International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS™ | Volume 8 | Issue 3 | JUL-SEPT | 2018 | 918-925
Research Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

SPECTRAL AUTOGRAPHIC CHARACTERISTICS AND PIGMENT COMPOSITION OF MARINE MICROALGAE

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ABSTRACT

Absorbance and quantification of microalgal pigments from southeast coast regions (Chennai, Cuddalore and Parangipettai) was monitored using visible spectrophotometer followed by HPLC. Eight microalgae named Chlorella marina, Nannochloropsis sp., Dunaliella Salina, Platymonas sp., Tetraselmis tetrathele, Tetraselmis chuii, Chromulina sp and Synechocystis sp. were morphologically identified and each individual species were isolated by serial dilution followed by quadrant streaking. The isolated strains were cultured under in-vitro condition using Guillard's f/2 medium. The exponentially grown cells from 2nd to 8th day were subjected to absorption with spectra at 2-day intervals for determining pigments in microalgae. The obtained absorption spectra for each individual strain showed corresponding peaks with the accumulation of different photosynthetic and photo-protective pigments viz. Chlorophyll a, Chlorophyll b, β-carotene and Diatoxanthin etc. Pigments synthesized by all strains were extracted using acetone and were quantified by HPLC-DAD. The study concludes that the process of acclimation and adaptation of microalgae under in-vitro condition induces many neutraceutically active pigments at a higher concentration which might be due to different phenotypical molecular organization. The results obtained are also helpful to identify bio-marker pigments from different groups of microalgae.

KEY WORDS

Microalgae; Pigments; Spectral absorbance; HPLC.

1. INTRODUCTION

In marine environment microalgae frequently experience intensive variations such as high and low light intensity and nutrient concentrations, which is important for their growth and productivity [4]. Light is one of the most prominent sources for photosynthetic organisms like plants, algae and cyanobacteria [5]. They harvest light energy and transformed into chemical energy by the presence of photosynthetic pigments. Light directly affects the photosynthetic rate in the cell. When lead to photo oxidative exceeds damage and affects the growth [9]. To cope with these changes in light regime, microalgae have evolved various adaptive processes of acclimation and adaptation. Here by the evolutionary fitness of species within the environmental constraints are increased [9].

Recently the studies on the spectral composition of light reveal the ability of microalgae to finely balanced light harvesting and photo protective capacity. Moreover, the spectral absorption contributes the information on total in vivo pigment absorption in microalgae [13]. The knowledge of the types and concentration of the pigments provides the mature information regards the photosynthetic process and the algal adaptability at various environments [6].

Photosynthetic pigments in microalgae appear as highly complex structures whose separation has been proven to be confronting for decades. Further, the diverse molecular structures possess different polarities, such as acidic chlorophylls to the non-polar hydrocarbon carotenes. It is to be noted that the species composition of microalgae spatially and temporally affects the quality and quantity of the pigments [10]. So far, no single technique or approach has been sufficient for determining the evidence of relevant structure and dynamics of phytoplankton community. High performance liquid chromatography has been serving as a gold standard for measuring pigment concentrations in al biotic sources [11]. Hence our study aimed to evaluate the distribution and abundance of photosynthetic pigments in microalgae of different species. Special attention was given to the culturing technique and pigment determination techniques, in order to determine the diversity of the pigments in micro algal population.

2. MATERIALS AND METHODS

2.1. Sample collection and cultivation

Sea water samples were collected from different landing centers in the Southeast coastal area of India, *viz.* Chennai (13° 7' 52.5576"N / 80° 18' 20.6064"E), Cuddalore (11° 18' 20.6064"N / 79° 47' 12.4512"E) and Parangipettai (11°29'58.9452"N / 79°46'39.6984"E). The samples were collected during pre-monsoon season in the month of June. Plankton nets (diameter -1.5 m) made up of bolting silk cloth (mesh size of 20 µm) was employed to collect the samples, which were stored in sterile conditions.

2.2. Identification of micro algae

The aliquots from the mixed species were collected and subjected to compound microscope examination. Microscopic identification of the algal species was performed under 100 x magnifications. The wet mounts of the species were taxonomically determined using identification manual [14]. Then the aliquots of mixed species were subjected to serial dilution followed by the quadrant streaking for pure cultures. Stock cultures of pure cultures were maintained in a special room adjacent to the mass culture and maintained in Guillard's f/2 medium and asses the growth potentials at regular spectral course.

2.3. Absorption spectra

Absorption spectra of unialgal species, which is obtained from the aseptic techniques were monitored by regularly/day. Spectral progression was measured using visible wavelength ranges from 400nm-700nm. Thus, enables the total pigments in algae with respective wavelength.

2.4. Identification of pigments

The algal cultures (each 10ml) were filtered using GF/F and stored at-20°C until further analysis. The presences of pigments were screened using HPLC analysis coupled with the DAD system to evaluate the pigments existence in the individual species with the help of pigment standards (Beta apo carotenoids).

2.5. High Performance Liquid Chromatography (HPLC)

2.5.1. Sample preparation for HPLC

The frozen samples were kept outside and thaw for seconds then mix with 90% acetone. (Here we use beta apo carotenoid as an internal standard) and sonicate the algal culture samples about 30 seconds, to break the hard-siliceous cell wall. Sonicated samples were centrifuged 10,000 rpm for 10 minutes at 4^0C. The supernatant was filtered through 0.22µm filter with syringe- to avoid the larger particles into the column. Finally, samples were injected to the instrument. Mobile phase: method Eluent A: Methanol 50ml: Acetone 30ml: Pyridine 20ml. Eluent B: Methanol 20ml: Acetone 60ml: Acetone 20ml.

3. RESULTS

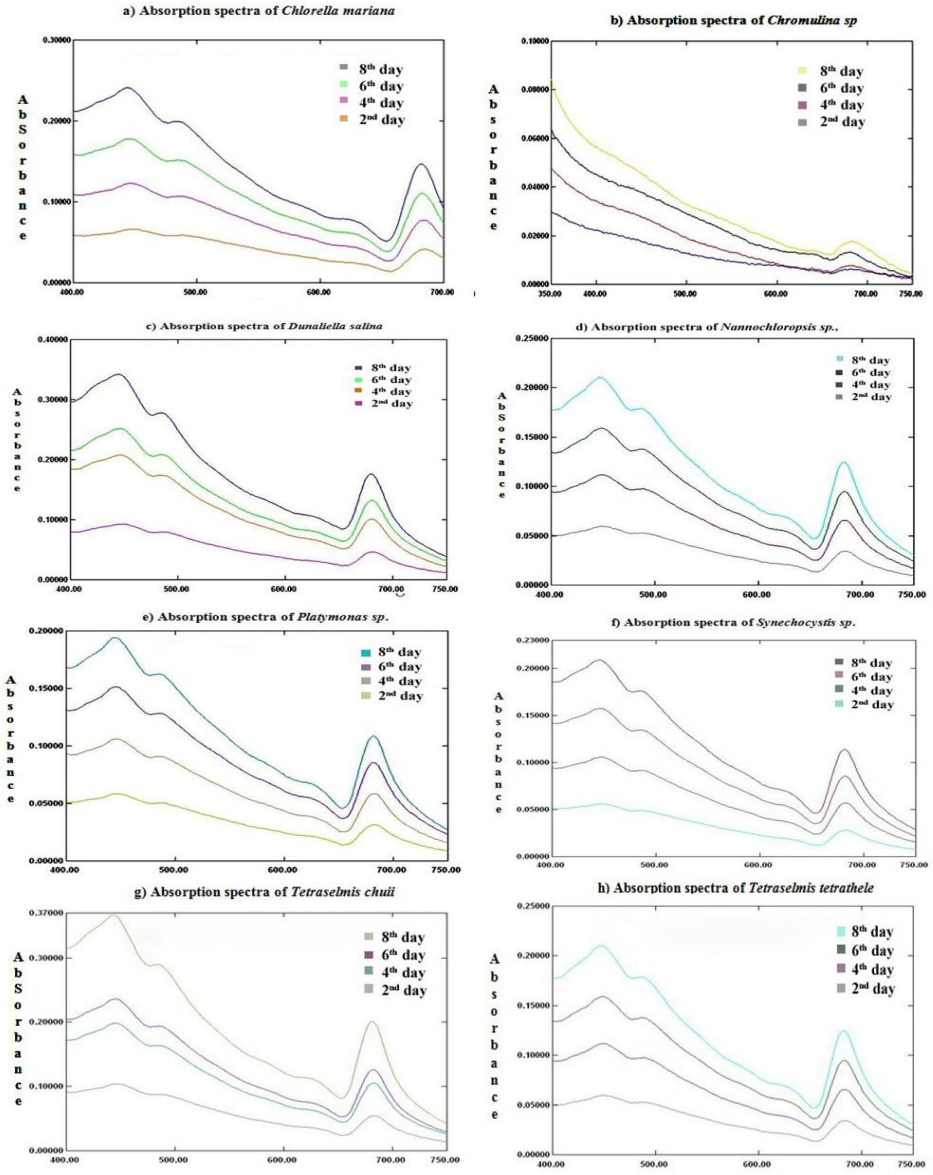
3.1. Identification of Micro algae

Totally 8 species have been collected from mixed cultures. The species collected, isolated and purified from seawater were identified as *Chlorella marina*, *Nannochloropsis sp*., *Dunaliella Salina*, *Platymonas sp*., *Tetraselmis tetrathele*, *Tetraselmis chuii*, *Chromulina sp*., *Synechocystis sp*.

3.1.1. Visible spectroscopy

The spectral images of the different microalgae members were as depicted in Fig. 1 (a-h).

Fig. 1. (a-h) Absorption spectra for the unialgal cultures of phytoplankton



Different pigments and their wavelength of 8 algal species are shown in Table 1.

Table 1. Different microalgae species wavelength (nm) and Pigments

S.No	Name of the species	Wavelength (nm)	Pigments
1	*Chromulina sp*	443,420,490,447,453	Chl a, Pra, αC, βC, An, Zea
2	*Dunaliella salina*	434,444,443	Hex-Fuc, Dt, Chlo a,
3	*Chlorella marina*	443,454,515,480	Chla, an, βc, Chl b
4	*Nannachloropsis sp.*	420,448,490	Per, Dn, βC
5	*Platymonas sp.*	448,670,685	Dn, Chla, Gyr
6	*Synechocystis sp.*	420,448	Per, Dn
7	*Tetraselmis chuii*	448,420,480,	Dn, Per, Chl b
8	*Tetraselmis tetrathele*	420,448	Dn, Per

(Chl a - Chlorophyll a, Pra – Prasinoxanthin, αC - Alpha carotene, βC – Beta carotene, An - Antheraxanthin, Zea – Zeaxanthin, Hex-Fuc – Hex Fucoxanthin, Dt - Diadinoxanthin, Chlo a, Chl b - Chlorophyll b, Per - Peridinin, Dn – Dinoxanthin and Gyr – Gyroxanthin)

3.2. HPLC chromatogram of different algal species and its pigments patterns

The pigment composition of individual species was analyzed by HPLC-DAD system and the results showed the presence of prominent pigments such as Chlorophyll-C2, Peridinin, Astaxanthin, Diadinoxanthin, Dinoxanthin and Alloxanthin. The HPLC chromatograms for native pigments of microalgae are illustrated in Fig. 2-10. Maximum and minimum concentrations of pigments present in the microalgal species are shown in Table 2.

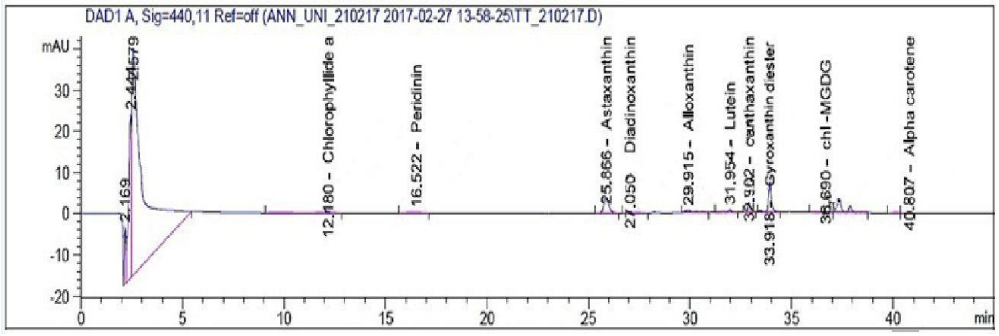


Fig. 2. HPLC spectrum of *Chromulina sp.* showing the presence of pigments Prasinoxanthin, Astaxanthin, Antheraxanthin, Zeaxanthin, Gyroxanthin, Chlorophyll b, Chlorophyll a, Pheophytin and Beta-carotene. Comparatively prasinoxanthin (237.59240ng/µl) was the most predominant, which is species specific in nature.

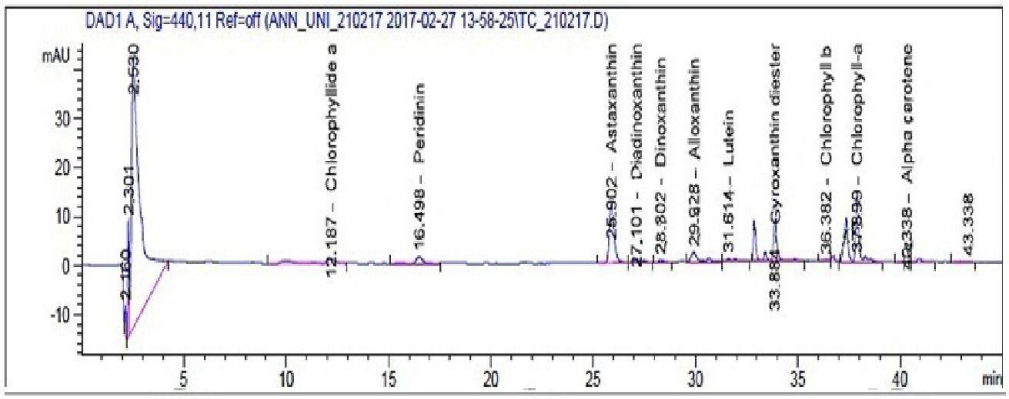


Fig. 3. HPLC spectrum of *Chlorella marina*showing the presence of pigments Perdinin, Astaxanthin, Diadinoxanthin, Dinoxanthin, Alloxanthin, Lutein, Canthaxanthin, Gyroxanthin, Chlorophyll b, Chlorophyll a and Pheophytin a. among the pigments present in chlorella species chlorophyll **a** (116.38128ng/µl) and **b** (3.67731ng/µl) is one of the dominant in Chlorophyceae. (Dos santos *etal.,*) Rest of them are accessory pigments especially dinoxanthin and diadinoxanthin are the photoprotective pigments when it encounters too much of light.

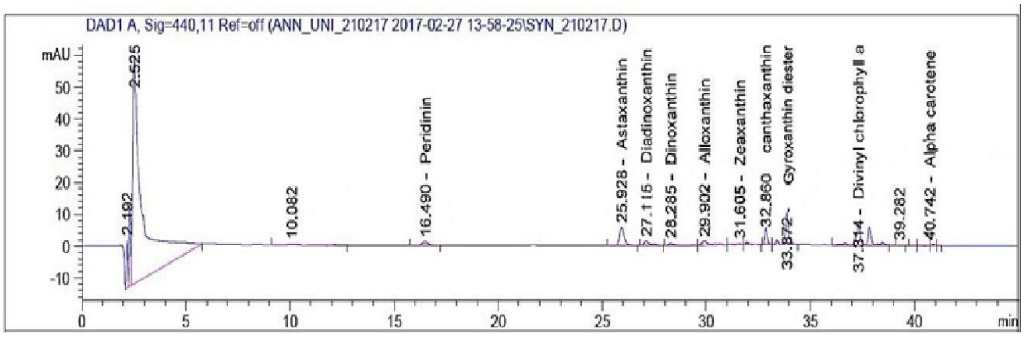


Fig. 4. HPLC spectrum of *Dunaliella Salina* explores the pigments of Neoxanthin, Hex-Fucoxanthin, Antheraxanthin, Diatoxanthin, Lutein, Canthaxanthin, Chl C2-MGDG, and Alpha-carotene. From the above results clearly shows the presence of light harvesting pigments (Chlorophylls) include the photosynthetic carotenoids (Fucoxanthin). Which

denotes the culture photo - acclimation condition when high light exposure. Here the highest concentration was found in diatoxanthin (953.66202ng/µl) whereas the unique Neoaxanthin of 9.76306ng/µl was found in same species.

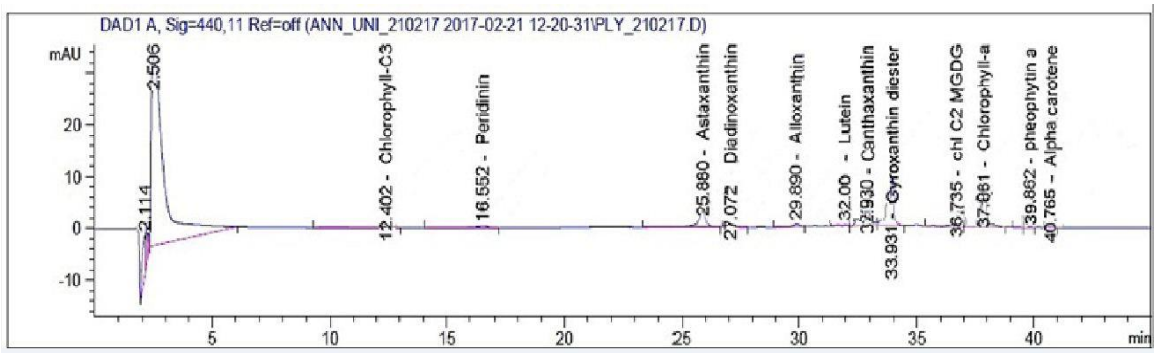


Fig. 5. HPLC spectrum of *Nannochloropsis sp*. show the pigments of Peridinin, Astaxanthin, Diadinoxanthin, Dinoxanthin, Alloxanthin, Leutin, Canthaxanthin, Gyroxanthin, Chlorophyll b and Beta-carotene. These are the photo synthetically active pigments are also called photoprotective carotenoids (PPC). Here the highest pigment concentration was measured at 166.84651ng/µl of Peridinin and the lowest were found in alpha carotene (6.80244ng/µl).

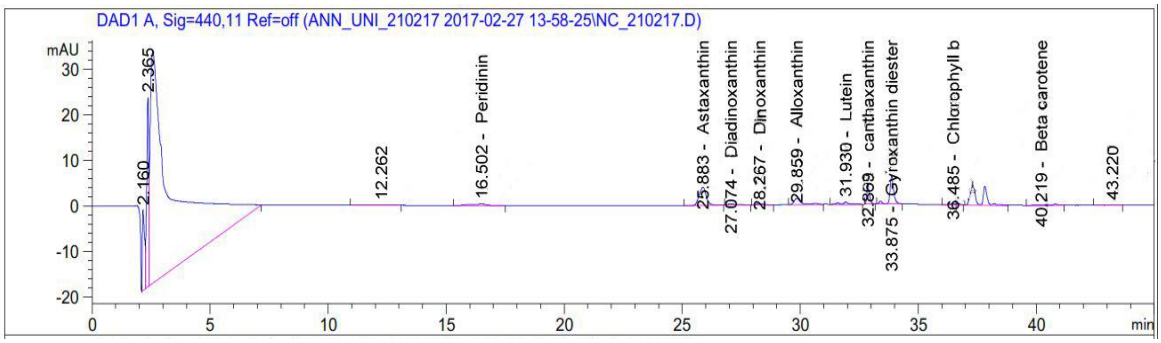


Fig. 6. HPLC spectrum of *Platymonas sp*. showing the presence of pigments Chlorophyll C3, Peridinin, Astaxanthin, Diadinoxanthin, Alloxanthin, Canthaxanthin, Chlorophyll C2, Chlorophyll-a, Pheophytin a and Alpha carotene. The highest pigment concentration was noticed at chlorophyll –a (315.04421ng/µl) and lowest concentration of alpha carotene at the concentration of 5.80595 ng/µl.

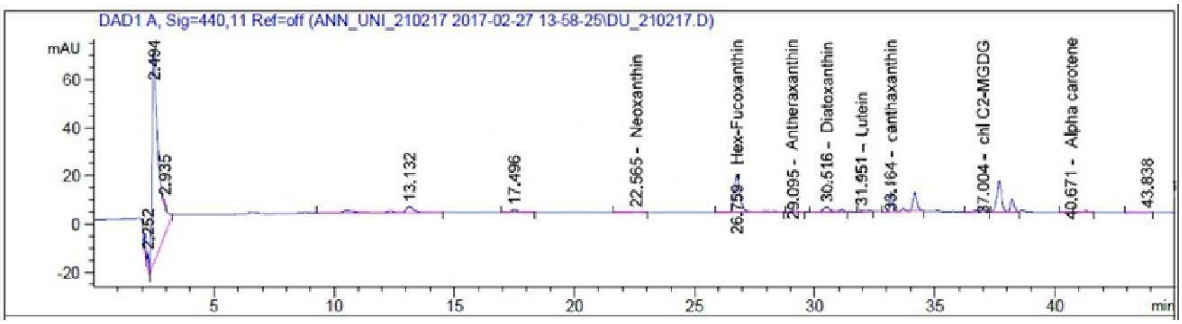


Fig. 7. HPLC spectrum of pigments present in *Synechocystis sp.* of Peridinin, Astaxanthin, Diadinoxanthin, Dinoxanthin, Alloxanthin, Zeaxanthin, Canthaxanthin, Gyroxanthin, Divinyl Chlorophyll-a and Alpha carotene. The highest pigment concentration was found in 651.72414 ng/µl of diadinoxanthin and the lowest concentration at 2.88902 ng/µl of Zeaxanthin.

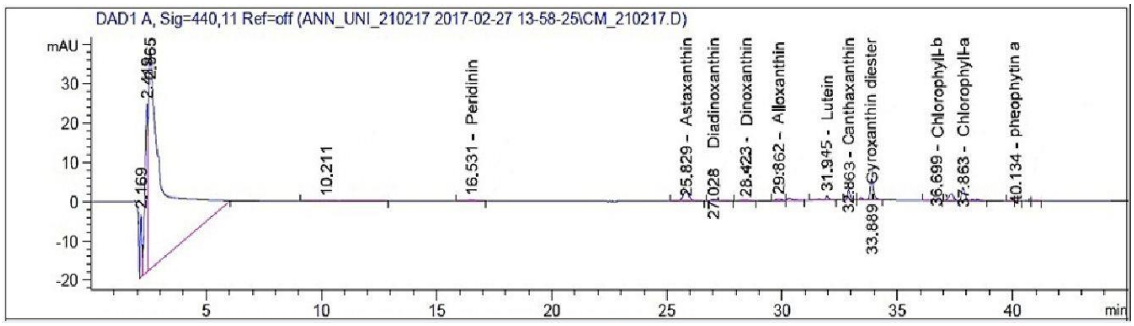


Fig. 8. HPLC spectrum of *Tetraselmis chuii* pigments of Chlorophyllide a, Peridinin, Astaxanthin, Diadinoxanthin, Alloxanthin, Lutein, Gyroxanthin, Chlorophyll-b, Chlorophyll-a andalpha carotene. The highest pigment concentration of Tetraselmis chuii was measured at 431.93715 ng/µl whereas alpha carotene was found at lower concentration of 5.88884 ng/µl.

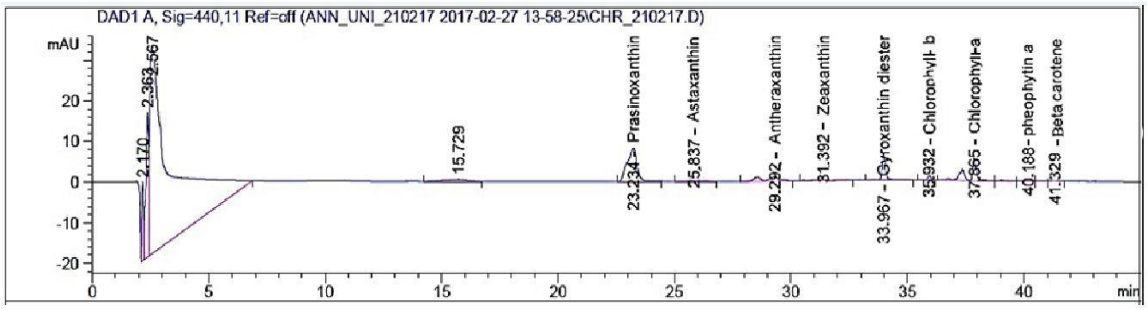


Fig. 9. HPLC spectrum of *Tetraselmis tetrathele* pigments are Chlorophyll a, Peridinin, Astaxanthin, Diadinoxanthin, Alloxanthin, Lutein, Gyroxanthin, Chlorophyll C2 and Alpha-carotene. Here the highest concentration was found in diadinoxanthin at 195.14550 ng/µl and the lowest was noted on chlorophyll b at 3.77144 ng/µl.

Table 2. Maximum and minimum concentrations of pigments present in the microalgae species.

S. No	Name of the Species	Name of the pigments	Concentration mg/µl	
			Maximum	Minimum
1	*Chromulina sp*	α-C, An, As, β-C, Chl-a, Chl-b, Pra, Zea.	237.5924 (Pra)	6.69032 (Zea)
2	*Dunaliella salina*	α-C, An, β-C, Can, Chl-a, Chl-C2, Dt, Hex-Fuc, Leu, Neo.	953.66202 (Dt)	9.76306 (Neo)
3	*Chlorella marina*	Al, α-C, As, β-C, Can, Chl-a, Chl-b, Dn, Div-chl a, Gyr, Hex-Fuc, Leu, Neo.	255.16056 (Dn)	3.67731 (chl-b)
4	*Nannachloropsis sp.*	Al, α-C, As, β-C, Can, Div-chl a, Dn, Dd, Gyr, Leu, Per.	237.7671 (Dd)	6.8024 (α-C)
5	*Platymonas sp.*	Al, α-C, As, Can, Chl-a, Chl-b, Dd, Gyr, Leu.	315.04421 (Chl-a)	5.80595 (α-C)
6	*Synechocystis sp.*	Al, α-C, As, β-C, Can, Div-chl a, Dn, Dd, Gyr, Leu, Per, Zea.	651.72414 (Dd)	2.88902 (Zea)
7	*Tetraselmis chuii*	Al, α-C, As, β-C, Chl-a, Chlo-a, Chl-b, Dn, Dd, Gyr, Per, Leu.	431.93715 (Chl-a)	5.88884 (α-C)
8	*Tetraselmis tetrathele*	Al, α-C, As, β-C, Can, Chlo-a, Chl-b, Div-Chl a, Dd, Gyr, Per, Leu.	145.72974 (Per)	3.77144 (Chl-b)

(Chl a - Chlorophyll a, Pra – Prasinoxanthin, αC - Alpha carotene, βC – Beta carotene, An - Antheraxanthin, Zea – Zeaxanthin, Hex-Fuc – Hex Fucoxanthin, Dt - Diatnoxanthin, Chl b - Chlorophyll b, Per - Peridinin, Gyr – Gyroxanthin diestor, As – Astaxanthin, Can - Canthaxanthin, $Chlc_2$ – Chlorophyll c_2, Leu – Leutin, Neo – Neoxanthin, Al – Alloxanthin, Dn – Dinoxanthin, Div-chla – Divinyl chlorophyll a, Dd – Diadinoxanthin and Chlo-a – Chlorophyllide a,)

4. DISCUSSION

Marine microbial communities are incredibly diverse and consisting of interconnected groups of cyanobacteria, heterotrophic bacteria, archaea, viruses, eukaryotic phytoplankton and protists [2]. *Chlorella marina*, *Nannochloropsis sp*., *D. Salina*, *Platymonas sp*., *T. tetrathele*, *T. chuii*, *Chromulina sp*., *Synechocystis sp*. were shown variety of pigment profiles.

According to [7], the organization and energy regulation mechanism of thylakoid membrane may change the light harvesting complex present in phytoplankton. Moreover, in contrast to other plankton groups, green algae possess both stacked and unstacked membrane region of thylakoids with equal proportions of PSI: PSII on both regions [1]. Except *chromulina sp*., all species belongs to *Chlorophyceae* having dinoxanthin, diadinoxanthin and diatoxanthin which explores the presence of photo protective carotenoids at high light exposure. Diadinoxanthin converted into diatoxanthin within 5 minutes [3] and diatoxanthin (953.66202ng/µl) present in *D. salina* comes under above process. Whereas in *Chromulina sp.* doesn't have lateral segregation of PSI and PSII [7]. Prasinoxanthin (237.59240ng/µl) followed by chlorophyll a (187.65103ng/µl) was the dominant pigment present *Chromulina sp.*

Remarkably, peridinin, prasinoxanthin, violoxanthin and fucoxanthin are the highly performed light harvesting pigments in most bloom forming phytoplankton species [15]. However, the peridinin was present in 4 species which include *Nannochlopropsis sp.* (166.84651 ng/µl), *T. tetrathele* (145.72974 ng/µl), *T. chuii* (213.13036ng/µl) and *Synechocystis* sp. (183.73088ng/µl) at increasing concentrations, respectively. It may due to the effect of light harvesting and utilization in chloroplast [12], as of the changes in intracellular self-shading and the optical signature from the chloroplast [8].

5. CONCLUSION

Microalgal pigments profiles were enable using analytical methods of spectrophotometer followed by HPLC which explore the micro algal pigments quantitatively. Hence the results clearly describe the species-specific pigments of different groups of micro algae (Chlorophyceae, Eustigmophyceae and Chrysophyceae) which were measured at visible wavelength from 400-700nm. Based on the results, *D. salina* showed the prominent pigments of diatoxanthin (953.66202ng/µl) and it shows peaks maxima at 454nm and minor peaks of chl c_2 (444nm) & $chlc_1$ (443nm) like diatoms. However the class of Eustigmophyceae, Cyanophyceae, Prasinophyceaeand Chlorophyceae with respective species *Nannochloropsis sp* (166.84651ng/µl), *Synechoystis sp* (183.73088ng/µl), *Tetraselmis tetrathele* (145.72974ng/µl), *Tetraselmis chuii* (213.13036ng/µl) has dominant pigments of peridinin respectively observed via HPLC as well as spectral absorbance at 420nm.Whereas *chromulina sp (ChryOphyceae)* shows absorption maxima at 420nm and HPLC quantification of 237.59240 ng/µl with the dominant pigment prasinoxanthine was measured. On the whole the results of the pigment level variation while photo acclimation were studied, which is related to the fact that the in-vitro environment is highly light encouraged. In this respect, diatoms seem to be a promising source of unique bioactive compounds, with fucoxanthin, diadinoxanthin and diatoxanthis as representatives of photosynthetic pigments. These kinds of studies will be helpful to identify bio-marker pigments from different group of phytoplankton in coastal waters and to identify their spectral absorbance peaks and otherwise their reflectance signals in linking with satellite sensor-based observations and to link with hyper spectral remote sensing.

ACKNOWLEDGMENTS

Authors are thankful to authorities of Space Application Centre (SAC-ISRO), Government of India, Ahmedabad for providing financial support during the study period as part of ISRO's MOP-3 Collaboration Project. Authors are thankful to Annamalai University, Parangipettai for providing facilities to carry out this study.

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Received:04.05.18, Accepted: 07.06.18, Published:01.07.2018

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