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MICROALGAL DIVERSITY (CHLOROPHYCEAE) AND IT'S CULTIVATION FOR BIOMASS USING AGRO-INDUSTRIAL WASTES

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ABSTRACT

The present study was focused on the diversity of microalgae and its cultivation governed by its physicochemical parameters from the lakes of Davangere district, India. Overall, 108 genera were documented including 29 from Chlorophyceae members (31%). Among them, *Scenedesmus* sp., *Chlorella* sp. and *Chlorococcum* sp. were frequently encountered with a high relative abundance and species number from the study area. Based on the diversity indices, the Chlorophyceae diversity was considerably high in polluted Bathi lake (21%), followed by Devarbelekere (18%) and Ayankere (14%) as these lakes were enriched with phosphate, nitrate, sulphate and organic constituents. Shanthisagara lake (8%) was documented with low nutrients and high D.O. Statistical analysis indicated that nutrients such as PO_4^-, NO_3^-, Cl^-, DO, pH, and temperature were the most important factors regulating the growth rate and its biomass productivity. The predominant isolates *Scenedesmus* sp., *Chlorella* sp. and *Chlorococcum* sp. were cultivated using Food Waste Hydrolyzed Broth (FWHB), Corn Steep Liquor (CSL) and Areca nut Husk Waste (AHW) and produced maximum biomass of 1.45 gm/L, 1.68 gm/L and 0.14 gm/L with specific growth rate (μd^{-1}) (0.362), (0.379) and (0.46) respectively. The mean nutrient uptake was observed maximum in *Scenedesmus* sp. (58%) followed by *Chlorella* sp. (38%), and *Chlorococcum* sp. (35%). Our study suggests that microalgae play a significant role as an ecological indicator and its application for the remediation of the aquatic ecosystem. Further, the biomass generated from the wastewater could serve as a feedstock for biofuel and biotechnological applications.

Keywords: Microalgae, Davangere district, Bio-indicator, Waste water, Phycoremediation, Biomass

1. INTRODUCTION:

Microalgae are primary producers in the food chain and possess strong adaptation to the any environment, thus used in the field of environmental application and in the generation of value-added products **[1, 2].** Microalgae have gained particular interest from researchers mainly because they can utilize organic matters as carbon and nutrient sources in wastewater. In addition, microalgae can treat wastewater, ultimately furnishing algal cultivation as cost-effective **[3].** A systematic study of microalgal diversity and its correlation with water constituents not only helps to assess the water quality but also to quantify the relative concentration of various pollutants in water and it helps in the implementation of rapid water quality management programmes. The use of microalgae growing autotrophically has been reported because of its capacity of utilizing P and N, at the same time amount of biomass of microalgae is obtained. Microalgae strains having specific properties need to be screened and characterized to achieve multiple applications **[4, 5].** Despite the enormous diversity of algae with approximately one million microalgal species, there are now only about 3,000 microalgal species available in algae culture collections **[6]**. The Chlorophycean (Green algae) has been among the most studied of all the microalgal groups and these members form the base of the food chain; they are directly or indirectly good sources of food for various animal groups **[7].** They are also a good source of oxygen for aquatic life and serve as a bioindicator for the aquatic ecosystem. It is a well-established fact that more than 75% of freshwater fishes feed on plankton at one or the other stage of their lifecycle **[8].** From a practical point of view, microalgae are easy to cultivate, can grow with little or even no attention, use water unsuitable for human consumption, and are easily inclined to provide nutrients. In recent years, the isolation of microalgae for its biomass generation and conversion products has received much attention. Media formulation and its optimization are the key factors in the development of bioprocesses that can produce affordable by-products. Interest in the recovery of waste or by-products has been increasing for both economic and ecological reasons. Waste generations through Agro-industrial were higher due to an increase in world population and wastes emanating from food and industrial sources which constitute about 40% of the total mass. The majority of this waste is often improperly disposed of and serves as pollutants; hence constitutes huge environmental problems **[9] [10].** Upcycling agro-industrial by-products and

their waste can be a dynamic option to address the cost problem of organic carbon sources, although many nutrients in food waste can be potentially useful in microbial cultivation **[11].** Nutrient removal from waste to acceptable levels for disposal or reuse is a major challenge in the treatment of wastewater, and many of the available conventional technologies based on chemical and physical methods have high costs **[12].** The use of agro-industrial wastewater appears to be a viable low-cost source for the cultivation of microalgae and cyanobacteria due to their excellent capacity to adapt to various conditions. A fundamental requirement needed to advance in the formulation of low-cost culture medium for the effective manufacture of value-added goods is the study of growth medium components that affect considerably growth rate, biomass output, pigments content, and biochemical composition of microorganisms. Microalgae are not only feedstock for fuel, and food for humans and animals, it's chemical compounds also serve as a wide source used in food technology, pharmaceuticals and cosmetics as well **[13].** Screening for indigenous strains is considered to be an excellent choice and such strains could potentially tolerate extreme wastewaters condition **[14]** altogether microalgae growth in wastewater is a cost-effective, highly productive, and environmentally friendly process. The object of this study was to evaluate the use of microalgae as bio-indicators in the lakes of the Davanagere district and to determine their effectiveness in growing on low-cost medium.

2. MATERIALS AND METHODS:

2.1. Study area, collection and analysis of water samples:

The Davanagere district is located in the mid-eastern region of the Karnataka state, between the 13° 45' 00" N to 14° 50' 00" N latitude and 75° 30' 00" E to 76° 30' 00" E longitude and its elevation is about 602.5 m above the mean sea level. The lakes selected for the study include Anaji (S1), Ayyankere (S2), Bathi (S3), Kondaji (S4), Kundwada (S5), Devarabelakere (S6) and Shanthisagar (S7) **(Fig-1).** The water samples were collected monthly from September 2018 to August 2019 from the different sampling sites and onsite temperature, colour, pH, conductivity, TDS and DO parameters were analyzed. Further, the water samples were collected in a sterile screw-capped container and carried to the laboratory for determining nutrient and organic constituents by following standard methods of APHA **[15].**

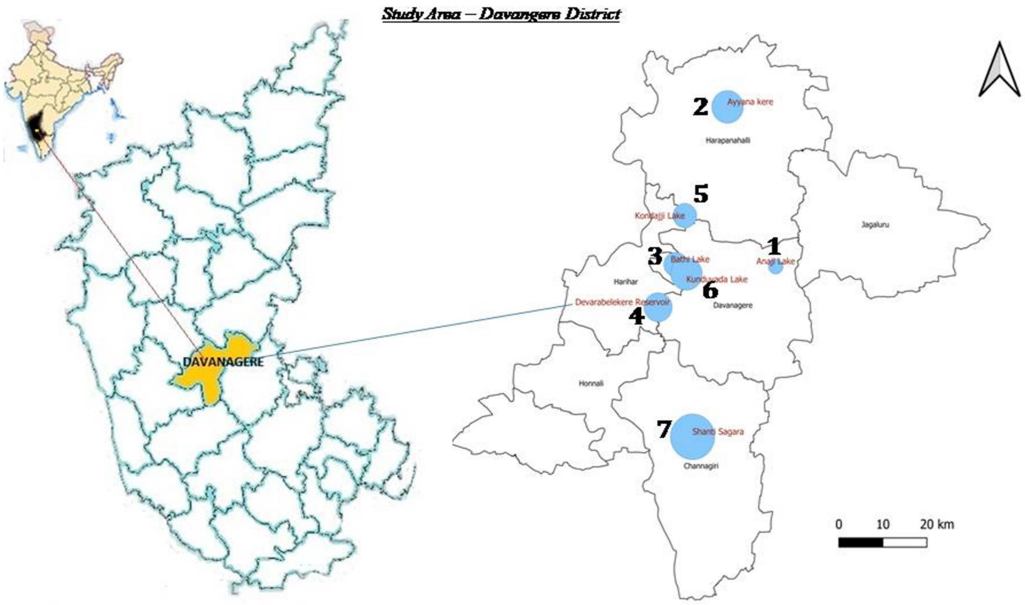

Figure 1:Seven Study area of Davangere District: 1.Anaji-(S1), 2.Ayyankere-(S2), 3.Bathi-(S3), 4.Kondaji-(S4), 5.Kundwada-(S5), 6.Devarabelakere-(S6) 7.Shanthisagar-(S7)

2. Isolation and identification of microalgae:

The microalgal study was conducted monthly from the selected sampling sites by using the modified Lackey's drop method. Here, the total number of microalgae present was calculated (cells/L) using a stereo-microscope (Lawrence and Mayo, India) with 40X magnification. Chlorophyceae members were identified using standard keys as provided by Fritsch and Desikachary **[16]**, Prescott **[17]** and Algae Base **[18].** The pure cultures were obtained by centrifugation, washing and streak plating technique **[19].** The water samples were collected aseptically from the sampling sites and 10 mL of it were transferred to a 500 mL conical flask containing 200 mL of sterilized Bristol, BG11, and BBM media and incubated on a rotary shaker at 150 rpm for 27°C and continuously illuminated using white fluorescent light at an intensity of 20 mol m^{-2} s^{-1} for four weeks under 16:8 h light/dark cycle. Further, subcultures were obtained by progressive dilutions of the original sample. A volume of 10 ml microalgal sample was taken aseptically from enrichment culture and centrifuged at 3000 rpm for 15 min. The supernatant was removed and the cells were washed in fresh sterile water. The cells were streaked onto BG11 agar medium upon centrifugation to obtain the pure culture.

2.3. Cultivation of microalgae in optimized wastewater:

Food waste was collected from the Cafeteria in Sahyadri Science College, Shivamogga. The Corn Steep Liquor was collected from Cargill Pvt. India Ltd.,

Harihar. Areca Husk waste (AHW) was prepared by areca nut husk which is a predominant crop of malnad area in Davanagere and Shivamogga districts. The samples were grounded, filtered & mixed with water. A 3% sulphuric acid was added to the mixture to hydrolyze the polysaccharides and proteins in the agro-industrial waste, which was then autoclaved at 121^{o}C for 30 min. The autoclaved mixture was neutralized with NaOH and the filtrate obtained is Food Waste Hydrolyzed Broth (FWHB) **[20]**, Corn Steep Liquor Waste (CSLW) and for Areca Husk waste (AHW), ripen husk was collected from farmers and dried sterilized optimized with 3% sulphuric acid, 5% Urea, 0.5% K_2HPO_4, 1% KH_2PO_4, 0.25 % $CaCl_2$ was added and pH was adjusted using 0.1N NaOH **[21, 22, 23]**. Lab-scale monoculture cultivation was performed using plastic containers with the following dimensions; 25cm (Width) X 50cm (Length) with a working volume of 20L and cultivation was supplemented with optimized wastewater (FWHB, CSLW, AHW) with pH 7.2±3 and it was inoculated with 5 % (v/v) seed culture of *Scenedesmus* sp. (GMDAj0519), *Chlorella* sp. (GMDBt0319) and *Chlorococcum* sp. (GMDKu0119) under 16:8h light:dark, continuous illumination of 3000 lm (36W) by using white LED lamps for 25 days at 25^{0}C **[24,25]**.

2.4. Growth studies:

The growth of microalgal species in the broth and wastewater was studied for every four days by measuring its optical density at 680 nm using a spectrophotometer and cell count was made using a haemocytometer with a suitable dilution. Further, cell biomass was harvested by centrifugation and dried at 400C and its weights of the wet and dry samples were measured. Specific growth rates were calculated using the formula described in equation 1, where N1 and N2 are defined as biomass at time 1 (t1) and time 2 (t2).

$$\boldsymbol{\mu = \frac{\ln(N1-N2)}{t2-t1}}(1)$$

Biomass productivity (B) was calculated using the equation below **[26, 27]**:

$$\boldsymbol{B = \frac{(B_1-B_0)}{(T_1-T_0)}}\textbf{(2)}$$

Where B_0 and $B_1$is the mean Dry biomass/Cell Number concentration at the times T_0 and T_1, respectively.

2.5. Statistical analysis:

The data were statistically analyzed using MS Excel, 2010; One-way ANOVA multivariate analyses were performed using Graphpad prism software. The Pearson correlation (P<0.05) was applied to study the correlation between specific growth rate and biomass productivity. The Hierarchical Cluster Analyses (HCA) was carried out with the h-cluster function of R using single-linkage Euclidean. The Canonical

Correspondence Analysis (CCA) was applied to establish the link between physicochemical factors and Chlorophyceae distribution in different stations using the PAST v 4.03. **[28]**.

3. RESULTS AND DISCUSSION:

The present study signified the distribution of Chlorophyceae members with the water quality based on its environmental parameters in the lakes of the Davanagere district, fluctuation in the physiochemical properties was noted in the seven sampling sites. (**Table-1)** shows the mean value of physicochemical parameters of the seven selected sampling sites, the district has spread into three agro - climatic zones, namely the central dry zone, southern transition zone, and northern dry zone, this climatic condition and anthropogenic activity play a vital role in describing the ecological status of Lake. Physical parameters like temperature, pH and nutrients such as nitrate, phosphate, sulphate and carbonates played a vital role in Chlorophyceae distribution, slight variation in the temperature was observed among the study sites with respect to seasons, the mean ambient temperature was 27.5 ^{o}C and pH was 7.3 ±0.6 which is optimum for the growth of microalgae. Nutrients like nitrate and phosphate are directly proportional to human activity and influence green algal growth. The S2-Ayiankere was highly concentrated with nitrate (2.7 mg/L) phosphates (2.3 mg/L), chloride (120 mg/L) and sulphates (75 mg/L), followed by S3-Bathi lake nitrate (1.8 mg/L), phosphates (1.2 mg/L), chloride (98 mg/L) and sulphates (55.3 mg/L), S4-Devarabelakere lake (1 mg/L), (0.8 mg/L), (71 mg/L) and (48 mg/L). S7-Shanthishagara Lake showed low nitrate (0.2 mg/L), phosphates (0.1 mg/L), chloride (18 mg/L) and sulphate (15 mg/L) concentration. The Dissolved oxygen concentration varied from 3-7 mg/L where S7-Shanthisagar (7), S4-Kondajji (6.5) and S1-Anaji (6) were less polluted and concentrated with high dissolved oxygen (>6) throughout the year. The S2-Lake was noted with 3 mg/L in the summer season. Lakes studied in the Davanagere district S2 site were stressed by human activities like agricultural runoff industries outlet and transportation and some migratory birds nearby have increased the nutrient level which favoured the growth of microalgae and highly diversified with Chlorophyceace members, this accumulation caused eutrophication with a decrease in oxygen concentration, similarly site S3 was contaminated from agricultural runoff and fishing, but the large quantity of water and its flow rate reduced the microalgal diversity.

Table 1: Water quality assessed in seven different lakes of Davangere district

Sl. No	Parameters	Tolerance Limit As per ISO	Water Sources						
			S1	S2	S3	S4	S5	S6	S7
			Mean± SD						
1	Temperature (°C)	30 ± 5	28.04 ± 02.0	28.50 ± 04.0	27.50 ± 02.0	27.1 ± 03.00	27.1 ± 01.00	27.55 ± 02.5	26.05 ± 03.0
2	pH	6.5 - 9.00	7.6 ± 01.5	7.9 ± 00.5	7.6 ± 00.50	7.7 ± 01.5	7.5 ± 02.00	7.6 ± 02.80	7.6 ± 01.00
3	E.C (µS/cm)	0.5 to 2500	283 ± 08.01	1242 ± 50.5	800 ± 24.5	452 ± 20.01	300 ± 10.05	190 ± 12.05	156 ± 06.00
4	Turbidity (NTU)	1	7 ± 0 3.00	15 ± 07.05	9 ± 02.04	6 ± 03.10	6 ± 2.1	4 ± 2.01	3 ± 1.2
5	DO (mg/L)	6	6.7 ± 03.00	5.4 ± 1.50	5.8 ± 1.4	6.2 ± 01.2	7.2 ± 01.02	7.2 ± 02.50	7.7 ± 2.01
6	BOD (mg/L)	30	4 ± 00.90	24.8 ± 4.0	9.5 ± 02.01	7.2 ± 01.4	4.5 ± 01.07	4.1 ± 01.50	3.7 ± 00.50
7	COD (mg/L)	250	18 ± 02.07	69.3 ± 22.2	43 ± 18.01	35.1 ± 04.02	22 ± 03.00	20 ± 07.00	15.3 ± 03.00
8	Calcium (mg/L)	5	48 ± 08.01	105.6 ± 10.8	86 ± 09.20	61 ± 08.07	58 ± 14.08	50 ± 10.01	46 ± 12.0
9	Magnesium (mg/L)	1	28 ± 07.01	68 ± 9.9	43 ± 07.09	37 ± 07.05	35 ± 02.90	30 ± 09.01	23 ± 09.02
10	Chloride (mg/L)	250	31 ± 04.7	121 ± 25.7	98 ± 15.02	55 ± 04.8	28 ± 03.4	24 ± 04.08	18 ± 03.00
11	Phosphate (mg/L)	10	0.56 ± 00.10	2.34 ± 0.4	1.1 ± 00.15	0.52 ± 00.19	0.22 ± 00.11	0.27 ± 00.17	0.15 ± 00.09
12	Sulphate (mg/L)	200	27 ± 03.07	75 ± 13.47	45 ± 07.09	31 ± 09.12	27 ± 03.9	21 ± 12.47	16 ± 07.00
13	Nitrate (mg/L)	10	0.64 ± 0.1	2.89 ± 1.04	1.40 ± 01.20	0.66 ± 00.19	0.39 ± 00.12	0.31 ± 00.11	0.51 ± 00.09
14	Potassium (mg/L)	5	2.1 ± 0.2	9.60 ± 0.7	3.37 ± 0.89	1.55 ± 0.8	0.97 ± 00.50	1.03 ± 0.2	0.61 ± 00.15
15	Sodium (mg/L)	50	13 ± 1.21	30 ± 3.88	20 ± 4.66	9.5 ± 3.6	5±2.28	5 ± 3.00	2 ±0.8

Chlorophyceae dominance has been attributed to the eutrophic nature of the lake **[29]**. The present study reported 110 microalgal genera belonging to six different classes viz., Chlorophyceae, Bacillariophyceae, Euglenophyceae, Cyanophyceae and Dinophyceae were identified from the study area. Among them, Chlorophyceae contributed 31% of green algae; including 29 genera and 202 species were documented in the seven study areas **(Table-2),** and site S3 was highly diversified with 21% of green algae followed by S4 (16%), S1 (15%) and least was S7 (8%) **(Fig-2A).** Based on its diversity and high relative abundance species of *Scenedesmus, Chlorella, Chlorococcum, Staurastrum, Coelastrum, Pediastrum and Cosmorium* served as a pollution indicator as they are predominant species in all the study sites and these species have proved to be pollutant tolerant, which were also proved in earlier studies **[30, 31]**. The *Chlorella* and *Scenedesmus* species were found to be pollution indicators as these species were found dominant in Mogan Lake, which is located quite close to a crowded urban area **[32]**.

Table 2: Chlorophyceae diversity observed in seven different lakes of Davangere district

Sl No	CHLOROPHYCEAE	S1	S2	S3	S4	S5	S6	S7	R.A%	Species
1	*Ankistrodesmus* sp.	1	1	3	2	2	1	1	3.57	5
2	*Arthrodesmus* sp.	0	0	1	1	0	0	0	0.65	1
3	*Botryococcus* sp.	0	0	1	0	1	0	0	0.65	1
4	*Chlamydomonas* sp.	1	1	2	2	1	1	1	2.92	2
5	*Chlorella* sp.	2	4	5	4	3	3	2	7.47	3
6	*Chlorococcum* sp.	2	2	4	3	2	2	1	5.19	2
7	*Chroococcus* sp.	1	1	2	1	1	1	1	2.6	1
8	*Coelastrum* sp.	3	4	4	2	2	2	1	5.84	5
9	*Cosmarium* sp.	3	1	3	2	1	1	1	3.9	18
10	*Crucigenia* sp.	0	1	1	0	0	0	0	0.65	1
11	*Cylindrocystis* sp.	0	1	1	0	0	0	0	0.65	1
12	*Dictyosphaerium* sp.	3	1	2	3	3	2	1	4.87	4
13	*Dimorphococcus* sp.	1	1	2	2	1	1	0	2.6	2
14	*Euastrum* sp.	0	0	2	1	0	0	0	0.97	2
15	*Eudorina* sp.	1	1	3	2	1	1	0	2.92	3
16	*Golenkinia* sp.	1	1	2	3	2	2	1	3.9	2
17	*Micractinium* sp.	1	1	2	4	3	3	1	4.87	4
18	*Monoraphidium* sp.	1	0	1	1	2	2	2	2.92	1
19	*Nephrocytium* sp.	0	0	1	1	1	0	0	0.97	2
20	*Oocysitis* sp..	2	1	2	3	3	3	1	4.87	3
21	*Pandorina* sp.	2	2	4	3	2	2	1	5.19	4
22	*Pediastrum* sp.	1	3	5	3	3	3	1	6.17	7
23	*Scenedesmus* sp.	3	3	5	4	4	3	2	7.79	15
24	*Selenastrum* sp.	2	1	1	3	2	2	1	3.9	5
25	*Sphaerocystis* sp.	0	1	0	0	0	0	0	0.32	1
26	*Staurastrum* sp.	2	2	5	4	3	2	1	6.17	6
27	*Tetraedron* sp.	2	1	3	2	2	2	1	4.22	10
28	*Volvox* sp.	0	1	1	1	0	0	0	0.97	1
29	*Westella* sp.	1	1	2	1	1	1	0	2.27	1

The distribution of Chlorophyceae and physico-chemical parameters showed statistically significant differences (P<0.05) for temperature, dissolved oxygen, conductivity, alkalinity, sulphate, calcium, nitrate, transparency, turbidity, magnesium and phosphate. The Pearson correlation studies between Chlorophyceae and the water constituents showed a positive correlation with DO and temperature, pH, specific conductivity, total alkalinity and Mg^{++}, Ca^{++} and a negative correlation with turbidity, phosphate, nitrate and BOD **(Table-3)**. Dissolved oxygen, temperature and pH, are important environmental parameter that decides the ecological health of a stream and protects aquatic life **[33]**. The present data have shown that light, temperature and pH are important factors for the growth and density of algae, which also confirms the data from Han *et al.* **[34]**. Nutrients like nitrate and phosphate, sulphates and chlorides are directly proportional to human activity and influence the growth of microalgae. The HCA plot of this study was based on Chlorophyceae

abundance, where sites S3 and S2 were placed in a close cluster with a high diversity of green algae. Site S4, S5 and S6 were distantly clustered with them, whereas Site S7 and S1 are out-group with the even microalgal diversity **(Fig-2B).**

Table 3: Values of Pearson correlation studies between water sample constituents and Chlorophyceae.

CHLOROPHYCEAEA	TEPM C	pH	TUR	D.O	BOD	SUL	CHL	SOD	POT	NIT	PHOS
R	-0.4896	-0.0890	0.7068	-0.275	0.6927	0.2632	0.2632	0.2632	0.2632	0.5225	0.5632
P (one-tailed)	0.0541	0.3931	0.0057	0.1927	0.0064	0.2047	0.2047	0.2047	0.2047	0.042	0.038
P-value summary	Ns	ns	**	Ns	**	Ns	Ns	Ns	Ns	*	*
Significant (α = 0.05)	No	No	Yes	No	Yes	No	No	No	No	Yes	Yes

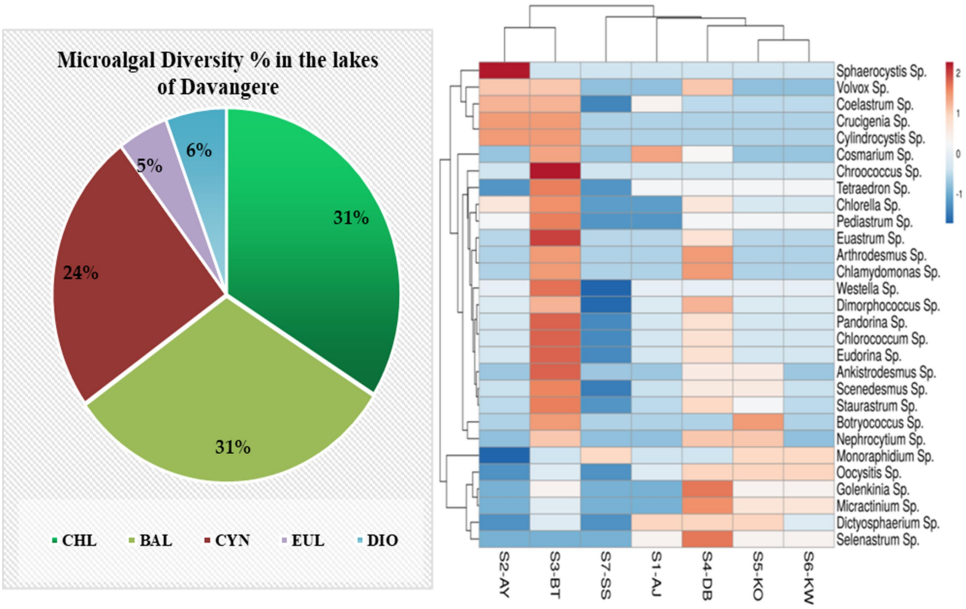

Figure 2A: Distribution of Microalgae in the lakes of Davangere District, 2B- A. Hierarchical clustering of 7 study sites according to Chlorophyceaea species composition using Ward's linkage as per Chlorophyceaea richness

The dynamics of microalgae have altered with the physicochemical variables and each species responds differently to environmental factors. Therefore, Canonical Correspondence Analyses (CCA) is used to determine this correlation. The results from a CCA analysis based on normalized environmental variables in 7 sampling sites, the Eigen values and the percentages of variance in axis-1 are found to be higher than on axis-2. Between physicochemical factors and Chlorophyceae species, the Eigenvalue for axis-1 (0.0432) explains 68.47% correlation, while axis-2 (0.0113) explicates 17.06% correlation. Totally, 29 Chlorophyceae species were documented among which 7 predominant species were highlighted as bio-indicator in the data

analysis using CCA **(Fig.3)**. *Chlorella* sp. *Chlorococcum* sp. *Coelastrum* sp. *Pandorina* sp. *Pediastrum* sp. *Scenedesmus* sp. and *Staurastrum* sp. indicate that they are positively correlated with the higher value of PO_4^-, NO_3^-, Cl^- in sites S3, S4 and S6. Specifically, these species are dependent on nutrients. Simultaneously, D.O, pH and temperature were correlated with few species where the lakes were less contaminated and were found to be least affected by the variation of physicochemical factors which corroborates with earlier findings **[35] [36]**.

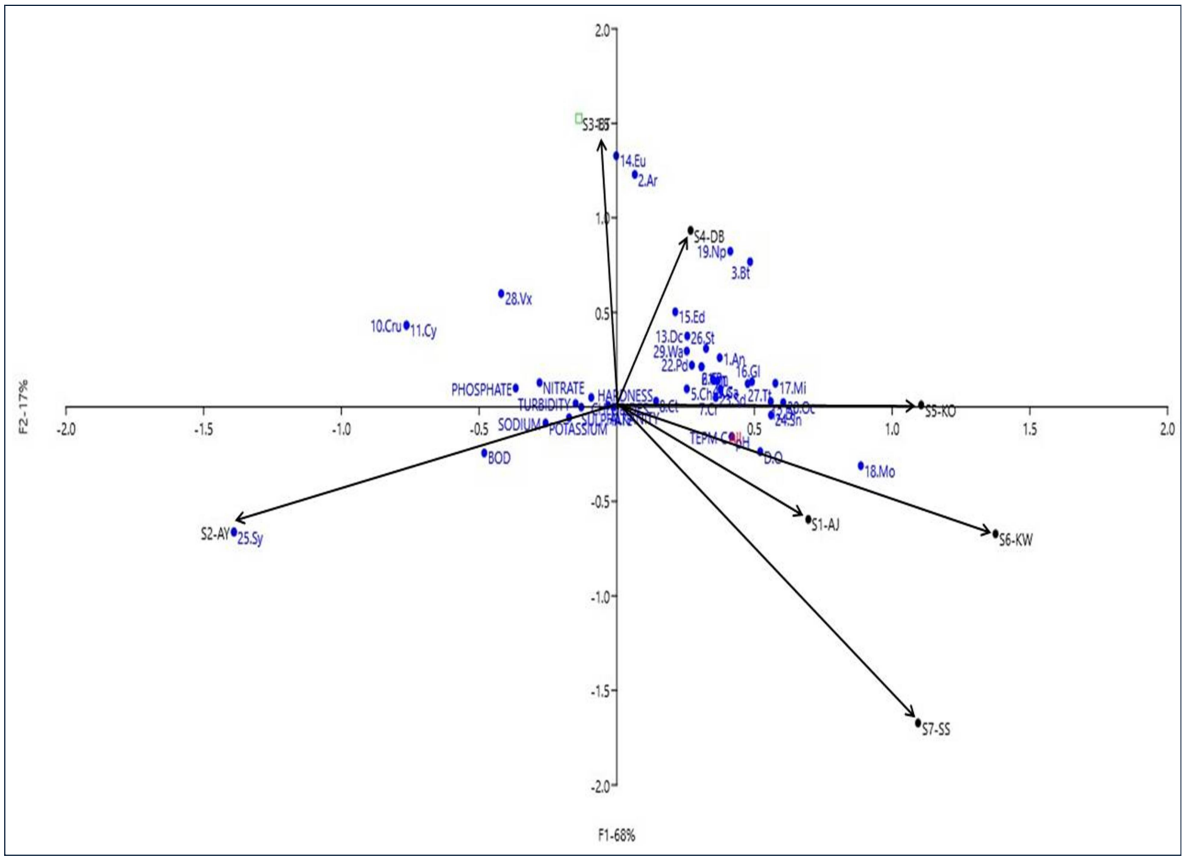

Figure: 3. Canonical correspondence analysis of Chlorophyceae diversity and water quality.

1. Ankistrodesmus sp. 2.*Arthrodesmus* sp. 3.*Botryococcus* sp. *4.Chlamydomonas* sp. 5.*Chlorella* sp. 6.*Chlorococcum* sp. 7.*Chroococcus* sp. 8.*Coelastrum* sp. 9.*Cosmarium* Sp. 10.*Crucigenia* Sp., 11.*Cylindrocystis* sp. 12.*Dictyosphaerium* sp. 13.*Dimorphococcus* sp. 14.*Euastrum* sp. 15.*Eudorina* sp. 16.*Golenkinia* sp. 17.*Micractinium* sp. 18.*Monoraphidium* sp. 19.*Nephrocytium* sp. 20.*Oocysitis* sp. 21. *Pandorina* sp. 22. *Pediastrum* sp. 23. *Scenedesmus* sp. 24. *Selenastrum* sp. 25. *Sphaerocystis* sp. 26. *Staurastrum* sp. 27. *Tetraedron* sp. 28. *Volvox* sp. 29. *Westella* sp.

After purification by streak plate technique, the isolated microalgae were maintained in the laboratory in BG-11 medium and subcultured every 10 days. Predominant *Scenedesmus* sp. (GMDAj0519), *Chlorella* sp. (GMDBt0319) and *Chlorococcum* sp. (GMDKu0119) isolates were cultured and cultivated on a laboratory scale using agro-industrial waste for the growth, biomass generation and the

incubation period for 25 days. Based on these diversity studies, the occurrence and adaptation of microalgae with the water constituent significantly helped in the formulation of low-cost media thus, cultivation of microalgae was carried out accordingly using optimized wastewater. The microalgae which were isolated from sewage have the inherent capacity to grow in heavy organic load **[37]**. Synthetic media are artificial and simplified media that are designed to provide nutrients for the growth of freshwater algae, as well as for maintaining algal strains. In microbial cell suspensions, cell turbidity can be determined easily by using the optical density method. The growth medium for algal cell cultures must constitute essential micronutrients such as nitrogen and phosphorus **[38]**. The wastewater contains high amounts of nitrogen and phosphorous that poses a serious concern to the environment in the form of eutrophication and the research has been focused on the utilization of nutrients from the wastewater before its discharge into the natural water bodies **[39]**. The results indicated that three species have shown similar trends and adhered to the standard growth curve in nutrient media and wastewater. The algal growth curves were studied at OD 680 nm and cell count as shown in **(Table-4).**

Table 4: Growth studies of microalgae in the media and different wastewater.

Cultivation Time in Days	*Scenedesmus* sp.			*Chlorella* sp.			*Chlorococcum* sp.		
	BG11 Broth	FWHB	CSL	BG11 Broth	FBHW	CSL	BG11 Broth	FBHW	CSL
1st	0.025	0.045	0.025	0.026	0.056	0.036	0.050	0.045	0.039
3th	0.17	0.12	0.1	0.14	0.38	0.12	0.49	0.34	0.22
6th	0.51	0.388	0.234	0.849	0.684	0.321	0.84	0.692	0.40
9th	0.966	0.730	0.54	1.160	0.920	0.650	1.08	0.713	0.63
12th	1.334	1.104	0.777	1.673	1.124	0.802	0.933	0.705	0.612
15th	1.200	1.090	0.64	1.42	0.95	0.681	0.73	0.68	0.536
Cell Count Cell/ml	$5.2x10^6$	$4.9x10^5$	$3.0x10^4$	$6.01x10^5$	$5.2 x10^5$	$3.2 x10^5$	$5.0x10^5$	$4.6x10^5$	$2.7x10^5$
Cell Weight g/L (Biomass)	1.45	1.29	0.889	1.58	1.20	0.87	1.24	0.849	0.56
Specific Growth Rate (μ, d^{-1})	0.362	0.322	0.293	0.379	0.304	0.279	0.329	0.273	0.250
Biomass productivity (B) g dw $L^{-1}d^{-1}$)	0.130	0.098	0.080	0.166	0.095	0.066	0.120	0.068	0.038
R^2	0.8432	0.8852	0.8833	0.7519	0.8266	0.9048	0.4734	0.6434	0.7485
Slope	0.08887	0.07570	0.05423	0.1031	0.06821	0.05833	0.04756	0.04153	0.03816

FWHB-Food Waste Hydrolyzed Broth, **CSL**-Corn Steep Liquor.

The cultivated microalgae underwent lag, exponential, and stationary phases when growing in BG11 and low-cost media FWHB and CSL, which supported microalgal growth positively as also shown by Moussa *et al.***[40].** AHW failed to

generate the biomass and there was no significant growth observed from the above-used species. Based on the growth curve represented in **(Fig-4),** the lag phase (μ≈0) of all the species was consistent from day 0 to day 5(μ<μmax), followed by (μ≈μmax) exponential phase (day 6 to day 12), and stationary phase (μ<μmax) (day 13 to day 18) and decline (death) (μ=0) phase which was achieved after 21 days. *Chlorococcum* sp. has achieved log phase on 6th day followed by *Scenedesmus* sp., 10th days, *Chlorella* sp. 12th day and the results were correlated with the cell number and biomass to determine Specific growth rate (μ, d^{-1}) and Biomass productivity (B). Specific growth rate refers to cell regeneration, which is the way how fast the cells are dividing in culture and some microalgal species grow by increasing their cell number (cell division), rather than in size. The rate of cell division was varied according to the species and their living conditions, fitting a straight line gives a slope of three species with respect to cultivation media, three species significantly showed exponential growth in Modified BG11 medium *Chlorella* sp. has the highest O.D 1.673 and 1.5 g/L dry weight similarly *Scenedesmus* sp. was having 1.33 O.D, 1.45 g/L dry weight, the least growth and productivity was observed in *Chlorococcum* sp. O.D 1.08 and dry weight 0.7 g/L. Comparatively, FWHB proved to be a good source for cultivation with O.D 1.04 *Chlorella* sp. *Scenedesmus* sp. 1.24, and 0.76 *Chlorococcum* sp. 1.29, 1.10, 0.84 and its dry weight g/L, and CSL growth was found to be average O.D ranged between 0.8-0.6 of all the three isolates with 0.8-0.5 g/L. specific growth rate and biomass productivity were summarized accordingly in (**Table-4)**. *C. vulgaris* is capable of adapting to different conditions, as observed with other organisms by Brynjolfsson *et al.* **[41].** Sibi *et al.* **[24]** have produced the highest specific growth rate 1.92 μ d^{-1} and biomass productivity in fruit waste hydrolysates than in vegetable waste hydrolysates using *C. vulgaris*. Wang *et al.***[42]** reported enhanced biomass, lipid, and lutein production from *Chlorella* sp. using food waste hydrolysate, Cultivation coupling with nutrients absorption is added sustainable process, results were in (**Table–5)**, which signifies the nutrient removal before and after cultivation, among these isolates *Scenedesmus* sp. found to be efficient as utilization of nutrients nitrate, phosphate and carbon source in both the wastewater rate is high with COD 54 %, sugars 63%, NO_3^- 59%, PO_4^- 54%, followed by *Chlorella* sp. 37%, 49%, 34%, 30% and *Chlorococcum* sp. 27%, 51%, 36%, 26% described in **(Fig- 5A, 5B)**. Mercado *et al.* **[43]** recently worked on *Scenedesmus* sp. which has achieved high percentages of

biomass utilizing nutrients in dairy wastewater 88.41% and 97.07% for nitrogen and phosphorus, respectively, our result correlates similarly by producing biomass in FWHB and CSL. Nitrate and phosphates were highly consumed in all experimental conditions, as they are the main contributors to sustaining microalgae growth **[44]**. The utilization of food processing byproducts as low-cost substrates for generating microalgal value-added compounds could enhance productivity simultaneously enhance productivity and reduce costs **[45]**. Further study is required with a focus on screening of potential strains and their cultivation by using a low-cost medium for the generation of biomass.

Table 5: Nutrient removal from the Agro-Industrial waste water before and after cultivation

Sl. No	Parameters	FWBH				CSL				The efficiency of removal of pollutants %		
		Before Cultivation	After Cultivation			Before Cultivation	After Cultivation					
		Control	Sd	Cl	Cm	Control	Sd	Cl	Cm	Sd	Cl	Cm
1	COD mg/L	2200	900	1300	1600	700	430	520	520	54.1	37.24	27.0
2	Sugars mg/L	5342	1800	2700	2480	480	300	260	340	63.9	49.1	51.6
3	Nitrate mg/L	496	176	228	280	360	160	200	260	59.8	34.7	36.6
4	Phosphate mg/L	240	110	160	180	120	70	90	90	54.11	30.2	25.5

Sd- *Scenedesmus*, **Cl**- *Chlorella*, **Cm**- *Chlorococcum.*

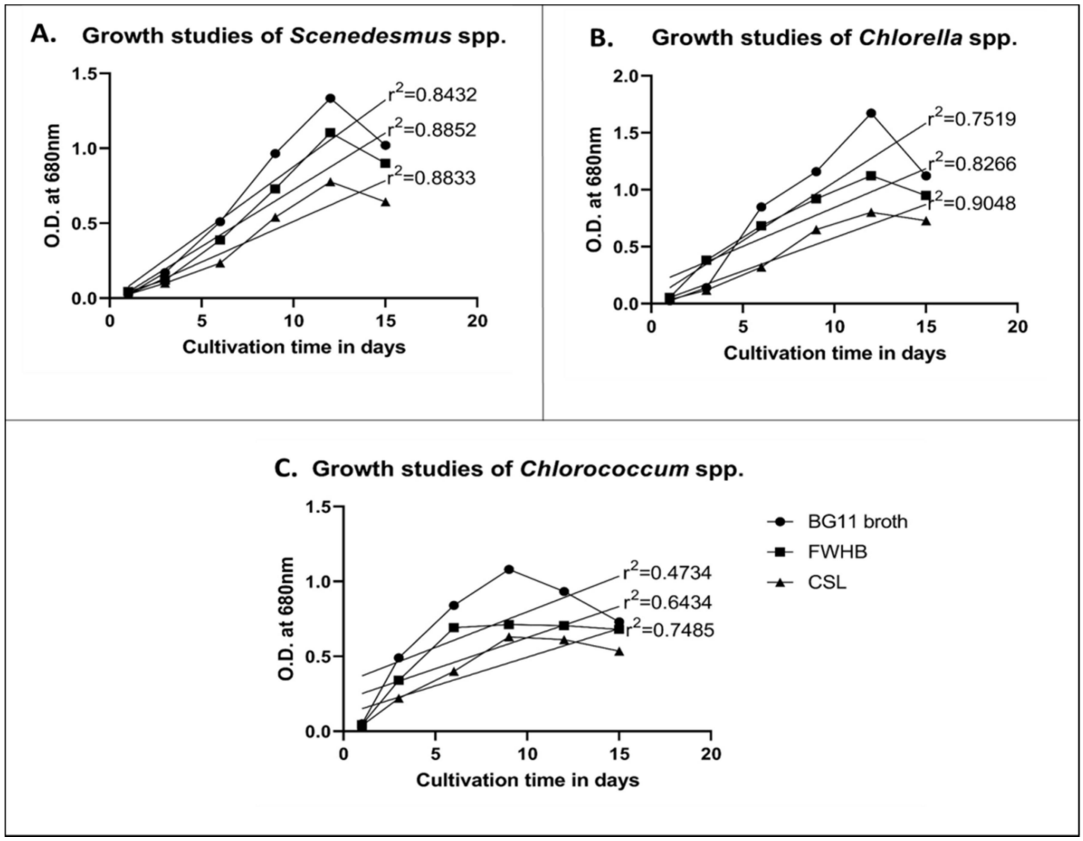

Figure 4: Comparative Growth Study of microalgae in different wastewater.

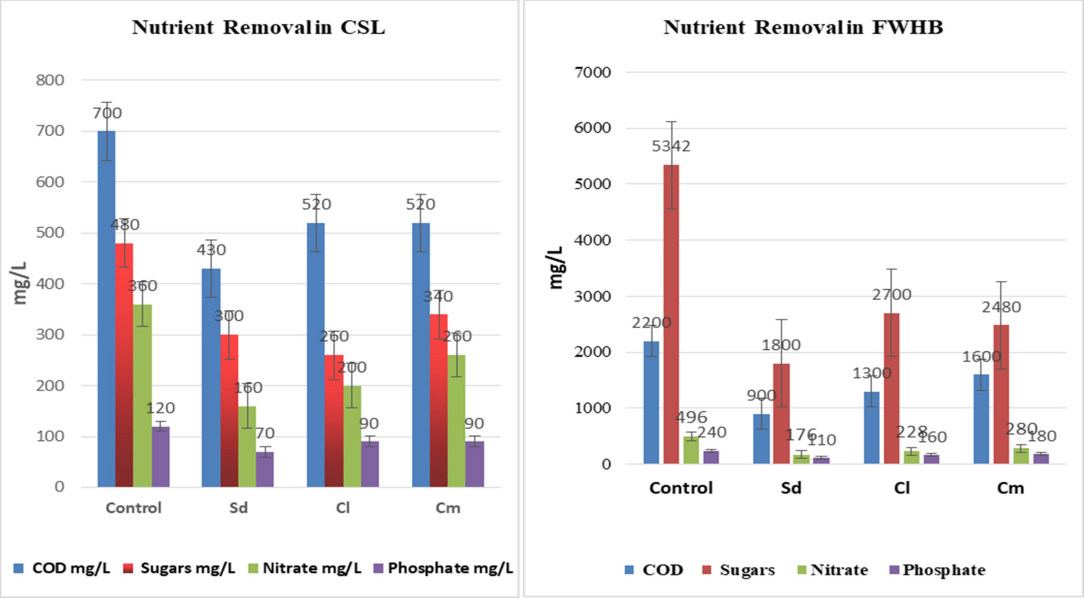

Figure 5: Efficiency of Microalgae in remediation of Pollutants from wastewater A) CSL, B) FWHB

4. CONCLUSION:

This study revealed that the Chlorophyceae strains documented in the lakes of the Davanagere district were due to variation in nutrient inputs caused by environmental stress and human activity. Three isolates *Scenedesmus* sp. (GMDAj0519), *Chlorella* sp. (GMDBt0319) and *Chlorococcum* sp. (GMDKu0119) were tested for the utilization of agro-industrial waste. The growth study and biomass productivity were measured and compared. Among the strains *Scenedesmus* sp. was found to be the best candidate for biomass generation by utilizing wastewater nutrients in a high absorption rate with the maximum specific growth rate was $\mu max=0.135\ d^{-1}$, ($r^2=0.957$), followed by *Chlorella* sp. The highest biomass yield was obtained from *Chlorella* sp. 1.5 g/L dry weight in 21 days of culture. Overall, the results suggest the cultivation of microalgae in wastewater is economical and eco-friendly as it contributes to mitigating the environmental problems, while biomass is generated to serve as feedstock for biofuel and biotechnological applications.

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