

Physicochemical studies of freshwater lakes in Dharwad and screening of algal species for biofuel production

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ABSTRACT

The world today is facing two major problems, of oil crisis and global warming; microalgae have been focused as living organisms that have high potential in solving both these problems by producing bio fuels and carbon sequestration. Aim of the study is to estimate physical and chemical parameters and to determine the algal diversity in ten lakes in the district of Hubli and Dharwad. Lipid content of algae will be analyzed. Physico-chemical analysis of lakes was done to check eutrophication of lakes by estimating the amount of physical factors like pH, turbidity, temperature, electrical conductivity and chemical parameters like alkalinity, calcium, magnesium, total hardness, potassium, sodium, phosphates, chloride, sulphate, nitrates, D.O, C.O.D, B.O.D present, which indeed serve as nutrients for the growth and proliferation of algal species. In this investigation, two lakes were found to be entropic and algal diversity present in all ten lakes were determined. About 20 different algal species were isolated and studied for lipid content. Out of this four species showed high potential for biofuel production.

Key words: Biofuel, physico-chemical analysis, microalgae.

INTRODUCTION

Ponds and lakes constitute an ecosystem, where proliferates a Huge array of organisms ranging from lower phytoplankton's to Higher plants and variety of zooplanktons. In developing nations, water sources are used for domestic purposes such as Washing of cloths, bathing and sometimes as a source of Drinking water (Chia *et al.*, 2009). The presence in Abundance of microalgae in ponds and lakes is controlled by a wide range of physico- chemical and biological factors. Trace elements are essential for metabolic processes in Phytoplankton (Monastersky 1995) which consists of sediment or bedrock of pond ecosystem. They are non-degradable and are bio accumulated along the food chain, transformed or complexed from one form to another in aquatic systems (Rai *et al.* 1981, Sunda *et al.* 2005). The extent to which micro algal species can tolerate trace metals potential indicators for the presence and levels of these metals. Trace elements act as micronutrients at low concentrations, while at high concentrations they become toxic. Macro nutrients and organic substances are added to the water bodies by human interference such as discharge of domestic sewage, running of inorganic fertilizers and pesticides for agricultural land, industrial effluents, and religious rituals.

The physico-chemical properties will also help in the identification of sources of pollution, for conducting further investigations on the exobiological impacts and also for initiating necessary steps for remedial actions in case of polluted water bodies. Therefore the nature and health of any aquatic community are an expression of quality of water. Phytoplankton constitutes the basic components of the aquatic food chain. The interplay of physical, chemical and biological properties of water most often leads to the production of phytoplankton, while their assemblage (composition, distribution, diversity and abundance) is also structured by these factors. One such phytoplankton is the algae which an indicator of polluted water body. Algae are a potential source for biofuel as it can grow at an exceptional fast rate: 100 times faster than terrestrial plants and they can double their biomass in less than one day with minimal requirement of nutrients, accumulating large quantity of lipids which is converted into biodiesel. The realistic value of microalgae biomass production is around 30 tonne/ha/year which is equivalent to a lipid production of 5.0–8.0 tonne/ha/year.

“Microalgae” have been considered as potential source of new renewable energy feedstock. Cultivation of microalgae provides many advantages for sustainable development as follows: 1) energy security (micro algal biomass is renewable feedstock), 2) food security (production of micro algal biomass uses smaller foot print and non-arable land, therefore, no competition with food crop on price and land used), 3) CO₂-fixation (from atmosphere or industrial flue gas, hence, reduces global warming), 4) Up take nitrogen and phosphorus from waste water (consequently reduce eutrophication in aquatic ecosystems) and 5) restoration of land (by fertilizer or soil conditioner obtained from biomass residue).

In our study we aim for isolation of fresh water algae from lakes of Hubli and dharwad for extraction of lipids and biodiesel production, growth parameters will also be studied, for this we need to evaluate the physical and chemical parameters of the source water i.e. of the lakes. As the micro and macro elements/nutrients are accumulated by the phytoplankton's, there growth, proliferation and lipid productivity is been effected by their concentration in the source water, hence these parameters are to be estimated for further growth studies

MATERIALS AND METHODS

Study area:

Following lakes of Hubli and Dharwad was selected for our study, their names and geographical coordinates are as follows (Table. 1) & (Figure.1).



Figure.1 Physical map of India showing Hubli-Dharwad location (Map not to scale)

Table 1: List of Lakes and their Geographical Co-ordinates

S. NO.	NAME OF THE POND/LAKE	PLACE	CO-ORDINATES
1.	Navalur lake	(Dharwad)	1. 15°25'55"N 75°2'14"E
2.	Someshwara lake	(Dharwad)	15°25'21"N 75°0'57"E
3.	Nuggikere	(Dharwad)	15°24'55"N 75°0'20"E
4.	Kelagere	(Dharwad)	15°27'28"N 74°58'21"E
5.	Sadhanakeri	(Dharwad)	15°27'51"N 74°59'12"E
6.	Mugad lake	(Dharwad)	15°26'26"N 74°54'57"E
7.	Unkal lake	(Hubli)	15°22'41"N 75°6'22"E
8.	Neer sagar	(Hubli)	15°19'26"N 74°58'34"E
9.	Rayanaal lake	(Hubli)	15°20'39"N 75°5'46"E
10.	Deveragudihal lake	(Hubli)	15°19'51"N 75°3'11"E

The study was carried out during the month of March, April and May 2014, algal density is increased during summer months, and is decreased during winter season, change in temperature influence the growth and proliferation of algae. The metabolism, physiology and behavior of aquatic organisms are related directly to the temp of the aquatic environment (Wetzel, 1990). Hence these Months were considered for the study.

Collection of samples:

Water Samples was collected periodically every month during morning hrs between 8.00 A.M and 9.00 A.M. Samples were collected in Polyethylene carbonyl bottles of 2 litre capacity, from 1 foot below the water surface for physico-chemical analysis. About 60 litres of surface water was filtered through standard plankton net. The final volume of the filtered sample was 200ml which were transferred to polyethylene bottles of 250 ml capacity and preserved by adding 8ml of 4% formalin.

Physico-chemical analysis:

Temperature (air and surface water) was recorded on the spot using Centigrade thermometer. The pH of the water samples was measured by using the gun pH meter on the spot. Physico-chemical analysis (electrical conductivity, alkalinity, salinity, phosphate, calcium hardness, magnesium hardness, total hardness, dissolved oxygen and biological oxygen demand) of the sample was done according to standard methods (APHA, 1998) and Indian standard (IS: 3025-1964).

Biological analysis:

Algal species were studied under light microscope and identified with the help of standard references. Quantitative analysis was made using a plankton-counting cell (Sedgwick rafter). Identification and enumeration of algae were done as per the methods described by Adoni et al., (1985), Agarker et al., (1994), Welch (1948), Hosmani and Bharathi (1980). Identification was done by consulting the monographs by Philipose (1967), Gandhi (1998) and Prescott (1998).

Screening of algal strains for lipid:

Samples were placed on glass slides using distilled water. Cells were obtained by micro pipette isolation method under a compound microscope, Nile red staining was done as proposed by Lee *et al.*, 1998 to all the isolated strains, to check the lipid content, pure cultures were obtained using CHU-13 agar medium, further cultivation for growth studies was done on BG-11 broth medium.

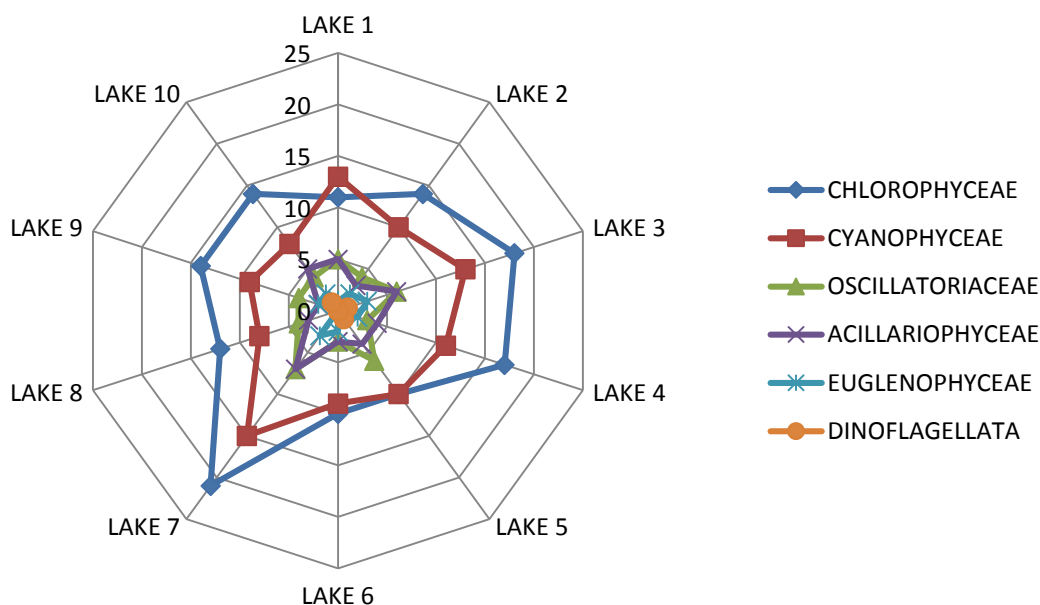
RESULTS

Results suggest the mesotrophication of three lakes ie. Navalur lake, Nuggikeri lake and Unkal lake with high mineral and salt composition; 4four lakes had moderate levels of nutrients and comparatively less concentration of algal biomass i.e. Someshwar lake, Kelageri, Sadhanakeri, Deveragudihal lake. Navalur lake hade high levels of potassium (85.8 mg/L), sulphate (70 mg/L), nitrate (2.1 mg/L) and chloride (122.5 mg/L) but very less dissolved oxygen (0.8 mg/L), Nuggikeri and Unkal Lake had high levels of salts along with good dissolved oxygen (12.7 & 7.3 mg/L) and low nitrate (0.6 & 0.5 mg/L) levels compared to other lakes, which indicates the presence of large number of phytoplankton's. Someshwar Lake, Kelageri, Sadhanakeri and Deveragudihal Lake, had moderate level of nitrates, sulphate, phosphates and D.O, with a good amount of algal diversity. Studies conducted on other lakes ie. Mugad Lake, Neersagar and Rayanaal Lake suggest that there are low levels of Compounds or mineral salts in these Lakes. Analyzed Physical and Chemical. Parameters are depicted in Table 2. Altogether 65 species of phytoplankton were identified, of which 28 species belonged to the class Chlorophyceae, 8 of class Oscillatoriaceae, 17 belonged to class

Cyanophaceae and 7 Belonged to class Bacillariophyceae and 3 species of Euglenophyceae and 1 of Dinoflagellata. Class wise distribution of algae is depicted in Graph 1. The list of algae isolated and identified from these lakes is depicted in Table 3. About 20 algal strains showed positive result for lipid content which were subjected for further growth studies using BG-11 medium. Out of the 20 strains, 4 strains AS-3, AS-6, AS-13, AS-18, showed high growth rate and high lipid productivity in *invitro* conditions. Results are given in Table 4. These strains were used for further studies. The pictures are given in Figure 2.

Table 2: Mean values of physicochemical parameters during months of March, April and May 2014

Parameters	Lake 1	Lake 2	Lake 3	Lake 4	Lake 5	Lake 6	Lake 7	Lake 8	Lake 9	Lake 10
pH	8.6	8.0	8.9	7.6	7.1	7.6	8.4	6.8	7.1	6.7
Temperature ° C	26.7°C	25.7°C	25.7°C	24.8°C	27.6°C	27°C	25°C	25.6°C	27.1°C	26.8°C
EC µs/cm	1152	530	1254	454.3	636	375	983.3	490	345	760
Turbidity NTU	216	85	177	56	67	94	130	112	46	66
Alkalinity mg/L	10.6	4.0	11.2	3.6	6.5	5.0	9.6	3.5	7.0	5.0
TDS mg/L	686	455	655	433	340	480	721	550	424	530
Calcium mg/L	145	45.2	68	36	13	32	55.6	36	32.7	24.6
Magnesium mg/L	76.3	13.6	17.5	14.72	40	26.7	43.5	26	20.2	16.8
Total hardness mg/L	256	87	145	96.6	202	190	218.4	200	165.3	98
Potassium mg/L	85.8	13.0	56.3	7.6	10.4	23.8	47.28	11.0	34.0	4.6
Sodium mg/L	155	57	45.1	69.4	23.4	38.5	108	44.5	56.2	80
Phosphates mg/L	7.3	0.06	5.4	0.02	1.4	0.5	3.7	0.6	0.01	1.8
Chloride mg/L	125	32.5	135	202	194	34.8	194	51.76	56.9	135
Sulphate mg/L	70	23.5	70.6	25	51.5	56	87.4	34.5	26.5	22.1
Nitrates mg/L	2.1	1.5	0.6	1.3	1.8	4.0	0.5	1.5	1.3	2.4
Do mg/L	0.8	2.0	7.5	8.0	3.4	3.6	7.5	12	0.6	14.2
BOD	7.0	5.8	6.5	3.0	4.7	2.3	5.8	4.2	2.5	0.3



Graph 1: Class wise distribution of total no. of algal species present in each lake

Table 3: List of algal species present in lakes (1-10) of Dharwad District. (+ Present, 0 absent)

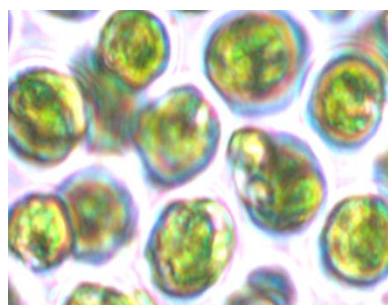
Sl. No.	ORGANISM	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
CHLOROPHYCEAE											
1	<i>Ankistrodesmus fusiformis</i>	0	+	0	0	0	+	+	0	0	+
2	<i>Closterium sp</i>	+	+	0	0	0	0	+	0	+	0
3	<i>Chlorella vulgaris</i>	0	+	+	+	+	0	+	+	+	+
4	<i>Chlorella sp</i>	+	0	+	+	0	+	0	0	0	0
5	<i>Chlorella pyrenoidosa</i>	0	0	+	0	0	0	+	+	+	+
6	<i>Cl. Porrectum</i>	0	0	+	+	0	0	0	+	0	+
7	<i>Cladophora glomarata</i>	+	+	0	+	+	+	+	0	+	0
8	<i>Cosmarium botrytis</i>	+	0	0	0	0	0	+	0	+	+

9	<i>C. auriculatum</i>	0	0	+	+	+	0	+	+	0	+
10	<i>C. phaseolus</i>	0	+	0	0	+	0	0	0	0	0
11	<i>C. javanicum</i>	+	0	0	+	0	+	+	0	+	+
12	<i>Coelastrum Microporum</i>	0	0	+	+	0	0	+	+	0	+
13	<i>Cosmarium sp</i>	+	+	+	0	0	+	0	0	0	0
14	<i>Crucigeninia tetrapedia</i>	0	0	0	+	0	0	+	+	+	0
15	<i>Crucigeninia crucifera</i>	0	0	+	0	0	0	+	0	0	0
16	<i>Dictyosphaerium sp</i>	+	+	+	+	+	+	0	0	+	0
17	<i>Kirchneriella lunaris</i>	0	+	0	0	0	0	+	0	+	+
18	<i>Pediastrum boryanum</i>	0	0	0	+	0	0	+	+	+	+
19	<i>Pediastrum simplex</i>	+	0	+	+	+	+	0	+	0	0
20	<i>Scenedesmus abundans</i>	0	+	+	+	0	0	+	+	0	0
21	<i>Scenedesmus sp</i>	0	+	+	+	+	0	+	0	0	0
22	<i>Scenedesmus dimorphus</i>	+	0	+	+	0	0	+	+	+	+
23	<i>Scenedesmus obliquus</i>	0	+	+	0	+	+	+	+	0	0
24	<i>Scenedesmus quadricauda</i>	0	+	0	+	0	0	+	0	+	+
25	<i>Schroederia setigera</i>	0	0	+	+	0	0	0	0	+	+
26	<i>Selenastrum gracile</i>	+	0	+	0	+	+	+	+	0	0
27	<i>Tetraedron sp</i>	0	+	+	+	+	0	+	0	0	+
28	<i>Westella botryoides</i>	+	+	+	0	0	+	+	+	+	0
CYANOPHYCEAE											
29	<i>Anabaena iyengarai</i>	+	0	+	+	0	0	+	0	0	+
30	<i>A. ambigua</i>	0	+	+	+	+	0	+	+	0	0
31	<i>A. vagincola</i>	+	+	+	0	0	+	0	0	+	0
32	<i>Anabaenopsis sp</i>	0	0	+	+	0	0	+	+	+	0
33	<i>Aphanozomenon sp</i>	+	+	0	+	+	+	+	0	0	+
34	<i>Calothrix membranacea</i>	+	+	+	0	+	+	+	0	+	0
35	<i>Gloeotrichia pizam</i>	+	0	0	+	0	0	+	0	0	+
36	<i>Hydrococcus sp</i>	+	+	+	+	0	0	0	+	+	0
37	<i>Merismopedia glauca</i>	0	+	+	0	+	0	+	0	0	0
38	<i>Microcystis aeruginosa</i>	+	+	+	0	0	0	+	+	0	+
39	<i>Nostoc linchia</i>	+	0	+	+	+	+	+	0	+	+
40	<i>N.ellipsoforum</i>	0	+	0	+	0	+	+	+	0	0
41	<i>Oscillatoria formosa</i>	+	0	+	+	+	0	0	+	+	+
42	<i>O. limosa</i>	+	+	+	0	+	+	+	0	0	0
43	<i>Spirulina platensis</i>	0	0	+	+	0	0	+	+	+	0
44	<i>Spirulina laxissima</i>	+	0	0	0	+	+	+	0	+	+
45	<i>Synechococcus sp</i>	+	0	+	0	+	+	+	0	+	+
46	<i>Trichodesmium sp</i>	+	+	0	+	0	+	+	+	0	0
OSCILLATORIAEAE											
47	<i>Oscillatoria nigra</i>	+	+	+	0	+	0	+	0	0	+
48	<i>Oscillatoria chlorina Kutz</i>	0	0	+	+	0	0	+	+	+	+
49	<i>Oscillatoria tenuis</i>	+	+	0	+	+	0	+	0	+	0
50	<i>Oscillatoria irrigua</i>	+	0	+	+	+	+	+	+	0	0
51	<i>Oscillatoria curviceps</i>	0	+	+	0	+	0	+	0	+	0
52	<i>Oscillatoria obscura</i>	+	0	0	0	0	+	+	+	0	+
53	<i>Oscillatoria proteus</i>	0	0	+	+	+	0	0	+	0	+
54	<i>Oscillatoria salina</i>	+	+	+	0	+	+	+	0	+	+
BACILLARIOPHYCEAE											
55	<i>Cyclotella meneghiniana</i>	+	+	+	0	+	0	+	0	0	0
56	<i>Cymbella tumida</i>	+	0	+	+	+	0	+	0	+	+
57	<i>Navicula sp</i>	0	0	+	+	0	+	+	0	0	0
58	<i>Nitzschia acicularis</i>	+	+	0	0	0	0	+	+	0	0
59	<i>Nitzschia longissima</i>	+	0	+	0	+	0	+	+	+	+
60	<i>Nitzschia sigma</i>	0	+	+	+	0	+	+	+	0	+
61	<i>Pinnularia sp</i>	+	0	+	+	+	+	+	0	0	+
EUGLENOPHYCEAE											
62	<i>Euglena sp</i>	0	0	+	+	0	+	+	0	+	+
63	<i>Phacus plueronectes</i>	+	+	+	0	0	+	+	0	0	0
64	<i>Phacus tortus</i>	0	+	+	+	0	0	+	0	+	+
DINOFLAGELLATA											
65	<i>Gonyaulax sp</i>	0	0	+	0	+	0	0	0	0	+

Table 4: list of algal isolates positive for lipid content

Sl. No.	Organism	Code	Source lake	Biomass mg/ml	Lipid mg/ml
1	<i>Ankistrodesmus fusiformis</i>	AS-1	L2	0.650	0.095
2	<i>Chlorella sp</i>	AS-2	L6	0.850	0.110
3	<i>Chlorella vulgaris</i>	AS-3	L7	2.200	0.800
4	<i>Chlorella vulgaris</i>	AS-4	L4	1.500	0.400
5	<i>Chlorella pyrenoidosa</i>	AS-5	L3	1.060	0.300
6	<i>Chlorella pyrenoidosa</i>	AS-6	L7	1.580	0.520
7	<i>Chlorella pyrenoidosa</i>	AS-7	L10	1.200	0.260
8	<i>Scenedesmus abundans</i>	AS-8	L2	0.740	0.135
9	<i>Scenedesmus abundans</i>	AS-9	L3	0.810	0.160
10	<i>Scenedesmus abundans</i>	AS-10	L4	0.759	0.115
11	<i>Scenedesmus abundans</i>	AS-11	L8	0.780	0.095
12	<i>Scenedesmus dimorphus</i>	AS-12	L1	1.200	0.215
13	<i>Scenedesmus dimorphus</i>	AS-13	L7	1.800	0.550
14	<i>Scenedesmus dimorphus</i>	AS-14	L10	1.150	0.220
15	<i>Scenedesmus obliquus</i>	AS-15	L3	0.675	0.140
16	<i>Scenedesmus obliquus</i>	AS-16	L5	0.650	0.130
17	<i>Scenedesmus quadricauda</i>	AS-17	L4	1.300	0.370
18	<i>Scenedesmus quadricauda</i>	AS-18	L9	1.322	0.410
19	<i>Scenedesmus sp</i>	AS-19	L2	1.400	0.300
20	<i>Spirulina platensis</i>	AS-20	L3	0.654	0.045

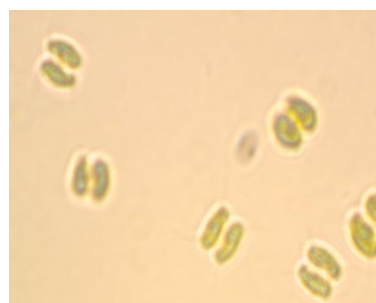
Figure 2: Algal strains A- *Chlorella vulgaris* (AS-3), B- *Chlorella pyrenoidosa* (AS-6), C- *Scenedesmus dimorphus* (AS-13), D- *Scenedesmus quadricauda* (AS-18), with high lipid and biomass



(A) *Chlorella vulgaris* (AS-3)



(B) *Chlorella pyrenoidosa* (AS-6)



(C) *Scenedesmus dimorphus* (AS-13)



(D) *Scenedesmus quadricauda* (AS-18)

DISCUSSION

Nutrients are used by algal metabolism in terms of nitrate, nitrite, ammonium and soluble reactive phosphorus. The high amount of nutrients may cause eutrophication and algal blooms. In entropic and hypereutrophic waters, cyanobacteria often dominate the phytoplankton (Bartram *et al.*, 1999). Physico chemical studies suggests that there is presence of pollutants in three of the lakes i.e. Navalur lake, Unkal lake and Nuggekeri. The water from these lakes is contaminated by domestic sewage, waste from religious rituals and agricultural flows these lakes are considered to be entropic. Similar results were obtained by Chaturvedi *et al.*, (1999), Hosmani.S.P (1980, 2010), S. B. Hulyal & B. B. Kaliwal (2008), Pandey, B.N. *et al.*, (2004). Hence water in these lakes is not fit for drinking. Thou a good number of algal species have been obtained from these lakes. Phytoplankton of most of the worlds lakes are subject to strong

seasonal influences. The growth of phytoplankton depends on ambient nutrient level more than other factors (Peerapornpisal, 1996). Navalur lake had high levels of potassium, sulphate, nitrate and chloride but very less dissolved oxygen which is not favorable for algal growth. Dissolved oxygen is used for photosynthesis and respiration. The solubility of oxygen decreases with increasing temperature (Mackenzie *et al.*, 2002). Nuggekeri and Unkal Lake had high levels of nutrients along with Good amount of dissolved oxygen, conductivity, calcium, Sulphates and low nitrate level was detected as it is accumulated by algae, such factors are most favorable for algal growth and proliferation. High levels of phosphates were also found in these two lakes. Ortho-phosphorus directly effects the distribution of algae as this phosphorus is used in metabolic process (Goldman and Horne, 1983). Hence these two lakes had vast algal diversity and biomass. Other lakes such as Someshwar Lake, Kelageri, Sadhanakeri and Deveragudihal Lake, had moderate level of pollution with a diversified amount of algal species, care have to be taken to limit the amount Of pollutants as soon as possible to prevent these lakes to enter the list of entropic lakes, these lakes had comparatively low level of conductivity, good pH levels, calcium, magnesium, sulphate, phosphates and D.O, with a good amount of algal biomass. The water of Neer sagar and Rayanaal Lake has low level of Pollutants, studies conducted on these lakes suggests that there are low levels of organic and inorganic Compounds in these Lakes, and less algal diversity, the water from these lakes can be used for drinking purposes. The algae isolated and identified from these lakes were grown using favorable conditions and the data obtained by the physicochemical analysis of the source lakes was used to modify the nutrient and mineral composition of the culture media for further growth studies. Altogether 63 species of phytoplankton were identified; similar results were obtained by Hutchinson, G.E. (1967), Hosmani, S.P. And S.G. Bharathi (1980), Gandhi. H.P. (1998), M.F. Ansari *et al.*, (2008), Giriappanavar. B. S (2011). About 28 species belonged to the class Chlorophyceae, this class of algae is known to contain good amount of chlorophyll, lipid and biomass, and most of the fresh water algal species selected for bio fuel production belong to this class. About 20 algal strains showed positive result for lipid content which were subjected for further growth studies using BG-11 medium Out of the 20 strains 4 strains *Chlorella vulgaris* (AS-3), *Chlorella pyrenoidosa* (AS-6), *Scenedesmus dimorphus* (AS-13), *Scenedesmus quadricauda* (AS-18), showed high growth rate and high lipid productivity in *invitro* conditions. These strains were used for further studies of biofuel production.

REFERENCES

- [1] Adoni, A., D. G. Joshi, K. Gosh, S. K. Chourasia, A. K. Vaishya, Mano Yadav and H. G. Verma: Work book on limnology. Pratibha Publishe Sagar. pp. 1-166 (1985).
- [2] Agarker, M. S., H. K. Goswami, S. Kaushik, S. M. Mishra, A. K. Bajpai and U. S. Sharma: *Bionature*, 14, 250-273 (1994)
- [3] Indian standard (IS: 3025-1964) Methods of sampling and Test (Physical and Chemical) for water used in Industry. Bureau of Indian standards, New Delhi. 122 pp.
- [4] APHA, (1998). *Adv. Appl. phycol.* II B:3-4.
- [5] Bartram, J. & Rees, G. 2000. Monitoring bathing waters –A practical guide to the design and implementation of assessments and monitoring programmes. World Health Organisation, Boundary Row, London.
- [6] Chaturvedi, R. K., K. P. Sharma, Kamayani Sharma, S. M. Bhardwaj and S. Sharma: *J. Environ. Pollut.*, 61, 77-84 (1999).
- [7] Chia, A. M., Abolude, D. S., Ladan, Z., Akanbi, O. & Kalaboms, A. 2009a. *Research Journal of Environmental Toxicology* 3:170-178.
- [8] Gandhi. H. P. (1998) Freshwater Diatoms of Central Gujarath. Bisen Sing Mahendra Singh Pal Singh pub. Deharadun.
- [9] Goldman, C. R. and A. J. Horne. 1983. Limnology. McGraw-Hill.
- [10] Giriappanavar. B. S. and Patil. R. R. *Proceedings of the National conference. Karnataka University Dharwad.* 2011. 104–108p.
- [11] Hosmani. S. P. (2010) *The Ecoscan* 4(1):53-57.
- [12] Hosmani. S. P. and S.G. Bharathi (1980) *PHYKOS* 19(1):27-43.
- [13] Hutchinson, G.E. (1967). A Treatise on Limnology. Vol. II. Introduction to lake biology and the Limnoplankton. John Wiley and Sons Inc. New York. Pp.1115.
- [14] Mackenzie, C. L., Morrison, A., Taylor, D. L., Burrell, V. G., Arnold, W. S., and Wakida-Kusunoki, A. T. 2002. Quahogs in Eastern North America: Part I, Biology, Ecology, and Historical Uses. *Marine Fisheries Review* 64:1–55.
- [15] M. F. Ansari, R. F. Ankalgi and S. R. Ankalgi (2008).
- [16] Monastersky, 1995. *science news* 148:220-222.
- [17] Palmer, M. A., Covich, A. P., Finlay, B. J., Gibert, J., Hyde, K. D., Johnson, R. K. and Kairesalo, T. 1997. 17. Biodiversity and ecosystem process in freshwater sediments. *Ambio*, 26(8).

- [18] Pandey, B. N., S. Hussain, O.P.Ambasta and S. K. Poddar, **2004**. *Environment and Ecology*, 22(4):804-809.
- [19] Philipose, M.T. (**1967**). Chlorococcales, ICAR Publication, New Delhi.
- [20] Peerapornpisal, Y.**1996**. Phytoplankton seasonality and limnology of three reservoirs in the Huai Hong Khrai Royal Development study Centre, Chiang Mai, Thailand. Ph.D. Thesis. University of Innsbruck, Austria, 133 pp.
- [21] Prescott G. W. (**1998**) *Algae of the Western Great Lakes area*. Otto. Kaetz, Science Publishers, W. Germany. Pp977.
- [22] Robert G. Wetzel (**1990**) *Limnol. Oceanogr.*, 40(8), **1995**, 1369-1380.
- [23] S. B. Hulyal & B. B. Kaliwal (**2008**). *Environ Monit Assess* (**2009**) 153:45–59
- [24] Welch, P.S (**1948**). *Limnological Methods*. Mc Hill Book Company, New Yoork.pp. 381.