Spatial and seasonal variations of phytoplankton species and their relationship to physicochemical variables in the Cochin estuarine waters, Southwest coast of India

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Received; revised

During the present study total 120 species of phytoplankton were identified and represents different distinct classes viz: Bacillariophyceae (73), Dinophyceae (24), Chlorophyceae (17), Cyanophyceae (4), and Chrysophyceae (2). Diatom formed the most dominant group. Phytoplankton density varied from 15,893 to 46,486 cells L$^{-1}$. Maximum density was found during pre monsoon season coinciding with the stable hydrographical conditions. Change in phytoplankton density and species composition during different seasons was significant and was found to be regulated by the change in water characteristics.

[Key words: Phytoplankton, food web, Marine, estuarine]

Introduction

Phytoplankton is one of the initial biological components from which the energy is transferred to higher organisms through food chain$^{1-3}$. They are microscopic unicellular plants originates from the base of the marine food web, fueling all of the higher organisms with the products of their photosynthesis$^4$. Phytoplankton are important components in the energy exchange process of the oceans and their tremendous involvement in reducing atmospheric carbondioxide would play a crucial role in controlling climatic changes and global warming$^5$. Phytoplankton species distribution shows wide spatio-temporal variations due to the differential effect of hydrographical factors on individual species and they serve as good indicators of water quality including pollution$^6$. Seasonal patterns include changes in phytoplankton diversity, composition, biovolume and importantly, the magnitude of primary production; but the photosynthetic
response to limiting factors might be regulated by changes in species composition and diversity of phytoplankton\(^7\).

Marine phytoplankton communities usually comprise several taxonomic groups, and contribute to primary production and interaction between trophic levels\(^8\). Primary production varies with season and this can be ascribed to variation in nutrient access, light and temperature\(^9\). Phytoplankton growth and abundance are primarily regulated by both biotic and abiotic interactions. The constant nutrient supply always supports the rich phytoplankton production, but generally nitrogen and phosphorus have been considered as the potentially limiting nutrients for phytoplankton growth in the aquatic ecosystems\(^10\). Several works have been carried out in the Cochin estuarine system for species composition, seasonal variation, physico-chemical and biological characteristics of phytoplankton\(^11-15\) and in other regions of Indian coastal waters most of the scientist were investigated\(^1,5,16-24\).

Present study initiates to investigate the identification of phytoplankton species and their biomass during the period of 2009 and 2010. Relationship between the changing environmental hydrographic parameters are also encountered. Phytoplankton communities have been characterized by quantifying and identifying micro algal taxa seasonally. Recently much emphasis of this kind of studies have been carried out in these estuarine areas, and gaining more insight.

**Materials and Methods**

The Cochin estuarine system is considered to be the most productive aquatic realm and a dwelling area for diverse kinds of flora and fauna, that extends between (9\(^0\)40’ & 10\(^0\) 12’N and 76\(^0\) 10’ & 76\(^0\) 30’E) with two permanent openings to the Arabian Sea and sustains high biological production. This 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 16 km wide in the Vembanad lake area and there are several narrow canals along with those emptying municipal waste and other particulate organic matter into the estuary. Several major rivers Periyar, Muvattupuzha and Pampa discharge freshwater into the tropical estuarine system. Estuarine character is also persuaded by the adjoining rivers, altogether giving rise to seasonal and tidal fluctuations of varying hydrographic conditions. This tropical estuarine environment shows multitude features characterizing freshwater and seawater mixing and endows a breeding ground for marine organisms.

Seasonal sampling was performed to record the physico-chemical and biological characteristics of phytoplankton. Three prominent seasons viz, Post monsoon (POM), Pre monsoon (PRM), and monsoon (MON) were selected. The study area is divided into three zones - south, middle and north zones. Middle zone is near to the coastal area is affected by tides and consequently the estuarine and oceanic waters are mixed. South and north part is fresh water area, during low tide fresh water mixed with estuarine water. Total eight stations were selected from 9°47.646’ and 9°04.993’N latitude 76°25.708’ and 76°17.906’E longitude (Fig.1).

Field data like temperature were measured using thermometer. Light penetration in the water column was measured with the help of secchi disc. Salinity and pH were measured by Mohr-Knudsen titration technique and using potable pH meter respectively. Dissolved oxygen was estimated by the modified Winkler’s method\textsuperscript{25}. For analysis of nutrients, water samples were collected in clean plastic bottles and kept in an ice box and immediately to the laboratory. Nutrients (nitrite, nitrate, phosphate and ammonia) were measured as per Strickland and Parsons methods.
For analyzing phytoplankton cell counts and their composition, water samples were filtered through phytoplankton net made up of bolting silk cloth (mesh size 20µm). Collected samples were preserved in 3% neutralized formalin for further analysis. A setting and siphoning procedure was followed to concentrate the samples from 250ml to 20ml by the method. Phytoplankton analyzed was assigned to major groups viz. diatoms, dinoflagellates, bluegreen algae and green algae. Planktonic micro algae filtered from 100 L of water was made up to a fixed volume concentrate. 1 ml of this sample was transferred to the sedge wick-Rafter counting cell (the volume of this chamber is 1 ml). The number of micro algae present in the cell 1000 grids was calculated. Repeated the counting for three times and considered the average. Total numbers of planktonic algal species present in the water sample were calculated using the formula,

\[ N = m \times \frac{v}{V} \]

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

For estimation of chlorophyll a (chl a) concentrations, 1L of each sample was filtered onto whatman GF/F filters. Chl a was extracted in 10ml of 90% acetone and kept in overnight at 4°C. After extraction, the crude extract was centrifuged at 4000rpm for 10 minutes, and thereafter the concentrations were measured on a spectrophotometer. The amounts of Chlorophyll a were based on the equations from Parsons et al. All the statistical analyses of correlation matrix and principal component analysis (PCA) were performed using SPSS statistical software.
Results and Discussions

The profound aspect of water quality is the assessment of physico-chemical parameters and they serve as visible tool for the investigation of water status. Temperature did not register any marked variation during the study period. Variation in temperature showed lowest (26.5°C) in MON and highest (33.5°C) in PRM, insignificant to make any influence on the distribution and standing crop of microalgae during the present investigation. Maximum temperature showed an increasing trend in summer season and was powered by the intensity of high solar radiation and thereby temperature decreased, which would be the result of loss of energy from the sea surface to the atmosphere by evaporational cooling due to the impact of strong monsoonal winds. Temperature pressurized the fluctuation and distribution of microalgae particularly diatoms in temperate latitudes. Statistical analysis showed a moderately positive correlation with salinity and a strong negative correlation with pH was observed (Table-2).

Hydrogen ion concentration, pH in the sampled water remained alkaline throughout the study period with PRM maximum (8.5) and MON minimum (6.8) (Table 1). Generally, their seasonal variation is attributed to factors like removal of carbon dioxide by photosynthesis through bicarbonate degradation, dilution of seawater by freshwater influx, low primary productivity, reduction of salinity and temperature and decomposition of organic matter. The recorded high summer pH might be due to the influence of seawater penetration and high biological activity and due to the occurrence of high photosynthetic activity.

Salinity is one of the most important hydrographical parameters, which considerably influences almost all other physical and chemical properties of sea water and thus significantly determines the distribution of biological communities. Salinity ranged from 1.8-28.0 psu, the highest salinity was observed in station 5, during the pre monsoon and the lowest was recorded from station 1 in
the monsoon period. Generally, salinity changes in the estuaries is due to the mixing of freshwater from Muvattupuzha and Periyar river and land run off, caused by monsoon or by tidal variations. Salinity acts as a limiting factor in the distribution of living organisms and its variation intern caused by dilution and evaporation, which mostly control the fauna density in the coastal ecosystem. Salinity yielded a strong negative correlation with DO was observed, which was manifested the fact that minimum DO was coincided with salinity maximum.

Dissolved oxygen varied between 2.9-6.8mg L⁻¹, the highest value of DO reported in MON season. The elevated dissolved oxygen content in PRM period was possibly contributed by the photosynthetic release of DO by the high phytoplankton density and similarly, the relatively high level of DO observed during MON was attributed to the influx of oxygen rich fresh water from precipitation, backwater and land runoff.

Distribution of nutrients is mainly based on season. The nitrate-nitrogen is one of the indicators of pollution of the water body, the highest value of nitrate (26.6µM) was observed in monsoon season at station 3 and the lowest value (3.0µM) was noted in same station in PRM season (April-May). Onset of south west monsoon was always accompanied by a general rise in the nitrate level and the concentration of nutrients was high during the monsoon months. Seasonally, Nitrite shows an elevation in PRM season than the other season. Seasonal average of nitrite concentration was low during monsoon months (0.2 µM), the highest value (3.3 µM) were reported at January to April. Recorded higher nitrite values during monsoon season could be due to the increased phytoplankton excretion, oxidation of ammonia and reduction of nitrate and by recycling of nitrogen with the addition due to bacterial decomposition of planktonic detritus present in the environment.
Higher concentration of ammonia (78.0 µM) was observed during monsoon season in Station 8 and a lower value of ammonia (1.3 µM) was found during the post monsoon season in station 1. Recorded higher concentration could be partially due to the death and subsequent decomposition of phytoplankton and secondly to the excretion of ammonia by planktonic organisms\textsuperscript{39}. As pointed out by Calliari\textsuperscript{40}, the function of nutrients should not be taken into account as a separate but could be considered as a primary triggering factor for biomass and distribution of phytoplankton in most of the investigations.

Both spatially and seasonally an abnormal pattern of was found for phosphate concentration. In the riverine station, the concentration of phosphate was reported a sharp increase in the post monsoon and which dropped in pre monsoon season. Spatially the value showed lowest (0.4 µM) at station 2 and highest (8.2 µM) at station 8, which situated near the industrial area. Moreover, the weatherings of rocks soluble as alkali metal phosphates, the bulk of which are carried into the estuaries are also responsible for the recorded higher values as reported earlier\textsuperscript{41}. Addition of super phosphates applied in the agricultural fields as fertilizers and alkyl phosphates used in households as detergents can be other contributing sources for these inorganic phosphates during the season and earlier works also supports this statment\textsuperscript{35,28}. Phosphate showed a positive correlation with DO and strong negative correlation with salinity and nitrate.

Chlorophyll-a is considered as the most reliable and important index of phytoplankton biomass. Concentration of chlorophyll a varied between 0.4 -35.3mg/m3 showing a wide range of fluctuation. At PRM, higher and at MON, lower values of chlorophyll a (chl a) was recorded. Chlorophyll a values were higher during the pre monsoon almost at all stations except 2, 6 and 8 and station 7 attained the high value of chl a (35.3 mg/m$^3$). The recorded low monsoon values could be due to the anthropogenic effects along with the freshwater discharges flushing out from the adjacent rivers which could cause the concurrent turbidity and less availability of light\textsuperscript{41-43}. 
Composition and Community structure of phytoplankton

The species composition, growth, proliferation and abundance of phytoplankton are collectively influenced by a variety of environmental variables especially, salinity, nutrients, transparency and temperature. Based on the two year investigation (2009-2010), total 120 species of phytoplankton were identified (Table 2) which represents different classes viz: Bacillariophyceae (73), Dinophyceae (24), Chlorophyceae (17), Cyanophyceae (4) and Chrysophyceae (2). However, a visible change was noticed where the abundance of chlorophyceae species were dominant in monsoon seasons both the year. Diatoms and dinoflagellates were the abundant groups in terms of species diversity and density than the other taxonomic groups. In general, the distribution and intensity of phytoplankton in tropical waters, varied remarkably owing to the seasonal environmental fluctuations, and these variations are well pronounced in the sheltered system of estuarine waters. Cochin estuarine system is proved one among them. Percentage contribution of each group of phytoplankton was in the following order: Diatoms>Dinoflagellates>Blue green algae >Green algae.

Mostly, diatoms were found to be numerically preponderant during the pre monsoon. However, Coscinodiscus sps and skeletonema sps was dominant both at station 3 and 6. This phytoplankton abundance during summer season could be attributed to the increased salinity, pH, high temperature and high intensity of light penetration during the season and earlier reports supports this inference. While Thalassiosira and Navicula were dominant species in station 2, 6 and 8 during the monsoon. Abundance of phytoplankton was lowest during monsoon months. Because of heavy rainfall and concurrent high turbidity, reduced salinity, lower temperature and pH, overcast sky and cool conditions remarkably stratified to a large extent in the water column. However, the freshwater algal forms like Ankistrodesmus spiralis, Coelastrum sp, Cosmarium sp, Euastrum sp, Stuarastrum sp, Asteroidesmus sp, Tetradon sp, pediastrum sp, selenastrum sp,
*Sphaerozoma sp, Chlorella sp, and Nostoc sp,* were also noticed in the same season. Estuarine stations in the southwest India was dominated by diatoms but a shift in Chlorophycean dominance was observed in some periods, particularly in the monsoon at station 6. The ranges of these phytoplankton population density (cells L$^{-1}$) were highest (av. 46486.17) at station 3 in pre monsoon and lowest (av.15892.86) at station 2 in monsoon season. The observed high density during summer could be attributed to the more stable hydrographical conditions prevailed during these period.

Several previous studies have been conducted in the Cochin estuarine system on the distribution and diversity of phytoplankton based on various hydrographical parameters. Perusal of literature stand in this area which 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. Madhu et al., reported out of 89 species of phytoplankton identified in PRM season and diatoms (85 species) were the most dominant groups. Recently, Sanilkumar (2009) total 285 species of planktonic microalgae within seven classes viz Cyanophyceae, Chlorophyceae, Bacillariophyceae, Dinophyceae, Chrysophyceae, Dictyochophyceae and Coccolithophorids were observed in both coastal and estuarine stations of southwest coast of India.

*Statistical Analysis*

Principal Component Analysis (PCA)

In the present study, principal component analysis of phytoplankton communities and water quality developed four principal components as can be observed from the eigenvalues (Table.2). The total variability represented by these four PCs was 61.9%. Eigenvalues of the four PCs represented of total variance (PC1-24.1%; PC2-14.5%; PC3-13% and PC3-10.3%).
Results of this analysis showed that most of the variables associated with each factor are well defined. Parameters for which cross loading observed were pH, temperature, DO, salinity, alkalinity, nitrate, nitrite, phosphate, ammonia, iron, chlorophyll a and phytoplankton density. While the parameters pH, salinity, alkalinity, nitrate, phosphate, iron and chlorophyll a were positively loaded to PC1, variables such as temperature, DO, nitrate, ammonia and phytoplankton density were found to be negatively loaded. The negative loadings of the DO showed that decomposition of organic matter that consumes DO might have led to high phosphate in the estuarine waters. On the other hand, the decrease in DO content in estuarine waters may be high at saline conditions, when respiration by phytoplankton also contribute to the reduction in DO, could be the reason for its negative loading on PC1. The high phosphate observed in this PC termed as the phosphate regeneration PC. Release of phosphate from the sediment and suspended matter take place at lower temperature, which would have resulted in relating high concentration of phosphate with low temperature conditions as observed during the present study.

In PC2, showed 14.5% of the total variance, was found to be almost all the variables as positively loaded except alkalinity, nitrate and phytoplankton density. In this PC, high nitrite was positively loaded and this PC can be termed as nitrite enrichment component. Nitrite is formed from the oxidation of ammonium in the aquatic environment. Nitrifying bacteria oxidize ammonium into nitrite then convert nitrite into nitrate. Nitrite concentrations may increase if oxidation rates of ammonia exceed oxidation rates of nitrite, or if the oxidation process of nitrite is inhibited. A secondary source of nitrite is anaerobic denitrification, converting nitrate into nitrite.

PC3 explained 13% of total variance. The variables such as pH, temperature, salinity, alkalinity, nitrite, nitrate and phytoplankton density were positively loaded and the remaining DO, phosphate, ammonia, iron and chlorophyll a were negatively loaded to PC3. This PC showed that
phosphate depletion observed during relatively high pH and temperature most probably by the process of adsorption into sedimentary particles and suspended matter. This PC can be termed as the phosphorous adsorption component and perhaps represented the state of during the monsoon period as the results observed the above trend.

PC4 was found to be the least loaded one, with positive loadings of pH, DO, salinity, alkalinity, nitrite, ammonia chlorophyll a and phytoplankton density. The negative loadings were noted for temperature, nitrate, phosphate and iron. This PC accounted for 10.3% of the total variability and it can be interpreted that the DO content of the water was relatively high. This could be due to better light penetration leading to enhanced primary production coupled with nitrate consumption. These evidence showed that high phytoplankton density and intern high chlorophyll a concentration. This PC may be termed as the phytoplankton growth component as the sign of the loadings was found to be closely associated with phytoplankton growth.

**Conclusion**

Hydrographical parameters are imperative aspects related to a water body for distinguishing its distinction. Estuaries serve as the most dynamic body, since intrusion from the fresh water and sea water regime varies in all seasons and keeps on oscillating and leading to a multifaceted environment throughout the time. The correlation supports either significant or extraneous points towards the fate of an estuary and the life pertaining to it. Recurrent transitory actions take place silently enhancing the risk of either an adaptable situation or a lackluster condition. Seasonal incongruity in the hydrographical parameters along this versatile estuarine system is an implication of the morphometry changes undergoing day to day.
Acknowledgement
Authors are grateful to the Ministry of Earth Science (MoES) for financial support, Dr. Bijoy Nandan help provided for statistical analysis and Dr. Sanilkumar M.G. for the help of identification of phytoplankton species.

References


**Table-1: Check list of phytoplankton species encountered during the present investigation (January 2009-August 2010).**

<table>
<thead>
<tr>
<th>Class-Bacillariophyceae (Diatoms)</th>
<th>Asterionella japonica</th>
</tr>
</thead>
<tbody>
<tr>
<td>Achnanthes sps</td>
<td>Bacillaria paradoxa</td>
</tr>
<tr>
<td>Amphiprora alata</td>
<td>Bacteriastrum sps</td>
</tr>
<tr>
<td>Amphora sps</td>
<td>Bacteriastrum hyalinum</td>
</tr>
<tr>
<td></td>
<td>Biddulphia sps</td>
</tr>
<tr>
<td></td>
<td>Biddulphia rhombus</td>
</tr>
</tbody>
</table>
Biddulphia mobilianis
Caloneis brevis
Cerataulina pelagica
Chaetoceros spp
Chaetoceros affinis
Chaetoceros decipiens
Chaetoceros danicus
Chaetoceros debilis
Chaetoceros densus
Cocconeis spp
Coscinodiscus spp
Coscinodiscus marginatus
Coscinodiscus radiatus
Coscinodiscus subtilis
Cosinodiscus granii
Cosinodiscus centralis
Cyclotella spp
Cyclotella meneghiana
Cyclotella striata
Cylindrotheca closteridium
Cymbella spp
Diploneis spp
Dityllum brightwelli
Dityllum sol
Eucampia spp
Gyrosigma spp.
Gossleriella tropica
Guinardia flaccida
Guinardia striata
Leptocylindrus danicus
Licomophora spp
Melosira spp
Mastogloia spp
Navicula spp
Navicula longa
Navicula lyra
Nitzschia closterium
Nitzschia spp
Nitzshia sigma
Nitzshia sigmoidea
Nitzschia marina
Nitzschia lorenziana
Nitzschia fasiculata
Nitzschia longissima
Pleurosigma spp
Pseudonitzschia spp
Rhizosolenia spp.
Rhizosolenia delicatula
Rhizosolenia hebitala
Syedra spp
Skeletonema costatum
Surirella spp
Surirella elegans
Thalassionema spp.
Thalassionema nitzschioides
Thalassiosira spp
Thalassiosira subtilis
Thalassiosira hyalina
Triceratium dubium
Triceratium favus
Triceratium affine
Triceratium spp
Tropidineis spp

Class-Dinophyceae
(Dinoflagellates)

Ceratium spp
Ceratium dens
Ceratium furca
Ceratium fusus
Dinophysys spp
Diplopsalis lenticula
Diplosalis acuta
Diplosalis crabo
Diplosalis notabilis
Diplosalis interrupta
Gonyulux spp
Gonyalux polygramma
Gonyaulax verior
Prorocentrum gracile
Prorocentrum maximum
Prorocentrum micans
Pyrophacux stenii
Protoperidinium spp
Protoperidinium oceanica
Protoperidinium depressum
Protoperidinium pellucidum
Trichodesmium spp
Heterocapsia spp
Scrippseula spp
**Class-Chlorophyceae**  
(Green algae)  
*Tetraedron trigonum*  
*Xanthidium antilopaeum*

*Anthrodesmus convergens*  
*Closterium sps.*  
*Coelastrum sps*  
*Coelastrum spaerium*  
*Cosmarium sp.*  
*Euastrium didelta*  
*Pediastrum tetras*  
*Scenedesmus quadicauda*  
*Scenedesmus arcuatus*  
*Selenastrum gracile*  
*Sphaerozosma granulatum*  
*Staurastrum asteroideum*  
*Staurastrum pingue*  
*Staurastrum gracile*  
*Staurastrum leptocladium*

**Class-Cyanophyceae**  
(Blue-green algae)  
*Oscillatoria brevis*  
*Oscillatoria chalybea*  
*Oscillatoria Formosa*  
*Merismomedia glauca*

**Class-Chrysophyceae**  
(Silicoflagellate)  
*Dictyocha fibula*  
*Dinobryon belgica*

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**Table-2: Correlation with hydrographical parameters**

<table>
<thead>
<tr>
<th>Variables</th>
<th>pH</th>
<th>Temperature</th>
<th>DO</th>
<th>Salinity</th>
<th>Nitrate</th>
<th>Nitrite</th>
<th>Phosphate</th>
<th>Ammonia</th>
<th>Chla</th>
<th>Phyto. density</th>
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<td>Temperature</td>
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<td>Salinity</td>
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<td>Nitrate</td>
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<td>-.508</td>
<td>.727*</td>
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<tr>
<td>Phosphate</td>
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<td></td>
<td>-.511</td>
<td>.722*</td>
<td>-.871**</td>
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<td>-.406</td>
<td>-.519</td>
<td>.364</td>
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<td>Chla</td>
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<td>-.522</td>
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<td>-.118</td>
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**Table-3: Principal Component Analysis (PCA) of the hydro-biological variables during the study period.**

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<td>Variability (%)</td>
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<td>13.006</td>
<td>10.343</td>
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<tr>
<td>Cumulative (%)</td>
<td>24.117</td>
<td>38.599</td>
<td>51.605</td>
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### Factor Loadings:

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<td>Nitrite</td>
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<td>0.015</td>
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<td>Nitrate</td>
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<td>-0.047</td>
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<td>Phosphate</td>
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<td>-0.039</td>
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<td>Ammonia</td>
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<td>0.309</td>
<td>-0.733</td>
<td>0.003</td>
</tr>
<tr>
<td>Iron</td>
<td>0.054</td>
<td>0.860</td>
<td>-0.092</td>
<td>-0.383</td>
</tr>
<tr>
<td>Chlorophyll a</td>
<td>0.189</td>
<td>0.257</td>
<td>-0.041</td>
<td>0.508</td>
</tr>
<tr>
<td>Phytoplankton density</td>
<td>-0.016</td>
<td>-0.148</td>
<td>0.145</td>
<td>0.802</td>
</tr>
</tbody>
</table>

**Fig-1:** Map showing the selected sites of Cochin Estuary.

**Graphs -1:** Graph showing the results of hydrographic variables.