BIOFUEL PROSPECTS OF MICROALGAL COMMUNITY IN URBAN WETLANDS

Ramachandra T.V [1,2,3,]*, **Alakananda B** [1], **Supriya G.**[1]

[1]Energy & Wetlands Research Group
Centre for Ecological Sciences
[2]Centre for Sustainable Technologies (astra)
[3]Centre for infrastructure, Sustainable Transportation and Urban Planning [CiSTUP]
Indian Institute of Science,
Bangalore – 560 012, INDIA
[1]cestvr@ces.iisc.ernet.in (*corresponding Author),
[2]alka@ces.iisc.ernet.in, [3]supriya@ces.iisc.ernet.in,

Abstract: Microalgae are emerging as one of the most promising sources of biofuel because of their high photosynthetic efficiency and faster replication as compared to any other energy crops. Although, the concept of using microalgal lipid as a source of fuel is very mature, its approach in benefiting both environmental and energy-related is a frontier research area today. Algal community for the production of lipid depends on the physical, chemical as well as biological variables of aquatic ecosystems. This communication focuses on achieving the lipid characterization of the microalgal community collected from four wetlands and one agricultural field of Bangalore, Karnataka with a wide range of environmental characteristics. Results reveal significant change in lipid component with change in algal community and chlorophyll content which was explained by community structure analysis and chlorophyll estimation. The presence of Triacyl glycerol (TAG) was examined through thin layer chromatography (TLC). The profile of TAG was further confirmed through Gas chromatography – mass spectroscopy (GC-MS). This study confirms the potential of algal community towards meeting growing demand for alternate sustainable fuel.

Keywords*:* Microalgae, Community structure, Lipid, Gas chromatography – mass spectroscopy.

I. INTRODUCTION

Water occupies most part of the Earth's surface amounting to a volume of 1.38X109 km^3 of which freshwater contributes to approximately 0.0013% of the global water[1]. Freshwater ecosystems encompass an extensive range of habitats viz., rivers, lakes, and wetlands, with constant interaction of biotic with abiotic components. Studies have revealed that the use of freshwater in agricultural purposes is ~ 4000 km^3 of water by 2050[2], for domestic purposes (during 1987– 2003) is estimated to be 325 billion cubic meter[3] while industrial consumption was 665 billion m^3 during the same period[4]. However, in the 21st century, freshwater ecosystems are vulnerable to and by climate change[5,6,7], increase in burgeoning human population coupled with growing food requirements, industrialization and urban sprawl[8]. This turns fresh water into wastewater polluting the environment and creating health hazards to the aquatic life in the freshwater bodies making it unfit for human consumption. These polluted aquatic ecosystems are neglected owing to decline in water quality and quantity, nutrient and hence impeding species' diversity, photosynthesis, chlorophyll and the biochemical composition which includes lipids, carbohydrates and proteins. This has directed towards the threshold of water crisis and the urgent need for developing appropriate water management plans. Along with water management the utilization of biotic components like macrophytes[9,10], micro[11,12] and macrolage[13] as sources of energy has gained prominence in recent years in an era of global warming in addressing production and utilization of renewable energy while dealing with the social and ecological problems.

Biodiesel is a proven fuel and the technology for more than a decade now[14]. Water is the primary factor in the development of biofuel feedstock production[15]. Numerous researches have been carried

out on the production of biodiesel through vegetable oils[16] and other plant oils[17,18]. But due to the high cost of these oleaginous materials, the commercial production of biodiesel is hindered. Therefore, finding cheaper way of producing biodiesel is the need of the hour.

Lipids, the important secondary metabolite owing to specific cell functions and cell signaling pathways play a role in biodiesel production[19]. Major feedstock of biodiesel include soybeans, canola oil, animal fat, palm oil, corn oil, waste cooking oil and jatropha oil[20]. These crop based biofuels have limitations like low biomass productivity (Table 1), requirement of large land area and its non renewability[21]. The other limitation includes the inadequacy of these crops and animal fats oil to meet the existing demand for fuels[21]. Micro algae are efficient biological factories capable of taking zero-energy form of carbon and synthesize it into a high density liquid form of energy (natural oil) and are capable of storing carbon in the form of natural oils or as a polymer of carbohydrates[22]. Microalgae as primary producers form the basis of the food web and play a significant role in the biotic and abitoic interactions of any aquatic ecosystem. The variation in water chemistry and biotic components of an aquatic ecosystem consequent to anthropogenic stress attributes to changes in the structure of microalgae at community level. The concept of using microalgal lipid as a source of fuel is very mature, but its approach in benefiting multiple needs—both environmental and energy-related is an upcoming area of research. Hence characterizing the microalgal community is critical for better understanding of the ecological as well as biogeochemical processes[23]. Over the past few decades, several thousand algae and cyanobacterial species have been screened for high lipid content of which several hundred oleaginous species have been isolated and characterized under laboratory and or outdoor conditions[12,21,24,25]. The current investigation focuses on lipid characterization of the micro algal community in Bangalore collected from 4 wetlands with a wide range of environmental characteristics and an agricultural field.

TABLE 1
BIOFUEL SOURCE COMPARISON[21, 37]

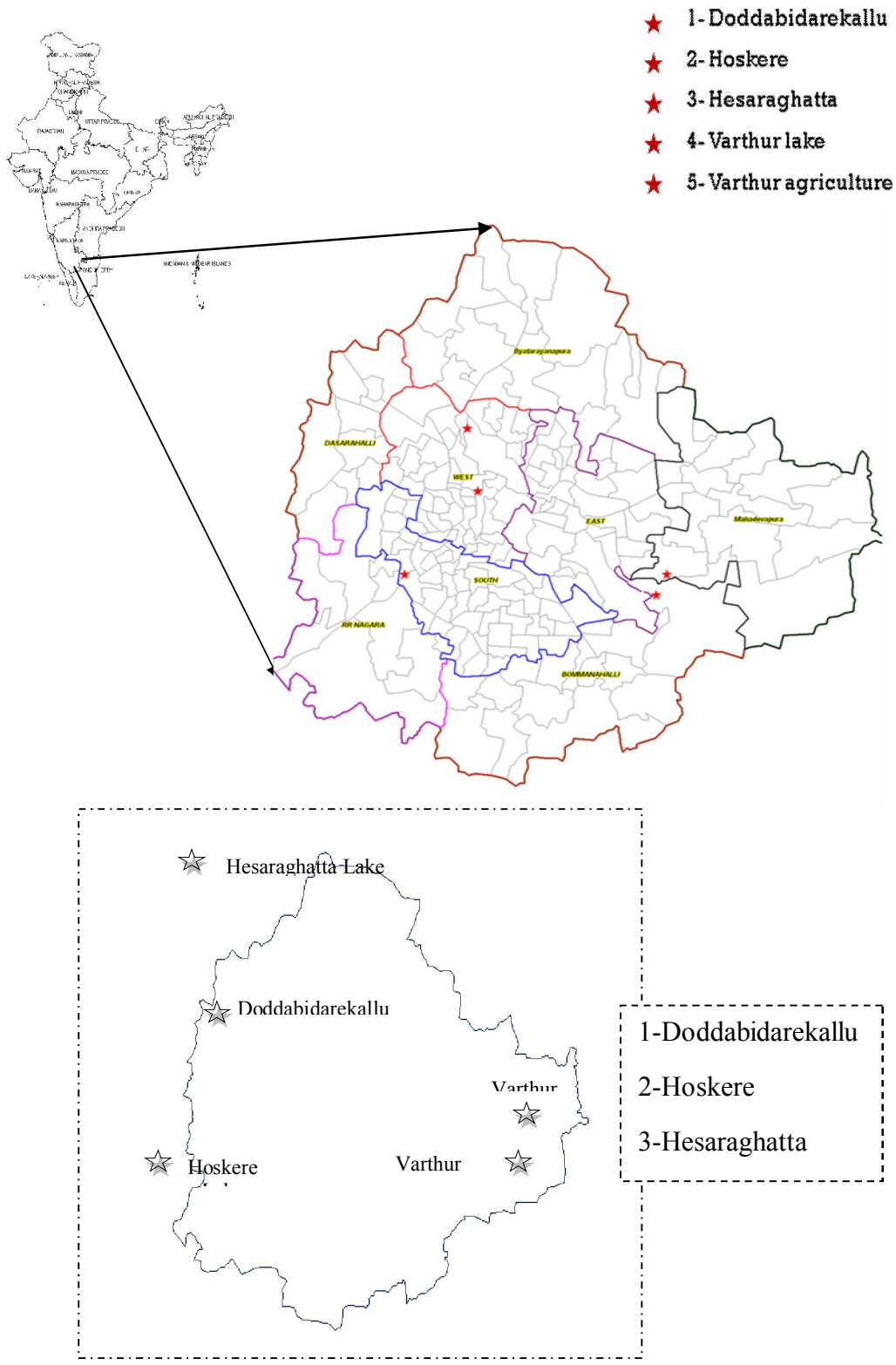
Feedstock	Oil Yield (l/Ha)
Corn	172
Soybean	446
Canola	1190
Jatropa	1892
Coconut	2689
Oil palm	5950
Microalgae a	136900
Microalgae b	58700

a= 70% oil (by wt) in biomass and b= 30 % oil (by wt) in biomass

II. STUDY AREA

Selected four lakes and an agricultural field for the Microalgal community and lipid characterization investigation fall within Bangalore (Fig. 1), the capital city of Karnataka and is the fifth largest city in India. Bangalore city is located at 12.940699^0N and 77.746596^0E geographic position, at an elevation of 900 meters and a surface area of 741 sq. km (as per 2007). Mean annual temperature being 24 ºC with extremes ranging from 15 ºC (in winter) to 37 ºC (in summer). The average annual rainfall is 859 millimeters[26].

The selection of lakes for the study was based on the levels of anthropogenic stress upon the lake, covering different environmental condition. Samples from each lake were sampled at inlet channel so as to record the pollution level on microalgal lipid content. The Doddabidarekallu lake (Nagasandra lake) with an area of 13.07 ha is situated in the industrial area (peenya industrial area), receives industrial effluents representing the industrial waste sample. Hesaraghatta (371.24 ha) and Hoskere lake (also known as Soolekere) with a surface area of 15.54 ha is situated on the city boundary (as per BBMP) and is relatively clean without any sewage and industrial waste into the lake channel while, Varthur with an area of 180.4 ha receives about 40% of the city's sewage. Agriculture field was selected near the Varthur lake to see the effects of inorganic fertilizers with high phosphates and nitrates.



Note: Sampling sites are 1- Doddabidarekallu, 2- Hoskere, 3- Hesaraghatta, 4- Varthur lake, 5- Varthur agriculture)

Fig 1. Sampling locations in Bangalore, Karnataka, India

III. MATERIALS AND METHODS

A. *Water sampling and analysis*

Four Lakes (Fig. 1) were selected based on the exploratory survey of 15 lakes during eight months (September 2009- April 2010) which includes water quality analysis of both Inlet and outlet channels. Water samples were collected from four lakes and one agricultural field in Bangalore during May 2010. They were selected based on the anthropogenic stress (industrial runoff, sewage runoff, unpolluted, high nutrient load) influencing on it. Triplicates were collected at each sampling point in 1L polythene bottle. On site physical parameters like pH, water temperature (WT), electric conductivity (EC), salinity and total dissolved solids (TDS) were analyzed using pH/EC probe. Dissolved oxygen (DO) was estimated following Wrinkler's method. Samples were brought to Aquatic ecology laboratory for further analysis of chemical variables such as Nitrates, Phosphates, Alkalinity, Total hardness, Calcium hardness, Magnesium hardness, Chlorides, Sodium, Potassium, Biological oxygen demand (BOD) and Chemical oxygen demand (COD). These variables were estimated as per standard procedure[27].

B. *Microalgae sampling*

Microalgae were sampled from aquatic plant at all sampling points by shaking vigorously and then squeezed in the plastic bag. The resulting brown suspension is transferred into a polythene sample bottle and preserved. Community structure analysis: 0.5 ml of the preserved microalgal sample was observed under light microscope (100X magnification). The entire coverslip was covered to record the presence/absence data of the taxa and photographed for identification.

C. *Chlorophyll estimation*

25 ml of the microalgal sample was centrifuged t 300 rpm and was filtered. The filtered sample was then processed for chlorophyll estimation following APHA method (APHA 10200 H).

D. *Lipid characterization*

25 ml of the microalgae sample was sonicated[28] in water bath for 2 hours at room temperature in order to disrupt the cell membranes, chloroform: methanol (2:1) was added as the extraction solvent. The chloroform layer was evaporated using rotary evaporator (Eppendorf Vacuum Concentrator 5301) to obtain lipids. Thin layer chromatography: All samples were reconstituted in chloroform to make stock solutions. The stock solutions were spotted in bands onto silica gel TLC plates (Merck KGaA). The mobile phase consisted of a solvent system of hexane/diethyl ether/acetic acid (70:30:1 by volume)[29]. The plates were developed by exposing the vapors of iodine crystals to stain the plates for visualizing neutral lipids. The samples were extracted and stored in -20 ºC until further analysis[30].

E. *Gas chromatography–mass spectrometry analysis*

After the initial thin layer chromatography (TLC) lipid screening, the extracts were converted into fatty acid methyl esters (FAME) using Boron trifluoride-methanol and was heated in water bath at a temperature of 60 ºC for 1 hour. The methylated sample was then purified further for GC-MS. The main focus of using GC-MS was purely for lipid identification rather than quantification. The injector and detector temperatures were set at 250 ºC while the initial column temperature was set at 40 ºC for 1 min. A 1μL sample volume was injected into the column and ran using a 50:1 split ratio. After 1 min, the oven temperature was raised to 150 ºC at a ramp rate of 10 ºC min^{-1}. The oven temperature was then raised to 230 ºC at a ramp rate of 3 ºC min^{-1}, and finally the oven temperature was increased to 300 ºC at a ramp rate of 10 ºC min^{-1} and maintained at this temperature for 2 min. The total run time was programmed for 47.667 min. The mass spectra were acquired and processed using Agilent Chem Station (5975 C; Agilent, USA).

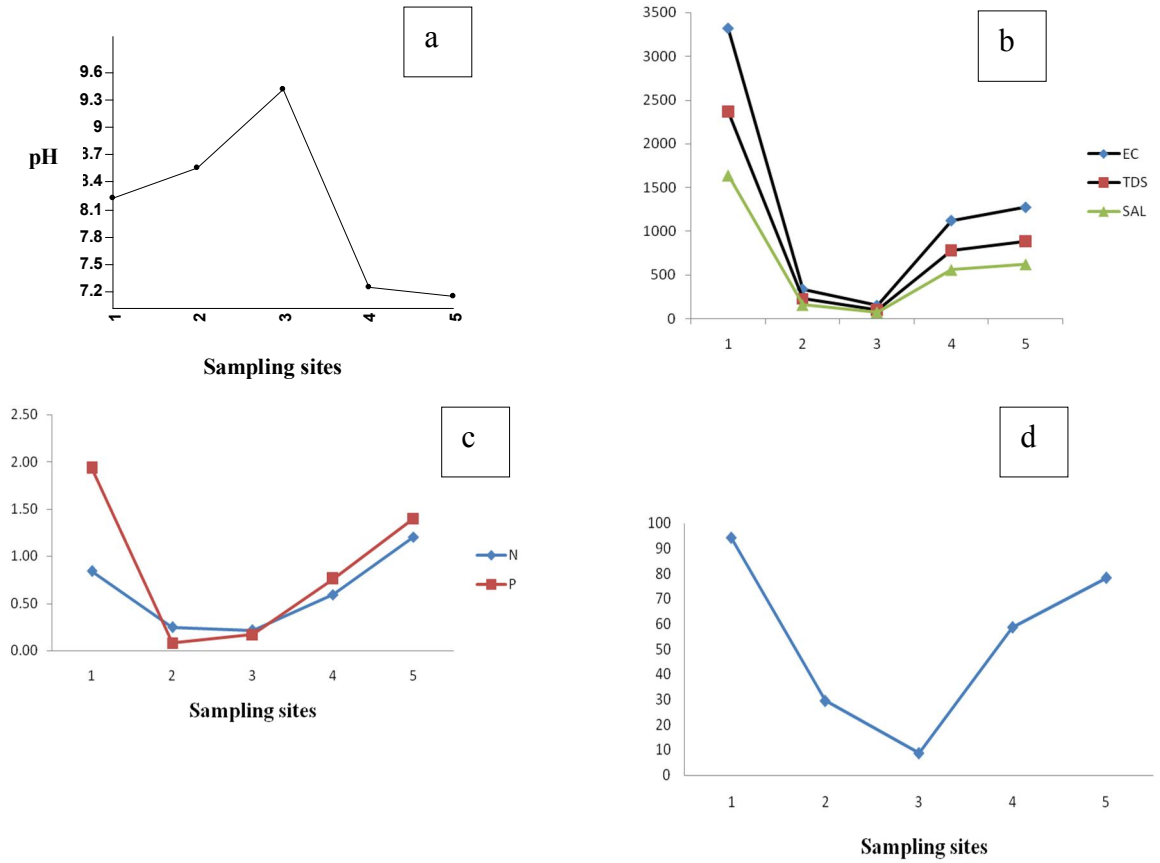


Fig. 2 Variation in water physical and chemical variables [a): pH, b)EC,SAL and TDS, c) N and P, d) chlorophyll composition across sites (mg/l)]

IV. RESULTS AND DISCUSSION

A. *Water quality*

Physical and chemical variables analyzed across the sampling sites (lakes) are listed in Table 2. The pH ranged from neutral to alkaline (7.13 – 9.42 as in Fig. 2a), highest being in the Hesaraghatta lake (9.42) due to the increased acid neutralizing capacity. Ionic concentration was low at Hesarghatta lake (150.7 µS), Hoskere lake (337 µS) and fairly high at Doddabidderakallu lake (3320 µS) owing the industrial pollution. Difference between Hesarghatta and Doddabidderakallu lakes was significant by EC, SAL and TDS (Fig. 2b). Among water chemistry variables, phosphates, chlorides, hardness and alkalinity showed a high value in Doddabidderakallu followed by Varthur akin to conditions in agriculture site while Hesarghatta and Hoskere showed low range reflecting clean water compared to the former sites. Nitrate levels of agricultural field (1.203 mgL^{-1}) encompassed the low range as observed in Doddabidderakallu (0.84 mgL^{-1}), Varthur (0.594 mgL^{-1}), Hoskere (0.246 mgL^{-1}) and Hesarghatta (0.215 mgL^{-1}) lakes. High amount of phosphate was sensible in Doddabidderakallu (1.93 mgL^{-1}) compared to other lakes (Fig. 2c). This high amount of nutrients and ionic concentrations, mainly alkalinity and hardness in Doddabidderakallu can be attributed to the untreated industrial effluents and sewage into the inlet channel. Even though Varthur showed moderate water quality, high amounts of contamination has been reported in the past[31]. Hoskere and Hesarghatta showed a negligible amount of anthropogenic activities except for few local disturbances. The elevated nitrate and phosphate concentrations in agriculture site were evident from the intrusion of fertilizers.

TABLE 2:
WATER QUALITY VARIABLES OF 5 SAMPLING SITES (1-5 AS DESCRIBED IN STUDY AREA)

	1	2	3	4	5
pH	8.21	8.55	9.42	7.23	7.13
WT (^{oC})	27.00	30.3	33.1	32.50	29.80
EC (µ S)	3320	337.	150	1122	127
TDS (mgL^{-1})	2370.0	230.0	102.7	781.00	886.00
SAL (mgL^{-1})	1640.0	159.0	75.70	560.00	623.00
TURBIDITY (ntu)	139.00	13.20	15.00	71.70	44.30
DO (ppm)	0.00	9.35	12.20	13.33	9.35
COD (mgL^{-1})	240.00	213.3	117.3	128.00	250.67
BOD (mgL^{-1})	1.5	6.2	5.5	2.53	3.52
N (mgL^{-1})	0.84	0.246	0.215	0.594	1.203
P (mgL^{-1})	1.93	0.08	0.17	0.76	1.40
Chlorides (mgL^{-1})	610.60	62.48	22.72	187.44	249.92
Total Ha (mgL^{-1})	680.00	96.00	80.00	232.00	332.00
Ca. Ha (mgL^{-1})	439.81	67.98	39.97	59.86	147.85
Mg (mgL^{-1})	107.31	16.59	9.75	14.61	36.08
Alkalinity (mgL^{-1})	1080.0	160.0	380.0	440.00	540.00

B. *Community structure analysis*

The community structure of microalgae through microscopic analysis resulted with 27 genus belonging to 4 classes with 2 unidentified filamentous algae (Table 3). The class Bacillariophyta (diatoms) and Chlorophyta dominated at Hoskere and Varthur lake as well as agricultural sample with *Achnanthidium* Kützing, *Gomphonema* Ehrenberg, *Nitzschia* Hassall, *Navicula* Bory de Saint-Vincent, *Chlamydomonas* Ehrenberg, *Scenedesmus* Meyen and *Anabaena* Bory de Saint-Vincent ex Bornet & Flahault accounting more in number (occurrence number in microscopic field). Dodabidarekallu was represented by *Nitzschia* sp. alone, whose presence justifies high ionic and organic nutrients load. Hoskere was well occupied by diatoms viz., *Fragiallria* Lyngbye, *Sellaphora* Mereschowsky, *Surirella* Turpin along with the former species. Significant relation of ecology of microalgae such as *Nitzschia* sp., *Sellaphora* sp., *Chlorella* M.Beijerinck and *Phacus* Dujardin (varthur and agricultural field samples) with the extent of pollution load was observed.

TABLE 3
COMMUNITY STRUCTURE OF 5 SAMPLING SITES (1-5 AS DESCRIBED IN STUDY AREA. + INDICATES PRESENCE AND – INDICATES ABSENCE OF SPECIES)

Sampling sites	**1**	**2**	**3**	**4**	**5**
BACILLARIOPHYTA					
Achnanthidium Kützing	-	+	-	-	-
Cyclotella (Kützing) Brébisson	-	+	-	+	-
Cymbella C.Agardh	-	+	-	-	-
Diploneis Ehrenberg ex Cleve	-	+	-	-	-
Fragillaria Lyngbye	-	+	-	-	-
Gomphonema Ehrenberg	-	+	+	-	+
Navicula Bory de Saint-Vincent	-	+	+	-	+
Nitzschia Hassall	+	+	-	+	+
Rhopalodia Otto Müller	-	-	+	-	-
Sellaphora Mereschowsky	-	+	-	+	+
Surirella Turpin	-	+	-	-	-
CHLOROPHYTA					
Chlamydomonas Ehrenberg	-	-	-	+	-
Chlorella M.Beijerinck	-	+	+	+	+
Chlorogonium Ehrenberg	-	-	-	+	-
Closterium Nitzsch ex Ralfs	-	-	+	+	-
Cosmarium Corda ex Ralfs	-	+	-	-	-
Monoraphidium Komárková-Legnerová	-	+	-	+	-
Pandorina Bory de Saint-Vincent	-	+	+	-	-
Scenedesmus Meyen	-	-	-	+	-
EUGLENOPHYTA					
Euglena Ehrenberg	-	-	-	+	-
Phacus Dujardin	-	-	-	+	+
Trachelomonas Ehrenberg	-	-	-	+	-
FILAMENTOUS ALGAE					
Filamentous algae 1	-	-	-	+	-
Filamentous algae 2	-	-	-	+	-
CYANOPHYTA					
Anabaena Bory de Saint-Vincent ex Bornet & Flahault	-	+	+	-	-
Cylindrospermopsis Seenayya & Subba Raju	+	-	-	-	-
Merismopedia Meyen	-	+	-	+	-

C. *Water Quality and Community structure*

Nitzschia sp. was prevalent in Doddabidderakallu with the high quantum of nutrients and ionic concentrations. Compared to this Varthur showed moderate water quality, while, Hoskere and Hesarghatta showed a negligible amount of anthropogenic activities except for few local disturbances. The elevated nitrate and phosphate concentrations is observed in agriculture sites. The class Bacillariophyta (diatoms) and Chlorophyta dominated at Hoskere and Varthur lake as well as agricultural sample. Occurrence of microalgae such as *Nitzschia* sp., *Sellaphora* sp., *Chlorella* M.Beijerinck and *Phacus* Dujardin with the extent of pollution load show significant correlation ($p<0.05$).

D. *Lipid analysis*

The neutral lipid profile of the microalgal community revealed characteristic profile of the given community. The neutral lipid profile of each lake which is characteristic feature of the thriving

microalgal community is given in Table 4. Agricultural field with *Gomphonema* sp. and *Nitzschia* sp. as dominant also reflected more fatty acids as of Doddabidarekallu sample due to inhibition of cell cycle and which causes TAG accumulation. Hoskere and Hesaraghatta (unpolluted water) had relatively less chlorophyll (Fig. 2d)and fatty acids in lipid profile. This is due to the inability of the diatoms to accumulate more TAG due to lack of any stress. In freshwater, lipid productivity, the mass of lipid that can be produced per day is dependent upon plant biomass production as well as the lipid content of this biomass[21]. The pattern of fatty acids varies according to the internal and external factors working on the algal cell[32,33] which concludes that growth rate and the mixed population which competes for the resources, influences on fatty acid composition. Although there are many microalgae as evident from Table 4 that have the ability to accumulate oils under some special cultivation, they have different prospects for biodiesel production in terms of oil yield lipid coefficient and lipid volumetric productivity[34]. However lipid production varies with variation in algal species with reference to both quantity and quality of lipids[35].

In the current investigation, Doddabidarekallu, Varthur and agriculture field samples were represented by diatoms, which are lipid-rich and have been demonstrated to be an important source for biodiesel[36,37]. *Nitzschia* species at Doddabidarekallu site (industrial waste) was prevailing with high organic and ionic content resulted with high lipid profile and chlorophyll content. This supports that environmental condition are decisive variables for lipid in microalgae. However, this has to be explored further through in situ experiments (like axenic culture, synchronous inoculums for bioreactors etc.). For further evidence, role of each keystone micoalgae species in the contribution towards lipid production with its ecological preference has to be studied.

V. CONCLUSION

The polluted lake water Doddabidarekallu supported growth of Bacillariophyceae members. The lipid profile obtained from this lake also had relatively higher proportion of fatty acid methyl esters (12 types) which highlights that diatoms are rich in TAG accumulation. The changes in neutral lipid emphasize the importance of knowing how nutrient levels play an important role in each of the microalgae for an enhanced accumulation of neutral lipids. Therefore this study proposes the use of lakes for sustained biodiesel production with the further research concentrating on transesterification process of lipid to biofuel characteristics.

ACKNOWLEDGMENT

We thank the Ministry of Environment and Forests, Government of India and Indian Institute of science for sustained finance and infrastructure support. We are grateful to Prof. Ram Rajashekaran, Lipid Lab, Department of Biochemistry, IISc for permitting us to use lab facilities.

TABLE 4
LIST OF POLYUNSATURATED FATTY ACIDS

POLYUNSATURATED FATTY ACIDS	Formula	1	2	3	4	5
9- octadecenoic acid (Z)- methyl ester	C18:1	-	+	-	-	-
10- octa decanoic acid methyl ester		+	-	-	-	-
Decanoic acid methyl ester	C10:0	+	-	-	-	+
Docosonoic acid methyl ester	C22:0	+	+	-	-	-
Dodecanoic acid methyl ester	C12:0	+	+	-	-	+
Dodecanoic acid, 1- methyl ethyl ester	C15:1	+	+	-	+	+
Eicosanoic acid methy ester	C20:4	-	+	-	+	+
Heptadecanoic acid methyl ester	C17:0	+	-	-	+	+
Hexadecanoic acid methyl ester	C16:0	+	+	+	+	+
Hexadecanoic acid 14 - methyl ester	C17:1	-	-	+	-	-
Isopropyl myristate		-	+	-	-	-
Isopropyl palmitate	C19:1	-	+	-	-	-
Methyl tetradecanoate	C14:1	+	+	+	+	+
Nona decanoic acid methyl ester	C20:1	+	-	-	-	-
Octadecanoic acid methyl ester	C18:0	+	+	+	+	+
Octanoic acid methyl ester	C8:0	+	+	+	+	-
Pentadecanoic acid methyl ester	C15:1	+	-	-	+	+
Tetradecanoic acid, methyl ester	C14:0	-	-	+	+	-

REFERENCES

[1]. D.C. Sigee, *Freshwater Microbiology. Biodiversity and dynamic interactions of microorganisms in the aquatic environment.* John Wiley and Sons ltd., 2004.

[2]. U. Amarasinghe, B. Sharma, N. Aloysius, C. Scott, V.Smakthin, C. de Fraiture, A. K. Sinha, A. K. Sukla, *Spatial Variation in Water Supply and demand Across River Basins of India*. IWMI Research Report 83. IWMI: Colombo, Sri Lanka, 2005.

[3]. World Mapper Project, Domestic Water Use Data. <http://www. worldmapper.org/>.2007a.

[4]. World Mapper Project, Industrial Water Use Data. <http://www. worldmapper.org/>.2007b.

[5]. A. Fischlin, G. F. Midgley, J. T. Price, R. Leemans, B. Gopal, C. Turley, M. D. A. Rounsevell, O. P. Dube, J. Tarazona, A. A. Velichko, *Ecosystems, their properties, goods, and services*. In: (eds M. L. Parry, O. F. Canziani, J. P. Palutikof, P. J.mvan der Linden, C. E. Hanson,) Climate change 2007: impacts, adaptation and vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge, UK. 2007, pp 211–272.

[6]. B. C. Bates, Z. W. Kundzewicz, S. Wu, J. P. Palutikof, (eds.,) *Climate change and water*. Technical paper of the intergovernmental panel on climate change, IPCC Secretariat, Geneva, **2008**, pp 210.

[7]. P. A. Harrison, P. M. Berry, C. Henriques, I. P. Holman, *Impacts of socio-economic and climate change scenarios on wetlands: linking water resources and biodiversity meta-models*, Climate Change, **2008**, 90, 113–139.

[8]. T. V. Ramachandra, *Spatial Analysis and Characterisation of Lentic Ecosystems: A Case Study of Varthur Lake, Bangalore*. International Journal of Ecology & Development, Vol. 9; No. W08; 2008, pp 39-56.

[9]. G. J. Lawson, T. V. Callaghan, *Primary productivity and the prospects for biofuels in the United Kingdom. Earth and Environmental Science.* International Journal of Biometeorology, **1983**, 27(3), 197-218.

[10]. M. P. Ciria, M. L. Solano, P.Soriano, *Role of macrophyte Typha latifolia in a constructed wetland for wastewater treatment and assessment of its potential as a biomass fuel.* Biosys. Eng., **2005**, 92 (4), 535–544.

[11]. V. Patil, K. Tran, H. R. Giselrød, *Towards Sustainable Production of Biofuels from Microalgae.* Int. J. Mol. Sci., **2008**, 9, 1188-1195.

[12]. L. Gouveia, A. C. Oliveira, *Microalgae as raw material for biofuels production*. J. Ind. Microbiol. Biotechnol., **2009**, 36, 269-274.

[13]. M. Aresta, A. Dibenedetto, G.Barberio, *Utilization of macro-algae for enhanced CO_2 fixation and biofuels production: development of computation software for an LCA study*. Fuel processing Technology **2005**, 86, 1679-1693.

[14]. B. K. Barnwal, M. P. Sharma, *Prospects of biodiesel production from vegetables oils in India*, Renew Sustain Energy Rev., **2005**, 9, 363–378.

[15]. S.Varghese, *Biofuels and Global Water Challenges, Institute for Agriculture and Trade Policy*, Minnesota, USA, 2007.

[16]. M. J. Haas, *Improving the economics of biodiesel production through the use of low value lipids as feedstock: Vegetable oil soapstock.* Fuel Proc. Tech., **2005**, 86(10), 1087- 1096.

[17]. A. K. Tiwari, A. Kumar, H. Raheman, *Biodiesel production from jatropha oil (Jatropha curcas) with high free fatty acid: An optimized process*. Biomass Bioenerg., **2007**, 31 (8), 569 -575.

[18]. A. B. Chhetri, K. C. Watts, M. R. Islam, *Waste cooking oil as an alternate feedstock for biodiesel production.* Energies **2008**, 1 (1), 3-18.

[19]. D. W. Hu, H. Liu, C.L. Yang, E.Z. Hu, The design and optimization for light-algae bioreactor controller based on Artificial Neural Network-Model Predictive Control. Acta Astronaut. **2008,** 63, 1067–1075.

[20]. Q. Hu, M. Sommerfeld, E. Jarvis, M. Ghirardi, M. Posewitz, M. Seibert, A. Darzins, *Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances*. Plant J., **2008a**, 54, 621-639.

[21]. Y. Chisti, *Biodiesel from microalgae*. Biotechnology Advances, **2007**, 25, 294-306.

[22]. J. Benemann, P. I. Oswald, *Systems and Economic Analysis of Microalgae Ponds for Conversion of CO_2 to Biomass*. Department of Energy, Pittsburgh Energy Technology Center, **1996**.

[23]. D. Siegel, *Resource competition in discrete environment: why are plankton distributions paradoxical?* Limnology and Oceanography 43, **1998**, pp 1133–1146.

[24]. E. W. Becker, *Microalgae — Biotechnology and Microbiology*, Cambridge University Press, Cambridge, **1994**.

[25]. P. K. Mohapatra, *Biotechnological approaches to microalga culture. In: Textbook of environmental biotechnology.* IK International Publishing House Pvt. Ltd, New Delhi, India, **2006,** pp167-200.

[26]. T. V. Ramachandra, *Energy alternatives: renewable energy and energy conservation technologies*, CES Technical Report No.88, Centre for Ecological Sciences IISc, Bangalore, **2000**, p 48.

[27]. APHA *Standard Methods for the Examination of Water and Wastewater*. American Public Health Assoc., American Waterworks Assoc., Water Pollution Control Federation, Washington, D.C., **1985**.

[28]. F. Pernet, R.Tremblay, *Effect of ultrasonication and grinding on the determination of lipid class content of microalgae harvested on filters*. Lipids, **2003**, 38, 1191.

[29]. M. Maloney, *Thin-layer chromatography in bacteriology*. In: (eds, Fried, B.; Sherma, J.) Practical thin-layer chromatography: a multidisciplinary approach. CRC, Boca Raton, 1996, pp 336.

[30]. M. P. Mansour, D. M. F. Frampton, P. D. Nichols, J. K. Volkman, and S. I. Blackburn, Lipid and fatty acid yield of nine stationary-phase microalgae: applications and unusual C24–C28 polyunsaturated fatty acids. J Appl Phycol., **2005**, 17:287–300.

[31]. T. V. Ramachandra, N. Ahalya, and M. Payne, Status of Varthur lake: opportunities for restoration and sustainable management. Technical report 102. Centre for Ecological Sciences, Indian Institute of Science, Bangalore, 2003.

[32]. P. A. Thompson, P. J. Harrison, J. N. C. Whyte, *Influence of irradiance on the fatty acid composition of phytoplankton*, J. Phycol. **1990**, 26, 278–288.

[33]. Z. Cohen, A. Vonshak, A. Richmond, *Effect of environmental conditions on fatty acid composition of the red alga Porphyridium cruentum: correlation to growth rate*. J. Phycol., **1988**, 24, 328–332.

[34]. Q. Li, W. Du, D. Liu, *Perspectives of microbial oils for biodiesel production*. Appl. Microbiol. Biotechnol., **2008**, 80, 749-756.

[35]. O. Berglund, P. Larsson, G. Ewald, L. Okla, *The effect of lake trophy on lipid content and PCB concentrations in planktonic food webs*, Ecology **2001**, 82, 1078–1088.

[36]. R. L. Lowe, R. W. Pillsbury, *Shifts in the benthic algal community structure and function following the appearance of zebra mussels (Dreissna polymorpha) in Saginaw Bay, Lake Huron.* Journal of Great Lakes Research, **1995**, 21(4), 558-566.

[37]. T. V. Ramachandra, Durga Madhab Mahapatra, B.; Karthick, R. Gordon Milking Diatoms for Sustainable Energy: Biochemical Engineering versus Gasoline-Secreting Diatom Solar Panels. Ind. Eng. Chem. Res. **2009**, 48, 8769–8788.