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# The seasonal variations in distribution of photosynthetic pigments in four edible species of Chlorophyceae and the effect of light, dissolved oxygen and nutrients on their distribution

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**ABSTRACT**

*Four edible species of Chlorophyceae collected from Visakhapatnam coast were analysed for their photosynthetic pigments such as Chl – a, Chl – b and carotenoids. The environmental variables such as light, dissolved oxygen, nitrite, phosphate and silicate were also measured for the same period at the same location. Interspecific variations were occurred in distribution of pigments. Of the pigments analysed, the Chl – b was high and it was followed by Chl – a and carotenoids. In Ulva fasciata higher content of chl – a and Chl – b were occurred during rainy season and carotenoids were higher in summer. In Enteromorpha compressa and Caulerpa racemosa the photosynthetic pigments were high during summer season. In Caulerpa sertularioides chl – a and chl – b were high during winter and carotenoids during summer season. The pigment ratios of Chl – b/ chl – a were high in Caulerpa racemosa among the four species and it varied from 0.84 to 1.85 among the these species. A significant positive correlation was found for pigments with their environmental variables at $P \geq 0.05$ level. In Ulva fasciata, phosphate correlated with Chl – b. In Enteromorpha. compressa the carotenoids correlated with light, phosphate and silicate. In Caulerpa racemosa the content of chl - b was correlated with contents of nitrite and phosphate. In Caulerpa sertularioides the carotenoids correlated with the dissolved oxygen.*

**Key words:** Carotenoids, chlorophylls, Chlorophyceae, environmental variables, macroalgae, seasonal distribution, Visakhapatnam

## INTRODUCTION

The variations in seaweed thallus colour are related to varying amount of pigments present in them. Pigments are the molecules strongly absorbing the visible light. The Chlorophyceae contain chlorophyll – a and chlorophyll – b, zeaxanthin, lutein together with minor proportion of less typical carotenoids. The chl – a and b have common basic structure. It is consisting of four pyrrol rings, a long chain of carbon atoms attached to the ring. The chl –a is a pigment present in all most all photosynthesizing plants. The green land plants and green algae contain Chlorophyll –b in addition to chlorophyll –a. The chlorophyll – b in solution is blue green while chlorophyll – a is yellow green. The third type of pigments in all photosynthesizing cells are the carotenoids. The carotenoids contain an open chain conjugated double bond system of pyrene type, ending with ionone rings. The carotenoids have their oxygen in the form of hydroxyl, carbonyl or carboxyl groups attached to the ionone rings. They are associated with photosynthetic membranes. They mainly function in light harvesting.

The plant pigments play an important role in human health. The quantity of macroalgae pigment is mostly used to define algal biomass. It is also affected by environmental factors such as salinity, temperature, nutrients, and intense irradiance that cause a high rate of pigment production (Ramus *et al*., 1972 Boussiba *et al*., 1999 and Zucchi and Necchi 2001). The algal pigments of east coast of India have been reported earlier by Reeta and Ramalingam (1993), Chakraborty and santra (2008), Sarojini and Lakshminarayana (2009), Chakraborty and B hattacharya (2012) and Saranya *et al*., (2013) but, the seasonal variations in distribution of these pigments and the effect of environmental factors on their distribution was not reported so far. So, in view of the economic importance of pigments and significance in taxonomic research and lacuna in study of their seasonal variations the present study was chosen to elucidate the present objectives in the four edible green algae along the Visakhapatnam, east coast of India.

## MATERIALS AND METHODS

The macroalgae were collected from Visakhapatnam coast, during the low tide period from February 2012 to January 2013 at monthly intervals. Simultaneously, the sea water samples were also collected from the same locality where the algae were grown for the above mentioned period. The algae were brought to the laboratory immediately, washed thoroughly with fresh water, shade dried at room temperature. The samples were pulverized, homogenized with 90% acetone by following the method of Jeffrey and Humphrey (1975). The extract was centrifuged and the optical density of the pigments was measured on Systronics spectrophotometer and the content was calculated and expressed as mg/l and µg/g dry weight. The light was measured using a lux meter. The dissolved oxygen was measured by Winkler's method, the nutrients by Strickland and Parsons Method (1972). The mean, standard deviation, correlation coefficients were calculated using standard statistical packages.

## RESULTS AND DISCUSSION

Four species of Chlorophyceae viz., *Ulva fasciata, Enteromorpha compressa, caulerpa racemosa and C. sertularioides* were analysed to estimate their photosynthetic pigments such as chl – a, chl - b and carotenoids. Inter specific variations were observed in distribution of pigments. Of the pigments analysed the chl – b was high among the pigments and it was followed by chl – a and carotenoids in the four species. The pigment chl – a was high with 14.82 mg/l in *C. racemosa* and it was low with 6.03 mg/l in *E. compressa*. The chl – b was also high in *C. racemosa* with 25.80 mg/l and it was low with 2.89 mg/l in *U. fasciata*. The carotenoids were high with 0.18 µg/g *in C. sertularioides* and low with 0.0040 µg/g in *U. fasciata*. The range and mean values of photosynthetic pigments was given in Table 1. In *U. fasciata* the chl – a ranged from 1.08 to 3.61 mg/l with a mean of 2.24 mg/l. The chl – b ranged from 1.46 to 4.76 mg/l with a mean of 2.89 mg/l. The carotenoids ranged from 0.0048 µg/g to 0.220 µg/g with a mean value of 0.040 µg/g. In *E. compressa* the chl – a ranged from 2.34 to 10.35 mg/l with a mean of 6.03 mg/l. the chl – b ranged from 2.34 to 11.86 mg/l with a mean of 6.34 mg/l. The carotenoids ranged from 0.013 to 0.182 µg/g with a mean of 0.065 µg/g. In *C. racemosa* the chl – a ranged from 10.08 to 17.4 mg/l, with a mean of 14.8 mg/l. The chl –b ranged from 15.2 to 31.2 with a mean of 25.80 mg/l. The carotenoids ranged from 0.023 to 0.248 µg/g with a mean value of 0.13 µg/g. In *C. sertularioides* the chl –a ranged from 10.98 to 19.12 mg/l with a mean of 14.46 mg/l. The chl – b ranged from 11.2 to 28.6 mg/l with a mean of 14.46 mg/l. The carotenoids ranged from 0.076 to 0.463 µg/g with a mean value of 0.183 µg/g. These results are in agreement with the earlier reports on macroalgae pigments of east coast of India (Sarojini and Lakshminarayana, 2009; Saranya *et al.*, 2013).

The annual distribution pattern of pigments of these algae showed three peaks for each species. The major peak was observed from July to October 2012 with little variation among the species and another peak was occurred in December 2012 to January 2013. The minor peak was occurred during March to May 2012 for chl a and chl – b pigments. The seasonal variations in photosynthetic pigments are given in Table 2. Interspecific variations were observed in seasonal distribution among these species. In *U. fasciata* the chl –a and chl – b were high with 2.7 and 3.6 mg/l respectively during rainy season. The carotenoids were high in summer with 0.048 µg/g. The chl – a and chl – b were low with 2.0 and 2.25 mg/l respectively during winter. The carotenoids were low with 0.0058 µg/g in summer. In *E. compressa* the pigments were high with 6.27, 8.97 mg/l and 0.138 µg/g for Chl – a, chl - b and carotenods respectively during rainy and winter seasons. It was low with 3.79, 3.97 mg/l and 0.035 µg/g for Chlorophylls and carotenoids respectively during summer. In *C. racemosa* the Chl – a, chl – b and carotenoids were high with 16.22, 28.27 mg/l and 0.23 µg/g respectively during summer. The chlorophylls were low with 13. 64 and 23.72 mg/l for chl –a and chl –b respectively during rainy season. The carotenoids were low with 0.064 µg/g in winter. In *C. sertularioides* the chl – a and chl – b were high with 14.45 and 20.462 mg/l respectively during rainy and summer and carotenoids were high with 0.208 µg/g during summer. The chl – a and chl – b were little low with 14.19 and 20.12 mg/l during summer and carotenoids with 0.159 µg/g during winter. The high salinity and high nutrient conditions due to run off during rainy season might have favoured the growth of Chlorophyceae (Sarojini *et al*., 2000; Sarojini and Lakshminarayana, 2009). The pigments in macroalgae varied depending on the species. The nutritional content of macro algae were observed to depend not only on season and geography (Flurence 1999, Harpoon et al., 2000) but also on the nutrient condition of the environment in which they grow (Bianchi *et al*., 1997).

The pigment ratio of Chl – a/ chl – b is presented in Table 3. The pigment ratio was high in *C. racemosa* and low in *E. compressa*. In *U. fasciata* the highest ratio of 1.3 to 1.76 was occurred in winter and lowest with 0.873 to 1.28 was occurred in summer. In *E. compressa* highest ratio with 1.91 was occurred in February 2012 and lower ratio with 0.81 in August 2012. In *C. racemosa* the higher ratio of 1.78 to 1.85 was occurred in April to July and lowest in August 2012. In *C. sertularioides* the higher ratio was observed in winter and lower ratio in rainy period. The typical range of Chl - a/ chl –b in higher plants and algae is about 3 to 1. The present values for all the four species of Chlorophyceae are in this range, agreeing with the above said ratio. The chl – b/ chl – a ratio of *Caulerpa* species was 0.15 and 0.12 reported from Okha coast, India (Kumar *et al.*, 2009). It was much lesser than the present values on *Caulerpa* species, which may be attributed to geographical and seasonal differences in environment.

The seasonal variations in environmental variables of Visakhapatnam coast is given in table 4. The environmental factors showed variations in different seasons. The atmospheric light was high with 60.75 K lux in winter and low with 44.0 K lux in rainy season. The dissolved oxygen of the sea water was high with 7.04 mg/l in summer and low with 4.34 mg/l in winter. The nitrite of the sea water was high with 1.90 µ moles in rainy period and low with 0.65 µ moles in winter. The phosphate and silicate of the sea water were high with 1.22 and 8.59 µ moles in rainy season and low with 0.55 and 2.71 µ moles in winter season. The pigment distribution was correlated positively with environmental variables of the sea weeds at $P \geq 0.05$ level. The carotenoids In *U. fasciata* correlated with phosphate. In *E. compressa* chl – a correlated with phosphates, chl –b with phosphate, carotenoids with phosphate, light and silicate. In *C. racemosa* the chl – b was correlated with nitrite and phosphate but not at significant levels. The carotenoids were correlated with dissolved oxygen, nitrite and phosphate but not at significant levels. In *C. sertularioides*, the carotenoids correlated positively with dissolved oxygen but not at significant level. Zucchi and Necchi (2001) reported that the physical factors such as light intensity, photoperiod and temperature can alter the pigment contents. The chlorophyll – a was correlated positively with nitrate (Pereira *et al*., 2003)

**Table 1: Range and mean values of photosynthetic pigments of Chlorophyceae**

| Name of the alga | Chl –a (mg/l) | | | Chl – b (mg/l) | | | Carotenoids (µg/g) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Range | Mean | SD | Range | Mean | SD | Range | Mean | SD |
| *Ulva fasciata* | 1.08 - 3.61 | 2.24 | 0.7 | 1.46 –4.76 | 2.89 | 0.9 | 0.004 -0.22 | 0.04 | 0.06 |
| *Enteromorpha compressa* | 2.34 - 10.35 | 6.03 | 2.7 | 2.3 -11.86 | 6.34 | 3.6 | 0.013-0.18 | 0.06 | 0.06 |
| *Caulerpa racemosa* | 10.08 – 17.4 | 14.8 | 2.2 | 15.2 -31.2 | 25.8 | 5.2 | 0.023 -0.24 | 0.13 | 0.08 |
| *C. sertularioides* | 10.98 -19.12 | 14.4 | 2.9 | 11.2 –28.6 | 20.2 | 7.0 | 0.076 -0.46 | 0.18 | 0.12 |

**Table 2: Seasonal variations in photosynthetic pigments**

| Name of the alga | pigment | Summer* | Rainy** | Winter*** |
|---|---|---|---|---|
| *Ulva fasciata* | Chl - a | 2.436 | 2.771 | 2.036 |
| | Chl - b | 2.85 | 3.601 | 2.252 |
| | Carotenoids | 0.048 | 0.0058 | 0.016 |
| *Enteromorpha compressa* | Chl - a | 3.796 | 6.525 | 6.276 |
| | Chl - b | 3.771 | 6.96 | 8.97 |
| | Carotenoids | 0.035 | 0.0469 | 0.138 |
| *Caulerpa racemosa* | Chl - a | 16.22 | 13.647 | 14.551 |
| | Chl - b | 28.27 | 23.72 | 25.416 |
| | Carotenoids | 0.203 | 0.127 | 0.0643 |
| *C. sertularioides* | Chl - a | 14.199 | 14.627 | 14.451 |
| | Chl - b | 20.026 | 20.233 | 20.462 |
| | Carotenoids | 0.208 | 0.187 | 0.159 |

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

**Table 3: Chl –b/ chl –a pigment ratios**

| Month | *U. faciata* | *E. compressa* | *C. recemosa* | *C. sertularioides* |
|---|---|---|---|---|
| Feb | 0.873 | 1.91 | 1.63 | 1.17 |
| Mar | 1.25 | 1.0 | - | - |
| Apr | 1.3 | 0.94 | 1.78 | 1.26 |
| May | 1.28 | 1.17 | 1.79 | 1.66 |
| Jun | 1.2 | 0.84 | 1.76 | 1.02 |
| July | 1.24 | 1.19 | 1.85 | 1.25 |
| Aug | 1.25 | 0.81 | 1.5 | 1.59 |
| Sept | 1.47 | 1.34 | - | 1.47 |
| Oct | 1.35 | 1.11 | - | 1.28 |
| Nov | 1.38 | 0.84 | 1.65 | 1.22 |
| Dec | 1.3 | - | 1.75 | 1.46 |
| Jan | 1.76 | 1.14 | 1.83 | 1.6 |

*n-No data*

**Table 4: The seasonal variations in environmental variables of coastal water**

| Parameter | Summer* | Rainy** | Winter *** |
|---|---|---|---|
| Light (K lux) | 46.5 | 44.0 | 60.75 |
| Dissolved oxygen (mg/l) | 7.04 | 5.49 | 4.34 |
| Nitrite (µ moles) | 1.36 | 1.9 | 0.65 |
| Phosphate (µ moles) | 0.71 | 1.2 | 0.55 |
| Silicate (µ moles) | 8.85 | 8.59 | 2.71 |

**Summer – February to May **Rainy – June to September*
****winter – October to January*

## CONCLUSION

The distribution of chlorophylls, carotenoids and their seasonal variations of four species of Chlorophyceae were described for their exploitation in food and industry. They are potential biomarkers of plant systematics. The study on seasonal variations helps in cultivatation strategies of seaweeds and marine products. The chlorophyll as a food colourant is found to exhibit antiimutagenic property. Most of the pigments have high nutritional value. They are eco - friendly and cause no pollution. The chlorophyll derivatives are used for dyeing of fabrics such as wool and cotton. The *Ulva* and *Caulerpa* are available in good quantities of biomass along the Visakhapatnam coast throughout the year and hence they can be exploited for their pigments and other economic products.

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