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STUDIES ON BIO-PHYSICO-CHEMICAL PROPERTIES OF A FRESHWATER ALGAL BLOOM: *SPIROGYRA* SP.

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ABSTRACT
Spirogyra bloom in the aquatic ecosystem is a natural process which is triggered by different anthropogenic activity. Excessive nutrients particularly phosphorus is the accruing agents for such type of bloom. An investigation was carried out in the drainage basin of IITG campus to generate baseline information about physico-chemical properties of *Spirogyra* blooms. *Spirogyra* is very common filamentous green algae that have high biochemical properties. The maximum total chlorophyll of 39.58 mg/g was uncounted with acidic pH of 5.98. This study revealed that *Spirogyra* blooms possess high biomass concentration i.e. 94.91%. Nitrogen (1.54%), total phosphorus (0.351 g/kg) and other micronutrient also show promising result. Present study concluded that utilization of this green algal bloom as an alternative food source is possible.

KEYWORDS: Algae, biomass, biochemical, blooms, ecosystem, pigments, *Spirogyra* sp.

INTRODUCTION
Algae a vital group of plants in aquatic ecosystems are an important component of biological monitoring programs for evaluating water quality. They are suited to water quality assessment because of their nutrient needs, rapid reproduction rate and very short life cycle. Algae are valuable indicators of ecosystem conditions because they respond quickly both in species composition and densities to a wide range of water conditions due to changes in water chemistry.

Recently, algae are receiving considerable attention due to their ability to synthesize valuable compounds e.g. pigments (Pacheco, *et al*., 2015). The pigments are characteristic of certain algal groups. There are four different kinds of pigments that are found in algae such as chlorophyll, carotenes, xanthophylls and phycobilins. Sorby (1873) classified blue chlorophyll as chlorophyll a, green chlorophyll as chlorophyll b and orange-yellow as xanthophyll according to the pigment colors. Chlorophyll is a key biochemical component in the molecular apparatus that is responsible for photosynthesis. Algae frequently live in extreme environments of light, salinity, and temperature. In order to adapt these extreme conditions, most of them produce a high variety of secondary metabolites that have potent biological activities. These secondary metabolites cannot be found in other organisms (Rodriguez, *et al*., 2010). Again, it has a remarkable ability to take up and accumulate heavy metals from their surrounding environment. The pollution of aquatic environments by metals is well documented worldwide. Metal contamination of the environment arises not only from natural sources but from anthropogenic activity (Deka and Sharma, 2012 and Sharma *et al*., 2007). Heavy metal ions have become an Eco toxicological hazard of prime interest and increasing significance, because of their accumulation in living organisms (Mane, *et al*., 2013). During algal growth, metals are removed from the surrounding habitat and accumulated in the cells by non-metabolic process called adsorption and by metabolic-dependent phase known as absorption.

Spirogyra is very common in relatively clean eutrophic water, developing slimy filamentous green masses and there are more than 400 species of *Spirogyra* in the world. The genus is one of the most ecologically important primary producers in aquatic food webs (Stancheva *et al*., 2013). In hot sunny day it grows under water but when there is enough sunlight and warmth they produce large amounts of oxygen, adhering as bubbles between the tangled filaments. The filamentous masses come to the surface and become visible as slimy green mats. The slimy free-floating mats of this type of algae occur frequently in stagnant water bodies during early spring and summer (Gerrath, 2003). *Spirogyra* is named for the helical or spiral arrangement of the chloroplasts that is diagnostic of the genus. It is commonly found in freshwater areas and comes under the division of Chlorophyta. It contains chlorophyll *a* and *b* which are responsible for its green color. However, in stress condition it appears yellow or orange due to the presence of secondary pigments i.e. carotenoids. Carotenoids are of high value as it is a potential antioxidant and could prevent cancer and cardio- vascular problems (Park *et al*., 2010 and Giordano *et al*., 2012). Spirogyra is used as a nutrient dense foods and it is a source of fine chemicals (Tipnee, 2015).Despite the widespread uses and claimed advantages of these algae only few investigations on the chemical composition of *Spirogyra* sp. have been reported. So, the objective of this study is to determine the bio-physico-chemical properties of these filamentous green algae and explore the ecology of its habitat.

MATERIALS AND METHODS

A survey of algae was carried out in the drainage basin of Indian Institute of Technology Guwahati campus, Assam. The geographical location of the study sites were recorded by GPS which was 26°11'14"N 91°41' 30"E at an elevation of 55.5 m from the sea level. It is situated in the banks of the Brahmaputra River and the foothills of the Shillong plateau. The climatic conditions of this region is highly humid which is coupled with heterogenic physiography make possible luxuriant growth of a number of plant communities imparting it a distinct identity of phytogeographic habitat with many endemic species. The work was done in the month of December, 2015 – January, 2016 when water level was low which was coupled with nutrient rich water condition and dense mat of algae.

Collection and analysis of Algae and Water samples

Algae and water samples were collected from different selected drain within the campus based on the input of nutrient inflow and growth of algal bloom in the month of February, 2016 when water level was low {Fig.1(a-b)}. Collected algal mats were washed 5-6 times with distilled water {Fig. (c-d)} and then oven-dried at 80°C for 24 hours. Dried algae were grinded and kept in polythene bags for future use. For other experiment after washing the algae fresh samples were used. Microscopic observations of both fresh and preserved samples were done. The cell morphology such as size of the organism, presence or absence of chloroplast, eyespot, flagella, paramylon, locomotary movement etc. were taken into account and identified following standard protocol as mentioned by Prescott (1951), Desikachary (1959). Pigment profile of the algal samples was estimated by analyzing chlorophyll (*a* and *b*) and carotenoid content by extracting the samples with 90% acetone following cold extraction method (Strickland and Parsons, 1968). Total carbohydrate and protein were estimated by Anthrone and Lowry's method. Biomass content was determined by weight loss of the algal samples (105° C for 24 h) using the gravimetric method (BIS, 1982). Particle size of the algae was determined by Laser particular size analyzer. The pH of the algal samples was measured using a pH meter. Volatile solids (VS) and Ash content were determined by the ignition method (550 °C for 2 h in muffle furnace). Total nitrogen was analyzed by using Kjeldhl method, total and available phosphorus were determined by using the stannous chloride method. Nutrients estimation of algae water samples were performed by using flame photometer and heavy metal were estimated by employing atomic absorption photometers (APHA, 2005). All tests were performed three times and data were expressed as mean ± S.D. (n==3).

RESULT AND DISCUSSION

Morphological Character

Spirogyra is an unbranched filamentous alga. It is commonly occurring in freshwater habitats of Assam. It is difficult to identify the *Spirogyra* species due to their variations in some features of vegetative filaments and the rarity of fertile filaments in nature. Vegetative filaments of the genus are common throughout the year but reproductive filaments are rarely found in short periods. The plants are unbranched filaments of cylindrical cells with plane end walls of adjacent cells (Fig. 2). The cells have four or five chloroplasts containing numerous disc-like pyrenoids and each chloroplast makes 1.5–2 turns. The vegetative cells are 50–53 µm in width and 80–90 µm in length. Kim, (2015) reported that this species is distinguished by its vegetative cell width, number of chloroplasts, cylindrical female gametangia and ovoid zygospores. Prescott (1951), Randhawa (1959), Vidyavati and Noor (1995), Kargupta and Jha (2004), Taft (2009) reported *Spirogyra* can be recognized by three characteristics i.e. types of cross wall (plane, replicate, semi replicate or colligate), cell length and width and by the numbers of chloroplast.

Biochemical properties of *Spirogyra* sp.

Generally, *Spirogyra* contains chlorophyll *a* and chlorophyll *b* which are responsible for its green color but due to stressful condition it produces some secondary pigments i.e. carotenoids. Chlorophyll is essential for photosynthesis because it has the ability to captured solar energy. It is also a necessary and important bioactive compound that can be extracted from algal biomass. In the present work (Table 1) maximum concentration of chlorophyll *a* and *b* was recorded in site I (12.29 mg/g and 27.29 mg/g). Least value of was 10.55 mg/g and 22.76 mg/g in site III. *Spirogyra* is not only used as an additive in pharmaceutical and cosmetic products but also as a natural food coloring agent. Again, it has antimutagenic properties (Hosikian *et al*., 2010). Carotenoids are compounds with pharmaceutical, high industrial and economical value and it is recognized as a potential antioxidant which can prevent cancer and cardiovascular problems (Giordano *et al*., 2012). Highest value of carotenoid was also observed in site I and lowest was in site III (15.67 mg/g and 13.87 mg/g). High concentration of carotenoid in plant cell directly related to its antioxidant activity. Chaudhuri *et al.*, (2014) reported that methanol extraction of *Euglena tuba* contains large amounts of bioactive phytochemicals, exhibits high antioxidant and free radical scavenging activities with high reducing power capacity. Deb (2016) again incorporated that the efficacies of antioxidants are associated with their ability to scavenge stable free radicals. The DPPH radical scavenging activity of methanol extract demonstrated its oxygen radical absorbance capacity and indicated its potent antioxidant nature. Algal extraction is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses which is also beneficial in prevention of various other human diseases as reported by Goodman *et al*., 2011.

Protein is the most important component contributing to the nutritional value of food Many algae have the ability to produce protein in reasonable amount of their dry weight, making them good protein sources for organic fertilizer, animal feed and human nutrition supplement (Dawczynski *et al*., 2007). In our study the range of protein concentration was found 24.52µg/ml – 40.95 µg/ml. Carbohydrates are molecules that are composed of carbon, hydrogen and oxygen, made up of sugars, starches, cellulose and lignin. Carbohydrate is an essential component for metabolism and metabolic processes. The concentration of carbohydrate was monitored within the range of 27.69µg/ml to 43.21. Wijesekara *et al*., (2011) stated that algal carbohydrates can provide human health benefits in the form of anticoagulants, antivirals, dietary fibers and antioxidants. The types and abundance of carbohydrates vary strongly between algae species (Chennubhotla, 1996). Our study shows high concentration of carbohydrate as compared to protein in all three study site as earlier observed by Deb (2015) in case of *Euglena tuba*, a freshwater algal bloom forming species.

Particle size analysis was done for finding out the particle size range of *Spirogyra*. Fig 4 shows the mean size of the algal sample was in the range of 10 – 1000 µm. The smaller the particle size the higher the surface area per unit weight of *Spirogyra* and hence higher percentage of metal removal is obtained.

Physico-chemical properties of *Spirogyra* sp.

Table 2 shows the physicochemical properties of the *Spirogyra* sp. pH of the algal sample was recorded in in the range of 5.62 - 5.98. The acidic nature of the sample is due to the release of toxic element of the algae. Biomass of the sample was high in site I (94.91 %) and least in site III which was 90.23 %. The experimental site was exposed by heavy sunlight with good input of nutrients. Volatile Solids (VS) of the sample was recorded high in site II (74.45 %) and less in site I (70.73%). Ash content (AC), Total organic carbon (TOC), Total Nitrogen (TN) were found high repeatedly in site I (29.27, 39.29, 1.54) % and low in site II which were 25.55%, 38.22% and 1.02%. Again total Phosphorus (TP) was recorded high in site I (3.092 µg/mL) and least in site III (2.011 µg/mL).

Comparison of macro and micro nutrient of Spirogyra sp. and water

Fig. 3 shows the comparison of nutrients in water and algae. Trace element such as copper, nickel, and zinc, are at very low concentrations are essential for life because they play important roles in metabolic processes taking place in living cells. Metals in the environment occur in different chemical forms (metal speciation): as ions dissolved in water, as vapors, or as salts or minerals in rocks, sand, and soils. The chemical form of a metal in the environment is constantly changing due to a wide spectrum of dynamic biochemical processes (Hafeburg & Kohe, 2007). It was found that resistant microalgae which isolated from metal contaminated sites have a higher capacity for accumulating heavy metals as compared with species isolated from non-contaminated sites.

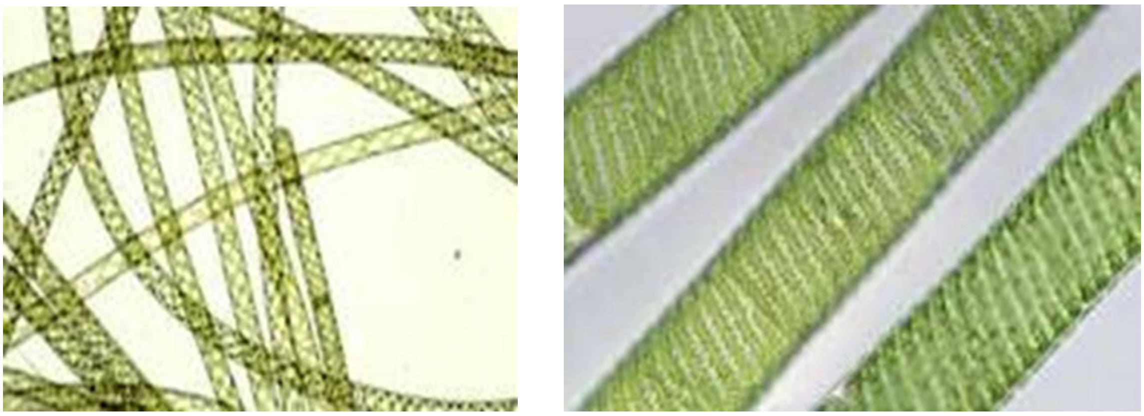
The effects of metals on aquatic organisms are difficult to determine as many physical and chemical properties such as flow rate contribute to the outcome. Also the size and the nature of particulates to which the metals are attached affect the toxicity of the metals. Various factors influence the metabolism and effects of metals. Those factors that include particular characteristics of the organisms exposed are known as host factors. Host factors include age, diet, immune status, sex, species and interphyletic and circadian biorhythms (Mane, *et al*., 2011).

(a)

(b)

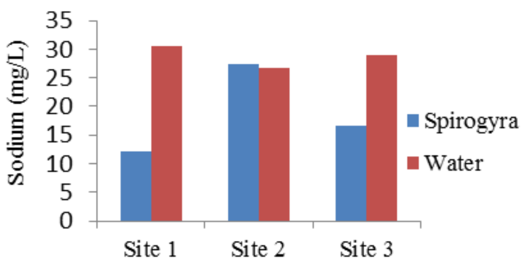
(c) **(d)**

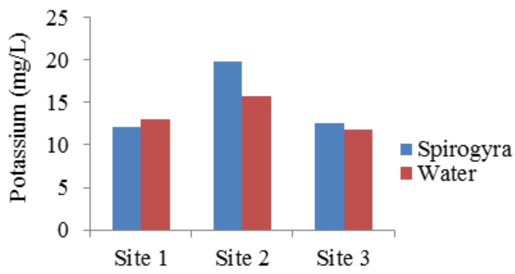
Fig. 1(a-d) shows the fresh *Spirogyra* bloom collected from the drainage basin in the IITG campus.

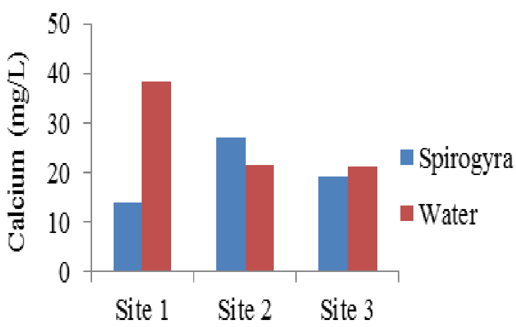


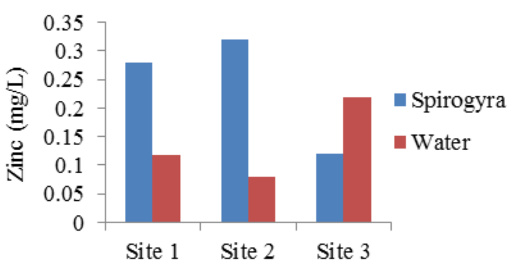
(a) **(b)**

Fig. 2(a) and (b) shows the microscopic profile of *Spirogyra* sp.









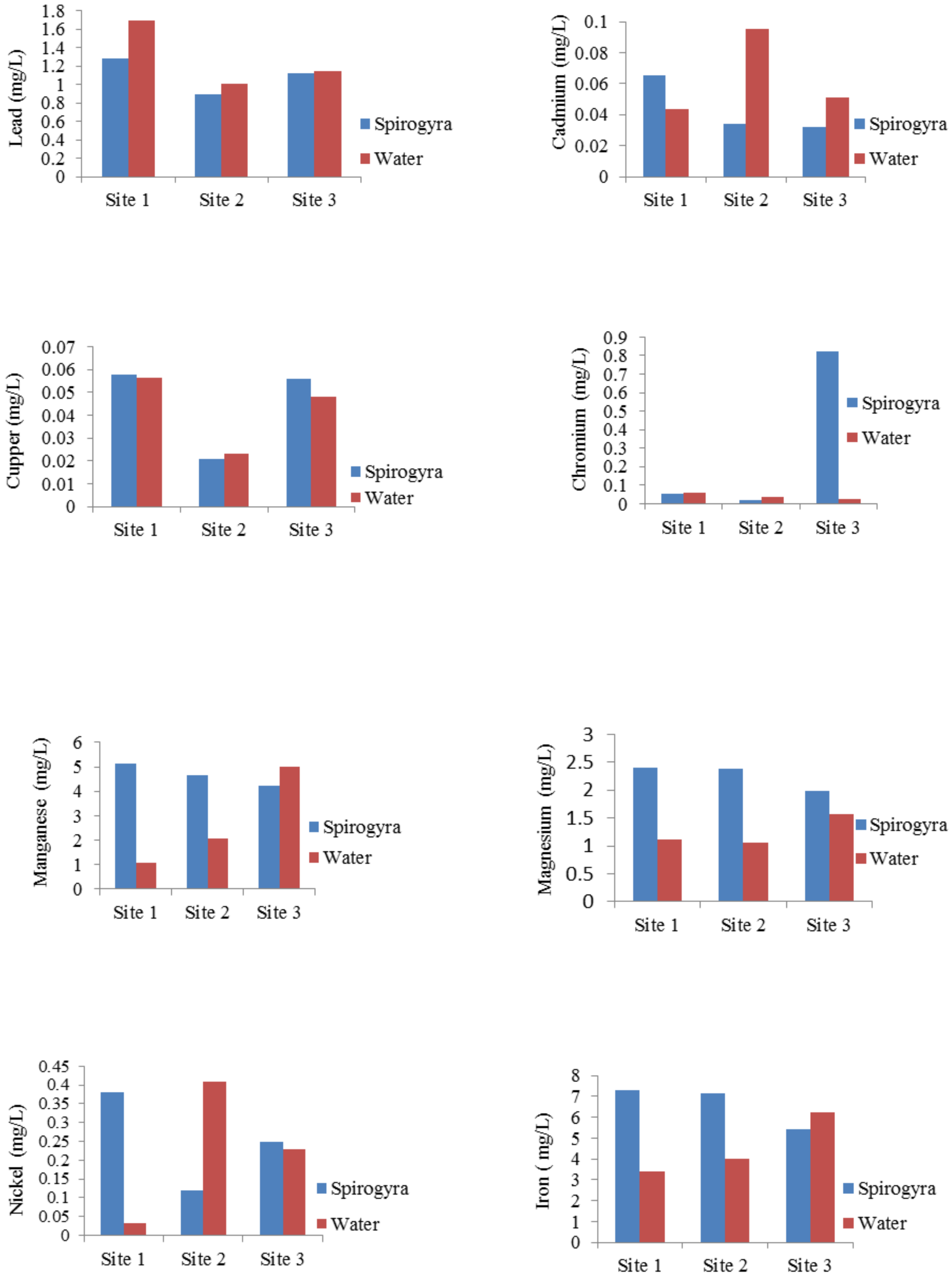


Fig. 3. Shows the comparison of macro and micro nutrient of *Spirogyra* sp. and water in different stratic location of drain ecosystem in the IITG campus

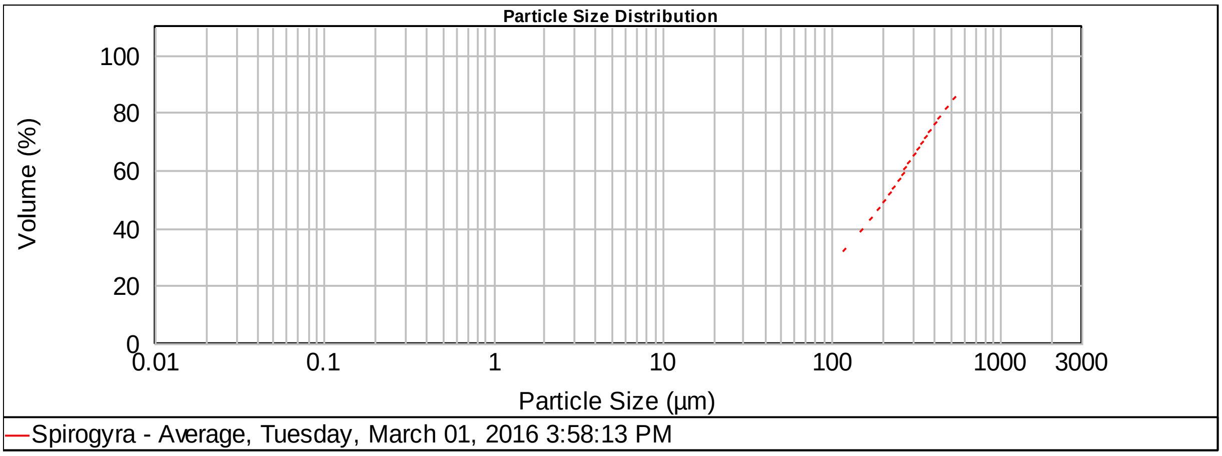


Fig. 4: Particle Size Analysis of Freshwater *Spirogyra* species

Table 1: Biochemical Properties of *Spirogyra* species.

Biochemical properties	**Site**		
	I	**II**	**III**
Chlorophyll a (mg/g)	12.29	12.02	10.55
Chlorophyll b (mg/g)	27.29	26.98	22.76
Carotenoid (mg/g)	15.67	14.54	13.87
Protein (µg/mL)	29.99	24.52	40.95
Carbohydrate (µg/mL)	43.21	27.69	29.65

Table 2: Physico-chemical properties of Freshwater *Spirogyra* species.

Parameters	**Site**		
	I	**II**	**III**
pH	5.98	5.67	5.62
Biomass (%)	94.91	91.84	90.23
Volatile Solids (%)	70.73	74.45	71.25
Ash Content (%)	29.27	25.55	28.75
Total Organic Carbon (%)	39.29	38.22	39.15
Total Nitrogen (%)	1.54	1.02	1.23
Total Phosphorus (mg/L)	3.092	2.35	2.011

CONCLUSION

The metals plays very important role in the growth and photosynthetic rate of the algae but at higher concentrations of the metals the algae shows toxic effects. Metals in the environment may have a profound impact on the physiology and general health of the exposed organism. In the present study the effects of the heavy metals concentrations on some physico-chemical parameters of *Spirogyra* sp. was shown. Present study conclude that *Spirogyra* sp. is a potential health food in human diets and may be of use to the food industry as a source of ingredients with high nutritional value. *S. varians* can provide a dietary alternative due to its nutritional value and its commercial value can be enhanced by improving the quality and expanding the range of freshwater macro algae based products.

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