

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 *Sonneratia alba* and 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 observed in Chirackal area and chlorophyll 'b'(0.595%) obtained 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 other mangrove species and some sediment samples 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 chl. a and chl. 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 islands, 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). This study was designed to investigate the pigment composition of selected mangrove species and water and sediment samples.

2. MATERIALS AND METHODS

Collection and preparation of samples : Fresh leaf samples were washed thoroughly first with 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 using spectrophotometer. Water sample were collected from three locations of Kattiparambu and Chirackal areas 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 liter of sample and kept in chilled condition, then used for analysis. Sediments also collected in polythene bags from three locations of these two areas, then they were dried, powdered and then used for the analysis.

Estimation of pigments

The amount of chlorophyll present in the leaves was estimated by the standard method. Five hundred milligrams of leaf tissues were ground well using kept in a pestle and mortar and pestle 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 used for pigment analysis. Pigments in water and finely powdered sediment samples, were extracted by adding volume of (10 ml) 90% acetone to the samples and was added, mixed well and kept for overnight at low temperature under dark condition. for extraction. Then 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 chlorophylls and carotenoids contents were determined by using the following formulas in fresh weight basis,

$$\text{Chlorophyll a (\% fr. wt)} = \frac{12.7 \times A_{663} - 2.69 \times A_{645}}{a \times 1000 \times W} \times V \times 100$$

$$\text{Chlorophyll b (\% fr. wt)} = \frac{22.9 \times A_{645} - 4.68 \times A_{663}}{a \times 1000 \times W} \times V \times 100$$

$$\text{Total Chlorophylls (\% fr. wt)} = \frac{20.2 \times A_{645} + 8.02 \times A_{663}}{a \times 1000 \times W} \times V \times 100$$

$$\text{Carotenoids (\% fr. wt)} = A_{480} + (0.114 \times A_{663}) - 0.638 \times A_{645} \times 100 \quad (13)$$

Where,

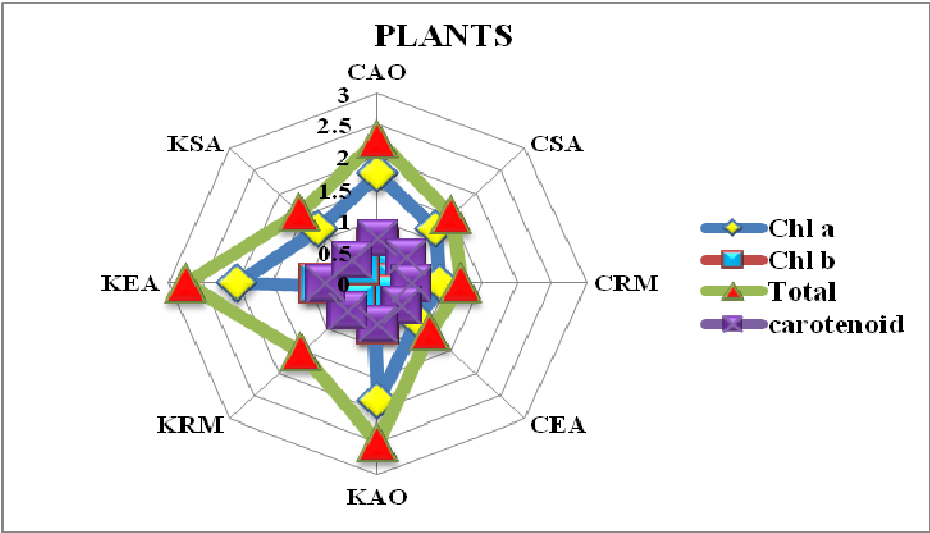
A - Absorbance at respective wave length

- a - Path Length of path in the cell
W - Fresh weight of the sample (g)
V - Volume of the extract (ml)

3. RESULTS AND DISCUSSION

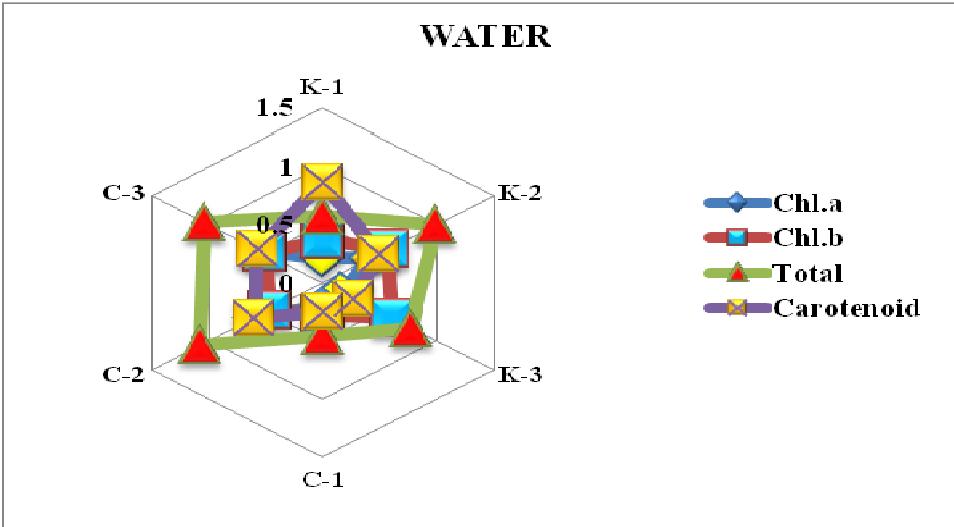
Chlorophyll a (chl. a) is a ubiquitous pigment and can be used as a global biomass indicator (ref????). In Angiosperms (most land plants), there are typically two types of chlorophyll (chl) molecules, namely, chlorophyll a (chl a) and chlorophyll b (chl. b). Both of these pigments absorb photons of light 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%) contents. 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% and 1.31%) 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 and 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 plants 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)



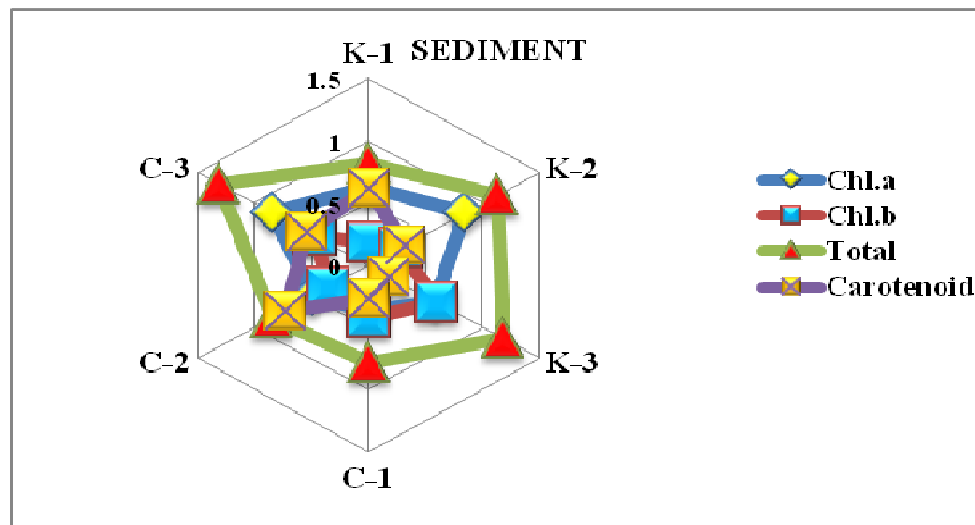
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Figure 2 :Pigment content in Water Samples (% value)



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



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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											

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172 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. Add one or two sentences to discuss your correlation results (with references).

183 Abbreviations

184 CAO- Chirackal *Avicennia officinalis*185 CRM-Chirackal *Rhizophora mucranata*186 CEA – Chirackal *Excoecaria agallocha*187 CSA – Chirackal *Sonneratia alba*

188 KAO – Kattipapambu *Avicennia officinalis*
 189 KRM – Kattiparambu *Rhizophora mucranata*
 190 KEA – Kattiparambu *Excoecaria agallocha*
 191 KSA – Kattiparambu *Sonneratia alba*
 192 SK-1 – Sediment of Kattiparambu-1
 193 SK-2 - Sediment of Kattiparambu-2
 194 SK-3 - Sediment of Kattiparambu-3
 195 SC-1 - Sediment of Chirackal-1
 196 SC-2- Sediment of Chirackal -2
 197 SC-3 - Sediment of Chirackal -3
 198 WK-1 – Water of Kattiparambu-1
 199 WK-2 - Water of Kattiparambu-2
 200 WK-3 - Water of Kattiparambu-3
 201 WC-1 - Water of Chirackal -1
 202 WC-2 - Water of Chirackal -2
 203 WC-3 - Water of Chirackal -3
 204 K-1 - Kattiparambu 1
 205 K-2 - Kattiparambu 2
 206 K-3 - Kattiparambu 3
 207 C-1 - Chirackal -1
 208 C-2 - Chirackal -2
 209 C-3 - Chirackal -3

210 211 4. CONCLUSION

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

221 222 REFERENCES

- 223 1. Shaikh S. D and Dongare M. Analysis of photosynthesis pigments in *Adiantum lunulatum*, Burm.
 224 At different localities of Sindhu durg District (Maharashtra). *Indian Fern J.* 2008; 25: 83–86.
- 225 2. Vicas S. I, Laslo V, Pantea S. and Bandict G. E. Chlorophyll and carotenoids pigments from
 226 Mistletoe (*Viscum album*) leaves using different solvents. *Fascicula Biol.* 2010; (2): 213–218.
- 227 3. Tripathi A. K and Gautam M. Biochemical parameters of plants as indicators of air Pollution. *J.*
 228 *Environ. Biol.* 2007; 28: 127–132.
- 229 4. Ritchie R. J. Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol
 230 and ethanol solvents. *Photosynth. Res.* 2006; 89: 27–41.
- 231 5. Schlemmer, M. R, Francis, D. D, Shanahan, J. F & Schepers, J. S. Remotely measuring
 232 chlorophyll content in corn leaves with differing nitrogen levels and relative water content.
 233 *Agronomy Journal.* 2005; 97(1): 106–112.

6. Rajalakshmi K and Banu N. Antioxidant capacity of chlorophyll in from *Mimosa pudica* by formation of a phosphomolybdenum complex. *International Journal of Frontiers in Science and Technology*. 2014; 2: 1-14.
7. Durgadevi, M and Banu, N. Study of antioxidant activity of chlorophyll from some medicinal plants. *Paripex Indian Journal of research*. 2015; 4(2): 6-8.
8. Morel A and Prieur L . Analysis of variations in ocean color *Limnol. Oceanogr*. 1977; 22 709–22
9. O'Reilly J E. *SeaWiFS Postlaunch Calibration and Validation Analyses (Part 3. NASA Tech. Memo. 2000-206892)* vol 11 (MD: NASA Goddard Space Flight Center). 2000; 49.
10. Carder K L, Chen F R, Cannizzaro J P, Campbell J W and Mitchell B G. Performance of the MODIS semi-analytical ocean color algorithm for chlorophyll-a *Adv. Space Res*. 2004; 33 1152–9
11. Dall'Olmo G and Gitelson A A. Effect of bio-optical parameter variability on the remote estimation of chlorophyll-a concentration in turbid productive waters: experimental results *Appl. Opt*. 2005; 44 412–22
12. Kupper, H, Kupper, F and Spiller, M. *In situ* detection of heavy metal substituted chlorophylls in water plants. *Photosynthesis Res*. 1998; 58: 123–133.
13. Arnon D. I. Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. *Plant. Physiol*. 1949; 24 : 1-5.
14. Rehman, A.M, Mohamed, M.I. Effect of cement dust deposition on physiological behaviors of some halophytes in the salt marshes of red sea. *Egyptian Academic Journal of Biological Sciences*. 2012; 3(1): 1-11.
15. Pangestuti, R., and S-K. Kim, (2011). Biological activities and health benefit effects of natural pigments derived from marine algae. *J. Func. Food.*, 3(4): 255-266.
16. Nakamura, A., T. Watanabe, (2001). Separation and determination of minor photosynthetic pigments by reversed-phase HPLC with minimal alteration of chlorophylls. *Anal. Sci*. 17: 503–508.
17. Zako S. M, W. Akht A. R, Khan S.A and Bhathy M.K. Characterization of *Cassia* seed oil. *Proc. Pakistan Acad. Sci*. 1986; 23 : 167-172.
18. Rao, A.V. and Rao, L.G. Carotenoids and human health. *Pharmacological Research*. 2007; 55: 207-216.
19. Kathiresan, K. & L. Kannan.. Photosynthetic productivity in species of *Rhizophora*. In: *The Mangroves*. Proc. Natl. Symp. Biol. Util. Cons. Mangroves Shivaji university, Kolhapur, India. 1985; 262-265.
20. Kathiresan, K. & P. Moorthy. Influence of different irradiance on growth and photosynthetic characteristics in seedlings of *Rhizophora* species. *Photosynthetica*. 1993; (29): 143-146.
21. Kathiresan, K. & P. Moorthy. Photosynthetic responses of *Rhizophora apiculata* Blume seedlings to a long chain aliphatic alcohol. *Aquat. Bot*. 1994; 47: 191-193.
22. Kathiresan, K. & P. Moorthy.. Hormone-induced physiological responses of a tropical mangrove species. *Bot Mar*. 1994a ;37: 139-141.
23. Krivosheeva, A., S.A. Shavnin, V.A. Kalinin & P.S. Venedikov. Effect of industrial pollutants on seasonal changes of chlorophyll content in scotch pine seedlings. *Fiziol. Rastenii*. 1991; 38: 162-168.
24. Moorthy, P.& K. Kathiresan. Physiological responses of a mangrove seedling. *Biol. Plant*. 1993; 35: 577-581.

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