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Estimation of photosynthetic pigments of green and brown seaweeds in relation to primary productivity and nutrients of polluted coastal waters of Tuticorin coast, Gulf of Mannar, Southeast coast of India

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ABSTRACT

The present study was made to evaluate the photosynthetic pigment concentrations from two groups of seaweeds such as green algae (*Ulva fasciata, Udotea indica* and *Halimeda macroloba*) and brown algae (*Stoechospermum marginatum* and *Colpomenia sinuosa*) collected from the Tuticorin coast, Southeast coast of India with relation to environmental conditions in terms of photosynthetic pigments (Chlorophyl- *a*, Chlorophyl- *b* and carotenoids), nutrients and productivity of water samples. The ammonia ranged from 0.40±0.25 to 0.45±0.39, nitrite from 0.37±0.22 to 0.15±0.12, nitrate from 0.04±0.02 to 0.15±0.12, phosphate from 0.39±0.13 to 0.87±0.38, silicate from 1.08±0.48 to 1.76± 0.37; The gross productivity ranged from 0.29±0.08 to 0.63±0.20 and net productivity from 0.12±0.03 to 0.39±0.18. The Pearson correlation revealed that Chl-*a* was strongly correlated with Chl-*b* (r=0.999; $p<0.05$ and NH_3 (r=999; $p<0.05$. Chlorophyl-*b* was strongly correlated with NH_3 (r=0.999; $p<0.05$); Gross productivity was strongly correlated with net productivity (r=0.999; $p<0.05$). The one way ANOVA results showed that nutrients and productivity was significant variations $p<0.05$ level. The highest chlorophyll-*a* and chlorophyll-*b* content was recorded in *Udotea indica* and *Halimeda macroloba* respectively while lowest Chlorophyll–*a* and Chlorophyl-*b* was recorded in *Halimeda macroloba* and *Colpomenia sinuosa* respectively. The maximum of carotenoid content was recorded in *Udotea indica* and minimum was recorded in *Halimeda macroloba and Colpomenia sinuosa*. The groupwise distribution of Chl-*a* and Chl-*b* showed an order: *U. indica* > *S. marginatum* > *U.fasciata* > *C.sinuosa* >*H. macroloba* ; Chl-*b* showed an order *H. macroloba* > *U. indica* > *U.fasciata* > *C. sinuosa* > *S. marginatum* and carotenoids showed an order: *Udotea sp* > *S. marginatum* > *C. sinuosa* > *U. fasciata* > *H. macroloba.* The ANOVA results showed that Chlorophyl-a, b and carotenoids revealed significant variations $p<0.05$ level in Tharuvaikulam and Threspuram, whereas the significant variation was not found at Tuticorin Port ($p>0.05$).

Introduction

In plants, algae and cyanobacterial pigments are the most responsible compounds by which the energy of sunlight is captured for photosynthesis. Hence the light energy is converted into chemical energy in all photosynthetic organisms (Strokes, 1864). In addition to their role in photosynthesis, natural pigments carry out various important biological functions. The basic classes of pigments are chlorophyll and carotenoids. Chlorophyll is the only precursor molecule which was developed to evaluate the trophic level of waters. Chlorophyll itself is not a single molecule but a family of related molecules, designated as chlorophyll a, b, c and d . Chlorophyll pigments are major supplement source in the production of nutraceutical products (Higdon, 2004). Chlorophyll a is most abundant in nature, although chlorophyll b is also common in fresh water organisms. The quantification of chlorophyll a is easier than the algal biomass itself and can be used as an indirect method of biomass quantification (Dere *et al.,* 1998). The main function of chlorophyll b is to gather the light energy, working together with chlorophyll a and carotenoids. The most

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important one in the carotenoids; they play vital role in the photosynthetic pathway and are called as accessory pigments because, they don't dissolve in water, and must attach within the pathway, but must pass their absorbed energy to chlorophyll. The carotene pigments are the most important photosynthetic pigments and they prevent the chlorophyll and thylakoid membrane from the damage of absorbed energy by photooxidation (Vechetel *et al.,* 1992). It was also determined that the various limiting factors such high light, lack of nitrogen and limited nutrient, influence the change in pigment level.

Seaweeds are the most abundant photosynthetic species that contain different groups of light harvesting and photo protective pigments. The pigments are important in classification, biodiversity and taxonomical studies of seaweeds and which is highly important for their value reserve food and other valuable products. There are three groups of photosynthetic pigments in the seaweeds. They are chlorophylls, carotenoids and phycobilins (Rowan, 1989). Based on pigments the macroalgae/seaweeds are classified into three major groups viz., Green algae (Chlorophyta), Brown algae (Phaeophyta) and Red algae (Rhodophyta). The lipid-soluble Chlorophyll a is found in all photosynthetic algae, while chlorophyll b is found in Chlorophytes. Carotenoids are separated into carotenes and xanthophylls (Rowan 1989). The carotene β, β-carotene is found in Rhodophytes (and to a certain extent in Phaeophytes and Chlorophytes) (Rowan, 1989). They absorb efficiently in the green to red part of the light spectrum (500-650 nm in vitro) (Rowan, 1989).

Over the past several decades, seaweeds extracts have been studied as novel sources which have been shown to produce a variety of compounds and some of them have been reported to possess biological activity of potential medicinal value (Moore, 1978; Konig *et al.,* 1994; Tutour *et al.,* 1998; Satoru *et al.,* 2003). Seaweeds are considered to be a rich source of antioxidants (Cahyana *et al.,* 1992). Recently, the potential antioxidant compounds were identified as some pigments (e.g. fucoxanthin, astaxanthin, carotenoid) and polyphenols (e.g. phenolic acid, flavonoid, tannins) (Heo *et al.,* 2005). Among marine organisms, marine algae are rich sources of structurally diverse bioactive compounds with various biological activities. Recently, their importance as a source of novel bioactive substances is growing rapidly and researchers have revealed that marine algal originated compounds exhibit various biological activities (Barrow and Shahidi, 2008; Wijesekara and Kim, 2010; Wijesekara *et al.,* 2010). Among functional ingredients identified from marine algae, natural pigments have received particular attention. These natural pigments exhibit various beneficial biological activities such as antioxidant, anticancer, anti-inflammatory, anti-obesity, anti-angiogenic and neuroprotective activities. Therefore, various natural pigments isolated from marine algae have attracted much attention in the fields of food, cosmetic and pharmacology (Pangestuti and Kim, 2011).

Materials and Methods

Sample collection

Five species of marine macroalgae, *Ulva fasciata, Udotea indica, Halimeda macroloba* of Chlorophyceae; *Stoechospermum marginatum* and *Colpomenia sinuosa* of Phaeophyceae were collected from the industrial and domestic sewage discharging areas from Tuticorin coast and the study areas are shown in Fig.1. The samples were washed thoroughly to remove adhering soil particles and immediately transported to the laboratory in ice box for analysis of various pigments.

Water sample collection and analysis

The nutrients such as NH_3, NO_2-, NO_3-, PO_4 AND SiO_3 and biological parameters such as gross productivity and net productivity were analyzed in the collected water samples at the three stations. The primary productivity was measured by using the light and dark bottle method (Vollenweider, 1974). Dissolved micronutrients such as NO2- was measured by a colorimetric method using sulfanilamide, NO3- by the cadmium reduction method, inorganic phosphorus (IP) and total phosphorus (TP) by the ascorbic acid method and reactive silicate by the molybdate method using a PC based double beam spectrophotometer (Systronics-2202).

Estimation of Chlorophyll

Five hundred mg of fresh leaf material was taken and ground with help of pestle and mortar with 10 ml of 80% acetone. The homogenate was centrifuged at 3000 rpm for 15 minutes. The supernatant was filtered and stored. The residue was re-extracted with 5 ml of 80% acetone. The extract was utilized for chlorophyll estimation. Absorbance was read at 645 and 663 nm in the UV-spectrophotometer (Arnon, 1949).

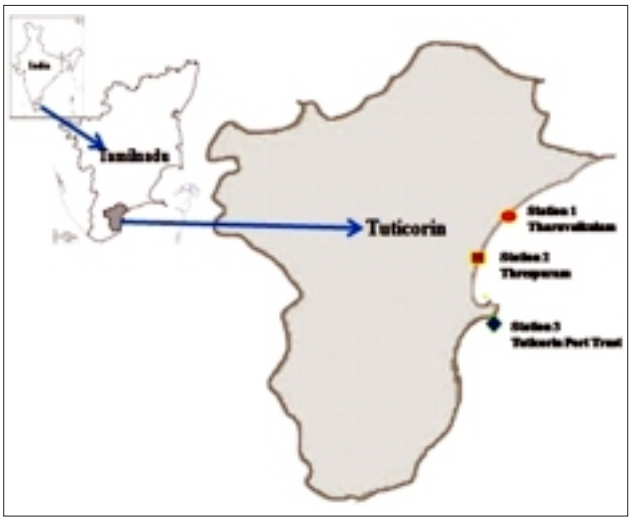

Fig. 1. *Map showing the study areas*

$$\text{Chlorophyll a (mg/g.fr.wt.)} = \frac{(12.7 \times \Delta A663 - 2.69 \times \Delta A645)}{a \times 1000 \times W} \times V$$

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

ΔA = Absorbance at respective wavelength

V = Volume of extract (ml)

W = Fresh weight of the sample (g)

Estimation of Carotenoid

The carotenoid content of seaweeds were determined by the method of (Kirk and Allen, 1965). The extract that was used for the chlorophyll estimation was used for carotenoid estimation also. The same chlorophyll extract was measured at 480nm in UV-spectrophotometer to estimate the carotenoid content.

Carotenoid (µg/g.fr.wt) = ΔA480 + (0.114 × ΔA663) – (0.638 × ΔA645)

ΔA = Absorbance at respective wavelength.

Results

The major photosynthetic pigments, chlorophyll-*a, b* and carotenoid content were estimated from fresh seaweeds. The chlorophyll-*a* ranged from 0.04±0.03 to 3.85±0.33mgg^{-1} with minimum in the green seaweed *Halimeda macroloba* and maximum in the green seaweed *Udotea indica* in the Tharuvaikilam (Station-1) (Fig. 2). Chlorophyll-*b* varied from 0.25±0.20 to 7.63±0.84mg g^{-1}. The minimum was recorded in brown algae *Colpomenia sinuosa* in Tharuvaikilam (Station-1) while the maximum was recorded in green seaweed *Halimeda macroloba* in Threspuram (Station-2) (Fig. 3). The carotenoid content ranged from 0.33±0.11 to 7.63±0.95 mgg^{-1} with minimum in brown algae *Colpomenia sinuosa* in Tharuvaikilam (Station-1) and maximum in green seaweed *Udotea indica* in Threspuram (Station-2) (Fig.4).

Ammonia ranged from 0.40±0.25 to 0.45±0.39 with minimum and maximum values in Station-3 and Station-2 respectively; Nitrite ranged from 0.37±0.22 to 0.15±0.12 with minimum and maximum in Station-3 and Station-1 respectively, Nitrate ranged from 0.04±0.02 to 0.15±0.12 with minimum and maximum in Station-2 and Station-1 respectively. Phosphate ranged from 0.39±0.13 to 0.87±0.38 with minimum and maximum in Station-2 and Station-3 respectively. Silicate ranged from 1.08±0.48 to 1.76± 0.37 with minimum and maximum in Station-2 and Station-3 respectively. Gross productivity ranged from 0.29±0.08 to 0.63±0.20 with minimum and maximum was in Station-3 and Station- 1 respectively. Net productivity ranged from 0.12±0.03 to 0.39±0.18 with minimum and maximum in Station-3 and Station-1 respectively. Pearson correlation revealed that Chl-*a* was strongly correlated with Chl-*b* (r=0.999; p<0.05 and NH_3 (r=999; p<0.05. Chlorophyll-*b* was strongly correlated with NH_3 (r=0.999; p<0.05). Gross productivity was strongly correlated with net productivity (r=0.999; p<0.05) (Table-2, Fig. 5).

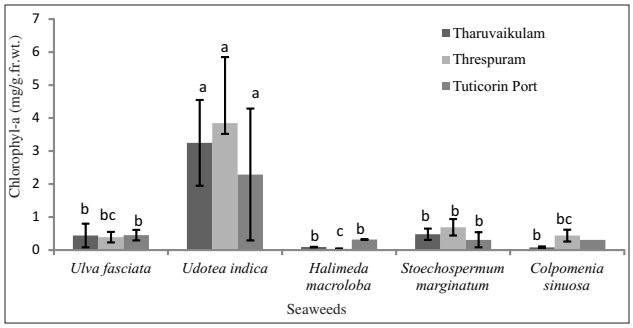

Fig. 2. *Level of Chlorophyl-a of the different species at three stations*

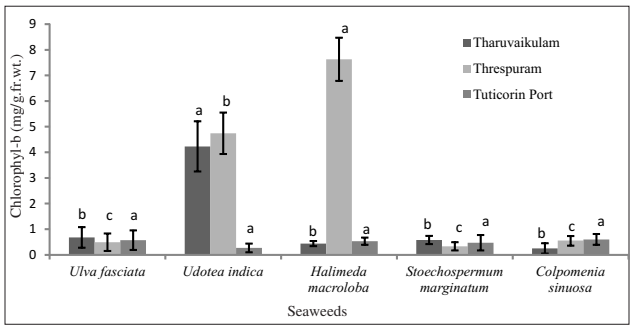

Fig. 3. *Level of Chlorophyl-b of the different species at three stations*

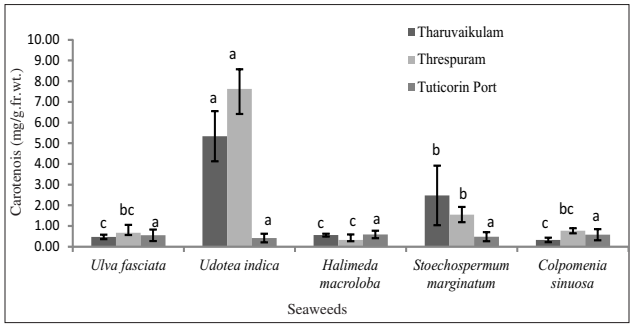

Fig.4. *Level of Carotenoid contents of the different species at three stations*

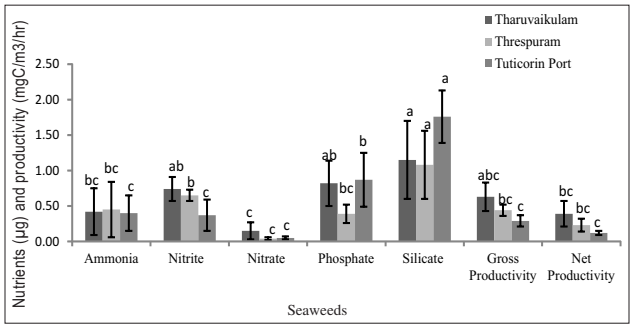

Fig. 5. *Variations of nutrients and productivity at three stations*

The statistical tool One-way ANOVA was used. It was significantly (p<.0.05) elevated in Chlorophyll a, b and carotenoids in Station-1 and Station-2. However insignificantly

was noted in Station-3 for all three pigments. In Station-1, Chlorophyll a was higher in *Udotea indica* and One way Anova was significantly higher (p <.0.05). In Station-2 Chlorophyll a was higher in *Udotea indica* followed by *Stoechospermum marginatum* with One way Anova significantly higher (p <.0.05). In Station-3, Chlorophyll a, b and carotenoid pigments were found as insignificant variation (p>0.05) in all five seaweeds. Among the five species of seaweeds, the highest chlorophyll-a and chlorophyll-b content was registered in *Udotea ap* and *Stoechospermum marginatum* respectively, while lowest chlorophyll-a and chlorophyll-b was registered in *Colpomenia sinuosa*. The maximum carotenoid content was recorded in *Udotea indica* and minimum in *Colpomenia sinuosa*. The three pigments was in the order of Chlorophyl-b>Chlorophyl-a>Carotenoid. The group wise distribution of Chl-*a* and Chl-*b* showed an order: *U. indica* > *S. marginatum* > *U.fasciata* > *C.sinuosa* >*H. macroloba* ; Chl-*b* showed an order *H. macroloba* > *U. indica* > *U.fasciata* > *C. sinuosa* > *S. marginatum* and carotenoids showed an order: *Udotea sp* > *S. marginatum* > *C. sinuosa* > *U. fasciata* > *H. macroloba.* The ANOVA results showed that, Chlorophyl-a, b and carotenoids revealed significant variations p<0.05 level in Tharuvaikulam and Threspuram, whereas the significant variation was not found at Tuticorin Port (p>0.05) (Table-1).

Table-1. *Chlorophyll a, b and carotenoid content of five seaweeds collected from Tuticorin coast*

Stations	Pigments	*Ulva fasciata*	*Udotea indica*	*Halimeda acroloba*	*Stoechospermum marginatum*	*Colpomenia sinuosa*	*P* Value
Tharuvaikulam	Chl-a	0.44±0.36^{b}	3.25±1.30^{a}	0.09±0.01^{b}	0.48±0.17^{b}	0.08±0.03^{b}	0.000
	Chl-b	0.68±0.40^{b}	4.23±0.98^{a}	0.44±0.10^{b}	0.33±0.16^{b}	0.56±0.17^{b}	0.000
	Carotenoids	0.47±0.11^{c}	5.34±1.21^{a}	0.56±0.07^{c}	2.48±1.44^{b}	0.33±0.11^{c}	0.000
Threspuram	Chl-a	0.39±0.16^{bc}	3.85±0.33^{a}	0.04±0.03^{c}	0.69±0.25^{b}	0.44±0.18^{bc}	0.000
	Chl-b	0.49±0.34^{c}	4.74±0.81^{b}	7.63±0.84^{a}	0.33±0.16^{c}	0.56±0.17^{c}	0.000
	Carotenoids	0.67±0.39^{bc}	7.63±0.95^{a}	0.33±0.26^{c}	1.55±0.37^{b}	0.77±0.12^{bc}	0.000
Tuticorin port Port	Chl-a	0.45±0.16^{b}	2.29±2.00^{a}	0.32±0.01^{b}	0.31±0.23^{bc}	0.31±0.06^{a}	0.086
	Chl-b	0.57±0.38^{a}	0.27±0.17^{a}	0.53±0.14^{a}	0.47±0.30^{a}	0.60±0.21^{a}	0.564
	Carotenoids	0.55±0.28^{a}	0.42±0.21^{a}	0.59±0.18^{a}	0.48±0.22^{a}	0.58±0.27^{a}	0.882

Discussion

In the present study, the highest chlorophyll a and b was recorded in the green seaweed *Udotea indica and Halimeda macroloba* respectively and highest carotenoids content was recorded in the *Udotea indica*. Pedersen (1964) reported highest total chlorophyll in the green alga *C. adharens* and minimum in the red alga *A. spicifera.* Similarly, Muthuraman and Ranganathan (2004) reported maximum chlorophyll in the green alga *Caulerpa scalpelliformis* among the 12 seaweeds tested which include phaeophycean and Rhodophycean member also. The successful analysis of algal pigments depends on the selection of an appropriate extraction procedure. Both acetone and methanol are widely applied in the extraction of algal pigments. Acetone is known to have a lower extractability of chlorophylls from the protein matrix (Nakamura and Watanabe, 2001). Acetone, on the other hand, provides a stable environment. While acetone and methanol have the same polarity index, acetone has greater eluotrophic strength than methanol for carbon-rich substrates (Stock and Rice, 1967). It can therefore be concluded that the environmental, seasonal and physicochemical properties of the seawater are the most important factors affecting the algae. Pinchetti *et al.* (1998), explained that the green macroalga *Ulva* has been widely used as a biofilter because of its high efficiency in the removal of nitrogenous inorganic compounds. In the present study, the green algae have highest contents of pigments especially in *Udotea indica.*

Table-2. *Variation of nutrients and productivity levels in water samples of three stations*

	Chlorophyl-a	Chlorophyl-*b*	Carotenoids	Ammonia	Nitrite	Nitrate	Phosphate	Silicate	Gross Productivity	Net Productivity
Chlorophyl-*a*	1									
Chlorophyl-*b*	.999*	1								
Carotenoids	.894	.872	1							
Ammonia	.999*	.997*	.908	1						
Nitrite	.617	.580	.904	.642	1					
Nitrate	-.228	-.272	.233	-.196	.626	1				
Phosphate	-.961	-.972	-.734	-.951	-.374	.489	1			
Silicate	-.838	-.812	-.994	-.855	-.946	-.341	.653	1		
Gross Productivity	.304	.259	.698	.334	.937	.859	-.027	-.774	1	
Net Productivity	.266	.222	.670	.298	.923	.878	.012	-.749	.999*	1

*Correlation is significant at the 0.05 level (2-tailed)

The decrease in chlorophyll content is due to the increased anthropogenic activities along the coastal region. During the period of study, the region where sample was collected was highly polluted by oil due to operation of boats. When there is oil and it spreads on the water surface and drift by wind and currents, the low-boiling fraction evaporates and the low-boiling aromatic fraction is easily dissolved in the water (Gunkel and Gassmann, 1980). Some algae have higher resistance to oil pollution such as, *F.vesiculosus* when compared with other macrophytes. This alga survives even by altering environmental factors such as desalination, negative temperature and ultra-violet radiation (Clendening and North, 1960; Eichenberger *et al.,* 1993). It is important to study the impact of oil and oil products on the coastal marine ecosystem. The effect of crude oil and other contaminates related to hydrocarbons on marine macro and micro algae can be assayed by growth measurements (Atlas *et al.,* 1976; Ukeles, 1965) or by means of metabolic or photosynthetic activities (Gordon *et al.,* 1973; Kusk, 1978; Soto *et al.,* 1975). Hence, it is important to study the chlorophyll content of the algae occurring in the coastal regions in respect to oil pollution. Further, studies regarding oil and chlorophyll interaction are to be assessed through continuous monitoring. In the present study, high pigment content of Chl a, b and carotenoid was found only in *Udotea indica* and all other species had lowest contents. This seaweed is benthic habitat and during high wave action it is drifted to the shore. In Tuticorin coast, industrial and domestic effluents were discharged in the coast and it may affect this species. Hence, further study is necessary to assess the impact of pollutants on seaweed pigments.

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