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SEASONAL DYNAMICS IN THE LEAF CHLOROPHYLL CONTENT OF THE MAJOR MANGROVE SPECIES OF PICHAVARAM

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Abstract: The present study aims to monitor the seasonal variability in the chlorophyll concentrations of eight major mangrove species recorded from Pichavaram mangroves of Tamil Nadu. Along with the leaf pigment variation, the study also concentrates on the prevailing soil conditions which are likely to affect the plant processes. Soil salinity, conductivity, presence of major and minor elements was recorded. A study of the leaf pigments reveals that the highest values of total chlorophyll (< 1) was observed during winter season for Bruguiera cylindrica, Excoecaria agallocha and Rhizophora mucronata and the least observed was for Excoecaria agallocha and Lumnitzera racemosa for the summer season. The Chlorophyll a/b ratio varied throughout the seasons. The ranges varied from 2.0-3.5 in summer to 1.0 -2.6 during post monsoon (expect for Aegiceras corniculatum and Excoecaria agallocha, which possessed higher ratio greater than 6.0) and typically around 1.0-2.0 (except Aegiceras corniculatum) for all the mangrove species under study. The highest carotenoid content observed was for Avicennia officinalis during winter season (22.5 mg/g). Such studies on seasonal dynamics aids the management, restoration and conservation practices of mangroves worldwide on a large scale. This provides a better aid to ecological assessment activities too. Mangroves are majorly found along the coast lines of tropical and subtropical regions, where the seasons are distinct and the climatic and environmental conditions are highly variable. Hence the leaf pigment variability among the seasons highly affects the physiological processes like productivity. Thus the research could be o greater aid to studies related to mangrove ecosystem functioning and productivity.

Keywords: *Chlorophyll a, chlorophyll b, Total chlorophyll, Carotenoids, Chlorophyll a/b ratio, Pearson's product moment correlation coefficient analysis*

I INTRODUCTION

Mangroves inhabit the intertidal shallow water coastlines (Hogarth 1999) worldwide, in the tropics and sub tropics (Nagelkerken et al. 2008). They harbor wide variety of flora and fauna and thus proves to be an essential resource for a wide variety of local activities (Walters et al. 2008). They are one among the highly productive (Komiyama et al. 2008) and biogenic ecosystems. Higher solar irradiation in addition to natural disasters (like flood, tsunami, cyclones) and anthropogenic disturbances (for timber, aquaculture and other forest resources like honey etc.) are more prevalent in these areas. Thus they can be considered as highly stressed canopies. Rapid and accurate exploration of such ecosystems has higher impacts when future conservation measures are considered. Monitoring the seasonal dynamics in the leaf phenology of mangroves has thus emerged as a major necessity for ecological assessment and management of mangrove ecosystems, where the seasons are distinct, and play a major role in driving the physiological processes. Higher salinity of these regions causes a decline in the concentration of Chl-*a* and *b* (Mitra et al., 2014, Panda et al., 2006). Studies conducted by Kathiresan and Bingham (2001) reveals that the mangrove chlorophyll and carotenoid levels are higher at the summer season. Major mangrove species like *Avicennia marina, Avicennia officinalis, Excoecaria* *agallocha*, *Suaeda monoica, and Sonneratia apetala* possess higher chlorophyll concentrations (Murty & Rao, 2014).

Plant photosynthesis is mostly affected with the variations in diurnal fluctuations in Photosynthetically active radiation (PAR), ambient temperature, ambient carbon dioxide, humidity and vapour pressure deficit (Schulze & Caldwell 1994). Physiological and bio-chemical plant processes are primarily dependent on the leaf chemical properties (Evain et al. 2004). In green plants, the photosynthesis takes place in the chloroplasts, which contain the light absorbing pigments- chlorophyll, embedded in the thylakoid membrane. The experiments conducted by Richard Willstätter and his collaborators led to the discovery of the formulas of chlorophyll-*a* and chlorophyll-*b* (Chl-*a.* & Chl-*b*). They occur in the ratio of 3:1 in plants. They are associated with numerous structurally related antenna proteins (Sathe et al., 2015). The basic structure constitutes a tetrapyrrole (porphyrin) ring. Chl-*a* contains a methyl residue in ring a, which makes it different from Chl-*b*, which has a formyl residue in ring b. Even this negligible structural dissimilarity accounts a larger variation in their light absorbing capacity. Chl-*a* is considered as the central photosynthesis pigment as it is a constituent of the photosynthetic reaction centers. Variations in its concentration can be used to monitor plant growth (Raven et al. 1992) and it also indicates disturbances from stressors (Blackburn 2007). However, it does not absorb light in a wide range of visible spectrum, and the non-absorbing region is termed as the "green window". In this case, the absorption gap is narrowed by the light absorption of Chl-*b*, with its first maximum at a higher wavelength than Chl-*a* and the second maximum at a lower wavelength. Thus Chl-*b* enhances the plant's efficiency for utilizing the light energy by transferring the light energy to Chl-*a* in an efficient manner. Hence alng with carotenoids they act as supportive accessory pigments (Bidlack & Jansky, 2011). Carotenoids provides protection to the plant from photo damage. The ability of the pigments to capture and utilize light energy (Evain et al. 2004) is also subjected to the seasonal variations and availability of water (Gilman et al. 2008).

The present study addresses the seasonal variation in the chlorophyll a, b and carotenoids of Pichavaram mangroves of Tamil Nadu. Extensive sampling was carried out to study the soil parameters along with the analysis of leaf pigments.

II STUDY AREA

The Pichavaram mangrove wetlands (20' N and 11º 30'N and longitudes 79º 45' E and 79º 55'E) (Fig 1), is one of the major mangroves of Tamil Nadu coast lying between the Coleroon –Vellar estuarine complex in the northernmost region of the Cauvery delta. The entire mangrove vegetation which was declared as a Reserve forest in 1987 covers an area of about 1471 ha including mangrove forests, mud flats, sand dunes and back water. The reserved area is divided into three divisions namely Pichavaram (1055 ha), Killai (327 ha) and Pichavaram RF Extension (89 ha). The mangroves had gathered considerable attention due to their ecology, flora and fauna and have been well studied for their water quality, pollution, fishery resources and much more. Flood plains, sedimentary plains and beach sand constitute its geomorphological terrain (Balasubramanian et al; 2002). The area is colonized by 13 true mangrove species (Selvam.V., 2002; Kathiresan, K., 2000), monospecifically dominated by *Avicennia marina* (Forsk.) when studied by community structure. While the distribution pattern is taken to consideration, two distinct zones, *Rhizophora* zone which is found along the fringes of the tidal creeks, and the *Avicennia* zone which starts just behind the *Rhizophora* zone are identified (Selvam.V., 2002). The climate is sub-humid with very warm summer and with an annual average rainfall of 1310 mm (70 years) and annual average rainy days up to 56.

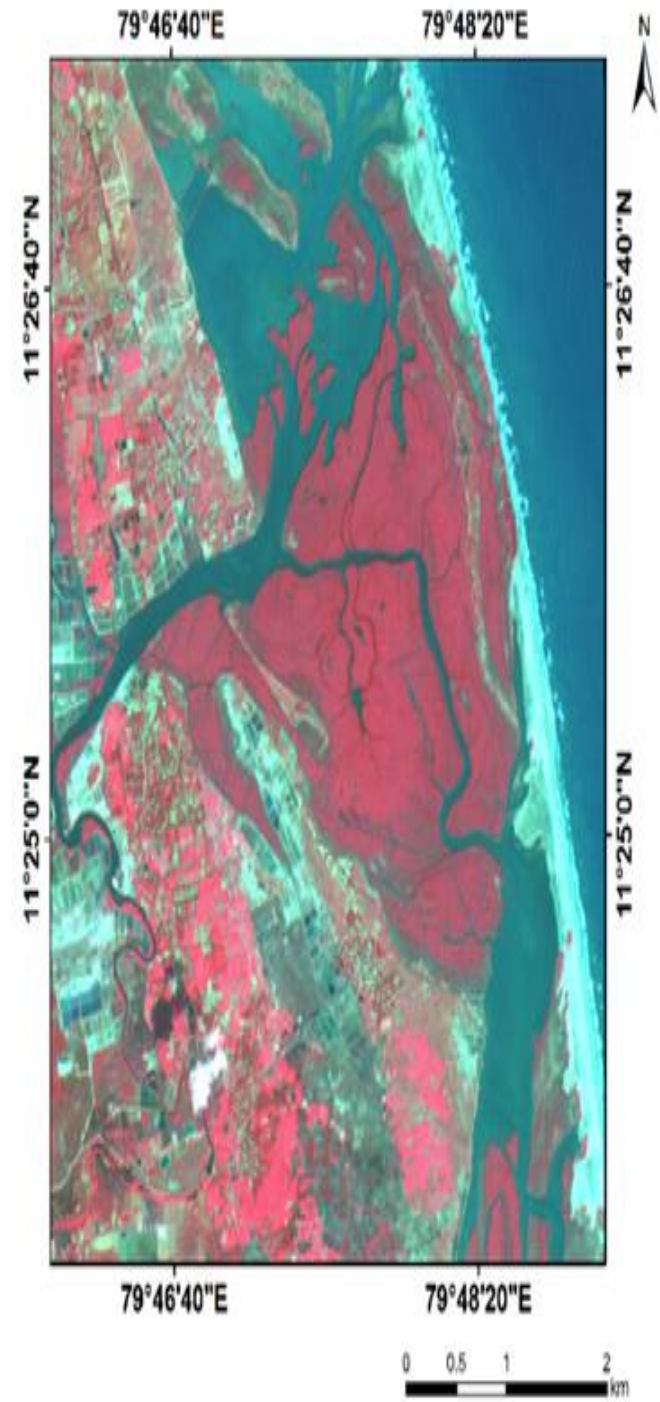

Figure 1: Pichavaram mangrove forest, Tamil Nadu

III METHDOLOGY

Field campaign and data collection

Field visits to the Pichavaram mangrove reserved forests were carried out during Summer (May 2015), Post monsoon (December 2015) and Winter (February 2016). A total of 8 major mangrove species were recorded from the field and were studied. The technique of randomization was used for sampling and plants in good growth conditions were selected and labelled. Measurements were taken for the same selected trees during the seasons. *Avicennia officinalis, Bruguiera cylindrica* and *Lumnitzera racemosa* was recorded from first quadrat (Latitude: 11.4166 Longitude:79.8070). From the second quadrat (Latitude: 11.4195 Longitude: 79.8031), *Avicennia marina, Rhizophora mucronata* and *Ceriops decandra* was identified. From the third quadrat (Latitude: 11.4284 Longitude: 79.7773) *Excoecaria agallocha* and *Aegiceras corniculatum* was studied. Soil samples from the respective quadrats of the occurrence of the species was also collected. From the soil samples, various parameters like pH, conductivity, texture, and salinity were estimated. Apart from that the presence of major elements like Nitrogen (N), Phosphorus (P) and Potassium (K) and trace elements like Iron, Manganese (Mn), Iron (Fe), Zinc (Zn) and Copper (Cu) were retrieved. Water samples nearest to the plants were also collected to study the variations in water parameters throughout the year.

In-vitro sample analysis

Fresh leaves collected from the labelled plants were collected, washed and dried with the aid of tissue paper. Further the leaves were cut into smaller pieces discarding major veins and fibrous tissues. 100 mg of the leaf tissues were weighed and incubated in 10 ml 80 % acetone in centrifuge tube for 48 hours in dark. The absorbance of the sample was taken at the wavelengths of 470nm, 645nm and 663nm with the aid of Spectrophotometer. Further the Chlorophyll-a, Chlorophyll-b, total Chlorophyll, and Carotenoid contents of the mangrove leaves were estimated using Arnon's equation (equation 1) (1949).

IV RESULTS AND DISCUSSION

Seasonal dynamics in the soil components

A remarkable change is noticed in the texture of the soil samples with regard to the changes in season (Table 1). In summer, the soil exhibited a silty clay nature, while during post monsoon, it changed to clay type.

The clayey nature of the first quadrat was retained even during the winter season, whereas the second quadrat regained its silty clay nature during winter. The soil pH exhibited a gradual increase throughout the year, i.e., least during summer, average during post monsoon and the highest during winter season. Similar trend was observed in the case of conductivity and soil salinity. Other parameters like the presence of Nitrogen, Potassium and Phosphorous exhibited a fluctuating trend over the seasons. Overall, an increase in the minerals was seen in the post monsoon season.

Seasonal dynamics in the water parameters

The water temperature as expected was highest at the summer season, followed by winter and the least in post monsoon. Overall the water temperatures ranged from 25°C to 35°C. The pH of the water was also highest in the month of summer and so was the salinity.

Post monsoon witnessed a decrease in water salinity. Again during winter, the water salinity slowly raised. The overall variations in the water parameters are summarized in table 2.

Seasonal dynamics in Chlorophyll content

A total of eight major mangrove species were studied from three quadrats. The co-existence of multiple species in these quadrats made the data collection and sampling much easier. With the aid of the fresh leaf samples collected from the field, further analysis was carried out in the laboratory for the estimation of Chlorophyll a, Chlorophyll b, Total Chlorophyll and Carotenoids (Table 3). At a glance, it is observable that the Chlorophyll a content varied from least during the summer season to moderate during the post monsoon and the highest during winter for all the mangrove species under study. Chlorophyll b concentration also followed a similar trend rather than for *Aegiceras corniculatum* and *Avicennia marina,* which showed a decreased chlorophyll b concentration during post monsoon. Similarly, other than for *Aegiceras corniculatum* and Ceriops decandra, which showed a decrease in the carotenoids during post monsoon, all other species exhibited a general pattern, i.e., highest during winter, moderate during post monsoon, and the least in summer . The Chlorophyll a/b ratio was highest during summer, followed by post monsoon and winter for all species except *Aegiceras corniculatum* (Highest in post monsoon and winter followed by summer) and *Excoecaria agallocha* (Highest in post monsoon and summer followed by winter). Among all the mangrove species, *Avicennia officinalis* exhibited the highest range of total chlorophyll and carotenoid throughout all the seasons. *Brugueira cylindrica* and *Lumnitzera racemosa* showed an increase in the chlorophyll content during post monsoon and winter. The least was observed for *Excoecaria agallocha* for summer and post monsoon and *Ceriops decandra* exhibited the least during winter. Field photographs displaying the major mangrove species collected from the field are displayed in figure.

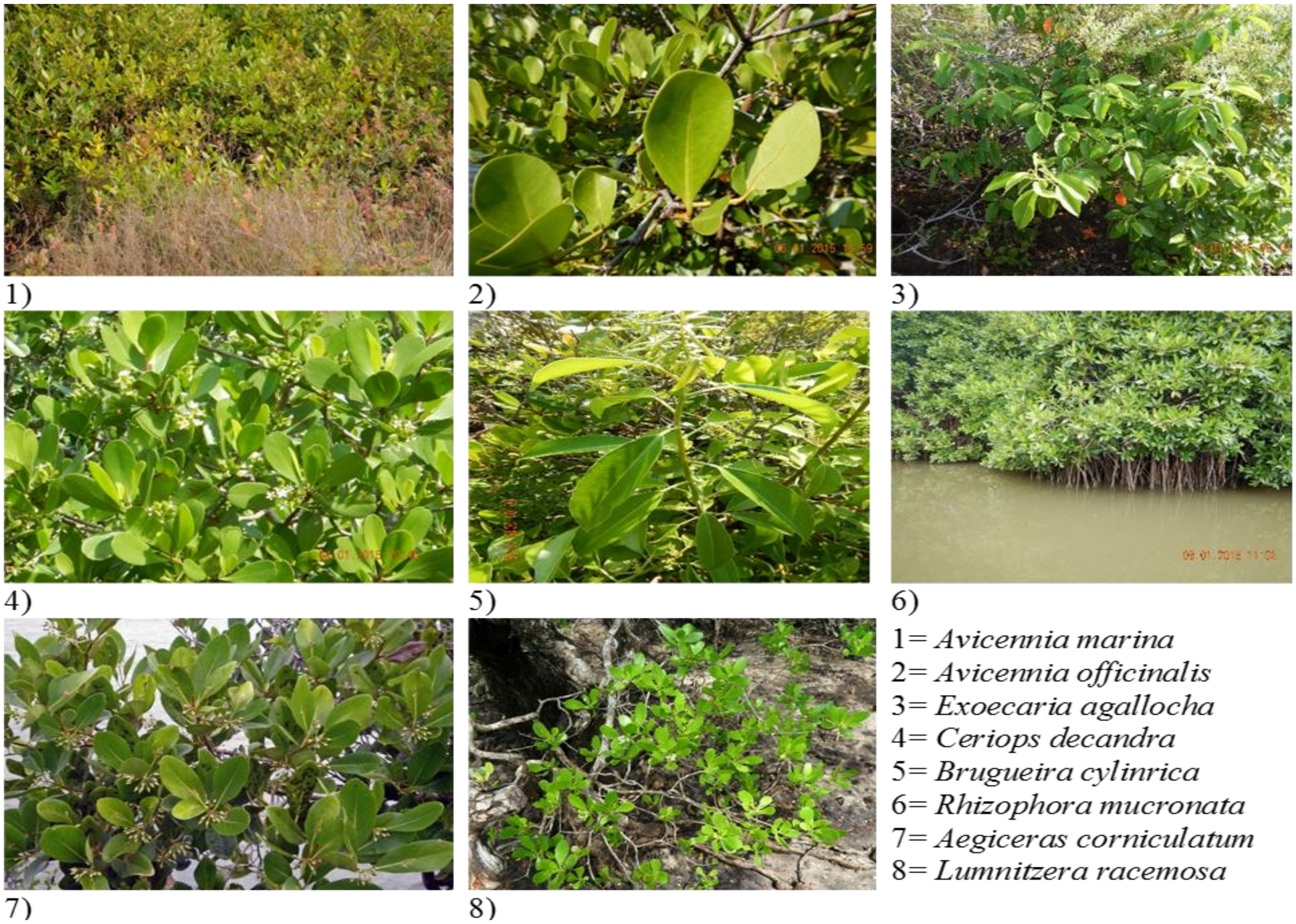

Figure 2: Field photographs displaying the major mangrove species collected from the field are display

Table 1: Various parameters studied from the collected soil samples

Quadrat	Soil Texture	Soil pH	Conductivity (m s /cm)	Salinity	N (Kg/ha)	K (Kg/ha)	P (Kg/ha)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
Summer											
Quadrat 1	Silty clay	6.7	3.73	23.8	77	278	36	14.16	6.42	0.66	2.90
Quadrat 2	Silty clay	7.2	3.82	24.4	67	500	50	16.04	8.2	0.38	2.54
Quadrat 3	Silty clay	7.7	4.54	29.0	88	257	31	14.88	7.04	0.63	1.81
Post Monsoon											
Quadrat 1	Clay	7.7	5.38	34.4	68	225	51	14.03	6.32	0.90	2.14
Quadrat 2	Clay	8.5	7.09	45.3	75	346	54	16.33	6.44	0.64	2.18
Quadrat 3	Clay	7.8	7.43	47.5	83	340	48	14.60	6.44	0.66	1.59
Winter											
Quadrat 1	Clay	8.5	6.94	44.4	78	241	50	16.83	7.68	0.64	2.14
Quadrat 2	Silty clay	8.4	8.36	53.5	81	318	47	16.64	7.94	0.43	1.15
Quadrat 3	Sandy clay	8.0	6.58	42.1	93	310	46	13.20	7.43	0.85	1.28

Table 2: Parameters derived out of water samples collected

Quadrat	Water temperature (°C)	pH	Salinity (ppt)	Conductivity(ms/cm)	TDS (ppt)
Summer					
Quadrat 1	35.7	7.7	29.65	14.6	4.3
Quadrat 2	30.2	7.2	23.45	12.4	7.6
Quadrat 3	25.0	7.7	29.45	5.3	4.5
Post monsoon					
Quadrat 1	28.4	7.5	26.45	13.8	7.2
Quadrat 2	28.9	7.0	25.95	10.9	8.7
Quadrat 3	28.8	7.5	27.85	6.6	7.1
Winter					
Quadrat 1	29.7	7.5	26.59	18.7	7.6
Quadrat 2	28.2	7.9	26.91	14.5	7.8
Quadrat 3	29.5	7.5	28.15	16.2	7.4

Table 3: Seasonal variation in the chlorophyll concentration of the major mangrove species of Pichavaram

Mangrove species	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll (mg/g)	Chl. a/b ratio	Carotenoids (mg/g)
Summer					
Aegiceras corniculatum	0.253	0.074	0.327	3.418	9.714
Avicennia marina	0.187	0.057	0.244	3.280	9.496
Avicennia officinalis	0.326	0.145	0.471	2.248	12.459
Bruguiera cylindrica	0.167	0.072	0.239	2.319	7.127
Ceriops decandra	0.237	0.112	0.349	2.116	10.544
Excoecaria agallocha	0.104	0.031	0.135	3.354	4.382
Lumnitzera racemosa	0.102	0.029	0.131	3.517	5.000
Rhizophora mucronata	0.214	0.069	0.282	3.101	8.744
Post monsoon					
Aegiceras corniculatum	0.281	0.046	0.327	6.108	7.509
Avicennia marina	0.375	0.139	0.514	2.697	13.998
Avicennia officinalis	0.403	0.203	0.606	1.985	20.138
Bruguiera cylindrica	0.250	0.147	0.396	1.700	20.155
Ceriops decandra	0.390	0.278	0.668	1.402	20.115
Excoecaria agallocha	0.328	0.049	0.377	6.693	5.529
Lumnitzera racemosa	0.423	0.398	0.821	1.062	19.047
Rhizophora mucronata	0.218	0.148	0.366	1.472	10.119
Winter					
Aegiceras corniculatum	0.542	0.118	0.660	4.593	8.835
Avicennia marina	0.534	0.418	0.952	1.277	15.897
Avicennia officinalis	0.543	0.364	0.907	1.491	22.505
Bruguiera cylindrica	0.614	0.446	1.060	1.376	20.299
Ceriops decandra	0.575	0.332	0.907	1.731	11.869
Excoecaria agallocha	0.681	0.358	1.039	1.902	12.565
Lumnitzera racemosa	0.302	0.276	0.578	1.094	21.103
Rhizophora mucronata	0.618	0.446	1.064	1.385	12.839

The increase in the chlorophyll content during the post monsoon and winter season can be attributed to the post rainfall conditions. The rainfall data collected from the nearest IMD center (Parangipettai) reveals that during November and December 2015, the maximum value recorded for rainfall was 7010mm and 2990mm from the North-East monsoon. The excessive rainfall was a resultant of El Nino effect (2015), which ultimately led to the Chennai floods. Under stress conditions, chlorophyll a is more susceptible for degradation than chlorophyll b which results in a reduction in Chlorophyll a/b ratio (Young, 1993).Similar studies conducted by Murthy and Rao (2014) reported a wide variation in the ranges of chlorophyll-a and b. Studies conducted by Basak et al. (1996) reported a variable range of chlorophyll a/b ratio but close to 3.0. MacFarlane (2002) studied the Leaf biochemical parameters in Avicennia marina (Forsk.) Vierh as potential biomarkers of heavy metal stress in estuarine ecosystems, where he reported chlorophyll a/b ratio close to 2.0 during October and November seasons, which coincides with our present study.

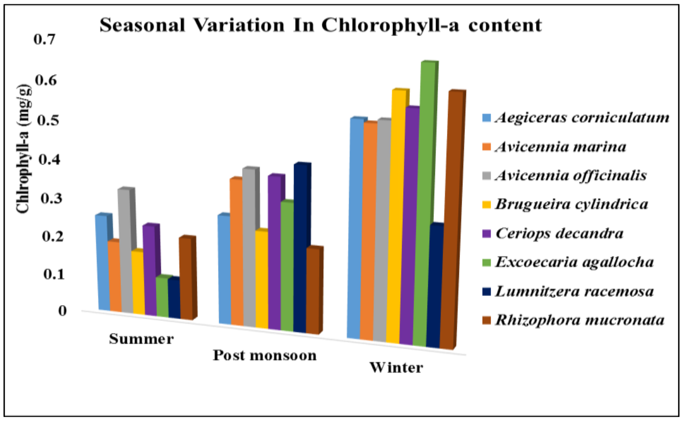

Figure 3 : Seasonal Variation In chlorophyll-Content

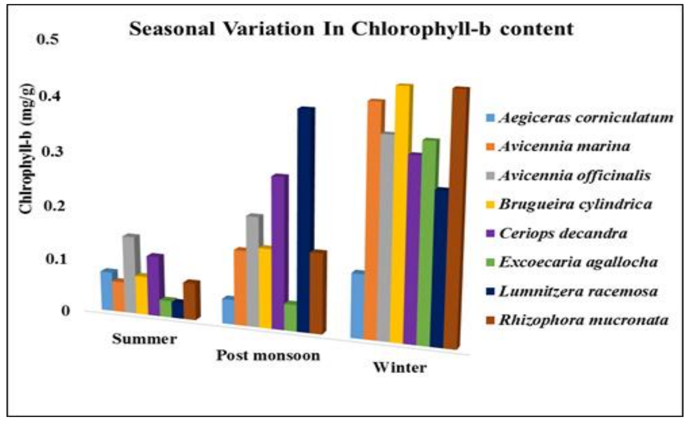

Figure 4: Seasonal Variation in Chlorophyll-b Content

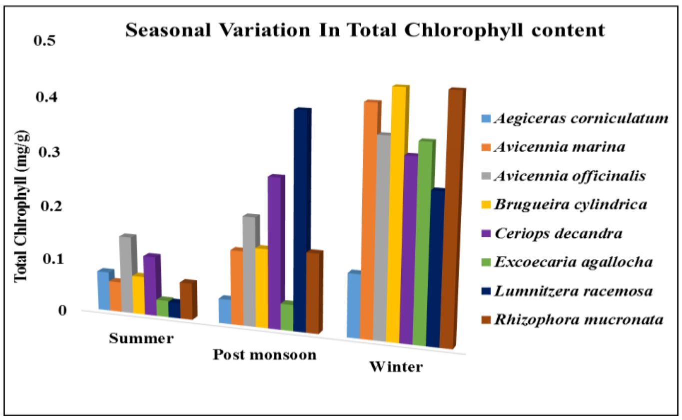

Figure 5:Seasonal Variation in Total Chlorophyll

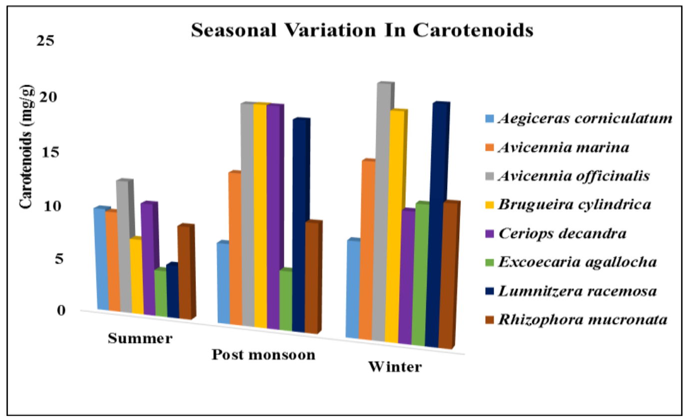

Figure 6 : Seasonal Variation in Carotenoids .

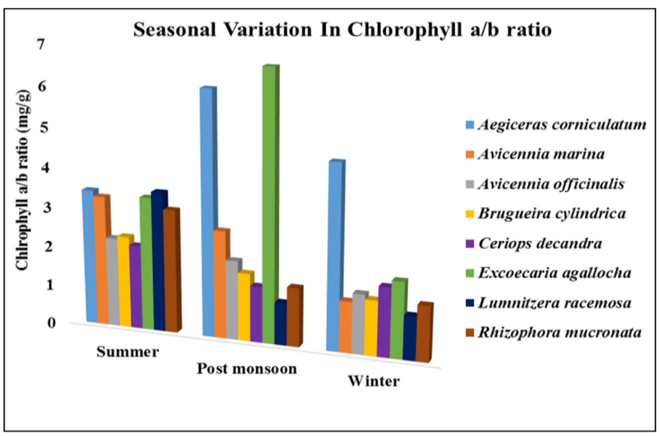

Figure 7 : Seasonal Variation in Chlorophyll a/b ratio

Statistical analysis

Pearson's product moment correlation coefficient analysis was carried out to analyze the statistical relation existing among the chlorophyll content (Table 4). The highest coefficient of correlation was obtained for the summer season. Chlorophyll-a exhibited the highest correlation with chl.-b (0.92), total chlorophyll (0.99) and carotenoids (0.96) during summer season. During the same season, chlorophyll b exhibited the highest correlation with the xanthophyll and carotene (0.89). Overall, total chlorophyll was related to carotenoids with an R^2 of 0.95. There was a decrease in the correlations during post monsoon season. The correlations between Chlorophyll a and carotenoids sharply decreased to 0.46. Similarly, chlorophyll b and carotenoids exhibited a lower value of 0.74. Winter season observed a much more decreased and even a negative correlation coefficient among the chlorophylls and carotenoids (-0.03).

Table 4: Pearson's Product Moment Correlation Coefficient analysis

Summer	*Chl. A*	*Chl. B*	*Total Chl.*	*Carotenoids*
Chl. A	1			
Chl. B	0.920	1		
Total Chl	0.990	0.965	1	
Carotenoids	0.962	0.897	0.957	1
Post monsoon	*Chl. A*	*Chl. B*	*Total Chl.*	*Carotenoids*
Chl. A	1			
Chl. B	0.623	1		
Total Chl	0.852	0.940	1	
Carotenoids	0.469	0.744	0.701	1
Winter	*Chl. A*	*Chl. B*	*Total Chl.*	*Carotenoids*
Chl. A	1			
Chl. B	0.378	1		
Total Chl	0.836	0.823	1	
Carotenoids	-0.441	0.396	-0.035	1

V CONCLUSION

Even though most of the studies emphasizes that the leaf pigments show an increase during the summer season which facilitates the use of wide range of irradiance and to protect the photosynthetic apparatus, it is noteworthy that seasonal dynamics, in conjunction with various environmental factors may affect the concentration in leaf pigments. Their concentrations are liable to changes depending upon their prevailing conditions. Thus seasonal monitoring of mangrove ecosystems has emerged as a vital necessity for the management and conservation of coast lines. Also, total chlorophyll concentration of 3 mg/g and above is a good indicator of healthy leaves and thus they aid high photosynthetic activity. Thus it would be effective to bring up highly photosynthetic plants to generate a productive and biogenic ecosystem along the shore.

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