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Morphology and Biochemical Study of a Microalga *Euglena tuba* Reported from the Aquatic Ecosystem of Cachar

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Research Article

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ABSTRACT

Euglena tuba is a fresh water microalga available throughout the year in the aquatic bodies of Cachar district. This unique organism shows immense potentiality in terms of morphological characteristic as well as biochemical properties. *E. tuba* is a model species which have high concentration of pigments like chlorophyll a, b and carotenoid, carbohydrate and also protein content in its cell. Maximum concentration of chlorophyll a, carotenoid, protein and carbohydrate (11.97 µg/ml, 12.87 µg/ml, 120.94 µg/ml, 188.52 µg/ml) values were recorded during the study period. Therefore, present study concluded that utilization of E. tuba for different type of medical base product like protein tablets, some high value byproduct like pro glucose, pigmentation cream, nutritive based products can be possible for the benefit of mankind.

INTRODUCTION

Euglena tuba is found in freshwater environment in high numbers with nutrient rich condition. It represents one of the earliest derived eukaryotic protist with both plant and animal like features. *E. tuba* requires H, C, N, O, Mg, P, S, Cl, K, Ca, Mn, Co, Zn and some other elements at very low levels [1]. It produces oxygen at a high rate, reduce carbon dioxide and breaks down organic matter [2]. *E. tuba* possess elongated cell with one nucleus that contain pigmented chloroplast which helps in photosynthesis, a contractile vacuole for excretion, an eye spot to spot sunlight and flagella for movement.

Chlorophyll a, chlorophyll b, carotenoids like xanthophyll, astaxanthin (euglenorhodone), zeaxanthin, and carotene mainly beta-carotene are the common pigments in *Euglena*. Chlorophyll and carotenoids are fat soluble molecules which are extracted from thylakoid membranes with the help of organic solvents such as acetone, methanol, etc. but phycobilins and peridinin are water soluble that can be extracted from the algal tissues after the organic solvent extracted from those tissues. In some cases red coloration of water occurs due to the presence of increase in the xanthophyll pigment called astaxanthin or euglenorhodone or hematochrome. *Euglena* contains xanthophyll pigments like diadinoxanthin and diatoxanthin but lutein, fucoxanthin and violaxanthin are not present [3]. At high light intensities *Euglena* lacked xanthophylls pigment [4]. Sorby [5] (1873) classified blue chlorophyll as chlorophyll a, green chlorophyll as chlorophyll b and orange- yellow as xanthophyll according to the pigment colour. Chlorophyll is a key biochemical component in the molecular apparatus that is responsible for photosynthesis. It is a metal-chelate which is bonded to a large organic molecule called a porphyrin. Pigmentation in Euglenophyta is found to be due to the presence of carotenoids such as carotene, zeaxanthin, neoxanthin and diadinoxanthin [6,7]. The role of carotenoid pigments in algae is not exactly known but it is suggested that they function as a passive light protecting filter and it has the role of accessory pigments transferring energy and oxygen [8-10]. Carotenoids are the secondary pigments which support the primary pigment i.e., chlorophyll in energy transfer during the process of photosynthesis and have high antioxidant potentiality by scavenging free radical [11]. In *Euglena*, carotenoids are found to play a major role in protecting chloroplasts against photosensitized oxidation [12]. It is the most important photosynthetic pigments and it prevents chlorophyll and thylakoid membrane from the damage of absorbed energy by

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photo-oxidation [13]. It contain a conjugated double bond system of the polyene type (C-C=C-C=C). Energy absorbed by carotenoids is transferred to chlorophyll a for photosynthesis.

Various studies have indicated that the carotenoids may have prevent or inhibit certain types of cancer, arthrosclerosis, age-related muscular degeneration and other diseases. At sufficiently high concentrations, it can protect lipids from peroxidative damage [14]. It also has anti proliferative effect on various cancer cell lines e.g., lycopene has been shown to inhibit cell cycle progression in breast, lung and prostate cell lines. β- Carotene has been shown to inhibit the expression of antiapoptotic protein Bcl-2 in cancer cells, thus it reduce the growth of cancer cells to some extend [15,16]. Paramylon is the characteristic carbohydrate reserve of the Euglenophyta [17] which is similar to starch. It accumulates in *Euglena* when it grown on an organotrophic medium in the dark and is consumed either in the dark when cells receive or at the end of the exponential growth phase or when cells are transferred to the light source [18- 21]. The chloroplasts found in *Euglena* contain chlorophyll which aid in the synthesis of carbohydrates which is stored as starch granules and paramylon. In *Euglena* paramylon is made in the pyrenoids. The eugenoids have chlorophylls a and b and they store their photosynthate in an unusual form called paramylon starch, a ß-1, 3-polymer of glucose. The paramylon is stored in rod like bodies throughout the cytoplasm. These are called paramylon bodies and are often visible as colorless or white rigid rods [22]. Protein or amino acids are the by-products of an algal process for the production of other fine chemicals or with appropriate genetic enhancement, microalgae could produce desirable amino acids in sufficiently high concentrations [23]. The high protein content of various algal species is one of the main reasons to consider them as an unconventional source of protein [24]. *Euglena* is an organism with a number of interesting characteristics like it has three membranes rather than two membranes surrounding its chloroplasts [25] which have implications for the targeting of nuclear-encoded chloroplast proteins.

The present work is an attempt to investigating the morphological character accompanied with biochemical potentiality of *E. tuba*.

MATERIAL AND METHODS

Study area

Cachar district in Southern Assam lies between latitude 90° 44' E and longitude 20° 22' N, encompassing a total geographical area of about 3,7861 km^2 and is drained by the River Barak and its tributaries. The climate is subtropical, warm and humid during the summer (July/August) and the temperature is generally recorded during December- January. Relative humidity ranges from 60-70%. The district receives about 3200-3500 mm rainfall during the year. 16 ponds were selected in different strategic location to carry out the experiment i.e., in north, south, east and west **[Figure 1]** direction and algal samples were collected bimonthly from July 2009 to May 2010.

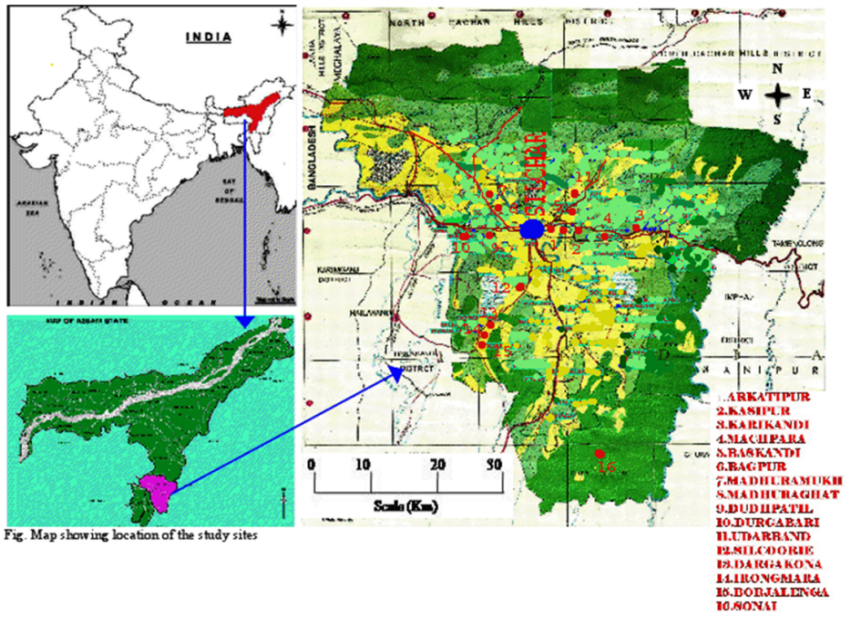

Fig. Map showing location of the study sites

Figure 1. Map showing the study sites.

Euglena species analysis

The samples were collected from different study sites by using plankton net made of No. 25 bolting silk. The strained samples were concentrated to a constant volume. Several drops from each sample were examined from 50 random fields of the mounds. Microscopic observations of the cell morphology such as size of the organism, presence or absence of chloroplast,

eyespot, flagella, paramylon, etc. were taken into account and identified by following standard protocol [26,27].

Estimation of biochemical properties

Chlorophyll (a and b) and carotenoid content were estimated by extracting the samples with 90% acetone following cold extraction method [28]. Total carbohydrate was estimated by Anthrone method [29]. Protein was measured by Lowry's method [30]. All tests were performed three times.

RESULT AND DISCUSSION

Algal characterization

The microscopic observation of cell morphology were taken into account and identified as *E. tuba* Carter. Microscopic observation of random samples after centrifugation assured that the samples were free from any other algae as well as phytoplankton contaminations which were supported by earlier researchers that the *Euglena* blooms occur at higher temperature, lower dissolved oxygen and acidic environment with higher nutrient concentration which have significantly inhibit the growth of other algal species and phytoplankton [31-35]. Identified characters of *E. tuba* was given in **Table 1**.

Table 1. Shows the characteristic of algal cell.

Sl.No.	Characters	Description
1	Color	Cells normally green (if red, only temporarily so and then brick – rather than blood red)
2	Periplast	Periplast without spiral rows of granules
3	chloroplast	Cells with other forms of or more than one chloroplast
4	Paramylon	Paramylon bodies are present but margins not convolute
5	Cell forms	Cells fusiform or somewhat cylindric , not produced into a long , fine point posteriorly
6	Motility	Cells highly metabolic, constantly changing shapes in movements
7	Cell Structure	Cells larger, longer than five times their diameter; elongate-fusiform or sub-cylindric, abruptly tapering posteriorly, forming a blunt tip.

Morphological structure of *Euglena tuba*

E. tuba is a freshwater protozoon which changes its body structure continuously. When the fresh sample was observed under microscope three different structural forms were found i.e. elongated, oval and spherical in one species. The size of elongated structure was about 65-105 µm long and 20-24 µm broad, oval form was the size of 60.086 µm long and 43.174 µm broad and the spherical structure was 50.158 µm long and 48.653 µm broad **[Figure 2.i.].** It change its shape and size in a continuous basis so it was very difficult to observed the ultra-structural forms, when it attain round and oval size, the whole mass of body become compact and the arrangement of organelles was not clearly understood. Basically the cells were motile, ovoid-pyriform to sub cylindrical, narrowed gradually posteriorly to a short, blunt tip, periplast with spiral striations. The whole structure of *Euglena* was irregular and spontaneously pulsating, i.e. exhibiting a rhythmic motion of expansion and contraction. It moves with the help of flagellum which was about the length of the body and located on the anterior end and twirls in such a way as to pull the cell through the water, it was attached to the bottom of the reservoir. The colour of the reservoir was grey and the flagellum was black. Chloroplast was visible as several disc-like structures throughout the cell with laciniate margins which trap sunlight used for photosynthesis and the colour of it was green. *Euglena* has an eyespot at the anterior end that detects light and was placed near the reservoir. Eyespot allows the cell to sense light direction and intensity according to this the cell respond to it by swimming either towards the light or away from the light (positive or negative phototaxis). Eyespot helps the cell in finding an environment with optimal light condition for photosynthesis. Eyespots are the simple form and composed of photoreceptors. The colour of the eyespot was red because of the presence of carotenoid pigments. It was composed of paraflagellar bodies which connect the eyespot to the flagellum. *Euglena* has a stiff pellicle outside the cell membrane that helps to keep its shape and was flexible in nature; the colour of the pellicle was blue. In the center of the cell nucleus was placed which contain cell´s DNA and control cell´s activities. The nucleolus was found within the nucleus and the colour of the nucleus was purple and the colour of the nucleolus was pink. In the interior portion of the cell a jelly like fluid substance was found which is known as cytoplasm and its colour was light yellow. Towards the posterior portion of the cell a star like structure called contractile vacuole was present which help *Euglena* to remove the excess of water. The colour of the contractile vacuole was orange. The whole structure of *E. tuba* was localized in a dark brown granular mass **[Figure 2.ii].**

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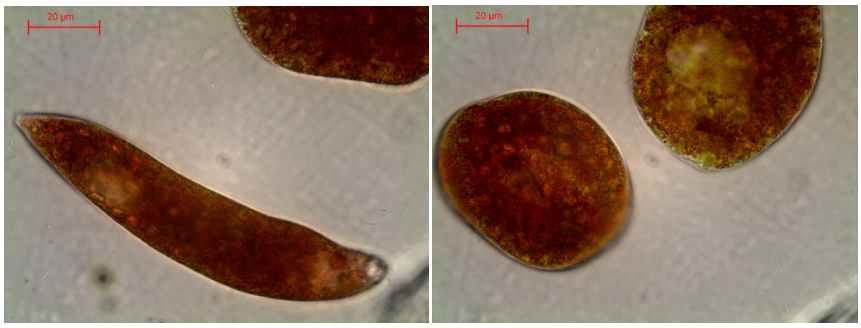


Figure 2(i). The elongated and spherical structure of *E. tuba*.

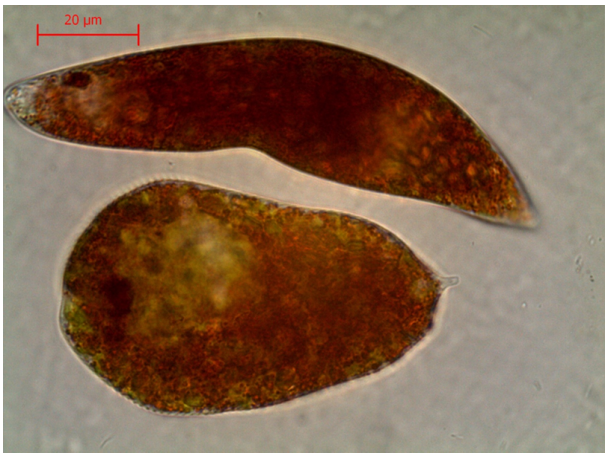
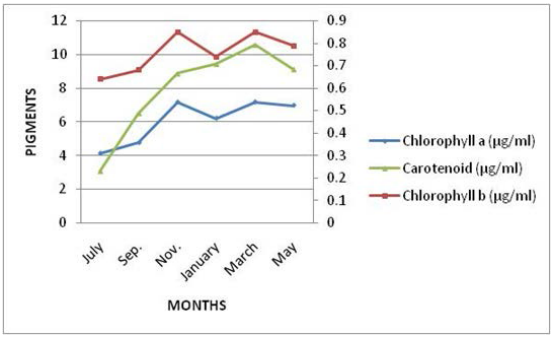


Figure 2(ii). Structure of *E. Tuba.*

E. tuba that are grown at different light intensities show remarkable changes in their chemical composition, pigment content and photosynthetic activity [36]. Biochemical properties and pigment concentration are largely depends upon the type of algae. Figure 3(i-xvi) shows the changes of pigments in *E. tuba*. In general carotenoid was found highest in almost all the ponds except Madhuraghat, Dudhpatil, Udarband, Barjalenja and Sonai. Chlorophyll b was always lower than other pigments. In Arkatipur, Chlorophyll a (7.18 µg/ml) and b (0.85 µg/ml) were highest in March. Both chlorophyll and carotenoids were low in July. The measurement of chlorophyll a is an index of water quality and phytoplankton biomass of any aquatic ecosystem [37-41]. Carotenoid gradually increased from the month of July then decreased with a fall in May. While chlorophyll a was found to be more or less stable. During month of March and May, in Baskandi carotenoid showed heavy fluctuation with its lowest concentration in



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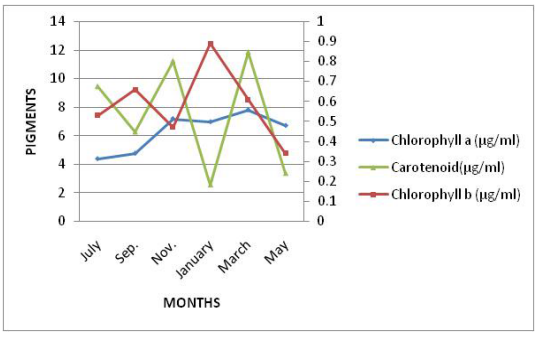
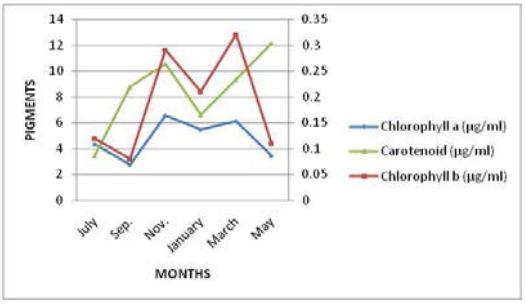
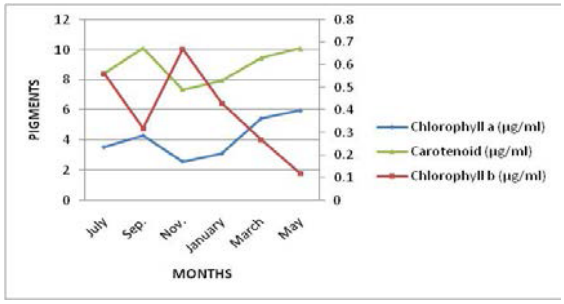


ii. Baskandi



iii. Karikandi



iv. Machpara

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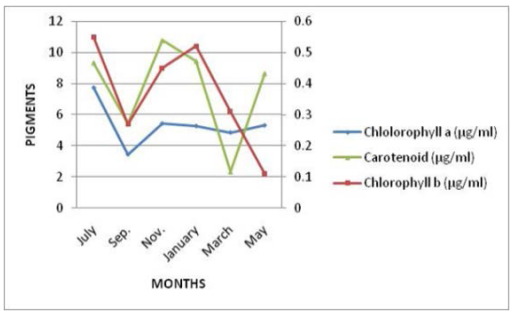
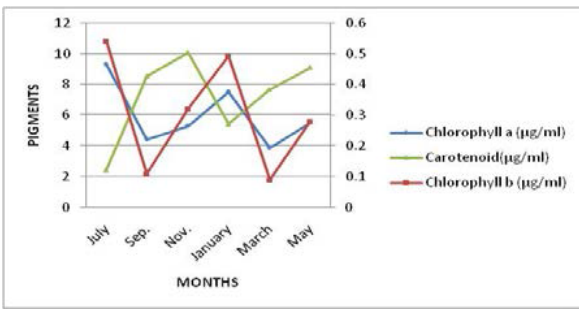


v. Kashipur



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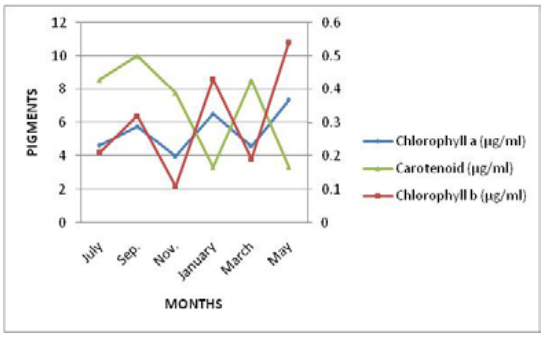
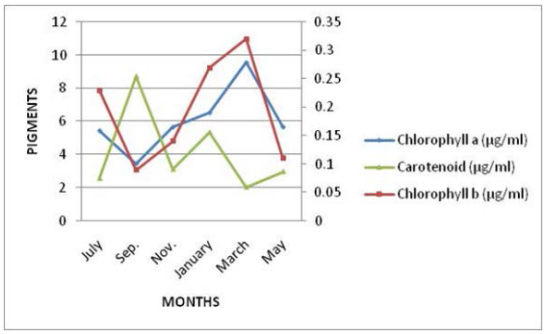
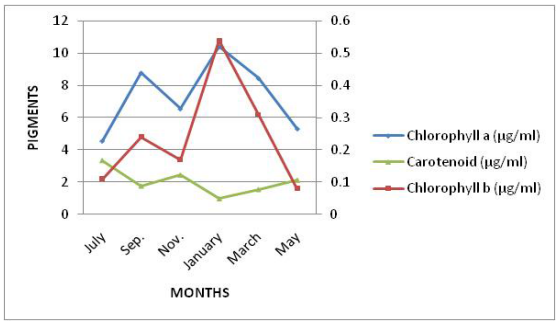


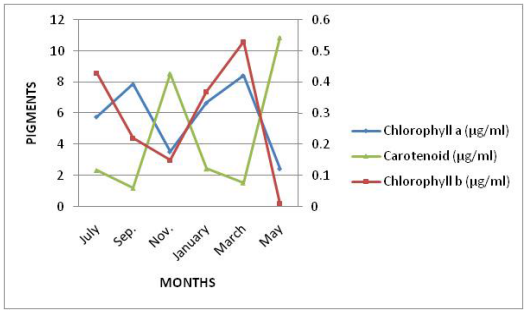
vii. Madhuramukh



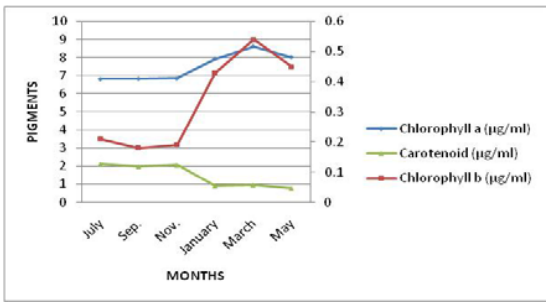
viii. Madhuraghat



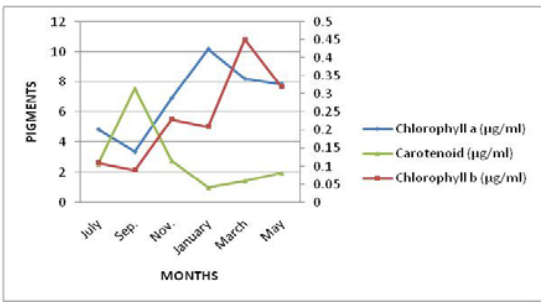
ix. Dudhpatil



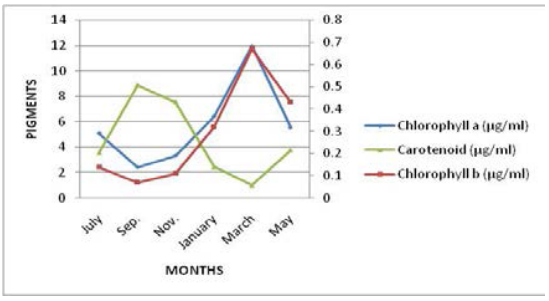
x. Durgabari



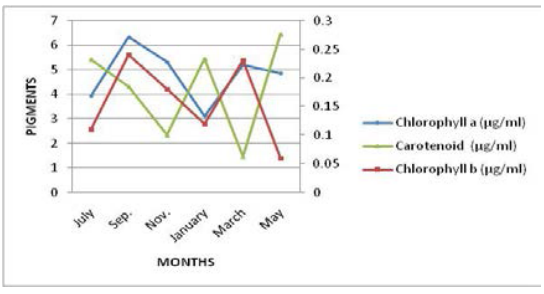
xi. Udarband



xii. Silcoorie



xiii. Dargakona



xiv. Irangmara

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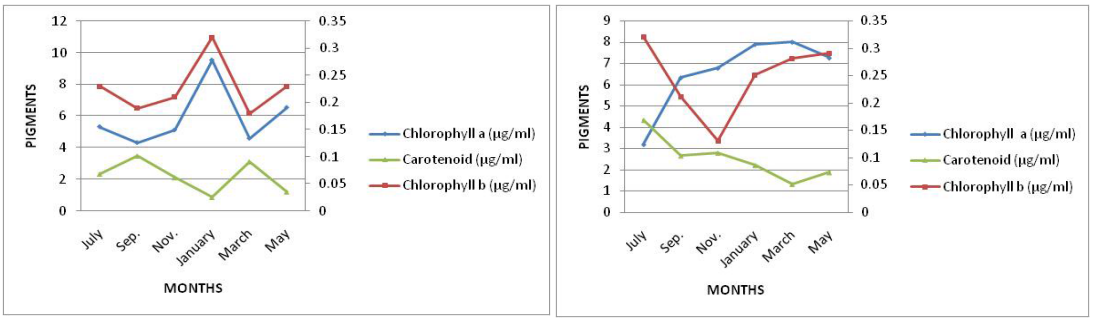

xv. Barjalenga **xvi.** Sonai

Figure 3(i-xvi). Variation of photosynthetic pigments of *Euglena tuba* cell in 16 aquatic bodies. All data are expressed as mean ± S. D. (n=3).

January (2.54 µg/ml) when chlorophyll b was highest (0.89 µg/ml). In Karikandi carotenoid was found to increase initially from the month of July to November with a sudden fall in January then gradually increased towards May. A decrease in chlorophyll a concentration from 6.12 µg/ml to 3.45 µg/ml was noticed in the month of May. In Machpara chlorophyll a and carotenoid shown a similar trend from July to May while chlorophyll b have a fall from November to May. Carotenoid concentration fell down to its lowest point in March (2.32 µg/ml) then again increased in May (8.65 µg/ml) in Kashipur while chlorophyll a did not differ much in its concentration. In Bagpur lowest carotenoid was observed in July (2.45 µg/ml) when both chlorophyll a and b were highest (9.32 µg/ml and 0.54 µg/ml). Chlorophyll b was greatly fluctuating in Madhuramukh pond with its highest value (0.54 µg/ml) in May when carotenoid was lowest (3.34 µg/ml). In Madhuraghat chlorophyll a was higher than the carotenoid from the month of November (3.11 µg/ml) to May (2.98 µg/ml). Increase in chlorophyll a (9.55 µg/ml) followed by decrease in carotenoid in March (2.03 µg/ml). Chlorophyll b gradually increased from September (0.09 µg/ml) to March (0.32 µg/ml) then fell down in May (0.11 µg/ml). Increases in chlorophyll a concentration in the water and pH is related to *Euglena* density whereas oxygen concentration changes are related to the changes in the density of euglenophytes [42]. Chlorophyll b had its peak in January in Dudhpatil. In this pond chlorophyll a was always higher than the carotenoid. In Durgabari carotenoid was lowest in September (1.21 µg/ml) and highest in May (10.87 µg/ml) when chlorophyll a and b reached it's lowest. Euglenophytes (*Euglena*) made up more chlorophyll a than diatoms, chlorophytes and especially cyanobacteria [42,43]. Carotenoid was found to be far lower than the chlorophyll a and b. Carotenoid was found to be more or less stable from July (2.11 µg/ml) to November (2.05 µg/ml) then decrease then increased again in Udarband. In Silcoorie lowest chlorophyll b was found in September (0.09 µg/ml) while highest in March (0.45 µg/ml) which lowers again in May (0.32 µg/ml). Generally, higher chlorophyll a concentration translate into higher individual cell counts and biomass of phytoplankton, though not always, as not all algal cells produce equal amounts of chlorophyll a [44]. In Dargakona highest chlorophyll a (11.97 µg/ml) and b (0.67 µg/ml) was followed by lowest carotenoid (0.99 µg/ml) in March. The pigment chlorophyll a, b and carotenoid were found to be fluctuating from July to May in Irangmara pond. Chlorophyll a (9.54 µg/ml) and b (0.32 µg/ml) were highest in Jaunary when carotenoid (3.12 µg/ml) was lowest in Barjalenga. In Sonai chlorophyll a increased gradually from the month of July than decreased in May. The amount of chlorophyll b is relatively less than chlorophyll a [3]. Figure 4(i-xvi) depicts the variation of carbohydrate and protein concentration in *E. tuba*. Carbohydrate concentration was found more than the protein concentration. *Euglena* gracilis shown to have high protein content as reported in ref. [2]. The protein content was about 40% for filamentous N_2 fixing cyanobacteria but carbohydrate content was about 3 times to that of protein in green alga Ulva rigida [45,46]. Algal cell mainly made up of proteins, carbohydrates, fats and nucleic acids in varying proportions but their percentages can vary with the type of algae, some types of algae are made up of up to 40% fatty acids based on their overall mass. It was the fatty acid that can be extracted and used as biofuel [47]. Proteins and carbohydrates concentrations were closely related to the media components like nitrate, phosphate and free copper ions [48,49]. Their report shows that in Chlorella vulgaris the protein production of 50% (7.0 $mg.L^{-1}$) in Chu and (6.8 $mg.L^{-1}$) in WC media and an equivalent of 33.74 g per 100 g growth in Chu medium. In Arkatipur carbohydrate was higher in all the months except March when protein (102.94 µg/ml) was higher than the carbohydrate (97.23 µg/ml) concentration. In Baskandi, Karikandi, Machpara, Kashipur, Bagpur, Madhuramukh, Madhuraghat, Dudhpatil, Udharband, Silcoorie, Irangmara and Sonai carbohydrate concentration was higher than the protein value. While in other ponds such as Durgabari, Dargakona and Barjalenga fluctuations in carbohydrate and protein concentrations were observed in between the months. Pigments concentrations most essentially the chlorophyll a concentration of a species which is a measure of biomass is associated with all other biochemical parameters.

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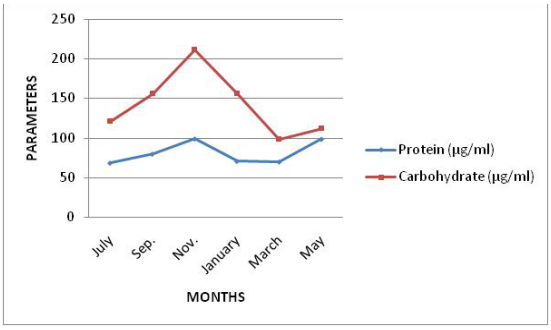
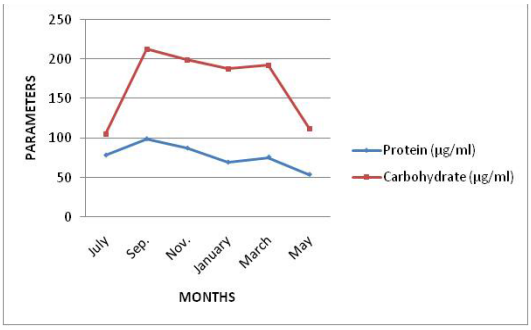


i. Arkatipur



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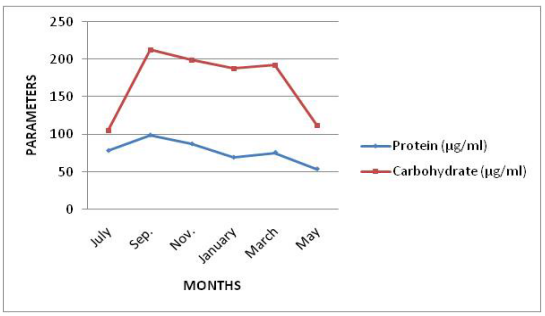
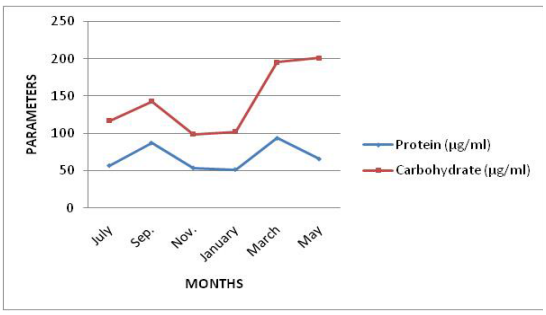


iii. Karikandi



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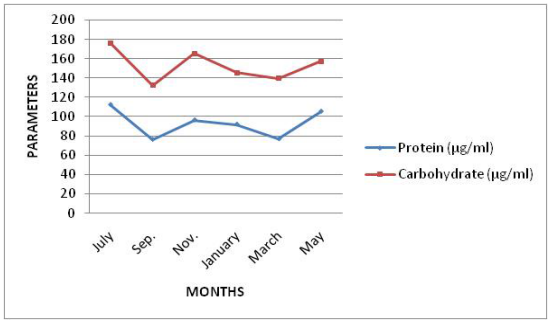

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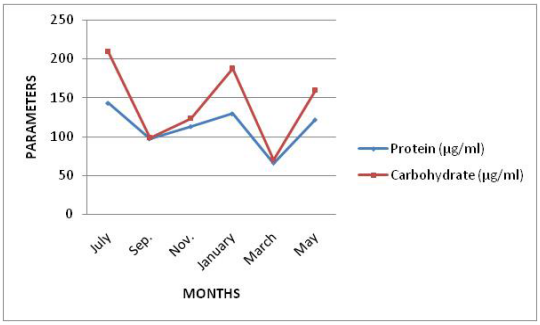

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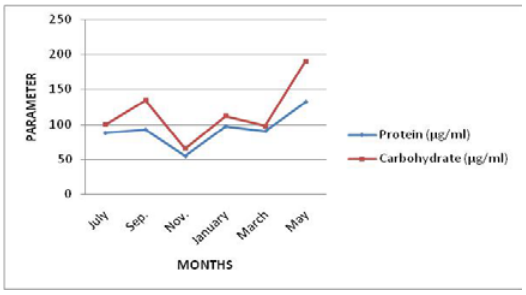
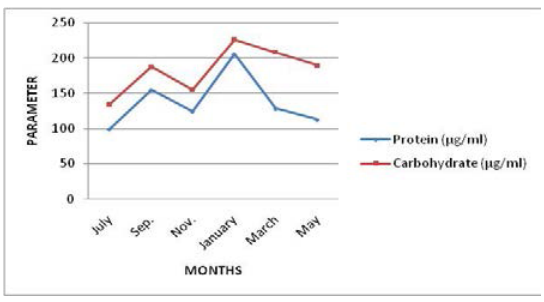
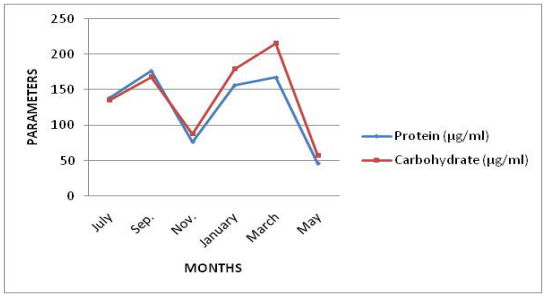


vii. Madhuramukh

viii. Madhuraghat



ix. Dudhpatil



x. Durgabari

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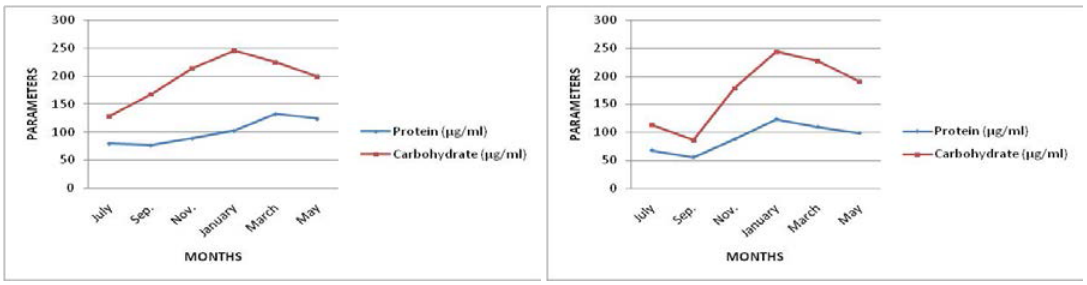

xi. Udarband **xii.** Silcoorie

Figure 4(i-xvi). Variation of Carbohydrate and protein concentration of *Euglena tuba* cell in 16 aquatic bodies. All data are expressed as mean ± S. D. (n=3).

CONCLUSION

In general for all the ponds carotenoid concentration was higher than the chlorophyll a and chlorophyll b pigments except Madhuraghat, Dudhpatil, Udharband, Barjalenga and Sonai with prominent bimonthly pigment fluctuations. Carotenoid pigment was rich and found to be dominant over the chlorophyll a and b pigments. Higher algal biomass in terms of chlorophyll a was associated with higher carbohydrate and protein value. From this study we can conclude that *E. tuba* showed high biochemical properties which can be utilized for the benefit of mankind. Morphological characterization along with biochemical approach of the organism can provide us some potential information for further research.

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