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# Quantitative analysis of photosynthetic pigments in five species belonging to Rhodophyceae and the effect of environmental variables on their distribution

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**ABSTRACT**

*Five species of Rhodophyceae collected for one year from Visakhapatnam coast were analysed for their Photosynthetic pigments. The environmental variables such as temperature, pH, salinity, light, dissolved oxygen, nitrite, phosphate and silicate were also measured for the same period at the same location of collection. Of the pigments analysed, Chl – a was higher in all the five species and it was followed by phycoerythrin in Pterocladia heteroplatos, Hypnea musciformis and Centroceras clavulatum and phycocyanin in Gracilaria corticata and Grateloupia lithophila. The distribution of pigments showed variations among the species. The higher content of pigments was found in April and June for G. corticata, it was in March for P. heteroplatos and January for C. clavulatum. The ratio of phycobilins to Chl – a also varied among the species. It was high in H. musciformis and C. clavulatum and P. heteroplatos and low in the remaining other two species. The environmental variables showed positive correlations with the pigments at significant levels. The Chl - a correlated positively with temperature, pH, salinity, light, nutrients and phycocyanin at P > 0.05 level. The phycoerythrin was correlated positively with pH, salinity, Phycocyanin and nitrite and phosphate at P > 0.05 level. The Phycocyanin was correlated positively with pH, salinity, dissolved oxygen, nitrate, phycoerythrin and Chl – a at P > 0.05 level.*

**Key words**: Distribution, Chlorophyll-a, environmental variables, phycobilins, photosynthetic pigments, Rhodophyceae

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## INTRODUCTION

The red algae contain a variety of pigments, including chl –a, phycobiliproteins, carotenes, lutein and zeaxanthin. The phycobiliproteins are characterestic pigments of Rhodophyceae, Cyanophyceae and Cryptophyceae. In all these groups the phycobiliproteins acts as photosynthetic accessory pigments. They are the potential biomarkers of plant taxonomy. Unlike Chlorophyceae and higher plants, the red algae use phycobiliproteins for the capturing light energy. They form particles called phycobilisomes on the surface of the thylakoids rather than being embedded in the membranes. The phycobilisomes are composed of phycobilin chromatophores (phycoerythrin, phycocyanin and allophycocyanin) associated with proteins. The phycobilin chromatophore is a linear tetrapyrole, somewhat similar in structure to the tetrapyrole ring portion of chlorophyll molecules, but linearised. Phycobilins do not possess a hydrophobic protein like phytol tail of chlorophyll molecules and therefore are water soluble. Phycoerythrins absorb in green region (495 – 570 nm) while phycocyanin absorb in green yellow region (550- 630 nm) and allophycocyanin in the orange red region (650 – 670 nm).

The Dyes and colorants from natural sources gaining importance due to health and environmental issues. Phycobiliproteins reported to exhibit variety of pharmacological activities, in fluorescent immunoassays for diagnostics and biomedical research [4]. They are also used as natural colorants in food, cosmetic and drug industries replacing the synthetic colorants. They are used in foods such as chewing gums and dairy products. They are used in lipstics and eyeliners [14]. The phycobilins are used as chemical tags in cancer research due to their distinctive fluorescence. They are used as photo sensitizers for treatment of tumors and have potential to substitute photofrin. The phycocyanins have been reported to have neuroprotective effects [11].

The phycobiliproteins of Rhodophyceae were reported earlier [10, 16, 6] from east coast of India. The phycobilin pigments of Rhodophyceae and their seasonal distribution was not reported so far from the Visakhapatnam coast. Therefore with this as an objective, in view of their economic importance the present study is conducted to estimate the seasonal distribution of phycobiliproteins in five species of Rhodophyceae in relation to their environmental variables.

## MATERIALS AND METHODS

The Rhodophyceae species such as *Gracilaria corticata, Grateloupia lithophila, Hypnea musciformis Pterocladia heteroplatos* and *Centroceras clavulatum* were collected for one year from February 2012 to January 2013, from the intertidal zone of Visakhapatnam coast during the low tide period. They were immediately brought to the laboratory, washed with fresh water, removed all the extraneous matter, dried in shade at 28 ◦C. They were pulverized and used for pigment analysis. The Chlorophyll – a, the Phycobilin pigments are measured by standard methods [3, 4].

The environmental factors such as temperature, salinity, pH, dissolved oxygen, light, nitrite, phosphate and silicate were also measured from the marine environment where the macroalge were growing. The temperature, salinity, pH, and light were measured using standard meters and dissolved oxygen was measured by Winkler's method. The nitrite, phosphate and silicate are measured by the analytical methods [15]. The mean and standard deviation are calculated using standard statistical packages.

## RESULTS AND DISCUSSION

The range and mean values of environmental variables are presented in Table 1. The temperature ranged from 28 to 32 ◦C with an annual mean of 29.5 ◦C. The pH ranged from 7.9 to 9.1 with an annual mean of 8.54. The salinity ranged from 20 to 32 ppt with an annual mean of 29 ppt. The light ranged from 26.8 to 68.6 K lux with an annual mean of 50.5 K lux. The dissolved oxygen ranged from 3.1 to 10.4 mg/l with an annual mean of 5.6 mg/l. Among the nutrients analyzed, silicate was high and it was followed by phosphate and nitrite. The nitrite ranged from 0.10 to 4.05 µ moles with an annual mean of 0.123 µ moles. The Phosphate ranged from 0.30 to 1.71 µ moles with an annual mean of 0.80 µ moles and the silicate ranged from 0.13 to 25.02 µ moles with an annual mean of 7.75 µ moles.

**Table 1. Range, mean and standard deviation of environmental variables**

| Variable | Range | Mean | Standard deviation |
|---|---|---|---|
| Temperature ◦C | 29.0 – 32.0 | 29.5 | ± 1.2 |
| pH | 7.9 – 9.1 | 8.5 | ± 0.40 |
| Salinity (ppt) | 20.0 – 32.0 | 29 | ± 3.4 |
| Light (K lux) | 26.8 – 68.6 | 50.5 | ± 15.4 |
| Dissolved oxygen (mg/l) | 3.10 – 10.4 | 5.6 | ± 0.48 |
| Nitrite (µ moles) | 0.10 – 4.05 | 1.23 | ± 0.16 |
| Phosphate (µmoles) | 0.30 – 1.71 | 0.8 | ± 0.43 |
| Silicate (µ moles) | 0.13 – 6.02 | 7.75 | ± 3.8 |

The seasonal variations in environmental parameters of Visakhapatnam coastal water, where the macro algae was collected is given in table 2. There are seasonal variations in environmental variables. The temperature of the sea water was high with 30.75 ◦C in summer and low with 28.87 ◦C in winter. The pH of the sea water was high with 9.0 in winter and low with 8.15 in rainy season. The salinity of the sea water was high with 30.75 ppt in summer and low with 25.5 ppt in winter. The atmospheric light was high with 62.07 K lux in winter and also in summer with 51.87 K lux and low with 46.25 K lux in rainy season. The dissolved oxygen of the sea water was high with 5.44 mg/l in summer and low with 4.83 mg/l in winter. The nitrite of the sea water was high with 1.53 µ moles in summer and low with 1.03 µ moles in winter. The phosphate and silicate of the sea water were high with 1.22 and

17.8 µ moles respectively in winter and low with 0.84 and 8.12 µ moles respectively in rainy season. The highest temperature and light are also reported in summer [9]. High nutrient content, high temperature and high salinity in pre – monsoon are also reported earlier [1]. The present study also agreed with these earlier reports from the other coastal environment

**Table 2. The seasonal variations in environmental variables of coastal waters**

| Parameter | Summer* | Rainy** | Winter *** |
|---|---|---|---|
| Temperature ◦C | 30.75 | 30.0 | 28.87 |
| pH | 8.47 | 8.15 | 9.0 |
| Salinity (ppt) | 30.75 | 30.75 | 25.5 |
| Light (K lux) | 51.87 | 46.22 | 62.02 |
| Dissolved oxygen (mg/l) | 5.44 | 5.2 | 4.83 |
| Nitrite (µ moles) | 1.53 | 1.05 | 1.03 |
| Phophate (µ moles) | 1.12 | 0.84 | 1.22 |
| Silicate (µ moles) | 8.55 | 8.12 | 17.8 |

**Summer – February to May: **Rainy – June to September: ***Winter – October to January*

Five species of Rhodophyceae were analysed for their photosynthetic pigments such as chl – a, phycoerythrin and phycocyanin. Of these pigments, Chl –a was found high in all the five species and it was followed by Phycoerythrin in *P. heteroplatos, H. musciformis and C. clavulatum* followed by phycocyanin in *G. corticata and G. lithophila*. The range and mean values of photosynthetic pigments of Rhodophyceae are presented in Table 3. In *G. corticata* the chl - a ranged from 2.01 to 3.8 mg/l with a mean of 2.7 mg/l. Phycoerythrin ranged from 0.00012 to 0.0027 mg/l with a mean of 0.0016 mg/l. The phycocyanin ranged from 0.0006 to 0.0034 mg/l with a mean of 0.0024 mg/l. In *G. lithophila* the chl – a was 0.089 mg/l, the phycoerythrin was 0.00024 and phycocyanin was 0.014105 mg/l. In *H. musciformis* the chl-a ranged from 1.21 to 1.34 mg/l with a mean of 1.27 mg/l. The phycoerythrin ranged from 0.00072 to 0.0072 mg/l with a mean of 0.0036. The phycocyanin ranged from 0.0012 to 0.0033 mg/l with a mean of.0023 mg/l. In *P. hetroplatos* the Chl – a ranged from 1.19 to 3.95 mg/l with a mean of 1.95 mg/l. The phycoerythrin was ranged from 0.00011 to 0.0018 mg/l with a mean of 0.0018 mg/l. The phycocyanin ranged from 0.00015 to 0.00045 mg/l with a mean of 0.00046 In *C. clavulatum* the Chl –a ranged from 0.80 to 2.66 mg/l with a mean of 1.73 mg/l. The phycoerythrin ranged from 0.00036 to 0.0072 mg/l with a mean of 0.0020 mg/l. The phycocyanin ranged from 0.00015 to 0.0017 with a mean of 0.00094 mg/l.

**Table 3. The range and mean values of photosynthetic pigments in Rhodophyceae**

| | Chl – a (mg/l) | | | Phcoerythrin (mg/l) | | | Phycocyanin (mg/l) | | |
|---|---|---|---|---|---|---|---|---|---|
| **Name of the alga** | **Range** | **Mean** | **SD** | **Range** | **Mean** | **SD** | **Range** | **Mean** | **SD** |
| *G.corticata* | 2.1 – 3.8 | 2.7 | 0.80 | 0.00012 – 0.0027 | 0.0016 | 0.0008 | 0.0006 -0.0034 | 0.0024 | 0.001 |
| *H.musciformis* | 1.21 -1.34 | 1.27 | 0.056 | 0.0007 -0.0072 | 0.0036 | 0.0034 | 0.0012 – 0.0033 | 0.0023 | 0.0008 |
| *P. heteroplatos* | 1.19 -3.95 | 1.95 | 0.470 | 0.00011 – 0.0018 | 0.0018 | 0.0043 | 0.00015 – 0.00045 | 0.00046 | 0.0001 |
| *C. clavulatum* | 0.80 - 2.66 | 1.73 | 0.603 | 0.00036 – 0.0072 | 0.0020 | 0.0023 | 0.00015 – 0.0017 | 0.00094 | 0.005 |

The seasonal distribution of pigments showed variations among the species. In *G. corticata* the pigments were analyzed during February to June 2012. They were high in April and June 2012 with 3.44, 3.89 mg/l Chl –a, 0.0013 and 0.0027 mg/l Phycoerythrin 0.0033 and 0.0034mg/l phycocyanin respectively. In March 2012 the pigments were low and chl – a was 2.13 mg/l, phycoerythrin with 0.0012mg/l and phycocyanin with 0.0006 mg/l. For the remaining period the pigments were slightly higher than the lower values. *H. musciformis* was analysed from October 2012 to January 2013. The pigments were high during December 2012. The chl – a was with 1.343 mg/l, phycoerythrin was with 0.006 mg/l and phycocyanin was 0.0025 mg/l. These pigments were low during October having chl – a with 1.2 mg/l, phcoerythrin 0.00072 mg/l and phycocyanin with 0.002 mg/l. For the remaining period the pigments were almost equal to the higher values. *P. heteroplatos* was analysed in February, May, June, December 2012 and January 2013. The chl –a was high with 2.87 mg/l in June 2012. The phycoerythrin and phycocyanin was with 0.010 and 0.00045 respectively in February 2012. These pigments were observed low in January 2013. For the remaining period the values varied from 1.19 to 1.84 mg/l for chl – a, from 0.00012 to 0.00072 mg/l for phycoerythrin and from 0.00015 to 0.00045 mg/l for phycocyanin. In *C. clavulatum* the pigments were analysed from April 2012 to January 2013. The chl – a was high in January 2013 with 2.663 mg/l and low in June 2012 with 0.805 mg/l. For the remaining period the chlorophyll pigment was varied from 1.24 to 1.934 mg/l: the phycoerythrin was high in December 2012 with 0.0018 mg/l and low in June 2012 with 0.00036 mg/l. For the remaining period this pigment varied from 0.0006 to 0.0014 mg/l. the phycocyanin was high in June 2012 with 0.0017 mg/l and low in May 2012 with 0.0015 mg/l. For the remaining period the pigment varied from 0.0006 to

0.0015 mg/l. In *G. lithophila* the pigments were analysed in January 2013 only. The chl –a was 0.089 mg/l, the phycoerythrin was 0.060 and phycocyanin was 0.00105 mg/l. It was also reported earlier that the photosynthetic pigments were more under higher salinities of sea water [17]. Similar trend was also observed in the present findings.

The variations in ratio of phycobilins to chl –a among the five species studied was presented in table 4. The higher ratio of 0.090 was observed for *G. lithophila* in January 2013 and the lower value of 0.00017 was found for *P. heteroplatos* in June 2012. In *G.corticata* the ratio was high with 0.0033 in February 2012 and low with 0.000338 in March. In *H. musciformis,* it was high with 0.00846 in January 2013 and low with 0.0013 in October 2012. In *P. heteroplatos* higher value was 0.0013 found in April 2012 and lower value was 0.00017 found in June 2012. In *C. clavulatum* it was high with 0.0061 in August 2012 and low with 0.00059 in May 2012. When algae were acclimated to low irradiance, the ratio between phycobiliproteins and chl – a was higher than when algae that were grown in high irradiance. The algae collected in the shaded site have higher phycoerythrin content and a higher lutein /carotene ratio [2]. The ratio of phycobiliproteins to chl – a significantly decreased when irradiance was increased from 40 to 500 µmol $m^2$ $S^{-1}$. The red algae changes its color in relation to chl - a/ phycoerythrin ratio [10]. The red algae with the little of phycoerythrin pigment may appear green or red blue than red due to dominance of the other pigments in these algae.

**Table 4. The ratio of phycobilins/Chl –a pigments**

*Gracilaria corticata* | *Hypnea musciformis*

| Month and year | Phycobilins / Chl - a | Month and year | Phycobilins / Chl - a |
|---|---|---|---|
| February 2012 | 0.00334 | October 2012 | 0.00137 |
| March 2012 | 0.000338 | November 2012 | 0.00248 |
| April 20012 | 0.0017 | December 2012 | 0.00632 |
| June 2012 | 0.0012 | January 2013 | 0.0084 |

*Pterocladia heteroplatos* | *Centroceras clavulatum*

| Month/ year | Phycobilins / Chl -a | Month and year | Phycobilins / Chl - a |
|---|---|---|---|
| April 2012 | 0.00086 | February 2012 | 0.0102 |
| May 2012 | 0.000599 | April 2012 | 0.0043 |
| June 2012 | 0.0025 | May 2012 | 0.000161 |
| August 2012 | 0.0061 | June 2012 | 0.00017 |
| September 2012 | 0.0011 | December 2012 | 0.00098 |
| October 2012 | 0.0012 | January 2013 | 0.000206 |
| December 2012 | 0.0011 | | |
| January 2013 | 0.00096 | | |

*Grateloupia lithophila*

| Month and year | Phycobilins / Chl -a |
|---|---|
| January 2013 | 0.090 |

The pigments of Rhodophyceae showed significant positive correlation coefficients with their environmental factors. In *G. corticata* the phycocyanin was correlated with chl –a and phycoerythrin. The temperature was correlated with chl – a, the pH with phycoerythrin and dissolved oxygen. The light with chl – a and temperature. The nitrite with chl – a, phycocyanin, temperature and phosphate with chl – a, phycocyanin and nitrogen at $P > 0.05$ level. In *H. musciformis* salinity was correlated with phycoerythrin, phycocyanin. The dissolved oxygen with phycoerythrin, light with Chl – a, Nitrite with phycoerythrin and salinity at $P > 0.05$. In *P. heteroplatos* Chl – a correlated with salinity, silicate, the phosphate with phycoerythrin at $P > 0.05$. In *C. clavulatum* pH correlated with chl – a, dissolved oxygen, phycocyanin, nitrite with phycocyanin and dissolved oxygen at $P > 0.05$. The salinity, temperature and biochemical components of algae are closely related to the growth of the species. Significant positive correlation between salinity and chl – a of macroalgae was also reported earlier from the east coast of India [12, 13]. The chlorophyll content in the red strains was related to water temperature, nitrate and orthophosphate [8]. The phycoerythrin content correlated significantly with nitrogen content [7].

## CONCLUSION

A sum of twenty species of Rhodophyceae was found growing along the coast of Visakhapatnam. The species like *G. corticata*, *G. lithophila* and *H. musciformis* are growing continuously with good annual biomass. These macro algae can also be exploited for their natural pigments, since phycoerythrin is very expensive, costing 50 Euros per milligram and is variously used in research and biomedical applications.

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## REFERENCES

[1] N. Balakrishnan Nair V. Sobha R. Chandran N. Rathi Ammal S. Maya and H. Suryanarayana, *Seaweed Res. Utiln.*, **1993**, 16, 183 – 197.
[2] G. Calabrese, Phycology, **1972,** 11, 141 – 146.
[3] L.V. Evans, Experimental *Phycology*, Lebban, C.S. Chapmann D.J. and Kremer B.P, (Eds), New York, **1988**, pp 123 – 134.
[4] A.N. Glazer and L. Stryer, Trends *Biochem. Sci.*, **1984**, 9, 423 – 427.
[5] S.W. Jeffrey and G.F. Humphrey, Biochem. *Physiol. Pflanz.* **1975**, 167, 191 –194.
[6 ] C. Manju R. Sreenivasan, H, Indu and S. Geetha *J. Appl. Pharma. Sci* . **2012**, 2, 100 – 107.
[7] H. Mizuta, Y. Shirakura and H. Yasui, *Algae*, **2002**, 17, 89 – 93.
[8] D.C. Pereira T.G. Trigueiro P. Colepicolo and E. Marinho – Soriana, *Revista Brasilera de farmacognosia,* **2012**, 22, 874 – 880.
[9] J.M. Rico and C. Fernandez, *Exp. J. Phycol.* **1996**, 31, 149 – 155.
[10] J. Reeta and V. Sally, *Indian J. Fish.* **2000**, 47, 365 – 369.
[11] C. Romay R. Gonzalez N. Ledon P. Remirez and V. Rimbau, *Curr. Protein Pept. Sci.*, **2003**, 4: 207 – 216.
[12] Y. Sarojini G. Subbarangaiah and E. Vanilla Kumari, *Indian Hydrohiology* **2000**, 3, 89 – 93.
[13] Y. Sarojini and K. Lakshminarayana, *Seaweed Res Utiln.,* **2009**, 31, 127 – 134.
[14] S. Sekar and M. Chandramohan *J. Appl. Phycol.* **2008**, 20, 113 -136.
[15] J.P.H. Strickland and T.R. Parsons, A practical hand book of sea water analysis. *Bull. Krish. Res. Bd. Canada,* **1972**, PP. 167 – 310.
[16] A. Vanitha and S. Chandra, *Int. J. Curr. Sci.* **2012**. 149 – 154.
[17] K. Velkanni and R. Rengasamy, *Mahasagar* **1992**, 25, 123 – 128.