Chapter 9

GENERAL DISCUSSION
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Algal bloom in lakes and ponds are usually composed of colony-forming, unicellular and filamentous blue-green alage, often contributing to one of the following genera: Microcystis, Anabaena, Aphanizomenon or Gloeotrichia (Reynolds and Walsby, 1975). Excessively eutrophic or polluted habitats are dominated by small green lage and euglenoids which form blooms. Certain blue green blooms occur when populations which have been growing unnoticed below the surface for several weeks and suddenly float to the surface during periods of calm water. The algae may remain trapped at their surface by excess buoyancy contributed by the gas vacuoles. Decreased rates of photosynthesis caused by nutrient and or CO2 deficiency and low levels of K+ and intense
sunlight in water causes senescence and death of the algae. Most of the euglenoids seems to concentrate along the shoreline by light wind with a smelly scum which lowers the water quality and disrupts the normal water use. Algal blooms therefore seem to be incidental events which occur when a period of calm weather happens to coincide with excess and uncontrollable buoyancy in a substantial population of cells. Nutrient level is enriched in lakes and ponds by anthropogenic activity promoting the blooms occur with increasing frequency. The *Euglena* species disappear from the water column for extended periods of time during the year in some ponds. It is possible that the organism is present but at such low concentrations that that it is undetectable. These ‘seed’ organism would serve as an inoculum for an increase in its population upon the return of favorable conditions. It is also possible that some cells survive in sediments of comparatively darker zone until the favorable environment returns them to the surface. The bloom event is a regular feature in the ponds and wetlands of Cachar district (Assam). Most of the aquatic bodies particularly lotic ecosystems appear red colored under high temperature, light and humidity condition. Normally during dry periods the water evaporation is more, thereby increasing the concentration of the nutrients in water bodies.

The present Ph.D. research work highlights the algal blooms from different pond ecosystems of Cachar District, Southern Assam, North-East India. The extensive exploration of algal blooms from this area describes the dominance of an euglenoid species and is identified to be *Euglena tuba* Ehrenberg colonized in most the pond ecosystems in this area. *Euglena tuba* is a dominant unicellular alga in the fishery ponds of Cachar district. Ecological characterization has been made and it indicated that the particular *Euglena tuba* bloom formation was the result of integrated effect of the number of environmental factors. In the present work, it was found that a pH of around 7 was most conducive to the heavy bloom of *Euglena tuba* as observed. Similar observation on bloom formations was made by Olveson and Nalewajko (2000) elsewhere. In the present case application of lime to the ponds by the owner increases the nutrient availability or buffer against pH fluctuations. In summer season among all the ponds, Arkatipur, Baskandi, Machpara, Bagpur, Madhuramukh, Dudhpatil, Durgabari, Udarband, Irangmara, Barjalenga, Sonai ponds were having highest dissolved oxygen content. Though the bloom aquiring fishery ponds were quite favorable in terms of dissolved oxygen content for the fishes which never fell below 5mg/l during the study, however, chlorophyll content was found to be negatively
correlated with dissolved oxygen with the significance level \( p \leq 0.05 \) (Chapter 4). This decrease in dissolved oxygen due to increase in *Euglena tuba* bloom is similar with the findings of Sorokin et al., (1996) where they found that high densities of algal blooms may cause oxygen depletion in the water bodies. It indicates that the though depletion of dissolved oxygen is the result of higher growth of algal bloom, blooms are also responsible for providing ample amount of oxygen in the aquatic body which reduced significantly the free CO\(_2\) content in the particular ecosystem by the process of photosynthesis (Kashipur pond, \( p \leq 0.05 \)). Changes in dissolved oxygen concentration due to *Euglena* bloom was supported by the findings of Pereira et al. (2001). They mentioned oxygen concentration changes are related to the changes in the density of euglenophytes. Again the decrease in the dissolved oxygen level during bloom period indicated a favorable condition for the growth of Euglenineae as is also supported by the findings of Singh(1960), Munawar (1970). During the tenure of the present study (2008-2010), mean free CO\(_2\) content in two years was found to be more or less similar in all the 16 ponds. The low dissolved oxygen value associated with the high conductivity and pH and free carbon dioxide and iron values is in accordance with the studies conducted at Jaisamand Lake, Udaipur, Rajasthan (Sharma and Sarang, 2004). SRP and nitrate concentrations were fluctuating in different time periods in the ponds studied. The reason behind this high fluctuation is attributed more to the allochthonous input rather than the autochthonous origin which transports the nutrients from the surroundings to the aquatic body mostly by anthropogenic activities. SRP concentration was found to be fluctuating in each pond and most of the ponds were having more than 0.05mg/l of SRP. This value was far lower than the phosphate concentration to indicate pollution as reported by Jain et al., (1996) wherein they mentioned that the phosphate concentration above 0.5mg/l indicated pollution. The higher value of phosphate in the ponds were due to the presence of agricultural wastes which was introduced into the water bodies with run-off. Similar finding was also reported by Prasannakumari(2000). While other workers such as Manna and Das (2004) reported low range of phosphate value in the *Euglena* bloom ponds. Individually a distinct bimonth variation of nitrate concentrations in all the 16 ponds during the study period could be observed. With the fluctuations of nitrate concentrations in the ponds a sudden increase in the concentration of nitrate was noticed in the month of
January in Machpara (0.068mg/l) and Madhuraghan(0.065mg/l) pond. Anthropogenic activity in the surrounding areas which lead to more input of nutrients as well as the fishery activities in the ponds might be the reasons behind the fluctuations. The nitrate range in the ponds were quite lower than the range reported by Verma (2002) where it was inferred that the algal bloom ponds have been found to be favorable for fish productivity if nitrate value of the ponds ranged between 0.1-3.0 mg/l. But in our findings nitrate range in the fishery ponds were far below than this limit. Low value of nitrate in our study was supported by Moss (1973) who reported that *Euglena* spp. were unable to utilize nitrate in high nitrate levels and that may have affected the growth of *Euglena*. In two years study, mean silica concentrations ranged from 8.57-10.64mg/l in Silcoorie and Barjalenga respectively. This may be due to the high level of phytoplankton growth regulating the silica level in the ponds. Seasonal growth of the *Euglena* bloom in terms of chlorophyll a concentration did not follow a similar trend. *Euglena* bloom could be noticed in the ponds independent of the seasons as it was observed in Kashipur that the chlorophyll a concentration (10.32µg/ml) was highest in autumn (October). In our findings the *Euglena* cell density along with its fresh as well as dryweight of the cells was not restricted to a particular season and topography as though Arkatipur, Baskandi and Karikandi are nearby areas they differed in their biomass. In five sampling sites, Udarband, Dargakona, Irangama, Barjalenga, Sonai it was observed that increase in the numbers of cells lead to increase in biomass. Similar trend was also noticed for other ponds like Durgabari, Dudhpatri, Madhuraghat, Madhuramukh and Bagpur. In the study ponds were subjected to corresponding changes in the chemical composition of the surrounding water, as well as with pH fluctuating through photosynthesis as mentioned by James *et al.*, (1993). Among the environmental factors studied in the sixteen pond ecosystems dissolved oxygen (*p*≤0.05), free CO₂ (*p*≤0.05), total alkalinity (*p*≤0.05), nitrate concentration (*p*≤0.001) and electrical conductivity (*p*≤0.01) were found to have significant differences between their means. All the ponds were found to be mesotrophic in nature according Lake Condition Index (LCI) value. The texture of the soil was found to be mostly sandy loam and acidic in nature. Brady and Weil (2004) mentioned that sandy clay loam of textural classes of soil has the greater water retaining capacity than sandy loam and loamy sand textural class. Principal component analysis identified that
the nitrate concentration, SRP, silica and total alkalinity are the important factors contributing to higher bloom formations. Hierarchical cluster analysis demonstrates that the ponds were clustered together on the basis of similarity between their geographical locations, topography and anthropogenic activities. Diurnal variation is the fluctuations in behavior that occurs each day. Eukaryotic algae are well known for serving as a model organism for diurnal periodicity. The study of diurnal variation of *Euglena tuba* and the mechanism responsible for this rhythm investigated revealed that the rate of photosynthesis was directly influenced by light intensity. Euglenophytes (*Euglena*) contain more chlorophyll *a* than the diatoms, chlorophytes and especially cyanobacteria (Reynolds, 1984, Pereira et al., 2001) in successive order. *Euglena tuba* bloom was found to follow a clear diurnal variation of its pigments (Chapter 5) in each pond studied viz. Baskandi, Udarband, Silcoorie and Sonai. Chlorophyll *a* which is a primary photosynthetic pigment was found to be lower in morning and evening which reached its highest during noon. Carotenoid was also found to follow the similar trend but generally exceeded chlorophyll *a* concentration due to its enhanced synthesis than the chlorophyll *a* during noon under bright light condition providing a characteristic brick-red colour to the organism while in evening carotenoid fell lower than the chlorophyll *a* when the unique organism reverts back its green colour. This diurnal behavior was found to be highly pronounced in *Euglena tuba* where the cells were observed to be green in lower light intensities both in the morning and evening. During noon when the light intensity was strong, the colour changed to red. Fresh samples of *Euglena tuba* collected from the ponds showed regular behavioral pattern in structure as well as in movement like continuous twisting and turning and retarded its regular behavioral pattern changing its shape to round when kept undisturbed under normal condition for prolonged period. Irritation provided to the organism by shaking was also found to arrest the regular twisting and turning behavior of the organism. (Hader and Liu, 1990) mentioned that the effects of artificial treatment on the orientation process of the *Euglena* are much more stronger than those of solar radiation. In our observations the pigments of *Euglena tuba* bloom showed clear fluctuations in each pond studied (Chapter 6). The pigment carotenoid was found highest in almost all the ponds except Madhuraghat, Dudhpatif, Udarband, Barjalenga and Sonai than the chlorophyll *a* and *b*. Chlorophyll *b* was the least
among the three pigments estimated. Cunningham and Schiff (1986) also had the similar findings where they mentioned that the amount of chlorophyll \(b\) was relatively lesser than chlorophyll \(a\). Solar radiation stimulates the production of both the pigments but after a certain period, more carotenoids are slowly synthesized in the \textit{Euglena tuba} cells as compared with chlorophyll \(a\) pigment. The bimonth study of the pigments indicated that the fluctuations of the pigments were not restricted to a particular month rather it fluctuated in all the ponds throughout the year. Statistical analysis revealed a significant negative correlation (\(p \leq 0.01\)) between chlorophyll \(a\) and carotenoid pigment. While generally carotenoid pigment was found to be dominant over chlorophyll \(a\) pigment, in Dudhpatal chlorophyll was dominant over carotenoid throughout the year. Geographical location, duration and intensity of sunlight due to topography might be the reasons behind these fluctuations. Guschina and Harwood (2005) also mentioned that \textit{Euglena} grown at different light intensities show remarkable changes in their chemical composition, pigment content and photosynthetic activity. Madronich (1992) and Kirk (1994) also reported that at the surface of aquatic ecosystems, these fluctuations are due to changes in a variety of factors, including solar zenith angle, atmospheric aerosols, amount of ozone in the stratosphere, density of cloud cover and elevation above the sea level. Biochemical parameters such as the estimation of carbohydrate and protein indicated higher carbohydrate concentration of \textit{Euglena tuba} than the protein in most of the ponds (Baskandi, Karikandi, Machpara, Kashipur, Bagpur, Madhuramukh, Madhuraghat, Dudhpatal, Udharband, Silcoorie, Irangmara and Sonai) while in other ponds (Arkatipur, Durgabari, Dargakona and Barjalenga) fluctuations of carbohydrate and protein could be observed in between the months. Another species of Euglenophyta, \textit{Euglena gracilis} is reported to have high protein content (Chae \textit{et al.}, 2006). Generally chlorophyll \(a\) which is a measure of biomass is associated with biochemical property of an organism and in our observations chlorophyll \(a\) pigment was associated with higher carbohydrate and protein value (\(p \leq 0.01\)). In our result \textit{Euglena tuba} bloom was found to be dominant in most of the ponds of the district by reducing the growth of other groups of algae. Hosmani, (1988) also noticed \textit{Euglena} sp. reducing significantly other groups of algae. The ponds were found to be quite favorable and conducive for the fish productivity. Blooms were found to supply ample amount of oxygen to the pond ecosystems allowing
dissolved oxygen to never fall below 5 mg/l. The district has not yet been reported to have any fish kill event. Despite the rich availability of the *Euglena tuba* bloom in the ponds, the application of the alga as a fish feed is uncertain. The gut contents of the fishes from the heavily bloomed ponds were analyzed and it was observed a very low number of *Euglena* in the 11 indigenous fish varieties. Fishes included Darkina (*Esomus danricus*), Jati puti (*Puntius sophore*), Chepta puti (*Puntius conchonius*), Puta (*Puntius sarana*), Japani(*Cyprinus carpio*), Goroi(*Chana punctatus*), Moka (*Amblypharyngodon mola*), Ghoria (*Labeo goria*), Rohu (*Labeo rohita*), Puti(*Puntius ticto*) and Chandhowa (*Chanda nama*). Lu *et al.* (2004) also reported that larval tilapia *Oreochromis niloticus* ingested significantly less *Euglena gracilis* but the ingestion was higher than the *Chlorella vulgaris*. Since the alga contains a high nutritive value, there is a prospect of increasing food selection of the fishes by formulation of the fish feed through blending *Euglena tuba* powder according to the fish food habit. Das *et al.* (2009) mentioned that intake of *Euglena viridis* powder in Rohu increases its immunity and makes *Labeo rohita* more resistant to *Aeromonas hydrophila*. This effort may increase the consumption rate of the fishes which would help in high and healthier growth rate of the fishes.

Free radicals - reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated in our body by various endogenous pathway, exposure to different physiochemical conditions or pathological states. A balance between free radicals and antioxidants is therefore essential for proper physiological function. Excess free radicals beyond the body's ability to regulate them lead to a condition known as oxidative stress. Under normal conditions, free radicals are produced through biological activities which are scavenged by various enzymes and antioxidants present in the organisms. When the critical balance between free radical generation and antioxidant defences is unfavourable, this may lead to some biological basis of several acute diseases. Various antioxidants (tocopherols, ascorbic acid, β-carotene etc.) in modifying the risk for conditions which may result from oxidative stress has stimulated research efforts about their bioavailability and synthesis in the cells (Kamat *et al.*, 1997; Palozza and Krinsky 1992). Traditionally, seaweeds have been used in the treatment of various infectious diseases (Hoppe, 1999), and reports of many active compounds have been isolated and their structure determined (Vairappan *et al.*, 2001; Mundt *et al.*, 2003). Among the features of
marine algae and their substance, several extracts were screened on an antioxidant capability (Latham, 2008), and their inhibitory activity on lipoxygenase enzyme (Mori et al., 2003), as well as by radical scavenging activity, using a stable free radical (Matsukawa et al., 1997). Among several algal genera, *Spirulina* and *Chlorella* are considered to be important source as nutrients in traditional diet of some population of Africa and Mexico. *Euglena*, in particular, was screened by Takeyama et al. (1997) who reported for their simultaneous production of more than a single antioxidant compound like β-carotene, vitamins C and vitamin E, rendering it a promising dietary supplement. Das et al.(2005) have described the production of antibacterials from the freshwater alga *Euglena viridis* (Ehren). Recently, *Euglena gracilis* Z wild type has already been considered a potential source of vitamin E (Kusmic et al. 1993). In the present research, screening of the phytochemicals and assessment of the total antioxidant property of this indigenous algal species from this region of Southern Assam has been made (Chapter 7). The phytochemical screenings of chemical constituents related to biological activity of the plant extract were summarized in the Table-7.1. The results revealed the presence of biologically active compounds such as alkaloids, carbohydrates, flavonoids, phenols, saponins, tannins, terpinoids. The quantitative phytochemical estimation indicate that the extract contains significant amount of phenolics, flavonoids, carbohydrates, tannins, ascorbic acids and alkaloids which confirms its potent scavenging property. The alga exhibits high antioxidant activities and found to be a very good free radical scavenger. It also chelates iron and possess high reducing power. These *in vitro* assays indicate that this algal extracts is a significant source of natural antioxidant.

Another aspect which is has drawn huge current attention is bionanomaterials. Design, fabrication and application of nanostructures, devices and systems is an attractive interdisciplinary agenda for current research (Pitkethly,2004; Holister and Harper, 2002; Jones and Mitchell, 2001; Rittner, 2001). This relatively new field of research that gained popularity in the last decade or so is thoroughly reviewed (Shen et al., 2006; Wang and Xie, 2006; Wang et al., 2006). Besides technological potentials, nanomaterials are also of academic interests. Low dimensionality combined with large surface area render them unique properties. Though physical methods are quite extensively used, chemical approaches involve mixing at molecular level and is thus considered to be versatile tool
for nanomaterial synthesis. Chemical methods are by and large less expensive and easy to scale up. Template based approaches for nano-material synthesis are also quite popular (Hung and Whang, 2003; Li et al., 2004). The carbon nanoflakes obtained through CVD method using *Euglena* algal biomass furnishes an innovative, inexpensive and convenient route for production of carbon nanomaterials. The pronounced antioxidant property shown by the CNM is not only an interesting find but also hold tremendous promises for a new generation of natural antioxidant substances with minimal toxicity. Carbon nanomaterials find interesting application in fields like pharmaceuticals, agriculture, medicinal, fast moving consumer goods (FMCG) products etc. (Yang et al., 2020; Semete et al., 2010; Sharon and Sharon, 2006). Organic chemicals like acetylene, methane, benzene, cyclohexane etc. are used as precursor for the synthesis of CNM. Besides being expensive, all of these precursors are related to fossil fuels that are nonrenewable. Alternative sources like plants that provide numerous fibrous materials, seeds and oils which could be a potential source for commercial access to nanomaterials (Xie et al., 2009; Myers et al., 2000).
SUMMARY

The bloom event is a regular feature in the ponds and wetlands of cachar district. Most of the aquatic bodies particularly lentic ecosystems appear a red coloration during the high temperature, light and humidity condition. Normally during dry periods the water evaporation is more, thereby increasing the concentration of the nutrients in water bodies. The present Ph.D. research highlighted on the extensive exploration of algal blooms of different pond ecosystems of Cachar district of Southern Assam, North-East India. The bloom forming alga is identified to be *Euglena tuba* imparting a green-orange-brick red colouration in the aquatic bodies in this region. The alga typical shows typical euglenoid movement and the taxonomic characterization and distribution pattern of a dominant bloom forming alga in relation to the different environmental factors operating in the pond ecosystems has been presented (Chapter 4). Water and algal sampling were done in bimonth interval for two years (June 2008 –July 2010) for various limnological attributes and twenty nine independent variables were estimated for physico-chemical characterization of water, soil and algal samples. The extensive exploration of algal blooms from the different pond ecosystems of the district describes on an euglenoid species. *Euglena tuba* is reported here for the first time from Barak Valley, North-east India. Other 6 *Euglena* species viz. *E. acutissima, E. tripteris, E. spirogyra, E. oxyuris, E. proxima* and *E. sanguine* were earlier reported from Assam( Phillipose, 1982) contributing to bloom formation either by single species or found associated with mixed populations of phytoplankton samples. The *Euglena tuba* cells are highly metabolic, twisting and turning continuously; elongate- fusiform, posteriorly tapering rather abruptly to a short, blunt tip; membrane finely striated; chloroplast numerous, disc-like; paramylon bodies several to many rods of various length; The size of the elongated structure was about 60-110 µm long and 18-24 µm broad. But nearly spherical ones 42-55×38-40 µm; cysts 40-80 long with flasks 40-44×30-32 µm, stalks up to 40-50 µm longer and 18-20 broad, base 25-27 µm in diameter.

Euglenoid movement is pronounced on irritation and cells frequently shed emergent flagellum. Ecological characterization of algal blooms revealed many factors to be responsible for the development of algal blooms. Temperature found to play an important
role in the growth and development of *Euglena* bloom. Air and water temperatures were
found to be highest in the summer months and lowest in winter months. In the present
work, it was found that a pH of around 7 provided the most conducive environment to the
heavy bloom of *Euglena* which might be due to the application of lime to the ponds by
the owner to increase the nutrient availability or to buffer against pH fluctuations in
fishery ponds. Free CO$_2$ content in two years among all the 16 ponds was found to be
more or less similar in all the ponds during the study period 2008-2010. During this study
period most of the ponds were having SRP concentration more than 0.05mg/l. The
agricultural wastes introduced into the water bodies with run-off from the surrounding
area might have contributed to higher value of phosphate in the sampling ponds. Bimonth
fluctuation of nitrate concentration was noticed in all the sixteen sampling ponds. The
reason might be attributed to the higher allochthonous inputs from the surrounding area.
The moderate nitrate concentration probably had enhanced the growth of algal blooms
which in turn produced more dissolved oxygen. High temperature, heavy rainfall before
the blooming, increased nutrients due fishery and other anthropogenic activities seemed
to favor bloom appearance in the ponds. High light intensity and large amount of organic
matter were also seemed to influence the growth of *Euglena tuba*. The variation of the
silica concentration in the sampling ponds might be due to the high level of phytoplankton growth regulating the silica level in the aquatic bodies. The amount of chlorophyll $a$ is an index of water quality and algal biomass. Seasonal changes in density
of algal blooms and total chlorophyll are known to grossly reflect each other. From our
study it was observed that the alga does not show a very strict seasonal pattern of growth,
rather even in rainy periods the alga is opportunist and contributes to a periodic bloom
condition for a shorter span of time if the habitat is not exposed to flood, incessant
rainfall, and other disturbances from various fisheries and agricultural activities. The
texture of the soil was mostly found to be sandy loam and acidic in nature. Acidic nature
of the soil might be due to the high litter decomposition in the bank of the ponds and
related decomposition due to microbial activity. Clay loam soil has the greater water
retaining capacity than sandy loam and loamy sand textural class. In the present work
colour of the soil was found to be pale brown, yellowish brown, light yellowish brown
and dark yellowish brown. Sediments were rich in nitrogen and it might be attributed to
the input due to runoff from the surrounding agricultural lands which consequently triggered the blooming of *Euglena tuba* in the ponds. However a high level of nitrogen and phosphorus might have induced Zinc and copper deficiency in the sediments respectively. Moisture content of the soils was dependent upon weather condition. In our observation, higher chlorophyll *a* concentration translated into higher individual cell count and biomass of the particular alga, though it is known that all algal cells do not produce equal amount of chlorophyll *a*. In Udarband, Dargakona, Irangmara, Barjalenga, Sonai it was observed that with increasing biomass the numbers of cells also increased. Similar trend was also followed by other ponds like Durgabari, Dudhpatil, Madhuraghat, Madhuramukh and Bagpur. Increase in *Euglena tuba* density lead to increased chlorophyll *a* concentration in the pond water where dissolved oxygen concentration changes might have related to the changes in the density of the particular Euglenophyte. In our findings the *Euglena* cell density along with its fresh as well as dry weight of the cells was also not restricted to a particular season and topography. It was clear from the observations of Arkatipur, Baskandi and Karikandi, the nearby areas differing in their biomass. One way ANOVA analysis for the different parameters studied in the ponds’ water was performed and among them dissolved oxygen (*p*≤05), free CO₂ (*p*≤05), total alkalinity (*p*≤05), nitrate concentration (*p*≤001) and electrical conductivity (*p*≤01) were found to have significant differences between their means. A number of significant correlations were obtained in between ecological attributes and Chlorophyll *a* concentration of *Euglena tuba*. Air temperature in our study showed significant negative correlation with chlorophyll *a* (*r*=-0.871, *p*≤0.01) in Baskandi pond. Similar significant negative correlation was obtained for Udarband pond (*r*=-0.644, *p*≤0.05). Air temperature was found to be an important factor in regulating chlorophyll *a* concentration during the study period with a significant negative correlation among all the ponds (*r*=-0.206, *p*≤0.01). The reason behind the fact might be that when temperature was high it stimulated *Euglena tuba* to produce more carotenoid by masking the chlorophyll *a* concentration giving a brick red coloration to the aquatic bodies. Dissolved oxygen content in our study showed significant negative correlation with chlorophyll *a* concentration (*r*=-0.180, *p*≤0.05) together in all the ponds and individually in Baskandi pond (*r*=-0.836, *p*≤0.01). High densities of algal blooms may cause oxygen depletion in
the water bodies indicating a favorable condition for the growth of *Euglena tuba*. Higher chlorophyll *a* which is a measure of biomass lead to higher dissolved oxygen content and lesser free carbon di oxide content (*r*=0.690, *p*≤0.05). Heavy growth of *Euglena tuba* in the form of bloom was one of the reasons which caused decay to a part of the algal population and release of substantial amount of carbon-di-oxide into the environment resulting into depletion of dissolved oxygen content in water body. Rich zooplankton abundance might be another reason which reduced dissolved oxygen availability in the aquatic environment through the process of respiration. Principal component analysis identified that the *Euglena tuba* biomass in terms of chlorophyll *a* was more influenced by nitrate concentration, SRP, silica and total alkalinity and formed a distinct group in the plot. The nitrate and phosphate load in the ponds are due to the fact that most of the selected ponds were the fishery ponds and the fecal wastes from the poultry and piggery farm contributed to a greater extent to increase the nutrients. Air temperature, water temperature and dissolved oxygen formed a separate group far away from the chlorophyll *a* concentration in the plot. Hierarchical cluster analysis was performed to identify relatively homogenous groups of sites based on physico-chemical parameters analyzed during the study period. Distance or similarity measures generated by proximity procedure demonstrated that two more or less similar topography and close geographical locations Arkatipur and Baskandi had highest similarity and clustered together. Ironmara and Sonai ponds were also resulted into a single cluster. Both the sampling sites were surrounded by tea plantations and paddy fields and similar anthropogenic activities were found to be associated with the ponds. Two geographically nearer ponds Kashipur and Madhuraghat formed a single cluster. Similar results were obtained for Silcoorie and Barjalenga, Karikandi and Machpara.

Diurnal variation is the fluctuations in behavior that occurs each day (Chapter 5). Circadian rhythms are known to be better exhibited by Eukaryotic algae serving as a model organism. Circadian rhythms or daily rhythms are the endogenous biological programmes that time number of behavioral events that occur at the optimal phases of the daily cycle. Though circadian rhythms are best characterized in *Euglena* yet with a very few reports are available on this interesting alga. To study the diurnal variation and circadian rhythm of *Euglena tuba* bloom four experimental sites were selected viz.
Baskandi, Udarband, Silcoorie and Sonai. Baskandi is about 16 km East of silchar town, Udarband is about 14 km North-East of Silchar, Silcoorie is about 20 km of South-West direction and Sonai is about 16 km South-East of Silchar. For observing the diurnal periodicity of the bloom four bright sunny days were selected. Experimental observation were made from 7 a.m. and concluded at 5 p.m. It included a total of 11 observations. The diurnal variation and circadian rhythm of Euglena tuba was observed and the possible mechanism behind this rhythm investigated showed that the rate of photosynthesis was directly influenced by light intensity. A similar trend was observed for the synthesis of chlorophyll and carotenoid in the cell of Euglena tuba. The circadian change in colour of the cells were more pronounced as the cell remained green in the morning when the intensity of light is less and during the day when the sun rays fell straight with strong intensity the colour of the cell became red. In the evening when the sun rays fell oblique and soft as in the morning the red colour of the cell began to disappear and the cell turned into green. This was because in the morning with the soft sun ray the production of chlorophyll and carotenoid were also less but the increase of solar radiation increased the production of both the pigments. Thus light certainly played an important role in changing the colour of the cell. Timing of increases and subsequent decreases in pigment concentrations are consistent. In our observations, it was found that the light-enhanced chlorophyll a formation but somewhat at a lower rate than the carotenoid. Solar radiation stimulates the production of both the pigment but after a certain period a gap occur where slowly more carotenoids are synthesized in the Euglena tuba cells as compared with chlorophyll a pigment. High intensity of solar irradiation had a significant effect on carotenoid synthesis by the cells which masked the chlorophyll a and consequently provided a characteristic brick-red colour to the cells and consequently to the pond. A systematic observation on Euglena tuba was done in laboratory set up under the microscope and behavioural changes were recorded. The Euglena tuba also showed a typical behavior in changing of shape and size of the cell. Fresh Euglena tuba collected from the field when observed under microscope, the organism showed its regular behavioral pattern in structure as well as in movement like continuous twisting and turning. The shape was seen as elongated to fusiform with continuous changes from elongated to triangular to oval and again become elongated. Same samples when applied
for shock treatment, all the cells became rounded in structure and their entire chloroplast arranged in a compressed mass of body.

Biochemical characterization and pigment profile of *Euglena tuba* and its intake in some indigenous fishes of Cachar district is described in **Chapter 6**. The photosynthetic pigments are characteristic of different algal groups. Among the four different kinds of pigments that are found in algae are chlorophyll, carotenes, xanthophylls, phycobilins of which phycobilins are not found but in *Euglena*. The chapter attempted to investigate the biochemical potential and pigment profiling of *Euglena tuba* including some informations on intake of *Euglena tuba* by the indigenous fishes to explore its innate potential as fish food. For the biochemical characterization and pigment profile analysis 16 ponds were selected and the algal samples were collected bimonthly from July-2009 to May- 2010. Information on intake of *Euglena tuba* by the fishes was obtained by analyzing the guts of some indigenous fish variety. Pigment analysis of *Euglena tuba* showed that in general carotenoid was found highest in almost all the ponds. Chlorophyll *b* was always found to be always lower than other pigments. In our observations, in general Carbohydrate concentration was found to be more than the protein content of the species. In Baskandi, Karikandi, Machpara, Kashipur, Bagpur, Madhuramukh, Madhuraghat, Dudhpatil, Udharband, Silcoorie, Irangmara and Sonai carbohydrate concentration was always 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. Pigment concentrations most essentially the chlorophyll *a* concentration of a species which is a measure of biomass was seemed to be associated with all other biochemical parameters. Statistical Analysis of the pigments and biochemical properties of *Euglena tuba* showed a significant negative correlation ($p \leq 0.01$) between chlorophyll *a* and Carotenoid pigment.

It thus reveals the fact that higher synthesis of carotenoid due to bright light conditions masked the chlorophyll *a* production by the *Euglena tuba* cell. Higher Chlorophyll *a* pigment was associated with higher carbohydrate and protein value ($p \leq 0.01$). *Euglena tuba* was found to be a dominant unicellular alga in the fishery ponds of Cachar district. Heavy blooms of *Euglena* spp. observed in the fishery ponds reduced significantly other groups of algae. In our study fish survivality in the ponds was quite favorable in terms of
dissolved oxygen content which never reached below 5 mg/l. Though many times blooms are known to deplete oxygen level in the aquatic body causing huge fish mortality but here in our study *Euglena tuba* blooms were not found to be harmful for the fish growth. In spite of a significant negative correlation in between chlorophyll a and dissolved oxygen, blooms were found to maintain the oxygen balance in the ponds by the process of photosynthesis. No fish kill event has been reported from the district earlier or during the study. Though *Euglena tuba* bloom formation in fishery ponds is a very common phenomenon, application of the alga as fish feed is uncertain. The gut contents of the fishes showed a very low number of *Euglena* in the 11 indigenous fish varieties. Fishes included Darkina (*Esomus danricus*), Jati puti (*Puntius sophore*), Chepta puti (*Puntius conchonius*), Puta (*Puntius sarana*), Japali (*Cyprinus carpio*), Goroi (*Chana punctatus*), Moka (*Amblypharyngodon mola*), Ghoria (*Labeo goria*), Rohu (*Labeo rohita*), Puti (*Puntius ticto*) and Chandhowa (*Chanda nama*). Food choice of the fishes might be another factor for the reducing the intake of the particular alga in their diet despite the huge abundance of *Euglena tuba* in the fishery ponds. However, irrespective of the food selection and consumption of the fishes *Euglena tuba* was found to have rich carbohydrate and protein content which might have a potential to increase the food value of the same since reports are available on *Euglena viridis* powder in Rohu increases its immunity and makes *Labeo rohita* more resistant to *Aeromonas hydrophila*. Thus high nutritive value of *Euglena tuba* opens a new area where a future prospect occurs in formulation of the fish feed by blending *Euglena tuba* powder according to the fish food habit that may ultimately increase the food selection and consumption rate of the fishes. This would again help in high and healthier growth rate of the fishes increasing the commercial output. Higher concentrations of macro and micronutrients observed in the species may have lead to higher amount of proteins and carbohydrate metabolism. Thus *Euglena tuba* has its every possibility to be used as a fish feed and a future prospect lies in the fish feed formulation according to fish food habit which would ultimately help the farmers to grow more fishes on commercial scale.

Different types of various types of phytochemicals and antioxidant properties (DPPH radical scavenging, hydroxyl radical scavenging, superoxide scavenging, nitric oxide radical scavenging, hydrogen peroxide scavenging, peroxynitrite scavenging,
singlet oxygen scavenging, hypochlorous acid scavenging, reducing power, lilipid peroxydation inhibition study) of methanolic extract of *Euglena tuba* has been explored. On the basis of the results obtained in the present investigation it is concluded that a 70% methanolic extract of *Euglena tuba*, contains large amounts of biologically active compounds such as alkaloids, carbohydrates, flavonoids, phenols, saponins, tannins, terpenoids. The alga exhibits high antioxidant activities and found to be a very good free radical scavenger. It also chelates iron and possess high reducing power. These *in vitro* assays indicate that this algal extracts is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Also described in the thesis (*Chapter 8*), the protential of *Euglena tuba* as a inexpensive and natural source for accessing carbon nano materials (CNM) through a convenient CVD technique. The carbon nano materials (CNM) accessed from the alga are of graphitic nature with carbon flakes possessing ‘spheres’ as well as ‘rhombohedral’ morphology with an average particle size 50 nm. The CNM from *Euglena* exhibited pronounced antioxidant activity against DPPH. These materials may serve as an viable alternative for synthetic substances in relation to mechanical , biochemical and therapeutic properties. The methodology uses an unconventional, inexpensive natural precursor, as a carbon source. Carbon nanomaterials (CNM) are used mainly in advanced composite materials to improve strength, stiffness, durability, electrical conductivity, or heat resistance CNM production cost are substantially less than carbon nanotubes (CNT) and therefore offer significant advantages over nanotubes for certain applications, providing a high performance to cost ratio.