QUANTIFICATION OF PHOTOSYNTHETIC PIGMENTS OF PLANTS, WATER AND SEDIMENT SAMPLES IN CHIRACKAL AND KATTIPARAMBU OF ERNAKULAM DISTRICT, KERALA

ABSTRACT

Aims : The present study intended to investigate the pigment composition of four selected mangrove plants viz., *Avicennia officinalis, Excoecaria agallocha, Rhizophora mucranata and Sonnaratia alba*, water and sediment samples. And to quantify the concentration of various pigments found in the above samples.

Place and Duration of Study: The samples were collected from the mangrove creeks of Chirackal and Kattiparambu of Ernakulam district, Kerala. Duration of the study was from 2013 December to 2015 December.

Methodology: The estimation of the total pigments, chlorophyll a, chlorophyll b and carotenoid concentration of the biotic samples, water and sediments were done using standard methods in Spectrophotometer.

Results: Plants showed high pigment concentration compared to water and sediments. High chlorophyll 'a'(2%), chlorophyll 'b'(0.8%) and total chlorophyll(2.74%) were observed in *Excoecaria agallocha* of Kattiparambu and carotenoids (0.72%) observed in *Rhizophora mucranata,* Chirackal. In sediment samples, high chlorophyll 'a'(0.85%), total chlorophylls(1.31%) and carotenoids (0.725%) were reported in Chirackal area and chlorophyll 'b'(0.595%) reported in Kattiparambu. Chlorophyll 'b'(0.6%) and carotenoids(0.86%) were reported high in the water samples of Kattiparambu region and chlorophyll 'a'(0.61%) and total chlorophylls(0.86%) in Chirackal. In Pearson's correlation coefficient studies, the content of KEA-chlorophyll was found to have a strong positive correlation(1,0.999,0.998 and 0.997) among themselves, with water and with sediment samples between the two media implying common source of plants and sediments.

Conclusion: Seasonal changes and local geological conditions are the major factors for variations in pigment concentrations in plants, water and sediment samples. Sediment pigments proved to be good indicators of lake-ecosystem response to climate change and long-term variability in the photo trophic community.

Keywords: Chlorophyll, Carotenoids, Pigments, Sediments, Mangroves, Correlation.

1. INTRODUCTION

Total leaf pigment includes chlorophyll-a(chl.a), chlorophyll-b(chl.b) and carotenoids that are necessary for photosynthesis process. Variation in leaf pigments (chlorophylls and carotenoids) and its relation can be due to internal factors and environmental conditions. Chlorophyll and carotenoids content varied with microclimatic conditions in species (1). The ratio of chlorophyll-a and chlorophyll-b in terrestrial plants has been used as an indicator of response to light shade conditions (2). The small proportion of chlorophyll a/b is considered as sensitive biomarker of pollution and environmental stress (3). Acetone gives very sharp chlorophyll absorption peaks and has great merit as the solvent for assay of chlorophylls (4). Chlorophyll is a pigment that has a clear impact on the spectral responses of plants, mainly in the visible spectrum portion. N is a key element in chlorophyll, therefore is usually a high correlation between them (5).

Previous studies indicated that chlorophyll pigments have antioxidant, anti inflammatory and wound healing properties. It has been observed that chlorophyll pigments contain chlorophyllin which is responsible for increasing the number and activity of dominant immune cells like Bcells, T- cells and macrophages essential to human health (6,7). Photoactive pigments such as chl-a cause distinct changes in the color of water by absorbing and scattering the light incident on water. In deep ocean waters, phytoplankton is usually the predominant constituent and the concentrations of other constituents covary with chl-a concentration. Thus, the optical properties of these waters are dominated by phytoplankton and the observed spectral features in the reflected light can be directly related to chl-a concentration (8,9). In most island, estuarine, and coastal waters, constituents such as suspended solids and dissolved organic matter occur in abundance and their concentrations do not co-vary with chl-a concentration (10,11).

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2. MATERIALS AND METHODS

Collection of Samples : Fresh leaf samples were washed thoroughly first in tap water followed by distilled water in the laboratory, kept to dry in room temperature and ground in an electric mixer (12). Then analyzed for the determination of chlorophylls (Chl-a and Chl-b) and carotenoids content. Water sample collected from three locations of Kattiparambu and Chirackal area in clean sampling bottles. For chlorophyll estimation, sample was collected from the sub surface water in sampling bottle and add 1 ml saturated $MgCl_2$ per litre of sample and keep in chill condition, then used for analysis. Sediment also collected in polythene bags from three locations of these two areas, dried, powdered and used for the analysis.

Estimation of pigments

The amount of chlorophyll present in the leaves was estimated by the standard method. 500 mg of leaf tissue was kept in a pestle and mortar with 10 ml of 80% acetone and it was ground well and the homogenate was centrifuged at 3000 rpm for 15 minutes and the supernatant was stored for analysis. In water and finely powdered sediment sample, 90% acetone was added, mixed and kept for overnight at low temperature under dark for extraction. The supernatant extract was centrifuged at 2000 to 3000 rpm to get clear solution and the solution was used for analysis. Absorbance of the samples was measured at 645nm, 663nm and 480nm in a spectrophotometer. The chlorophyll content was determined by using the following formula,

$$\text{Chlorophyll a (\%.fr.wt)} = \frac{12.7 \times A663 - 2.69 \times A645}{a \times 1000 \times W} \times V \times 100$$

$$\text{Chlorophyll b (\%.fr.wt)} = \frac{22.9 \times A645 - 4.68 \times A663}{a \times 1000 \times W} \times V \times 100$$

$$\text{Total Chlorophylls (\%.fr.wt)} = \frac{20.2 \times A645 + 8.02 \times A663}{a \times 1000 \times W} \times V \times 100$$

$$\text{Carotenoids (\%.fr.wt)} = A480 + (0.114 \times A663) - 0.638 \times A645 \times 100 \quad (13)$$

Where,

A - Absorbance at respective wave length

a - Length of path in the cell

W - Fresh weight of sample (g)

V - Volume of extract (ml)

3. RESULTS AND DISCUSSION

Chloropyll a (Chl a) is a ubiquitous pigment and can be used as a global biomass indicator. In Angiosperms (most land plants) there are typically two types of Chlorophyll (Chl) molecules, namely, chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*). Both of these pigments absorb photons of light in the blue and red spectral regions, but the specific wavelengths of light they absorb are different. These natural pigments exhibit various beneficial biological activities such as antioxidant, anticancer, anti-inflammatory, anti-obesity, anti-angiogenic and neuroprotective activities(14). Therefore, various natural pigments isolated from plants have attracted much attention in the fields of food, cosmetic and pharmacology(15).In the present study, pigment level of plants gave good results when compared to water and sediments. *E. agallocha* in Kattiparambu showed high range of Chl.a and Chl.b (2.01% and 0.804%). Total chlorophylls were found to be higher in *E. agallocha* (2.74%) of Kattiparambu, and lower in *E. agallocha* (1.09) of Chirackal. Similarly, carotenoids were measured to be higher in leaves of *A. officinalis* (0.72%) and *E. agallocha* (0.76%) of Chirackal and Kattiparambu respectively, minimum levels of caretenoid was present in *R. mucranata* (0.48%) of Chirackal compared to other plants (Figure-1). Acetone is known to have a lower extractability of chlorophylls from the protein matrix (16).The change in the carotenoids and tocopherols during seed maturation of *Cassia* species is studied (17). Water and sediment samples of Chirackal showed high chl.a (0.61% and 0.83%) and total chlorophyll (1.074% and1.31%) contents. High range of chl.b in water (0.61%) and sediment (0.85) was reported from Kattiparambu. High range of carotenoids (0.86%) reported in Kattiparambu water and sediment carotenoids (0.73%) from Chirackal (Figures 2 &3). Chlorophyll capture sunlight and make it available to plant system for its cultivation on photosynthesis (18). Chlorophyll a/b ratio is an index for determining the photosynthetic efficiency of the mangrove plant system (19). But, in this study, we claim that ratio between the bound and free forms of chlorophylls can be used as an index for determining the photosynthetic efficiency of the mangrove species. Similar reports have been made earlier in mangrove species, such as R. apiculata, R. mucronata and Avicennia marina (20,21,22) and pine species (23). Higher content of chlorophyll in reaction centre might enhance the light - induced photosynthetic activity of the chloroplast, thereby high energy transfer (24) and energy production could be assumed.

Figure 1 :Pigment content in Plant Samples (% value)

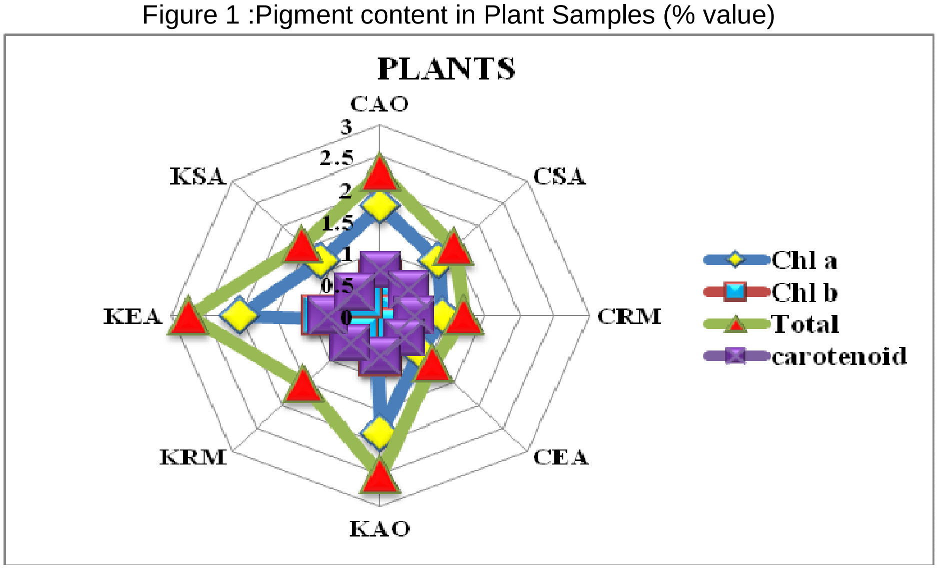

Figure 2 :Pigment content in Water Samples (% value)

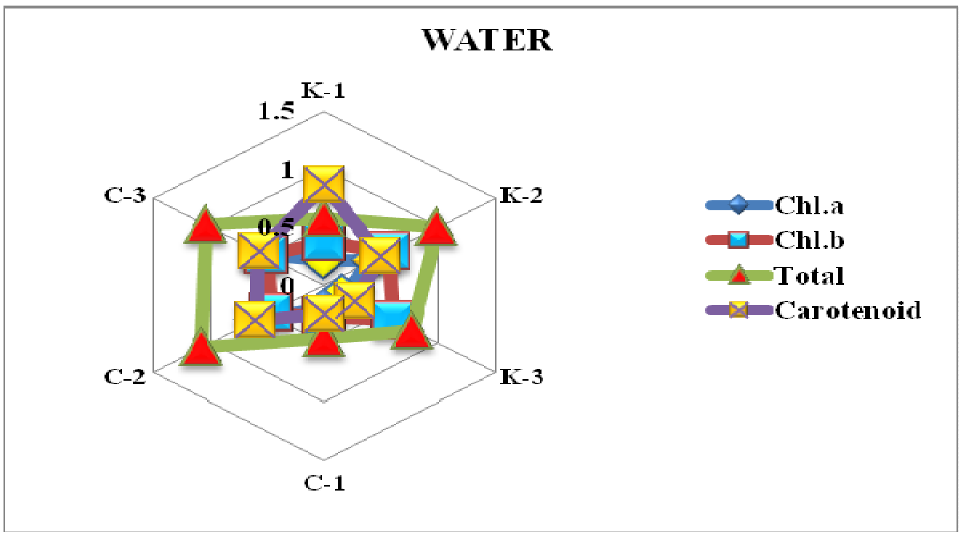

Figure 3 :Pigment content in Sediment Samples (% value)

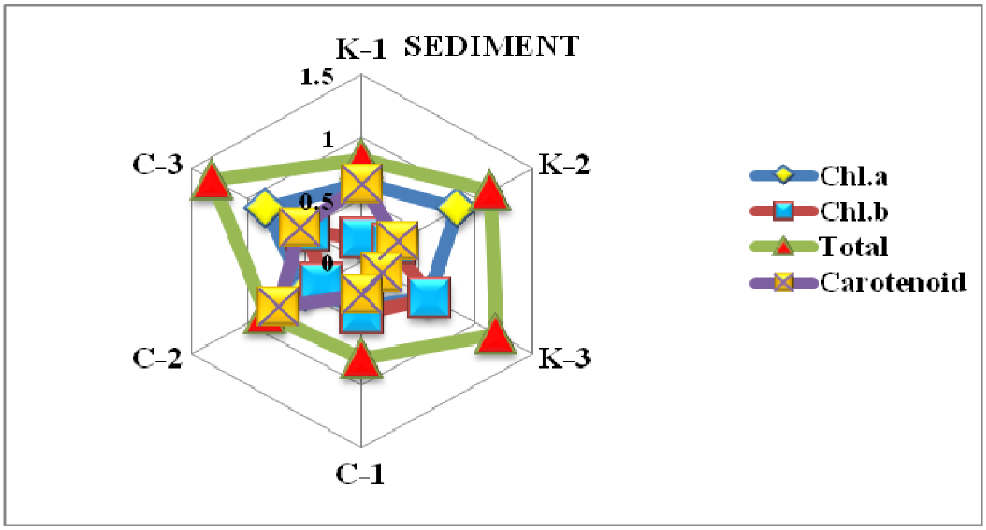

Table 1: Correlation Analysis of plants, water and sediments

	CAO	CSA	CRM	CEA	KAO	KRM	KEA	KSA	SK1	SK2	SK3	SC1	SC2	SC3	WK1	WK2	WK3	WC1	WC2	WC3
CAO	1																			
CSA	0.994**	1																		
CRM	0.994**	0.998**	1																	
CEA	0.984*	0.994**	0.997**	1																
KAO	0.995**	0.978*	0.981*	0.964*	1															
KRM	0.536	0.518	0.575	0.588	0.545	1														
KEA	0.993**	0.975*	0.979*	0.962*	1.000***	0.562	1													
KSA	1.000***	0.994**	0.995**	0.985*	0.995**	0.536	0.993**	1												
SK1	0.793	0.851	0.851	0.886	0.731	0.522	0.726	0.795	1											
SK2	0.999**	0.987*	0.989*	0.975*	0.999**	0.545	0.998**	0.998**	0.763	1										
SK3	0.787	0.720	0.751	0.716	0.838	0.713	0.850	0.785	0.357	0.815	1									
SC1	0.745	0.689	0.732	0.710	0.786	0.861	0.801	0.743	0.425	0.769	0.970*	1								
SC2	0.563	0.613	0.644	0.692	0.510	0.792	0.514	0.565	0.865	0.539	0.342	0.513	1							
SC3	0.970*	0.958*	0.975*	0.969*	0.970*	0.724	0.974	0.970*	0.787	0.972*	0.855	0.861	0.677	1						
WK1	-0.224	0.159	-0.123	0.058	0.282	0.458	0.275	-0.221	0.316	0.251	0.295	0.056	0.680	0.063	1					
WK2	0.612	0.569	0.624	0.618	0.643	0.966*	0.660	0.611	0.431	0.631	0.862	0.959*	0.645	0.780	0.217	1				
WK3	0.247	0.169	0.230	0.205	0.314	0.789	0.336	0.244	0.061	0.284	0.758	0.830	0.252	0.437	0.078	0.874	1			
WC1	0.589	0.552	0.609	0.607	0.615	0.981*	0.633	0.589	0.450	0.606	0.827	0.939*	0.684	0.765	0.283	0.998**	0.862	1		

WC2	0.846	0.845	0.879	0.890	0.837	0.892	0.846	0.847	0.802	0.845	0.783	0.869	0.848	0.945*	0.249	0.882	0.543	0.886	1	
WC3	0.763	0.745	0.790	0.795	0.769	0.955*	0.781	0.763	0.672	0.769	0.829	0.926*	0.798	0.897	0.264	0.958*	0.698	0.961*	0.980*	1

Correlation studies

The result of Pearson's correlation coefficient studies conducted between the pigment contents in Plants, Water and Sediments in Table 1. Plants showed strong positive correlation among themselves, with water and sediment samples and also very strong correlation (0.998 and 0.997) with sediment between the two media implying common source of plants and sediments. The content of KEA-chl was found to have a high positive correlation with the photosynthetic efficiency of mangrove species. The correlation coefficient between the KEA-chl and assimilation rate was 1,0.999,0.998 and 0.997. There was a strong negative correlation between water and plants (-0.224,-0.221 and -0.123). This suggests that in plants there is less production of pigments in the presence of certain sediments and water or vice versa in a particular condition.

Abbreviations

CAO- Chirackal *Avicennia officinalis*
CRM-Chirackal *Rhizophora mucranata*
CEA – Chirackal *Excoecaria agallocha*
CSA – Chirackal *Sonneratia alba*
KAO – Kattipapambu *Avicennia officinalis*
KRM – Kattiparambu *Rhozophora mucranata*
KEA – Kattiparambu *Excoecaria agallocha*
KSA – Kattiparambu *Sonneratia alba*
SK-1 – Sediment of Kattiparambu-1
SK-2 - Sediment of Kattiparambu-2
SK-3 - Sediment of Kattiparambu-3
SC-1 - Sediment of Chirackal-1
SC-2- Sediment of Chirackal -2
SC-3 - Sediment of Chirackal -3
WK-1 – Water of Kattiparambu-1
WK-2 - Water of Kattiparambu-2
WK-3 - Water of Kattiparambu-3
WC-1 - Water of Chirackal -1
WC-2 - Water of Chirackal -2
WC-3 - Water of Chirackal -3
K-1 - Kattiparambu 1
K-2 - Kattiparambu 2
K-3 - Kattiparambu 3
C-1 - Chirackal -1
C-2 - Chirackal -2
C-3 - Chirackal -3

4. CONCLUSION

Results from the above analysis clearly indicate that extraction of photosynthetic pigments depend on chemical nature of bio-molecules (cholorophyll-a, chlorophyll-b and carotenoids). The pigment content were influenced by environmental parameters. Temporal and seasonal changes and local geological conditions are the reasons for variations in pigment concentrations in plants, water and sediment samples. Sediment pigments proved to be good indicators of lake-ecosystem response to climate change and long-term variability in the photo trophic community, which is needed for predicting possible effects of future climate change. It was also recognized that the quality of the pigment record is highly dependent on the preservation regime in the sediment and water. Therefore further study in this context is recommended.

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