	0.34	0.01
2S	0.41	0.27	0.41	0.02	0.02	0.04	0.29	0.23	0.00
2B	0.38	0.34	0.38	0.02	0.03	0.02	0.23	0.22	0.04
3S	0.03	0.03	0.03	0.02	0.06	0.19	0.01	0.02	0.17
4S	0.22	0.36	0.22	0.20	0.06	0.43	0.10	0.02	0.16
4B	0.00	0.11	0.31	0.21	0.01	0.42	0.33	0.16	0.10
5S	0.13	0.22	0.13	0.04	0.19	0.02	0.10	0.53	0.60
6S	0.17	0.16	0.17	0.20	0.19	0.16	0.00	0.28	0.07
6B	0.22	0.29	0.22	0.05	0.20	0.05	0.00	0.23	0.18
7S	0.20	0.35	0.20	0.11	0.16	0.09	0.15	0.30	0.35
7B	0.07	0.24	0.07	0.04	0.22	0.14	0.16	0.36	0.45

Phosphate mg/L									
Stations	Nov-05	Jan-06	Apr-06	Jun-06	Aug-06	Oct-06	Jan-07	Apr-07	Sep-07
1S	0.27	0.12	0.17	0.10	0.05	0.06	0.02	0.03	0.09
1B	0.24	0.10	0.05	0.03	0.05	0.05	0.01	0.02	0.09
2S	1.74	2.62	1.42	1.07	1.98	3.70	3.62	2.84	1.21
2B	2.11	2.12	1.35	0.27	2.02	4.41	3.22	2.66	1.33
3S	0.12	0.10	0.22	0.23	0.56	1.13	0.12	0.05	0.22
4S	0.11	0.10	0.21	0.14	0.55	1.28	0.14	0.02	0.09
4B	0.00	0.16	0.42	0.17	0.38	1.14	0.05	0.03	0.03
5S	0.17	0.08	0.47	0.22	0.21	0.12	0.05	0.26	0.09
6S	0.13	0.11	0.54	0.33	0.24	0.07	0.05	0.15	0.01
6B	0.13	0.05	0.59	0.18	0.21	0.02	0.07	0.12	0.02
7S	0.17	0.10	0.60	0.20	0.33	0.02	0.07	0.11	0.03
7B	0.13	0.13	0.61	0.16	0.30	0.22	0.05	0.07	0.02

Total Dissolved Solids mg/L									
Stations	Nov-05	Jan-06	Apr-06	Jun-06	Aug-06	Oct-06	Jan-07	Apr-07	Sep-07
1S	.....	34.56	23.50	23.52	14.40	18.33	18.00	28.20	20.00
1B	.....	44.04	39.20	24.00	14.40	17.10	18.80	28.20	24.00
2S	.....	1232.00	765.00	826.80	55.20	114.22	972.90	874.20	160.00
2B	.....	1480.00	761.94	858.60	58.80	27.55	1057.50	860.10	180.00
3S	.....	236.80	75.35	72.00	32.00	50.40	42.30	84.60	40.00
4S	.....	73.28	87.42	66.24	32.80	65.80	112.80	211.50	32.00
4B	.....	81.28	278.40	115.20	38.00	76.10	233.20	249.35	84.00
5S	.....	1980.00	1734.00	2173.00	11.40	2793.00	1325.40	1353.60	320.00
6S	.....	1884.00	1555.30	1961.00	10.80	2805.00	1739.00	1598.00	360.00
6B	.....	1904.00	1577.20	2385.00	22.00	2626.00	1645.00	1598.00	360.00
7S	.....	1328.00	1582.00	1966.30	16.00	1189.00	1927.00	1880.00	160.00
7B	.....	1448.00	1616.50	2597.00	16.00	1479.00	2021.00	1927.00	280.00

Total Hardness mg/L									
Stations	Nov-05	Jan-06	Apr-06	Jun-06	Aug-06	Oct-06	Jan-07	Apr-07	Sep-07
1S	.....	16.00	28.42	14.21	11.12	8.72	20.00	12.00	15.00
1B	.....	50.00	36.54	13.20	9.14	10.15	20.00	16.00	20.00
2S	.....	2670.00	1571.22	150.22	42.63	50.75	940.00	1652.00	55.00
2B	.....	2822.00	1522.50	121.80	229.39	50.75	1150.00	1812.00	60.00
3S	.....	200.00	28.42	50.75	26.39	40.60	200.00	450.00	25.00
4S	.....	580.00	22.33	40.60	22.33	30.45	300.00	500.00	25.00
4B	.....	630.00	26.28	44.90	42.63	35.53	345.00	660.00	30.00
5S	.....	5650.00	3877.30	4334.05	237.51	588.70	2850.00	4260.00	735.00
6S	.....	6810.00	2994.30	5135.90	241.57	563.33	2150.00	3720.00	850.00
6B	.....	7250.00	3126.20	5399.80	513.59	492.28	2300.00	3860.00	925.00
7S	.....	4900.00	3187.10	4343.50	407.60	238.53	2400.00	4500.00	395.00
7B	.....	5300.00	3319.10	203.00	422.63	294.35	2600.00	5550.00	725.00

Chlorophyll a mg/m^3									
Stations	Nov-05	Jan-06	Apr-06	Jun-06	Aug-06	Oct-06	Jan-07	Apr-07	Sep-07
S1	.....	1.40	12.43	1.18	2.23	6.17	1.43	14.06	1.65
S2	.....	1.97	29.75	17.26	2.60	2.44	2.44	11.52	5.14
S3	.....	1.23	11.40	5.48	3.35	1.05	1.16	12.22	1.41
S4	.....	3.59	8.91	4.77	2.96	0.84	1.06	6.86	0.92
S5	.....	8.91	15.13	5.74		6.93	6.93	14.11	4.80
S6	.....	6.79	17.97	4.84	5.53	7.50	7.50	11.66	7.40
S7	.....	2.75	8.86	5.60	6.48	5.40	5.40	13.64	6.00

Chlorophyll b mg/m^3									
Stations	Nov-05	Jan-06	Apr-06	Jun-06	Aug-06	Oct-06	Jan-07	Apr-07	Sep-07
S1	.....	1.60	2.78	0.62	2.27	3.25	1.07	3.60	1.24
S2	.....	8.33	9.98	7.36	2.38	0.59	0.59	4.62	1.89
S3	.....	1.92	5.10	1.85	2.66	0.38	0.47	6.40	1.23
S4	.....	0.58	3.11	1.47	3.63	0.70	0.62	3.47	0.12
S5	.....	1.30	2.55	2.43	0.00	2.95	2.95	1.67	1.89
S6	.....	5.73	3.90	2.65	2.82	3.06	3.06	5.57	3.30
S7	.....	2.60	9.62	5.75	3.60	2.19	2.19	10.42	2.91

Chlorophyll c mg/m^3									
Stations	Nov-05	Jan-06	Apr-06	Jun-06	Aug-06	Oct-06	Jan-07	Apr-07	Sep-07
S1	.....	4.60	6.97	5.60	5.55	1.17	1.84	11.10	2.29
S2	.....	2.10	10.36	6.75	5.01	0.16	0.16	11.70	2.15
S3	.....	5.04	14.13	2.02	7.68	0.75	0.98	12.20	3.31
S4	.....	1.39	2.63	1.63	0.93	0.94	1.08	1.97	3.39
S5	.....	2.70	2.62	2.63		2.22	2.22	0.98	1.78
S6	.....	2.20	8.80	3.24	3.55	2.55	2.55	5.72	3.38
S7	.....	6.46	10.67	6.06	4.72	1.95	1.95	8.15	4.15

Pheophytin a mg/m^3									
Stations	Nov-05	Jan-06	Apr-06	Jun-06	Aug-06	Oct-06	Jan-07	Apr-07	Sep-07
S1	.....	5.40	15.43	9.10	6.10	14.53	3.63	4.50	8.08
S2	.....	4.40	15.83	21.79	17.70	4.99	4.99	19.97	6.81
S3	.....	8.62	26.40	6.81	7.26	22.70	17.70	18.09	9.08
S4	.....	5.45	6.77	2.27	3.70	2.20	2.10	8.62	1.82
S5	.....	12.68	11.80	5.45		10.44	10.44	14.07	6.35
S6	.....	15.87	15.50	4.99	2.27	13.16	13.16	14.54	11.80
S7	.....	9.99	14.47	15.89	1.82	2.72	2.72	13.63	9.53

Bio mass mg/m^3									
Stations	Nov-05	Jan-06	Apr-06	Jun-06	Aug-06	Oct-06	Jan-07	Apr-07	Sep-07
S1	.....	93.80	832.92	79.23	149.28	413.40	95.82	942.02	110.52
S2	.....	131.99	1992.96	1156.60	174.17	163.47	163.47	771.68	344.51
S3	.....	82.34	763.80	367.14	224.63	70.10	78.04	818.73	94.32
S4	.....	240.86	596.96	319.50	198.19	56.18	71.08	459.85	61.95
S5	.....	596.89	1014.02	384.87	0.00	464.58	464.58	945.44	321.83
S6	.....	454.92	1204.20	324.55	370.36	502.77	502.77	780.93	496.07
S7	.....	184.26	593.74	375.20	434.15	361.48	361.48	913.74	401.73

Iron µg/L								
Stations	Nov-05	Jan-06	Apr-06	Jun-06	Aug-06	Oct-06	Jan-07	Apr-07
S1	177.00	250.67	142.75	67.00	.....	50.98	16.60	41.90
S2	78.00	58.80	269.50	127.50	.....	156.50	35.33	59.50
S3	245.59	283.24	48.25	197.00	.....	344.55	28.65	140.68
S4	56.00	125.00	178.00	183.25	.....	100.20	41.23	162.40
S5	266.75	73.16	96.00	742.50	.....	203.95	213.73	116.60
S6	150.53	100.00	147.25	390.00	.....	193.28	131.63	251.48
S7	22.89	112.79	73.00	314.50	.....	323.55	19.73	109.85

Manganese µg/L								
Stations	Nov-05	Jan-06	Apr-06	Jun-06	Aug-06	Oct-06	Jan-07	Apr-07
S1	2.20	0.97	0.70	1.58	1.60	.....	1.50	0.50
S2	1.20	11.65	1.35	1.83	1.35	.....	3.50	6.50
S3	1.15	1.85	1.65	1.53	1.58	.....	0.50	1.25
S4	1.55	4.70	3.15	2.25	1.28	.....	nd	1.25
S5	2.00	2.63	1.33	2.20	1.55	.....	nd	3.75
S6	11.66	3.20	1.38	1.33	1.85	.....	nd	2.50
S7	2.26	0.32	1.20	1.60	1.25	.....	nd	3.50

Zn µg/L								
Stations	Nov-05	Jan-06	Apr-06	Jun-06	Aug-06	Oct-06	Jan-07	Apr-07
S1	21.20	16.15	12.55	7.13	18.50	.....	4.13	7.13
S2	60.68	19.79	36.20	10.35	8.75	.....	4.38	2.63
S3	7.56	25.24	38.88	11.53	15.95	.....	5.95	12.23
S4	44.85	92.40	11.90	21.08	35.20	.....	10.48	10.90
S5	27.50	20.42	44.13	19.25	12.78	.....	24.45	27.55
S6	26.21	17.50	5.70	10.90	11.48	.....	6.18	15.35
S7	17.24	2.52	9.15	9.38	11.73	.....	3.98	12.08

Salinity psu						
Stations	Post monsoon	Pre monsoon	monsoon	Post monsoon	Pre monsoon	monsoon
1S	0.06	0.06	0.10	0.01	0.07	0.01
1B	0.17	0.09	0.08	0.02	0.06	0.02
2S	8.00	11.60	0.62	0.14	13.97	0.24
2B	7.83	10.19	0.61	0.17	10.80	0.20
3S	0.08	1.42	0.15	0.02	1.30	0.02
4S	0.08	0.09	0.64	0.03	2.49	0.02
4B	2.75	3.09	1.08	0.08	5.66	0.10
5S	24.69	34.36	12.21	4.24	24.10	5.14
6S	23.17	31.66	11.81	3.22	21.25	3.66
6B	27.99	32.56	11.89	5.03	20.93	4.99
7S	23.18	27.47	9.27	2.03	25.64	2.31
7B	24.18	21.98	9.38	4.05	27.27	3.99

Stations	Carbohydrate mg/L			Protein mg/L		
	Pre monsoon	Monsoon	Post monsoon	Pre monsoon	Monsoon	Post monsoon
1S	2.91	1.90	5.56	5.10	39.92	17.52
1B	3.32	2.16	6.39	1.33	50.12	15.97
2S	13.09	2.84	9.35	52.78	41.81	24.73
2B	12.55	1.74	16.33	65.20	43.36	32.49
3S	2.96	3.96	3.46	22.62	37.71	59.90
4S	3.73	29.71	13.51	90.26	91.10	94.02
4B	4.32	27.35	15.65	95.06	87.49	107.90
5S	7.64	6.57	17.14	14.64	40.36	38.99
6S	3.64	6.88	16.77	.....	33.60	41.47
6B	2.55	9.77	16.26	.....	54.11	13.08
7S	6.55	10.94	14.70	33.04	34.60	40.37
7B	7.64	10.02	12.16	35.26	58.11	43.61

Stations	Sulphate mg/L		
	Pre Monsoon	Monsoon	post monsoon
1S	100.02	12.55	96.89
1B	95.00	11.47	26.78
2S	2314.00	196.30	1478.75
2B	1984.20	248.60	1415.35
3S	446.20	34.42	236.51
4S	568.60	48.45	529.10
4B	2018.00	68.85	1341.80
5S	2387.00	487.00	1936.50
6S	3422.00	688.50	2489.50
6B	3349.00	379.90	2431.50
7S	3342.00	136.40	3067.50
7B	3387.00	132.60	2128.95

We are in the cutting edge of a new era of development without leaving any promises to next generation. But the scale and size of the problem are only partially blamed. The juggernaut of Globalisation has trampled upon whatever little hope we might have had making a quick transition to a less energy –intensive world. "Environment friendliness begins at home". Our quest for productivity and profitability should progress simultaneous with our cooperative responsibility of leaving behind a clean and green earth for the generation to come. Climate change is the most pressing global environmental challenge being faced by humanity, with the quest for better productivity for our fragile ecosystem. It is too late to rely solely on reduction in Green house gas emissions to mitigate climate change although this is undoubtedly crucial. Coastal belts are more prone to these devastating impacts and its protection is an intensive filed of research. The present study describes how the colourful Carotenoids and Chlorophylls can be used in rapid hand on tool in conjunction with molecular biology to open sources and it also explores the fate of organic matter in the aquatic system and underlying sediments.