



ISSN : 2350-0743

www.ijramr.com



International Journal of Recent Advances in Multidisciplinary Research

Vol. 02, Issue 09, pp.0755-0761, September, 2015

RESEARCH ARTICLE

BIOMASS NUTRIENT PROFILE OF THE GREEN ALGA *GOLENKINIA RADIATA* CHODAT

¹Minhaj Akhtar Usmani, ^{1*}Suseela, M. R., ¹Kiran Toppo, ²Sarita Sheikh and ¹Sanjeeva Nayaka

Algology laboratory, CSIR-National Botanical Research Institute, Lucknow- 226 001, Uttar Pradesh, India
Ethelind School of Home Science, SHIATS, Allahabad, Uttar Pradesh, India

ARTICLE INFO

Article History:

Received 16th June 2015

Received in revised form

09th July, 2015

Accepted 28th August, 2015

Published online 30th September, 2015

Keywords:

Golenkinia radiata,
Nutritional profiling,
Carbohydrate
Protein
Lipid
Pigments
Fish food.

ABSTRACT

The nutrient profile of the green alga *Golenkinia radiata* Chodat includes- carbohydrate (40.39%), protein (13.3%), lipid (12.9%) 64.9 mg total lipid per 500 mg biomass, energy 1385.24 kJ per 100 gm biomass and pigments- chlorophyll a (1.44 ± 0.047 mg/g), chlorophyll b (0.64 ± 0.027 mg/g), total chlorophyll (2.09 ± 0.073 mg/g) and total carotenoids (0.17 ± 0.006 mg/g) (with acetone method) and chlorophyll a (1.76 ± 0.026 mg/g), chlorophyll b (0.45 ± 0.021 mg/g), total chlorophyll (2.21 ± 0.046 mg/g) and total carotenoids (0.20 ± 0.001 mg/g) (with DMSO method). Biomass was rich in carbohydrate content which might have influenced the fish growth in the pond rich of *Golenkinia*. Green alga *Golenkinia* has been recorded from different countries of World like - Europe, N. America, S. Africa, Ceylon, Japan and India (Philipose, M.T 1967). *Golenkinia radiata* has been reported from different water reservoirs but there were absolutely no report on nutritional profile of this alga (Priya Gopinath and Ajit Kumar, 2015, Pathmanapan et al., 2010, Tripathi, 2013, Singh and Balasingh 2012, Tiwari and Chauhan, 2006). Healthy fishes growing in an artificial pond with substantial amount of *Golenkinia* biomass inspired the idea of its nutritional profiling. Biomass was collected and processed for its proximate composition like carbohydrate, protein, lipid and also the pigments (chlorophyll and carotenoids). This is the first report from Indian species of *Golenkinia radiata*.

INTRODUCTION

Algae have a long history of use as foods, feeds and for the production of food ingredients. Nutritional aspects of several algal species like *Spirulina*, *Chlorella*, *Scenedesmus*, *Nostoc*, *Haematococcus*, etc. have been studied (Bishop and Zubeck, 2012, Biller and Ross, 2014). There was continuous search for newer nutritious species. In the year 1885, Sir Ray Lankester described a minute chlorophyllogenous organism with the genus "Archerina." and named the species "A. boltoni." Nine years later Professor Chodat, of Geneva, described the same organism under the name "*Golenkinia radiata*," in 1894. Professor Chodat obtained his specimens from a small duck-pond in the public park at Geneva.

However, Sir Ray Lankester's specimens were sent to him in a bottle-full of living material gathered by Mr. Thomas Bolton, of Birmingham from the duck pond of the gardens of the Royal Botanical Society in Regent's Park (Sir Ray Lankester, 1908). There are very few reports for the presence of *Golenkinia*. *Golenkinia radiata* (Chod.) Wille (Class: Chlorophyta, Order: Chlorococcales.) have been reported in Kodaikanal Lake, Tamil Nadu, India (Singh, and Balasingh, 2012). Philipose (1967) reported *Golenkinia radiata* from Puri and Cuttack of India. *Golenkinia* has been reported as the rare genera. 8.33% of annual occurrence of

*Corresponding author: Suseela, M. R.,

Algology laboratory, CSIR-National Botanical Research Institute,
Lucknow- 226 001, Uttar Pradesh, India

Golenkinia radiata was recorded in Ganga River of Kanpur (Tripathi, 2013). Similar to the earlier studies, fishes were found growing healthily in an artificial pond at CSIR-National Botanical Research Institute (NBRI), Lucknow, India with substantial amount of *Golenkinia* biomass. This observation inspired the idea of its nutritional profiling. In the nutritional value of any algal species, especially as fish feed protein is always the major organic constituent, followed by lipid and then carbohydrate.

This is the first report of nutrient profiling of Indian species of *Golenkinia radiata* although; studies on growth and chlorophyll synthesis by the green alga *Golenkinia* have been done. Its growth ceases after approximately 60-hr incubation in 0.01 M sodium acetate. Acetate alters the cells so that they become sensitive to high concentrations of OH⁻ ions and then the algal cell division is inhibited and they get bleached (Ellis, 1970). Studies have shown that glucose and sodium acetate are the only carbon sources able to support heterotrophic growth of *Golenkinia minutissima* Iyengar & Balakrishnan. Heterotrophic growth is maximal at a concentration range of 20–40 mM of either carbon source; however, growth is significantly more rapid and higher yields are obtained with acetate (Ellis, 1977). Detailed nutritional analysis of this rare alga has not been done yet. Keeping in view the above references the present study was done to estimate the proximate composition of green alga *Golenkinia radiata*.

MATERIALS AND METHODS

Sample Collection

The study was carried out on the fresh biomass of artificial fish pond at CSIR-National Botanical Research Institute, Lucknow, India. The floating algal bloom was directly and carefully collected by collecting spoon from the pond in conical flask and kept for sedimentation. Microscopic observation and morphological taxonomic identification. The cells were observed under Leica DM 500 light Microscope attached with Leica EC3 Camera with computerized image analysis system. Alga was identified by using the standard publications (Philipose, 1967).

Sample processing

The sedimented algal biomass was pressure filtered using motor with Whatman filter paper No. 42 and then dried in the oven at 60°C temperature for overnight. The dried pellets were grinded with pestle mortar to a fine powder which was further utilized for nutrient estimations (Fig.1).

Tubes are centrifuged at 10,000 rpm for 10 min. The absorbance of the supernatant was recorded at two different wavelengths (663 and 645 nm) using Chemito Spectroscan UV-2700 Spectrophotometer (Thermo Scientific) by keeping 80% acetone as blank.

$$\text{Chlorophyll 'a' (mg g}^{-1} \text{ fw)} = \frac{[(12.7 \times A_{663}) - (2.69 \times A_{645})] \times V}{1000 \times W}$$

$$\text{Chlorophyll 'b' (mg g}^{-1} \text{ fw)} = \frac{[(22.9 \times A_{645}) - (4.68 \times A_{663})] \times V}{1000 \times W}$$

$$\text{Total Chlorophyll 'a-b' (mg g}^{-1} \text{ fw)} = \frac{[(8.02 \times A_{663}) - (20.2 \times A_{645})] \times V}{1000 \times W}$$

Where, A= Absorbance at given wavelength; V= Final volume of 80% acetone in ml and W = Weight of the algal samples in grams. The carotenoids were quantified by using the equation followed by (Price, and Hendry, 1991, Venkatarayappa et al., 1984).

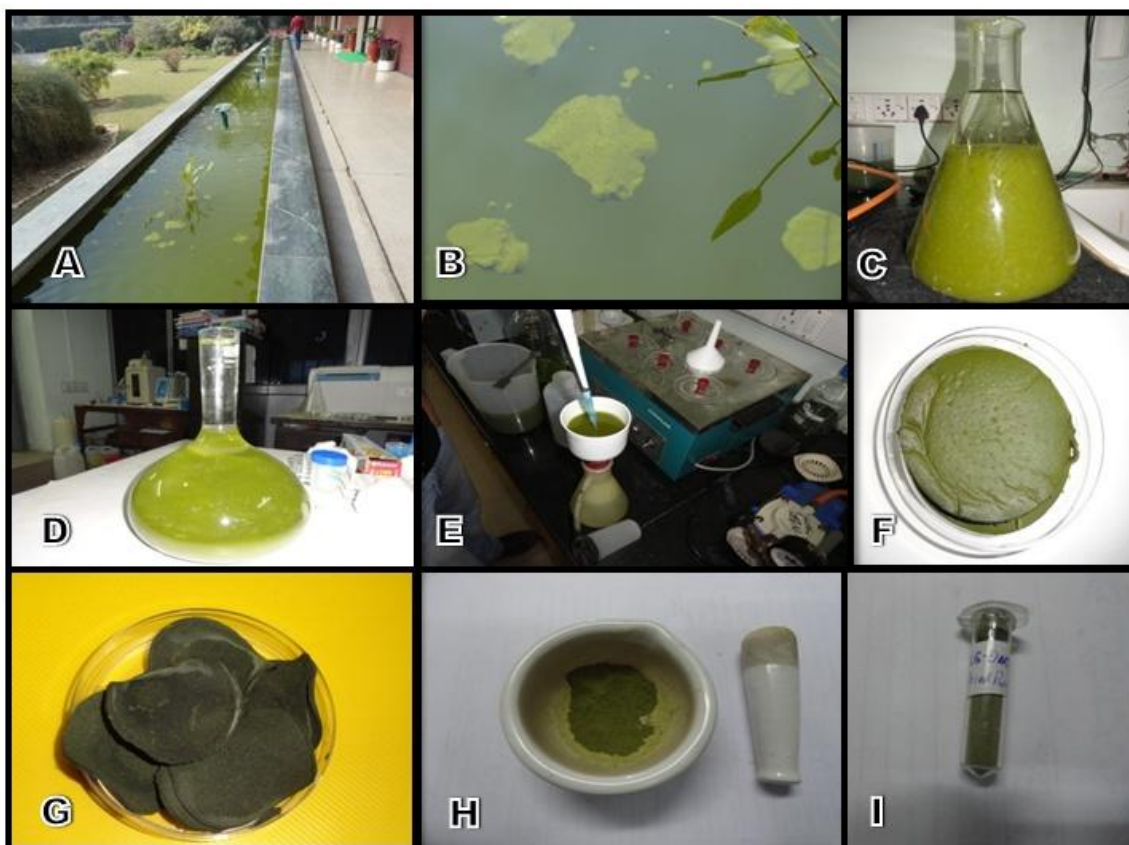


Fig. 1. (A) Fish pond having algal bloom. (B) Floating *Golenkinia* bloom patches. (C) Collected algal biomass. (D) Sedimentation of the algal biomass. (E) Filtration of algal biomass. (F) Filtered wet biomass. (G) Dried pellets of algal biomass. (H) Powdered algal pellets. (I) Powdered algae stored in eppendorf

Pigments Estimation

Total chlorophyll, chlorophyll ‘a’, ‘b’ content of the dried biomass was estimated by Acetone method (Arnon, 1949) and DMSO method (Hiscox, and Israelstam, 1979). In Acetone method, 10 mg dried algal powder was taken in triplicates in centrifuge tubes. 4 ml of 80% Acetone was added to each tube and kept at 4°C in refrigerator covered with aluminum foil for overnight.

$$\text{Total Carotenoids (mg g}^{-1} \text{ fw)} = \frac{[A_{480} - (0.114 \times A_{663}) - (0.638 \times A_{645})] \times V}{1000 \times W}$$

Where, A, V and W are as described above.

In DMSO method, 10 mg dried algal powder was taken in triplicates in centrifuge tubes. 4 ml of Dimethylsulphoxide DMSO (C₂H₆O₅) in each tube and covered with aluminium foil to avoid photo oxidation of pigments and were kept in oven at

65°C for 5 hours. The absorbance of the solution was recorded at 663, 645 and 480 nm using DMSO solution as blank. The Total Chlorophyll, Chlorophyll a & b and total carotenoids were determined by using the same formulas mentioned above as given by Arnon (Arnon, 1949) and results expressed in terms of mg g⁻¹ fw.

Carbohydrate Estimation

The carbohydrate content of the dried biomass was estimated by Anthrone Method (Hedge and Hofreiter, 1962). The following reagents were required for the Carbohydrate estimation

- 2.5 N-HCl
- Anthrone Reagent: 200 mg Anthrone dissolved in 100 ml of ice cold 95% H₂SO₄. Always prepared fresh.
- Standard Glucose: Stock- 100mg Glucose dissolved in 100 ml water. Working standard 10 ml of stock diluted to 100 ml with distilled water. Stored in refrigerator after adding few drops of toluene.

Total Carbohydrate estimation:

Weighed 100mg of the sample into a boiling test tube. Then hydrolyzed by keeping in a boiling water bath for three hours with 5ml of 2.5 N-HCl and then cooled to room temperature. Then it was neutralized with solid sodium carbonate until the effervescence ceases. Volume is made up to 100 ml and then centrifuged. The supernatant was collected and 0.5 to 1ml aliquots were taken for analysis. Standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard. "0" served as the blank. Volume was made up to 1ml in all the tubes including the sample tubes by adding distilled water. Tubes were cooled on ice. Then 4ml anthrone reagent (ice cold) was added and left for 15 min. After that it was heated for 8-10 min in a boiling water bath. It was rapidly cooled and the absorbance was read at 630nm of the samples producing green to dark green colors. The standard graph was plotted using concentration of the standard on the X-axis versus absorbance on the Y-axis. The amount of the carbohydrate present in the sample tubes was calculated using graph.

The formula used:

$$\text{Amount of carbohydrate present in 100 mg of the sample} = \frac{\text{mg of glucose} \times 100}{\text{Volume of test sample}}$$

Protein Estimation

The total protein content of the dried biomass was estimated by Lowry Method (Lowry *et al.*, 1951). The following required reagents were used for the protein estimation

- Solution A- 2% sodium carbonate in 0.1 N sodium hydroxide solution. 20g Na₂CO₃ and 4g NaOH was dissolved in 500 ml distilled water and the volume made up to one litre.
- Solution B- 1% Copper sulphate solution and 2% Sodium potassium tartarate (Rochelle Salt) solution were mixed in equal volume just prior to use.
- Solution C- Alkaline cupric tartarate solution prepared just prior to its use by mixing 50 ml of solution A and 1 ml of solution B.

- Solution D- 1N Folin Ciocalteu Reagent is prepared just prior to its use. Normality of Folin Ciocalteu Reagent is 2N so diluted 50% with distilled water.

Preparation of the sample solution

500 mg algal powder and 50 ml of distilled water is homogenized by sonication for 10-20 mins. The tube containing the homogenate is covered with aluminum foil and kept for overnight. The supernatant is taken for protein estimation.

Estimation of soluble protein

The above supernatant sample was taken in test tubes from 0.1 to 0.5 ml. The volume was made up to 1ml with the water and a tube containing 1ml distilled water was served as a blank. 5 ml of the solution C was added to all the tubes and mixed well by vortexing and then left for 10 min. Then 0.5 ml of the solution D (1N Folin Reagent) was mixed well using vortex mixer. Blue color develops immediately. All the tubes were kept for incubation at room temperature for 30 min covered with aluminum foil. The absorbance of the blue colour was read at 660nm using spectrophotometer. By using the standard curve, the protein content in the sample was calculated and result expressed as µg protein ml⁻¹ sample solution.

Standard curve for protein

Standard stock solution of Bovine serum albumin (BSA) (250 µg per ml) was prepared by dissolving the 6.25 mg of BSA in 25 ml distilled water, gently, avoid frothing. Then it was serially diluted with water in test tubes to get 25 to 125 µg of BSA per 1ml. A tube containing 1ml distilled water was served as a blank. 5 ml of the solution C (mentioned above) was added to all the tubes and mixed well by vortexing and then left for 10 min. Then 0.5 ml of the solution D (1N Folin Reagent) was mixed well using vortex mixer. Blue color develops immediately. All the tubes were kept for incubation at room temperature for 30 min covered with aluminum foil. The absorbance of the blue colour was read at 660nm using Chemito Spectroscan UV-2700 Spectrophotometer (Thermo Scientific). The readings obtained were then plotted on graph taking BSA concentration on X axis and absorbance on Y axis. The standard curve obtained was used for protein estimation.

Lipid Estimation

The total lipid content of the dried biomass was estimated by Folch Method (Folch *et al.*, 1957) 500 mg algal powder was homogenized with chloroform/methanol (2/1) to a final volume 20 times the volume of the sample (1 g in 20 ml of solvent mixture). After dispersion, the whole mixture was agitated during 35-40 min in a vortex shaker at room temperature so as to disrupt the cell walls with glass beads. The dark green color appears it was further shaken for 20 min. The homogenate was centrifuged at 8,000 rpm for 12 min in small tubes to recover the liquid phase in supernatant. The pellets were discarded and the process was repeated thrice to get the clear supernatant. 3ml of 0.9% NaCl solution was added to wash the supernatant, then shaken using vortex for 10 sec till the color changes. The mixture was transferred to pre-weighed empty centrifuge tubes and then centrifuged for 10 mins at low speed (2000 rpm) to separate the two phases. The upper phase was removed by siphoning and the lower chloroform phase containing lipids was evaporated, kept in oven overnight at 60°C. The

chloroform methanol mixture was evaporated and the remaining was lipid. The centrifuge tubes were reweighed to deduce the lipid weight.

The following formula was used

$$\% \text{ Lipid} = \frac{W_2 - W_1}{W} \times 100$$

Where,

W=Pre-weight of biomass

W₁= Final weight of centrifuge tube with lipid.

W₂= Pre-weight of empty centrifuge tube.

Energy Estimation

The energy content of the biomass was determined by multiplying the values obtained for protein, available carbohydrates and fat by 4.00, 3.75 and 9.00, respectively, and summing the results (Guil-Guerrero, 1999).

Water Quality Analysis

Physico-chemical parameter analysis of the pond water was done. Water parameters such as temperature, dissolved oxygen, pH, and conductivity were measured at sampling site with multiparameter analyser (HQ 40d multi, HACH). Other parameters viz. nitrate, phosphate, chlorine, iron and sulphate were analysed in lab by adding NitraVer5, PhosVer3, DPD Total Chlorine Reagent, FerroVer Iron Reagent and SulfaVer4 reagents respectively and silicate by silicomolybdate method.

RESULTS AND DISCUSSION

Identification

The cells observed microscopically were found to be a green algae *Golenkinia radiata* (Fig.2)

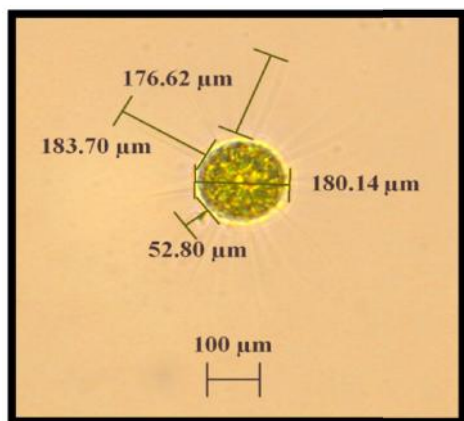


Fig. 2. Microphotograph of *Golenkinia radiata*

Classification

Class- Chlorophyceae

Order- Chlorococcales

Family- Micractiniaceae

Genus- *Golenkinia*

Species- *radiata*

Taxonomic description

Cells were usually solitary, free-floating, spherical and with a thin cell wall covered by long hyaline setae or bristles which

were not thickened at the base. Chloroplast was single, parietal and with a single pyrenoid. Till now only one species have been recorded from the Indian region (Philipose, 1967). Chloroplast was cup-shaped and with a pyrenoid. Cells were 180.14 μ in diameter. Bristles 52-183 μ long. Habitat of the *Golenkinia* was planktonic mostly found in rock pool and tank, as previously reported in Ceylon (Crow, 1923). Proximate Composition of the biomass of the green algae *Golenkinia radiata*

Pigments Estimation

The pigments profile of the *Golenkinia radiata* biomass was estimated with two methods namely-Acetone method and DMSO method. Total Chlorophyll, Chlorophyll a & b and total carotenoids were determined. The experiment was conducted in triplicates and the mean and standard deviation of results were taken (Table 1) (Fig.3).

Table 1. Comparative values of pigments content of *Golenkinia radiata* biomass using two different estimation methods

Pigments	Pigment contents in <i>Golenkinia radiata</i> (mg/g dry weight biomass, mean±SD)	
	Acetone Method	DMSO Method
Chlorophyll a	1.44±0.047	1.76±0.026
Chlorophyll b	0.64±0.027	0.45±0.021
Total Chlorophyll	2.09±0.073	2.21±0.046
Total Carotenoids	0.17±0.006	0.20±0.001

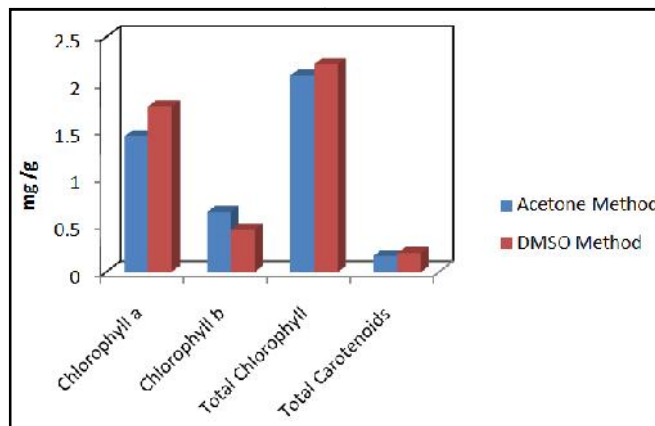


Fig. 3. Comparative values of pigments using two different estimation methods

Carbohydrate Estimation

The carbohydrate content of the biomass of *Golenkinia radiata* was found to be 40.39% (Fig. 4).

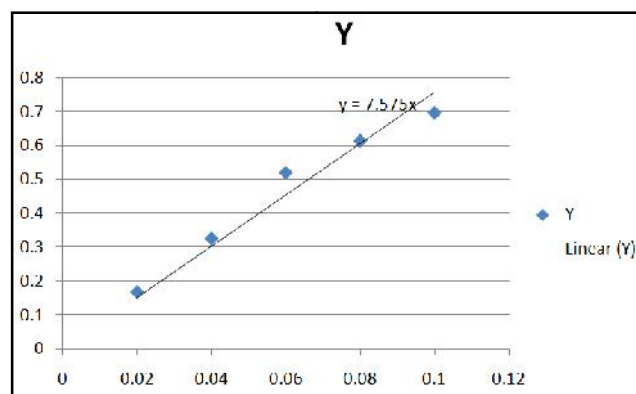


Fig. 4. The standard curve of the glucose

Protein Estimation

The protein content of the biomass of *Golenkinia radiata* was found to be 13.3% (Fig. 5).

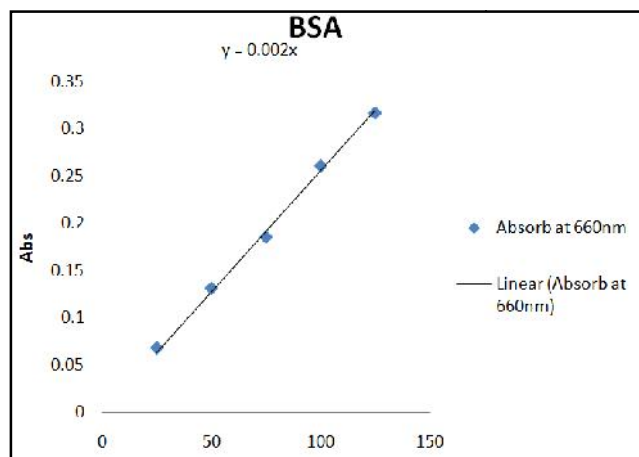


Fig.5. The standard curve of the BSA

Lipid Estimation

Total lipid % of the biomass was found to be 12.9%. 500mg of the biomass yielded 64.9 mg of total lipid (Fig. 6).

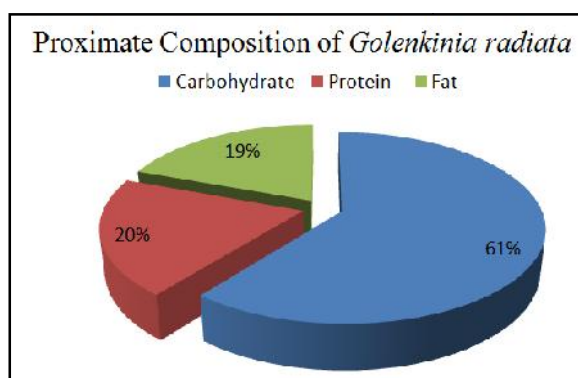


Fig. 6. Proximate Composition of *Golenkinia radiata* biomass

Energy Estimation

Total Energy of the biomass was found to be 1385.24 kJ per 100 gm biomass.

Water Quality Analysis

The results for the water quality parameters were following:

pH= 8.81, Temp= 29.7°C,
Dissolved Oxygen (DO) = 8.55mg/L,
Conductivity = 1150 μ S/cm.

- i) Nitrate (NO_3^-) = 1.35mg/L
(NO_3^-) = 5.8mg/L
- ii) Phosphate (PO_4^{3-}) = 8.1mg/L
(P) = 2.65mg/L
(P_2O_5) = 6.08mg/L
- iii) Iron (Fe) = 0.03mg/L
- iv) Sulphate (SO_4^{2-}) = 265 mg/L
- v) Chlorine (Cl_2) = 0.07mg/L
- vi) Silicate (SiO_2) = 32.7mg/L
(Si) = 15.7mg/L

DISCUSSION

The unconventional micro algal sources have been extensively utilized for the production of feed, food, food additive, pharmaceutical, and fine chemicals.

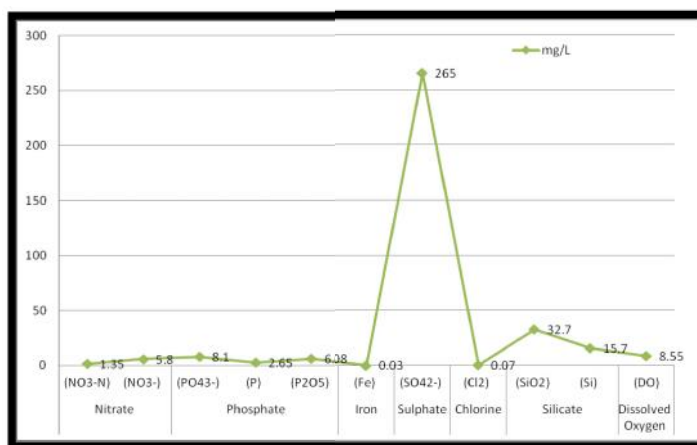


Fig. 7. Water quality parameters of *Golenkinia radiata* pond water

Numerous algal biomass like marine microalgae *Dunaliella salina*, *Nannochloropsis* spp., microalga *Phaeodactylum tricorutum*, *Porphyridium cruentum*, *Spirulina platensis*, *Chlorella vulgaris*, and *Isochrysis galbana* have been analyzed for proximate composition (Muhaemin, and Kaswadji, 2010, Reboloso Fuentes *et al.*, 2000, Tokusoglu, and Unal, 2003). On average, the biomass of the marine eustigmatophyte *Nannochloropsis* spp. was reported to contain 37.6% (w/w) available carbohydrates, 28.8% crude protein, and 18.4% total lipids (Reboloso Fuentes *et al.*, 2001). The microalga *Phaeodactylum tricorutum* cultured under various conditions in an external tubular photobioreactor and in a bubble column have also been analyzed for the nutrient content. On average, the biomass was found to contain 36.4% crude protein, 26.1% available carbohydrates, 18.0% lipids, 15.9% ash and 0.25% neutral detergent fiber, on a dry weight basis (Reboloso Fuentes *et al.*, 2001). *Golenkinia* have been earlier studied for its chlorophyll content but the present study is novel in reference to detailed nutrient profiling. The proximate composition of the *Golenkinia radiata* includes- carbohydrate (40.39%), protein (13.3%), lipid (12.9%) 64.9 mg total lipid per 500 mg biomass, energy 1385.24 kJ per 100 gm biomass and pigments- chlorophyll a (1.44 ± 0.047 mg g^{-1}), chlorophyll b (0.64 ± 0.027 mg g^{-1}), total chlorophyll (2.09 ± 0.073 mg g^{-1}) and total carotenoids (0.17 ± 0.006 mg g^{-1}) (with acetone method) and chlorophyll a (1.76 ± 0.026 mg g^{-1}), chlorophyll b (0.45 ± 0.021 mg g^{-1}), total chlorophyll (2.21 ± 0.046 mg g^{-1}) and total carotenoids (0.20 ± 0.001 mg g^{-1}) (with DMSO method). The better yields of chlorophyll and carotenoids were observed with the DMSO method of estimations.

Earlier studies on the effect of different compounds on the chlorophyll production in Green Alga *Golenkinia* have been done. Chlorophyll synthesis in *Golenkinia* is inhibited 10-fold by growth in darkness on acetate or by growth on elevated concentrations of acetate in the light, particularly if the growth medium contains low levels of nitrogen (Tiwari and Chauhan, 2006). Glucose has no such inhibitory effect. 5-aminolevulinic acid, with a maximal effect at 0.01 M, but not its precursors,

overrides the inhibitory effect of acetate and darkness, restoring chlorophyll synthesis. Glycine, succinate, and -ketoglutarate, the precursors tested, all enter the cell. Cyclic AMP has no effect on chlorophyll synthesis (Ellis *et al.*, 1975). The water quality analysis of the sample water from the fish pond revealed that the pH, dissolved oxygen and nutrients like nitrates and phosphates are within the permissible limits and supporting the both the fishes and algal growth. The pH was 8.81 in present study; however the optimum pH for fish growth and health is between 6 and 9. If pH is outside this range, fish growth will be reduced. Mortalities will occur when pH values are less than 4.5 or greater than 10 (Sallenave, 2012). Dissolved oxygen (DO) is probably the single most important water quality factor for pond. Oxygen is needed by fish and other aquatic organisms, and levels of DO will determine the ability of ponds and other water bodies to support aquatic life. Oxygen dissolves in water at very low concentrations measured in parts per million (ppm, which can be used interchangeably with milligrams per liter [mg/L]). Most oxygen in water is produced by algae and green plants through photosynthesis, the process whereby green plants use solar energy to convert water and carbon dioxide (CO₂) to oxygen and carbohydrates. Ponds will rarely have more than 10 ppm DO. Normal oxygen content in a healthy pond will range from 5 to 10 ppm (Sallenave, 2012, Gandhi, 2012).

The dissolved oxygen (DO) in present study was 8.55mg/L which has again proved to be sufficient for growth. The most important nutrients in aquatic systems are phosphorus (P) and nitrogen (N) in the forms of phosphates (PO₃) and nitrates (NO₃). These nutrients are critical to the growth of algae and fishes in aquatic systems. These nutrients in present study were also found to be present in sufficient amount i.e. Nitrate (NO₃⁻ N) = 1.35mg/L, (NO₃⁻) = 5.8mg/L, Phosphate (PO₄³⁻) = 8.1mg/L, (P) = 2.65mg/L, (P₂O₅) = 6.08mg/L (Sallenave, 2012). In the present study, carbohydrate was found to be the dominant nutrient, so it can be hypothesized that all these nutrients may have promoted fish growth. There have been extensive researches done earlier on algae being used in aquaculture. *Spirulina* was studied as a feed supplement for the giant freshwater prawn (*Macrobrachium rosenbergii*), and found to significantly improve growth, survival, and feed utilization.

The supplementation range was 5–20 percent and results were similar at any of the ranges added to the feed (Nakagawa, and Gomez-Diaz, 1975). China is using *spirulina* as a partial substitute of imported feed to promote the growth, immunity and viability of prawns (example *Penaeus monodon*). *Spirulina*-containing feed was found to reduce the cultivation time and mortality, and increase shell thickness of scallop. The survival rate of abalone (*Haliotis midae*) was improved by 37.4 percent. Feeding on *spirulina* helped to improve disease resistance of high value fish resulting in an improvement in their survival rate from 15 to 30 percent. Abalone (*Haliotis midae*) showed good growth when fed a diet containing *spirulina* meal (Britz, 1996). Abalone showed a significantly higher growth when fed diets based on fishmeal and *spirulina* than that fed diets prepared with soybean meal, torula yeast, casein and dried *Ecklonia maxima* (Habib *et al.*, 2008). Protein efficiency ratios of abalone fed formulated diets ranged from 3.3 for torula yeast to 6.5 for spirulina based diet. It was found that fishmeal and *spirulina* are the most suitable proteins for

inclusion in practical diets for Abalone. The replacement of artificial diet for post-larvae of abalone, *Haliotis discus discus* (Reeve) using *spirulina* gave good growth performance (Stott *et al.*, 2004). The metamorphosis rate of abalone post-larvae was increased by using *spirulina*.

The reproductive performance of Nile tilapia (*Oreochromis niloticus*) was tested using freshly harvested *Spirulina platensis* in comparison with control parent fish and progeny fed on commercial diets (Lu and Takeuchi 2004). There were no significant differences in the relative fecundity, spawning intervals and egg size among the various size groups. The fertilization rate and the hatching rate of the fertilized eggs, as well as the survival time of larvae from the parents fed the two types (*spirulina* and commercial diet) were similar. There was an increase in the synthesis of essential lipids when fed solely on *spirulina*. Significant differences were found in the fatty acid profile of fish eggs fed *spirulina* supplements containing more linoleic acid, -linolenic acid, eicosatrienoic acid, eicosatetraenoic acid, docosapentaenoic acid than those of fish fed solely on *spirulina*. Mozambique tilapia (*Oreochromis mossambicus*) was cultivated in artificial ponds with relatively high stocking density and fed with a mixture of solar-dried *spirulina* that had been cultivated and processed using low-cost technology and added to groundnut cake. The resulting average food conversion ratio was lower than that observed using control fish fed with the usual fishmeal-based ration. Furthermore, the yield of tilapia fed on *spirulina* mixed with groundnut cake was 4–5 higher than that of fish fed on groundnut cake alone (Vonshak, 1997). All these studies support the idea of using *Golenkinia radiata* biomass in making fish meal in near future and doing extensive studies in aquaculture.

The *Golenkinia* biomass was found rich in carbohydrate content which may have influenced the fish growth. The nutritious algal biomass has been extensively used for feed for animals like chicks and fishes. *Pavlova*, *Phaeodactylum*, *Chaetoceros*, *Skelotenma*, *Thalassiosira*, *Tetraselmis* [Pulz] are being used as feed for fish in aquariums. *Haematococcus pluvisialis* is being used as a colourant for living fishes due to the presence of astaxanthin (Enzing *et al.*, 2014). Carbohydrate content and good lipid sources of *Golenkinia* biomass can also be exploited for fish feed source. Further histological studies of the fishes breeding in the same pond can confirm the utility of the *Golenkinia* biomass for fish feed and even for human consumption.

Acknowledgement

Authors are thankful to the Director, CSIR-National Botanical Research Institute, Lucknow, India for his constant encouragement and laboratory facilities. The researcher at the CSIR-National Botanical Research Institute would like to thank the UGC for financial support and acknowledges the support provided in the form of UGC-SRF Fellowship.

REFERENCES

- Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts. Polyphenol oxidases in *Beta vulgaris*. *Plant Physiol.* 24(1): 1-15.

- Billar, P. and Ross, A. B. 2014. GC–MS Pyrolysis as a novel analysis technique to determine the biochemical composition of microalgae. *Algal Research*.6, 91–97.
- Bishop, W. M. and Zubeck, H. M. 2012. Evaluation of Microalgae for use as Nutraceuticals and Nutritional Supplements. *J Nutr Food Sci*. 2(5): 1-6.
- Britz, P. J. 1996. The suitability of selected protein sources for inclusion in formulated diets for the South African abalone, *Haliotis midae*. *Aquaculture*. 140, 63–73.
- Crow, W. B. 1923. Freshwater plankton algae from Ceylon. *J. Bot., Lond.* 61110-14, 138-45 and 164-71.
- Ellis, R., Spooner, T. and Yakulis, R. 1975. Regulation of chlorophyll synthesis in the green alga *Golenkinia*. *Plant Physiol*. 55, 791-795.
- Ellis, R. J. 1970. Effects of acetate on the growth and chlorophyll content of *Golenkinia*. *Journal of Phycology*. 6(4): 364–368.
- Ellis, R. J. 1977. Heterotrophic nutrition and its effects on chlorophyll synthesis in *Golenkinia* (chlorophyceae). *Journal of Phycology*. 13(3): 304–306.
- Enzing, C., Ploeg, M., Barbosa, M. and Sijtsma, L. 2014. Microalgae-based products for the food and feed sector: an outlook for Europe, JRC Scientific and Policy Reports, 19-37.
- Folch, J., Lees, M. and Stanley, G.H.S. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226 (1): 497-509.
- Gandhi, T. K. 2012. A study of water quality parameters to better manage our ponds or lakes. *International Journal of Latest Research in Science and Technology*. 1(4): 359-363.
- Guil-Guerrero, J. L., Gimenez-Martinez, J. J. and Torija-Isasa, M. E. 1999. Nutritional Composition of Wild Edible Crucifer Species. *Journal of Food Biochemistry*. 23: 283-294.
- Habib, M. A. B., Parvin, M., Huntington, T. C. and Hasan, M. R. 2008. A review on culture, production and use of *spirulina* as food for humans and feeds for domestic animals and fish, FAO Fisheries and Aquaculture Circular. No. 1034. Rome, FAO. 21.
- Hedge, J. E. and Hofreiter, B. T. 1962. Carbohydrate Chemistry 17. (Whistler R.L. and Be Miller, J.N. Eds.) Academic Press, New York.
- Hiscox, J. D. and Israelstam, G. F. 1979. A method for extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany*. 57(12): 1332-1334.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with Folin reagent. *J Biol. Chem.* 193: 265-273.
- Lu, J. and Takeuchi, T. 2004. Spawning and egg quality of the tilapia, *Oreochromis niloticus* fed solely on raw *Spirulina platensis* throughout three generations. *Aquaculture*. 234, 625–640.
- Muhaemin, M. and Kaswadji, R.F. 2010. Biomass nutrient profiles of marine microalgae *Dunaliella salina*. *Jurnal Penelitian Sains*. 13 (3D) 13313-64 -13313-67.
- Nakagawa, H. and Gomez-Diaz, G. 1975. Usefulness of *Spirulina* sp. meal as feed additive for giant freshwater prawn, *Macrobrachium rosenbergii*, *Suisanzoshuku*, 43: 521–526.
- Pathmanapan, A., Subramani, N. and Narayanswamy, A. 2010. Biodiversity of fresh water algae from temple tanks of Kerala. *Recent Research in Science and Technology*. 2(6): 58-71.
- Philipose, M. T. 1967. CHLOROCOCCALES, Indian Council of Agricultural Research, New Delhi. pg. 98,101-103.
- Price, A. H. and Hendry, G.A.F. 1991. Iron-catalyzed oxygen radical formation and its possible contribution to drought damage in nine native grasses and three cereals. *Plant Cell Environ*. 14, 477-484.
- Priya Gopinath, T. and Ajit Kumar, K.G. 2015. Microalgal diversity of the fresh water lake in Thiruvananthapuram district, Kerala. *International Journal of Plant, Animal and Environmental Sciences*. 5(1): 288-291.
- Reboloso Fuentes, M.M., Acien Fernandez, G.G., Sanchez Perez, J.A. and Guil Guerrero, J.L. 2000. Biomass nutrient profiles of the microalga *Porphyridium cruentum*. *Food Chemistry*. 70, 345-353.
- Reboloso-Fuentes, M. M., Navarro-Pérez, A., García-Camacho, F., Ramos-Miras, J. J. and Guil-Guerrero, J.L. 2001. Biomass Nutrient Profiles of the Microalga *Nannochloropsis*. *J. Agric. Food Chem.* 49(6): 2966–2972.
- Sallenave, R. 2012. Understanding water quality parameters to better manage your pond, Las Cruces, NM, Guide W-104, 1-4.
- Singh, R. P. and Balasingh, G. S. R. 2012. Contribution of algal flora in Kodaikanal Lake, Dindigul district, Tamil Nadu. *Indian Journal of Fundamental and Applied Life Sciences*. 2(4): 134-140.
- Sir Ray Lankester, K.C.B., F.R.S., 1908. Memoirs: On Archerina, Golenkinia and Botryococcus, *Quarterly Journal of Microscopical Science* 52(3): 423-430. (As accessed from <http://jcs.biologists.org/content/s2-52/207/423.full.pdf> on 31/07/2015)
- Stott, A. E., Takeuchi, T. and Koike, Y. 2004. Performance of a new artificial abalone hatchery culture system in terms of settlement of larvae and growth and survival of post-larvae *Haliotis discus* (Reeve). *Fish. Sci.*, 70, 1070–1081.
- Tiwari, A. and Chauhan, S. V. S. 2006. Seasonal phytoplanktonic diversity of Kitham lake, Agra. *Journal of Environmental Biology*. 27(1): 35-38.
- Tokusoglu, O. and Unal, M. K. 2003. Biomass nutrient profiles of three microalgae: *Spirulina platensis*, *Chlorella vulgaris*, and *Isochrysis galbana*. *Journal of Food Science: Food Chemistry and Toxicology*. 68(4): 1144-1148.
- Tripathi, S.K. 2013. Chlorococcales of river Ganga and their impact on water pollution. *Review of Research*. 2(6): 1-6.
- Venkatarayappa, T., Fletcher, R. A. and Thompson, J.E. 1984. Retardation and reversal of senescence in bean leaves by benzyl adenine and decapitation. *Plant Cell Physiology*. 25(3): 407-418.
- Vonshak, A. 1997. *Spirulina platensis* (Arthrospira). In Physiology, Cell Biology and Biotechnology. Basingstoke, Hants, London, UK, Taylor and Francis.