Journal of Algal Biomass Utilization
J. Algal Biomass Utln. - Copyright © PHYCOSPECTRUM
ISSN: 2229 – 6905

Diversity study of freshwater microalgae of some unexplored water bodies of a rapidly developing industrial region in India

Alifha Severes[1*], Shashikiran Nivas[1], L D'Souza[1], Smitha Hegde[2]

[1]Laboratory of Applied Biology, Dr Kuppers Biotechnology Unit,
St Aloysius College, Mangalore – Karnataka
[2]Nitte University Centre for Science Education and Research (NUCSER), Paneer campus, Deralakatte, Mangalore, Karnataka
Email: alifha.severes5@gmail.com

Abstract
The Western Ghat region of India has a favourable habitat, ample sunlight and adequate rainfall for supporting a richness of freshwater algae. The present study was aimed to assess the microalgal diversity of 6 water bodies of Dakshina Kannada region, Mangalore, India and also determine their distribution and abundance. The water samples collected were preserved and the physico-chemical parameters were analysed using standard protocols. Microalgae belonging to *Bacillariophycea, Chlorophycea, Cyanophycea and Euglenophycea* were identified. Bacillariophytes were dominant in all sites and *Navicula* and *Nitzchia sp.* were in abundance. The Shannon's diversity index in the range of 1.74 – 2.45, Simpson's Index, 0.08 – 0.16 while Pielou's evenness, 0.56 – 0.79 revealed a moderate to highly stable ecosystem with slight to moderate pollution values. Principle Component Analysis showed a positive correlation between salinity, TDS, conductivity, BOD, phosphates and nitrates at Karnad and Sultan Battery while a negative correlation with dissolved oxygen. Canonical analysis depicted positive correlation of *Nitzschia, Amphora, Oscillatoria, Aphanocapsa, Spirulina* and *Scenedesmus* species with the above parameters particularly at Gurupura lake and Baikampady effluent site. The findings provide an important piece of information of microalgal distribution and reflect the resourcefulness of the potential ecosystems of Dakshina Kannada that had not been explored earlier.

Keywords*:* Microalgae, diversity studies, Principal component analysis, Biresources.

INTRODUCTION:

The Western Ghats of the Indian peninsular, a unique biodiversity niche, with its undulating landscapes, ample sunlight and adequate rainfall is a favourable habitat for supporting a rich diversity of flora, fauna as well as freshwater and marine plankton. The heritage unit of UNESCO has granted the region with a "rich heritage tag" while the Western Ghats Ecology Expert Panel (WGEEP) has designated the Entire Western Ghats as an Ecologically Sensitive Are (ESA) (Nampoothiri *et al.* 2013).

The Western Ghats encasing an area of about 1, 80, 000km^2 ranging from Tapti River in Southern state of Gujarat to the south tip of Kerala state is enriched with a diversity of more than 4000 species of flowering plants while among the lower plants, 320 species of Pteridophytes, 200 species of bryophytes, 300 species of algae and 800 species of lichens are known. Among the vast range of organisms, microalgae are a diverse group which could be explored for a number of potential applications. One of the biosphere hotspots of the Western Ghats in Karnataka- the Kudremukh National Park spreads over an area of 600.32 km^2, encompassing regions in the districts of Dakshina Kannada, Udupi and Chikmagalur. Parts of the river flowing in different regions of Dakshina Kannada serve accessible points for microalgal distribution studies.

Microalgae represent an extraordinarily diverse but highly specialized group of microorganisms adapted to various ecological habitats. Their main habitats are freshwater, brackish and marine ecosystems. They are oligeonous microorganisms whose biomass could be converted to potential products such as pigments, fine chemicals, bioactive molecules, and most importantly for biofuels (Spolaore *et al.* 2006). Microalgae biomass is considered as a better alternative renewable energy source than terrestrial oil crops. Some species of green microalgae (e.g., *Chlorella vulgaris*, *Chlorococcum littorale, Botryococcus braunii, Nannochloropsis*) and diatoms (e.g., *Chaetoceros muelleri*) have been considered to be candidate strains for production of neutral lipids for conversion to various types of biofuels (e.g., biodiesel, kerosene, gasoline) (Liu *et al.* 2011). Those microalgae with an oil content ranging from 15 to 68% with a large portion of C18 fatty acids are known as potential species for the production of oil.

Among the vast diversity of algae, only a few hundred have so far been investigated for their chemical composition with biofuel prospects. Thus, the isolation and characterization of algae from unique aquatic environments is a continuing effort for screening of strains with potential for biodiesel production. Screening of Microalgae needs to be carried out in local habitats because it is expected to have a competitive advantage under the local geographical, climatic and ecological conditions (Ramachandra *et al.* 2011) Aquatic environments that undergo fluctuating and/or occasional adverse conditions provide a higher chance of isolating high lipid accumulating microalgae (Mubeen *et al*. 2011; Duong *et al.* 2012). Also, according to the recommendations of Aquatic Species Program the best species as a potential candidate for biofuel production would be the native algal species with rapid growth potential which can adapt to the native environmental conditions efficiently.

Correlation of the ecological parameters with the physicochemical characteristics of the sampling site greatly influences the morphophytic diversity of algae in the water bodies (Sharma *et al.* 2013). Elevated values of Physico-chemical parameters (Temp., pH, TDS, Alkalinity, Nitrate, Phosphate, DO etc.) supported by phytoplanktons of the nutrient rich waters give an idea about the trend of water body in the direction of eutrophication (Naik *et al.* 2010). In any aquatic ecosystem, the growth, density and abundance of phytoplankton are primarily governed by interactions between environmental factors and biotic entities. Influx of freshwater and a tidal activity are abiotic interactions which can play crucial roles on phytoplankton growth and their abundance in the estuary (Cloern 1987). The constant nutrient supply always supports the rich phytoplankton production but generally nitrogen (N) and phosphorus (P) have been considered as the potentially limiting nutrients for phytoplankton growth in aquatic ecosystems (Neill 2005). Phosphorus is being attributed as limiting nutrient in fresh water dominated waters, whereas Nitrogen is being attributed in coastal waters (Neill 2005; Fisher *et al.* 1992).

Based on the above considerations, an attempt was made to study the freshwater microalgal distribution in water bodies along with the physico-chemical parameters in different parts of Mangalore in Dakshina Kannada district of Karnataka so as to explore the presence and occurrence of native microalgae as promising strains for biofuel.

MATERIALS AND METHODS

Study area: The study was carried out at 6 different locations in the Mangalore region of Dakshina Kannada, Karnataka, India during the late monsoon period. The locations include the Sultan Battery river (12°53'20"N and 74°49'13" E), Baikampady effluent flow (12°94'54" N and 74°83'51" E), Shambhavi estuary, Karnad (13°76'33" N and 74°78'72" E), Kateel temple river (13°76'32" N and 74°78'38" E), Gurupura lake (12°56'25" N and 74°55'25" E) and Bairede kere, Padil (12°87'25" N and 74°88'86" E) (Fig. 1).Three sampling points were selected from each location for the study.

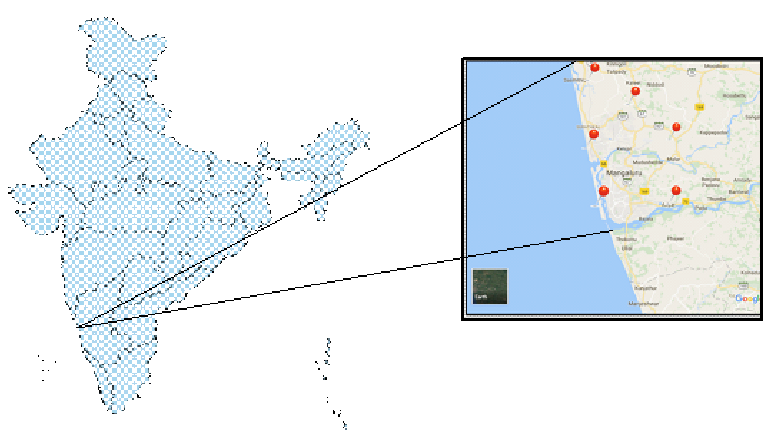

Fig 1: Geographical location of the study area

Sample collection and analysis : The algal sample collection was conducted one meter away from the shore at about 20-30cm depth . Onsite measurements such as pH, temperature and Dissolved Oxygen were conducted. Physicochemical parameters such as salinity, TDS, conductivity, etc. were measured using Systronics water analyzer model 371. For phosphate and nitrate analysis, polyethylene bottles were used for collection and storage of water samples and stored at -20 °C until analysis. Clean labeled polythene zipper bags were used to sample 50ml of concentrate sampled using 20-40microns phytoplanktonic mesh to obtain algal cells which were fixed immediately with Lugol's solution for preservation (APHA, 1998: APHA, 2005).

The algal cell density (cells ml^{-1}) was determined using drop count method (Bartram and Rees 2000). The algae were identified based on their morphological characteristics and enumerated using Olympus light microscope BX41-TF, Japan. The algae were classified based on different classes. Among phytoplankton, four groups were selected (blue-green algae, green algae, diatoms and Euglenoids) to study their distribution and abundance.

Data analysis : The physiochemical parameters were analyzed using ANOVA at $P < 0.005$. Diversity indices such as Shannon diversity index (Shannon 1948); Simpson's dominance index (Simpson 1949); and Pielou's species evenness index (Pielou 1966) were calculated.

Multivariate analyses were carried out using XLSTAT software. Principal component analysis (PCA) was used to determine the parameters that are strongly associated with each other and canonical correlation analysis (CCorA) was used to assess the correlation of environmental factors of the various water bodies to the observed microalgal species.

RESULTS

The physicochemical variables across the six sampling sites are listed in Table 1. The pH was slightly acidic, ranging from 5.1 to 6.4 except at Baikampady which was alkaline (8.1) attributed to high photosynthetic activity of the algae in the effluent flow. The ionic concentration at Shambhavi estuary, Karnad was fairly high (3990µS) while other locations had a low (Baikampady, Kateel, Gurupura, Padil) to moderate (Sultan Battery) concentration. A similar trend was noted in the salinity and dissolved solids measurements, where highest concentration (21 g l^{-1}) was obtained at Shambhavi estuary, Karnad.

Table 1: Physico-chemical parameters of water samples of the study areas

Parameters	Site 1 Sultan Battery river	Site 2 Baikampady river	Site 3 Shambhavi estuary,Karnad	Site 4 Kateel temple river	Site 5 Gurupura lake	Site 6 Bairede kere Padil
pH	5.43 ± 0.09	8.10 ± 0.10	5.97±0.09	5.10±0.06	6.47±0.03	5.47±0.15
Temp °C	31.67±0.33	32.67±0.33	32.67±0.33	30.33±0.33	30.67±0.33	30.67±0.33
BOD (mg l^{-1})	4.30± 0.28	2.4±0.42	4.96 ± 0.23	1.76±0.17	1.86±0.04	3.03± 0.07
Salinity (g l^{-1})	9.84±0.02	3.05±0.03	21.00±0.03	0.58±0.08	0.06±0.00	0.13±0.00
TDS (g l^{-1})	10.07±0.03	3.42±0.07	21.10±0.12	2.30±0.24	0.06±0.00	0.13±0.00
Conductivity (µS)	1847±0.28	683±0.01	3990±0.10	680±0.38	120±0.00	260±0.00
DO (mg l^{-1})	8.27±0.17	6.33±0.15	5.33±0.26	8.33±0.24	7.93±0.24	8.27±0.17
Phosphates(mg l^{-1})	0.43±0.16	0.64±0.22	0.67±0.18	0.38±0.14	0.15±0.14	0.18±0.03
Nitrates(mg l^{-1})	6.14±0.16	4.37±0.22	9.4±0.18	3.12±0.14	2.12±0.14	2.11±0.34

In an aquatic ecosystem, the amount of dissolved oxygen is chiefly influenced by temperature, photosynthetic activity, respiration and richness in organic matter. The higher values (8.33 mg l^{-1}) at Sultan Battery and Kateel Temple River could be credited to the circulation and mixing of water due to the gushing of water across the rocks. An inverse relationship was seen between dissolved oxygen and salinity in all the sampling locations. Srivastava *et al.* (2014)

also observed a similar correlation when studying physicochemical variables across seasons. The low levels (5.33 mg l^{-1}) of DO at Shambhavi estuary suggests higher density of aerobic microorganisms and micro-fauna which is also a sign of moderately polluted waters and hence the reason for slight turbidity with anoxic state of the water body. Also, the comparatively high levels (9.4 mg l^{-1}) of nitrates could be possibly due to presence of nitrifying bacteria which convert the ammonia to nitrites and nitrates. The range of phosphate content at all the six sites lay between 0.15 – 0.67 mg l^{-1} which indicates a suitable trigger for eutrophication process.

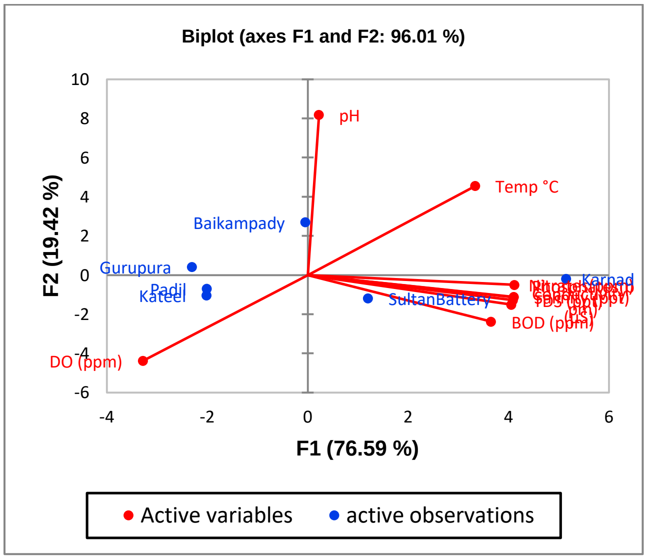

Fig 2: PCA analysis of the physicochemical parameters analyzed for the selected water bodies

The correlation between various parameters is seen in the PCA chart (Fig. 2). The PCA analysis of the first component F1 axis with 76.59% correlation reflects the positive correlation between TDS, conductivity, BOD, salinity, phosphates and nitrates especially at Sultan Battery and Shambhavi estuary which also had higher values compared to Baikampady, Gurupura, Kateel and Padil water bodies. However, the above parameters did not have a correlation to DO at the sampling sites which was exceptionally low (represented in the second component F2 axis). The PCA overall showed an independent relation between DO, Temperature and pH.

A total of 22 fresh water algal genera belonging to Class *Cyanophyceae, Chlorophyceae, Bacillariophyceae and Euglenophyceae* have been observed from the study areas (Fig. 3, Table 2). Members of *Bacillariophyceae and Chlorophyceae* were found to be the most diverse represented by eight genera each, *Navicula, Cymbella* and *Spirogyra* being the most dominant species (Fig. 5, Fig. 6 and Fig. 7).

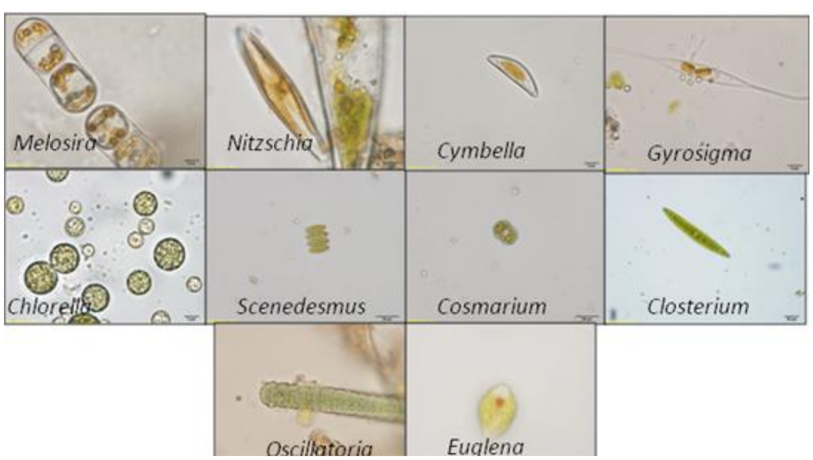

Fig 3: Photomicrographs of dominant microalgae found at the selected water bodies

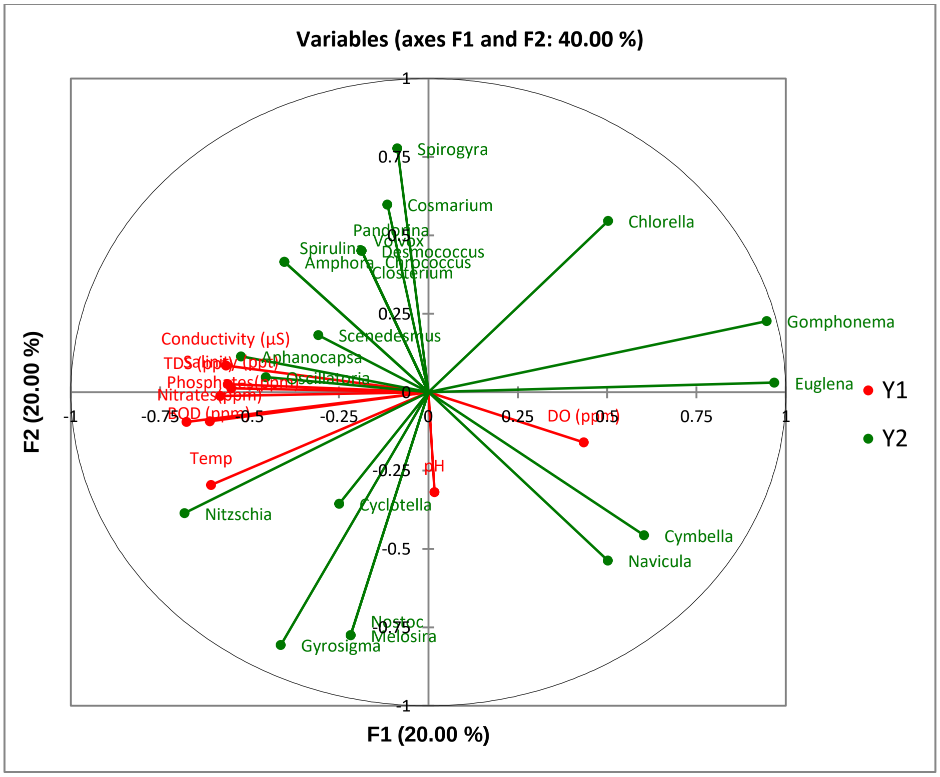

Fig 4: Canonical correlation analysis of the physicochemical parameters analyzed for the selected water bodies in Mangalore

Results of Canonical correlation analysis showed Eigen values as 1 for both the axis F1 and F2. Hence Eigen value for F1 (1) explained 20.00% correlation and axis 2 (1) explained 20.00% correlation between physico-chemical parameters and microalgal species. The CCA plot shows that phosphate, nitrate, salinity, conductivity, TDS and BOD are strongly correlated with the occurrence of *Nitzschia, Amphora, Oscillatoria, Aphanocapsa, Spirulina* and *Scenedesmus* (F2 axis). The plot also depicts the higher abundance and correlation of species such as *Spirogyra, Spirulina, Cosmarium,Volvox, Scenedesmus, Pandorina, Closterium* (represented in F2 axis) and *Chlorella, Cymbella, Navicula* (represented in F1 axis). The data represents a relationship of dissolved oxygen with the presence of *Cymbella and Navicula* which could be regarded as the “pollution indicators” specially in Shambhavi estuary, Karnad and Sultan Battery region (Fig 2 and 4). Hence, the CCA plots revealed descriptive information

Table 2: Distribution of phytoplankton in the 6 sampling locations

Phytoplankton	Site 1 Sultan Battery river	Site 2 Baikampady river	Site 3 Shambhavi estuary, Karnad	Site 4 Kateel temple river	Site 5 Gurupura lake	Site 6 Bairede kere, Padil
Bacillariophyceae						
Gomphonema	-	+	+	+	++	+
Navicula	++	++	+	++	++	+
Cymbella	+	+	++	+	++	+
Gyrosigma	++	+	+	-	-	-
Cyclotella	-	+	-	-	-	-
Melosira	+	-	-	-	-	-
Amphora	-	-	+	-	-	-
Nitzschia	+++	++	+++	+	-	++

Chlorophyceae						
Scenedesmus	-	+++	-	+	-	+++
Chlorella	-	+++	++	++	++	+++
Desmococcus	-	-	-	-	-	++
Pandorina	-	-	-	-	-	+++
Closterium	-	-	-	-	-	+++
Cosmarium	-	-	-	+	-	+++
Spirogyra	+	++	+++	+	+	++
Volvox	-	-	-	-	-	+
Cyanophyceae						
Chrococcus	-	-	-	-	-	++
Spirulina	-	-	+	-	-	-
Nostoc	+	-	-	-	-	-
Oscillatoria	+++	+	+++	++	-	+
Aphanocapsa	-	+	+	-	-	-
Euglenophyceae						
Euglena	-	-	-	+	++	+

(– =absent; + = sparse; ++ = abundant; +++ = dominant)

regarding the association and relationship of the studied physico-chemical parameters and the occurrence of species.

The phytoplankton sample from Sultan Battery was majorly represented by *Bacillariophytes* whose presence justifies occurrence of a high ionic and organic load of nutrients including silicates. The prominent Chlorophytes and Cyanophytes were from the genera *Spirogyra, Nostoc and Oscillatoria.* A very low density of *Cyanophyceae and Chlorophyceae* species along the sandy shore could be possibly due to rough tides, absence of adequate substratum and moderate ionic content of water (Thajuddin and Subramanian 2005)

The Baikampady River and Shambhavi estuary at Karnad showed a good distribution of genera from *Bacillariophyceae, Chlorophyceae* and *Cyanophyceae* with high ionic concentrations that possibly supported the microalgal growth. These backwaters also contained a stretch of mangrove forest along the river banks. The sampling site was therefore well guarded from severe action of waves, while a muddy substratum and the mangroves sheltered the niche. Considering the above factors along with the richness of ionic concentration and moderate salinity, the environment supported a good growth of notable microalgae. The Baikampady site showed a rich patch of *Spirogyra* along with *Chlorella* and *Scenedesmus* sp in the water sample. The nearby rocky surrounding also supported thriving *Oscillatoria* and *Aphanocapsa sp*. Among the Bacillariophyceae, *Navicula, Gomphonema, Cymbella, Gyrosigma and Nitzschia* were dominant at either places.

The phytoplankton species at Kateel Temple River and Gurupura Lake were commonly inhabited by *Gomphonema*, *Navicula* and *Cymbella* from Bacillariophyceae, *Chlorella* and *Spirogyra* from Chlorophyceae.. *Euglenophyceae* were also present in these two sampling sites. The highest diversity of 8 species of Chlorophytes was observed in the Padil, which according to Descy 1987, indicates relatively good health of a water body. This water body was also occupied by four diatom species, two Cyanophytes and Euglena. (Fig. 5, Fig. 6, Fig. 7)

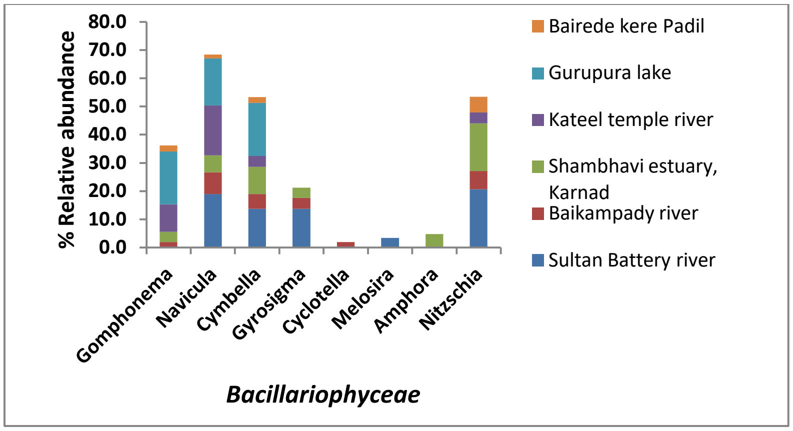

Fig 5: Comparative percentage relative abundance of the observed *Bacillariophyceae* species across the sampling sites

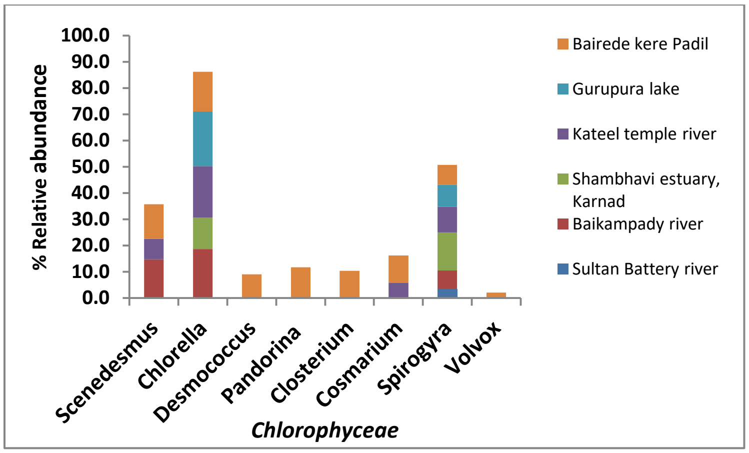

Fig 6: Comparative percentage relative abundance of the observed *Chlorophyceae* species across the sampling sites

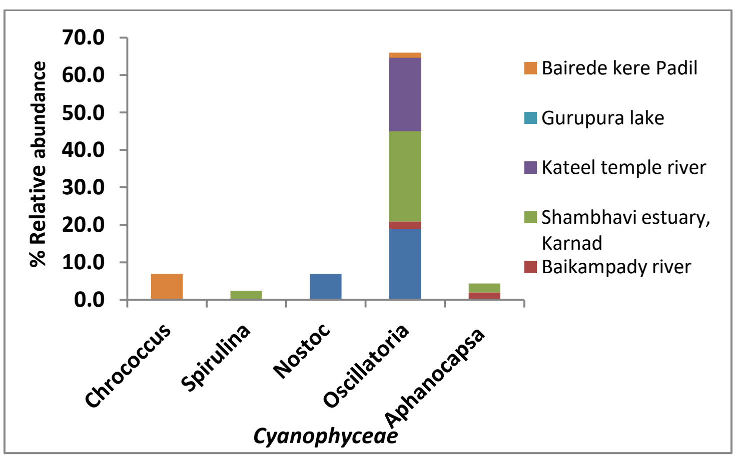

Fig 7: Comparative percentage relative abundance of the observed *Cyanophyceae* species across the sampling sites

Table 3: Micro algal community structure indices for the different water bodies studied

Water body	Diversity indices		
	Shannon Wiener index	Simpson index	Pielou's evenness
Sultan Battery river	1.92	0.16	0.62
Baikampady river	1.74	0.08	0.56
Shambhavi estuary	2.14	0.14	0.69
Temple pond, Kateel	2.10	0.14	0.68
Gurupura Lake	1.76	0.18	0.57
Bairede kere, Padil	2.45	0.10	0.79

The results of diversity indices depicted a moderate diversity of species across the studied sites. Highest value of Shannon Weiner index (2.45) was obtained at Bairede Kere, Padil which showed a rich diversity of *Chlorophyceae* along with *Bacillariophyceae* and *Cyanophyceae*. The diversity indices depict a highly stable community at Bairede kere, Padil while the rest of the sites possess a moderately stable community (Heffni *et al.* 2016). These values of Shannon Wiener index relate to the low values of Simpson's (dominance) index. Since the values of dominance are low there has been no significant dominance of any particular species within or between the sampling locations. However, the values indicate overall moderately polluted waterbodies since the Shannon – Wiener index lay in the range of 1-3 (William and Dorris 1968). Species evenness (ranged from 0-1) showed values between 0.56-0.79 indicating moderate to even distribution of species across the water bodies (Table 3).

DISCUSSION

In the present study, among the 22 freshwater algal genera, the most dominant species are *Navicula, Cymbella, Spirogyra followed by Cholrella, Ghomphonema, Nitzschia, Scenedesmus and Ocsillatoria*. Song *et al.* (2013) screened various genera of species for the biofuel prospective and pointed out *Selenastrum capricornutum, Chlorella vulgaris, Scenedesmus obliqnus, Phaeodactylum tricornutum* to be highly potential strain. Considering that Western Ghats have an enormous wealth of biodiversity, a systematic study of microalgae in various parts of the Western Ghats recorded 29 taxa of algae belonging to 19 *Chlorophyceae*, one *Cyanophyceae*, four *Bacillariophyceae* and five *Eugleneaphycea* (Chandra and Rajashekar 2011; Nampoothri *et al.* 2013). However, in spite of extensive research and analysis to explore its potential having been done, there still exists a huge gap to be bridged between its biological richness and ecological benefits.

Studies show that Chlorophyta, Bacillariophyta and Cyanobacteria are the most favored algae for biofuel production due to their high lipid content. In the past years, some algal strains from freshwaters (e.g., *Chlorella, Scenedesmus, Cylindrotheca, Dunaliella, Isochrysis, Nannochloris, Nannochloropsis, Neochloris, Phaeodactylum, Porphyridium, Schizochytrium, Tetraselmis, Crypthecodinium*) usually have been reported to have oil contents of 20%−50% of the dry biomass (Griffiths and Harrison 2009; Mata *et al*. 2010; Bozbas 2008; Liu *et al*. 2011). Chisti (2007) & Gouveia (2011) listed Oil content (% dry weight) of Microalgae which ranged from *Botryococcus braunii* 25–75%, *Chlorella emersonii* 63%, *Nannochloropsis sp*. 31–68 % to *Schizochytrium sp*. 50–77%. The major fatty acids to indicate the potential of biofuels in green algal strains are myristic acid (14:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1) and linolenic acid (C18:3) (Sathya and Srisudha 2013).

Temperature, pH and nutrient concentration have been emphasized to be a significant factors controlling distribution of *Cyanophyceae and Chlorophyceae* (EEA, 1999). Various physical, chemical and biological circumstances must be simultaneously taken in to the consideration for understanding the fluctuations of plankton population in any study area. The source of nitrogen for freshwaters includes drainage from the surrounding areas, precipitation bringing in dissolved free ammonia from atmosphere and release of nitrates from the dead fauna (Singh 1961; Thajuddin and Subramanian 2005). Considering the fact that major nutrients such as Nitrogen, Phosphorus, and Silica play an important role in the microalgal density of a system, these factors strongly influence the presence, quality and quantity of essential lipids in microalgae for biofuels.

Nevertheless, the screening of Microalgae for its prospects for biofuels remains as a continuous effort worldwide to overcome the ever- depleting fossil fuel reserve threat. Considering the results obtained from the present study, the species such as *Chlorella, Scendesmus* and diatoms could be upscaled and tested for their fatty acid profiles to best suit the biodiesel prospects. The present study shows a substantial richness in microalgal communities in the six sampling sites. The Padil water body stands out to be enriched with a good diversity of freshwater green algae out of which *Chlorella, Scendesmus* are chalked out by researchers as having biofuel prospects. However, Bacillariophyceae was considered to be the most diverse and relatively abundant class possessing a wide range of environmental variables for its occurrence. The diversity indices of the microalgae revealed a moderate – highly stable ecosystem with slight to moderate pollution values. The physico-chemical relation with the species diversity through the PCA and CCA analysis could explain the probability of generic existence of the algal communities and could be used as an indicator of the ecological status. Such diversity reflects the resourcefulness of the ecosystems. The findings provide an important piece of information of microalgal distribution in the six sampling sites of Dakshina Kannada that had not been explored before.

ACKNOWLEDGEMENT
The authors thank the MJES and UGC-minor Project for the financial support towards the study. Alifha Severes would like to thank the team of Laboratory of Applied Biology specially Mr. Shivananda for his technical assistance during sampling and for the constant cooperation.

REFERENCES
Bozbas, K. 2008 Biodiesel as an alternative motor fuel: Production and policies in the European Union. *Renew Sust Energ Rev*., 12(2): 542−552.

Chandra, S and Rajashekhar, M. 2011 Total Lipid And Fatty Acid Composition In Some Freshwater Cyanobacteria, *J. Algal Biomass Utln*., 2 (2): 83– 97.
Chisti, Y. 2007 Biodiesel from Microalgae. *Biotechnology Advances.* 25: 294–306.

Cloern, J.E. 1987 Turbidity as a control on phytoplankton biomass and productivity in estuaries, Continental Shelf Research. 7: 1367-1381.

Descy J. P. 1987 Phytoplankton composition and dynamics in the river Meuse (Belgium). Arch. Hydrobiol., Suppl., 78 (2): 225-245

Duong, V. T., Li,Y., Nowak, E., Schenk, P. M. 2012 Microalgae Isolation and Selection for Prospective Biodiesel Production. *Energies*, *5*: 1835-1849.

Fisher, T.R., Peele, E.R., Ammerman, J.W., Harding, L. 1992 Nutrient limitation of phytoplankton in Chesapeake Bay, Marine Ecology Progress Series 82: 51-63.

Gouveia, L. 2011 Microalgae as a feedstock for biofuels. *Springer Briefs in Microbiology*, 6: 7.

Griffiths, M. J., Harrison, S. T. L. 2009 Lipid productivity as a key characteristic for choosing algal species for biodiesel production. *J Appl Phycol.*, 21(5): 493−507.

Hefni E., Mujizat K., Dea F. L., Mursalina , Tri P. 2016 Distribution of phytoplankton diversity and abundance in Mahakam Delta, East Kalimantan. *Procedia Environmental Sciences*. 33: 496 – 504

Liu, An-Y., Chen, Wi., Zheng, L., Song, Li-R. 2011 Identification of High-Lipid Producers for Biodiesel Production from Forty-Three Green Algal Isolates In China. *Progress in Natural Science: materials International*, 21: 269 -276.

Mata, T. M., Martins, A. A., Caetano, N. S. 2010 Microalgae for biodiesel production and other applications: A review. *Renew Sust Energ Rev.*, 14(1): 217−232.

Mubeen, U., Hussain, W., Ul-Haq, I., Malik, A. 2011 Study of Native Algal Species for Growth Potential and Lipid Yield. *Current Research Journal of Biological Sciences,* 3(3): 240-245.

Naik, U. G., Nayak, V., Kusuma, N., Naik, U. G., Vinod, V. and Kusuma, N., 2010 Relationship between abundance of Micro Algae and Ecosystem of Sunkeri Backwaters, Karwar. In Proceedings of International Conference – Lake

2010: Wetlands, Biodiversity and Climate Change, Centre for Ecological Sciences, Indian Institute of Science, Bangalore.

Nampoothiri, K. M., Ramkumar, B., Pandey, A. 2013 Western Ghats of India: Rich Source of Microbial Biodiversity. *Journal of Scientific and Industrial Research*, 72: 617-623.

Neill, M. 2005 A method to determine which nutrient is limiting for plant growth in estuarine waters at any salinity. Marine Pollution Bulletin 50: 945-955.

Ramachandra, T.V., Alakananda, B., Supriya, G. 2011 Biofuel Prospects of Micro algal Community in Urban Wetlands. *International Journal of Environmental Protection,* 1 (2): 54-61.

Sathya, S and Srisudha, S. 2013 Isolation and Identification of Freshwater Microalgal Strains – Potential for Biofuel Production. *International Journal of Recent Scientific Research,* 4(9): 1432-1437*.*

Sharma, P., Patil, P., Rao, N., Swamy, K. V., Khetmalas, M. B., Tandon, G. D. 2013 Algal Database – Bioprospecting Indigenous Algae for Industrial Application. *Indian Journal of Biotechnology*, 12: 548 -549.

Shrivastava, A. K., Bharadwaj, M., Shrivastava, R. 2014 Algal Biodiversity in Fresh Water Reservoir of Durg. *IndianJ.Sci.Res*. 4 (1): 121-126.

Singh, R. N. 1961 *Role of Blue–Green Algae in Nitrogen Economy of Indian Agriculture*, ICAR, New Delhi.

Song, M., Pei, H., Hu, W., Ma, G. 2013 Evaluation of the potential of 10 microalgal strains for biodiesel production. *Bioresour Technol.,* 141:245-51.

Spolaore, P., Joannis-Cassan, C., Duran, E., Isambert, A. 2006 Commercial applications of microalgae. *Journal of Bioscience and Bioengineering*. 101: 87-96.

Thajuddin, N., and Subramanian, G. 2005 Cyanobacterial biodiversity and potential applications in biotechnology. *Current Science*, 89 (1).

William, J. L and Dorris T. C. 1968 Biological parameters for water quality criteria. Bioscience. 18: 477 -81.

APHA – American Public Health Association. 1998 Standard methods for the examination of water and wastewater. American Water Works Association/Water Environmental Federation, Washington.

APHA: Standard Methods for the examination of Water and Waste Water. 2005 American Public Health Association. Inc. New York.

Bartram J, Rees G. 2000 Monitoring bathing waters – A practical guide to the design and implementation of assessments and monitoring programmes. World Health Organization, London: Boundary Row.

EEA (European Environment Agency), 1999 Nutrients in European ecosystems. Environment Assessment Report No.4 EEA, Copenhagen (ISBN: 92-9167-163-0).