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Research Article

Occurrence and diversity of microalgae in phytoplankton collected from freshwater community ponds of Hooghly District, West Bengal, India

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Abstract

A study on diversity, seasonal occurrence, distribution percentage of microalgal taxa and physico-chemical parameters of five community ponds, located in Chinsurah town, Hooghly district of West Bengal, has been carried out. Correlation between occurrence of microalgal genera and some parameters of environment, physico-chemical nature of natural water bodies were explored by Canonical Correspondence Analysis (CCA). Diversity indices have been calculated using PAST software program. A total of 23 microalgal taxa belonging to four algal classes were recorded and the study indicated that the microalgal diversity vary with variation in physico-chemical parameters of water and light intensity. Out of these genera, occurrence of *Chodatella* sp. in late summer and *Tetrallantos* sp. and *Synechocystis* sp. in winter season were specific. In CCA, multiple variables (dissolved oxygen, water temperature, electrical conductivity, pH, light intensity and inorganic phosphorous) played a significant role in occurrence of microalgal taxa. Observation concluded that the Chlorophycean members were dominant throughout the study period and the Shannon–Wiener diversity index was highest for a site with large number of Chlorophycean member. This study will help in future to assess water quality.

Keywords: Microalgae; Community pond; Seasonal occurrence

Abbreviations

L.I, Light intensity; **W.T**, Water temperature; **pH**, Water pH; **E.C**, Electrical conductivity; **D.O**, Dissolved oxygen; **I.P**, Inorganic phosphorous; ***Sce.***, *Scenedesmus* sp.; ***Sel.***, *Selenastrum* sp.; ***Chl.***, *Chlorococcum* sp.; ***Tet.***, *Tetraedron* sp.; ***Tetr.***, *Tetrallantos* sp.; ***Sch.***, *Schroederia* sp.; ***Tetra.***, *Tetrastrum* sp.; ***Act.***, *Actinochloris* sp.; ***Cos.***, *Cosmaium* sp.; ***Clo.***, *Closterium* sp.; ***Cru.***, *Crucigenia* sp.; ***Coe.***, *Coelastrum* sp.; ***Chlo.***, *Chlorella* sp.; ***Ooc.***, *Oocystis* sp.; ***Ped.***, *Pediastrum* sp.; ***Cho.***, *Chodatella* sp.; ***Ank.***, *Ankistrodesmus* sp.; ***Syn.***, *Synechocystis* sp.; ***Mer.***, *Merismopedia* sp.; ***Nav.***, *Navicula* sp.; ***Pin.***, *Pinnularia* sp.; ***Eug.***, *Euglena* sp.; ***Pha.***, *Phacus* sp.

Citation

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Introduction

Unicellular and colonial microalgae form a very important component of freshwater phytoplankton. These organisms have a critical role in primary production, nutrient cycling and food web in the aquatic ecosystem (1). Apart from that, microalgae in the aquatic ecosystems are indicators of changes that take place due to anthropogenic activities and environmental pollution. The diversity of these microalgae occurring as phytoplankton is influenced by various biotic and abiotic factors. Considering these aspects extensive studies have been carried out in India on the microalgae occurring as phytoplankton. These include seasonal distribution (2-4), diversity of fresh water algae (4,5-14) physico-chemical parameters of aquatic system (3,4,15-17) and algal blooms (4,18). These works have been carried out with respect to both lotic and lentic ecosystems.

Studies concerning role of microalgae in water quality monitoring shows that changes in taxon diversity and composition indicate not only changes in physical and chemical variables of water, but also changes in biotic interactions (19). Ecological studies include seasonality of microalgal occurrence, natural variation of physico-chemical factors in water bodies and the relationship between occurrence and diversity of algal taxa and environmental factors (19,20-22). To cope up with the changes, microalgal taxa have developed morphological and physiological adaptive strategies for surviving in different environments (19,23,24). Defined functional groups of microalgae with defined morphological and physiological traits were also studied in a given environment (19). It is also important to mention here that fresh water ecosystems are associated with human activity in the rural India. These are an important source of water for daily use and sustaining the livelihoods. The present study has been undertaken in some fresh water bodies, not studied earlier, in a town Chinsurah of district Hooghly of state of West Bengal. These fresh water bodies are located in the middle of the town and exposed to various pollutants. The prime objective of this study, on the occurrence and diversity of the microalgal component and their correlation with physico-chemical parameters, was to form a data bank of species diversity and growth condition for future prospect of using them in pollution monitoring.

Materials and Methods

(a) ***Study area***: The study area is located in Chinsurah town of Hooghly district, West Bengal, India. Five fresh water ponds were selected in this study area and their latitude and longitude were obtained from GPS data (Table 1). The five sites were marked as Site I, Site II, Site III, Site IV, Site V. The ponds were almost of the same size and located in area inhabited by people. They are used for household purposes and they received effluent through the drainage system (Photo plate 1).

(b) A random sampling method was used for the collection of samples from the selected sites over two different seasons- Late Summer and Winter. The samples were collected using a sampling bottle.

(c) After collection, three aliquots of sample each of 12 ml centrifuged and pellets were collected for species diversity study. Voucher sample was preserved in 4% formaldehyde for qualitative and quantitative analysis in laboratory. Microalgal species were identified on the basis of morphological features following published works (25-28). 10% glycerine was used as mounting material for microscopic study.

(d) The organisms were photographed using Olympus CH20i.

(e) Shannon-Wiener index of diversity [H'(log_e)], Simpson's dominance index (D_{SIMP}), Pielou's evenness index (J/), and Margalef 's richness index (D_{MARG}) were calculated using PAST statistical software version 2.07 to show microalgal community structure.

(f) Temperature was noted by Eurolab ST9869B multi-thermometer, light intensity by LT Lutron LX-101 lux-meter, pH by Eutech instruments pH 700, for Electrical conductivity, Systronics conductivity meter 304 was used.

(g) For Inorganic phosphorous estimation, 50ml of water sample were taken and added 2 ml of ammonium molybdate followed by 5 drops of stannous chloride solution. After 5 min but before 12 min of the addition of the last reagent the absorbance was measured at 690 nm by UV-Visible spectrophotometer (UV-1800 Shimadzu).

(h) Dissolved oxygen was calculated by Winkler's Iodometric method.

(i) ***Canonical Correspondence Analysis***: Canonical Correspondence Analysis (CANOCO version 4.5; terBraak, 1986) was used to understand the correlation between physico-chemical parameters of fresh water and fresh water microalgal component in late summer and winter seasons. Biplot scores of the environmental parameters were noted. CCA ordination was tested

Table 1. List of sampling sites in Chinsurah, Hooghly the state of West Bengal India

Name of sampling stations	Name of the location	Latitude and longitude
Site I	Bonokalitala in Chinsurah,Hooghly	N-22°53.141´ E-88°23.231´
Site II	Bonokalitala in Chinsurah,Hooghly	N-22°53.171´ E-88°23.142´
Site III	Dharampur in Chinsurah,Hooghly	N-22°53.434´ E-88°23.238´
Site IV	Bonokalitala in Chinsurah,Hooghly	N-22°53.178´ E-88°23.142´
Site V	Khadinamore in Chinsurah,Hooghly	N-22°53.228´ E-88°22.968´

Table 2. Diversity of microalgal component in sampling sites in Chinsurah in Hooghly district of West Bengal. (+: Present; -: absent) (L.S: Late summer, W: Winter)

Sl. No.	Phytoplankton	Sampling Stations Pond									
		Site I		Site II		Site III		Site IV		Site V	
		L.S	W	L.S	W	L.S	W	L.S	W	L.S	W
1.	*Scenedesmus* sp.	-	+	-	-	+	-	+	+	+	+
2.	*Selenastrum* sp.	-	-	-	-	+	-	-	+	-	+
3.	*Chlorococcum* sp.	-	+	-	+	-	-	+	-	-	+
4.	*Tetraedron* sp.	-	-	-	-	-	-	+	+	-	+
5.	*Tetrallantos* sp.	-	-	-	+	-	-	-	-	-	-
6.	*Schroederia* sp.	-	+	-	+	+	-	+	+	+	+
7.	*Tetrastrum* sp.	-	-	-	-	+	-	+	-	-	-
8.	*Actinochloris* sp.	-	+	+	+	-	-	-	+	-	+
9.	*Closterium* sp.	-	-	-	+	+	-	+	+	-	+
10.	*Cosmarium* sp.	-	+	+	+	-	+	+	+	+	+
11.	*Crucigenia* sp.	-	+	-	-	-	+	-	-	+	+
12.	*Coelastrum* sp.	-	-	-	-	+	-	-	-	-	-
13.	*Chlorella* sp.	-	-	+	+	-	+	+	+	-	-
14.	*Oocystis* sp.	-	-	-	+	-	-	+	-	-	-
15.	*Pediastrum* sp.	+	+	-	-	-	+	+	+	+	+
16.	*Chodatella* sp.	-	-	-	-	-	-	-	-	+	-
17.	*Ankistrodesmus* sp.	-	-	-	-	-	+	-	-	+	-
18.	*Synechocystis* sp.	-	+	-	-	-	-	-	-	-	-
19.	*Merismopedia* sp.	-	+	-	-	+	+	+	+	+	+
20.	*Navicula* sp.	+	+	-	-	-	+	+	+	-	-
21.	*Pinnularia* sp.	+	+	-	-	-	+	+	-	-	-
22.	*Euglena* sp.	-	-	-	-	+	+	+	-	-	+
23.	*Phacus* sp.	-	-	-	-	-	+	-	+	+	-
Total Microalgal component =23		3	11	3	8	8	10	14	12	9	12

Table 3. Physico-chemical parameters in late summer (L.S) and winter (W) of sampling sites

Physico-chemical factor	Site I		Site II		Site III		Site IV		Site V	
	L.S	W	L.S	W	L.S	W	L.S	W	L.S	W
Light intensity (×100 Lux)	678±3.5	65±1.5	665±5.5	8±0.58	217±2	45±2	285±1	18± 1.4	415± 0.58	26±1
Water temp. (°C)	30.3±0.2	20.6±0.6	29.8±0.2	18.5±0.3	29.4±0.15	19.9±0.1	29.8±0.1	20.1±0.06	29.8±0.1	21.6±0.7
pH	7.6± 0.02	8±0.12	7.2±0.23	7.1±0.19	7.5±0.06	7.7±0.03	7.6± 0.05	7.8± 0.06	7.5± 0.05	7.7± 0.02
Electrical conductivity (ms/cm)	0.7± 0.03	0.6± 0.03	0.6±0.02	0.8±0.006	0.5±0.03	0.5±0.03	0.6± 0.05	0.5± 0.03	0.5± 0.03	0.6± 0.04
Dissolved oxygen (mg/l)	4.87±0.01	6.49±0.1	4.87±0.006	1.62±0.1	3.24±0.006	1.22±0.07	2.43±0.02	3.24±0.4	4.05±0.01	2.84±0.04
Inorganic Phosphorous (mg/l)	0.16±0.006	0.08±0.003	0.63±0.01	0.40±0.02	0.35±0.007	1.15±0.02	0.1± 0.01	0.07±0.006	0.15±0.006	0.85±0.02

for significance with Monte Carlo test (500 runs) using CANOCO software.

(j) Venn diagrams were generated by using the tool http://www.interactivenn.net to represent the unique and common microalgal taxa in different sites in late summer and winter seasons.

Results and Discussion

The microalgal components of phytoplankton community, collected from five ponds in the study area, were diverse. A total number of 23 taxa, identified up to genus, were recorded. These microalgae belong to 4 different classes, namely Cyanophyceae, Chlorophyceae, Bacillariophyceae

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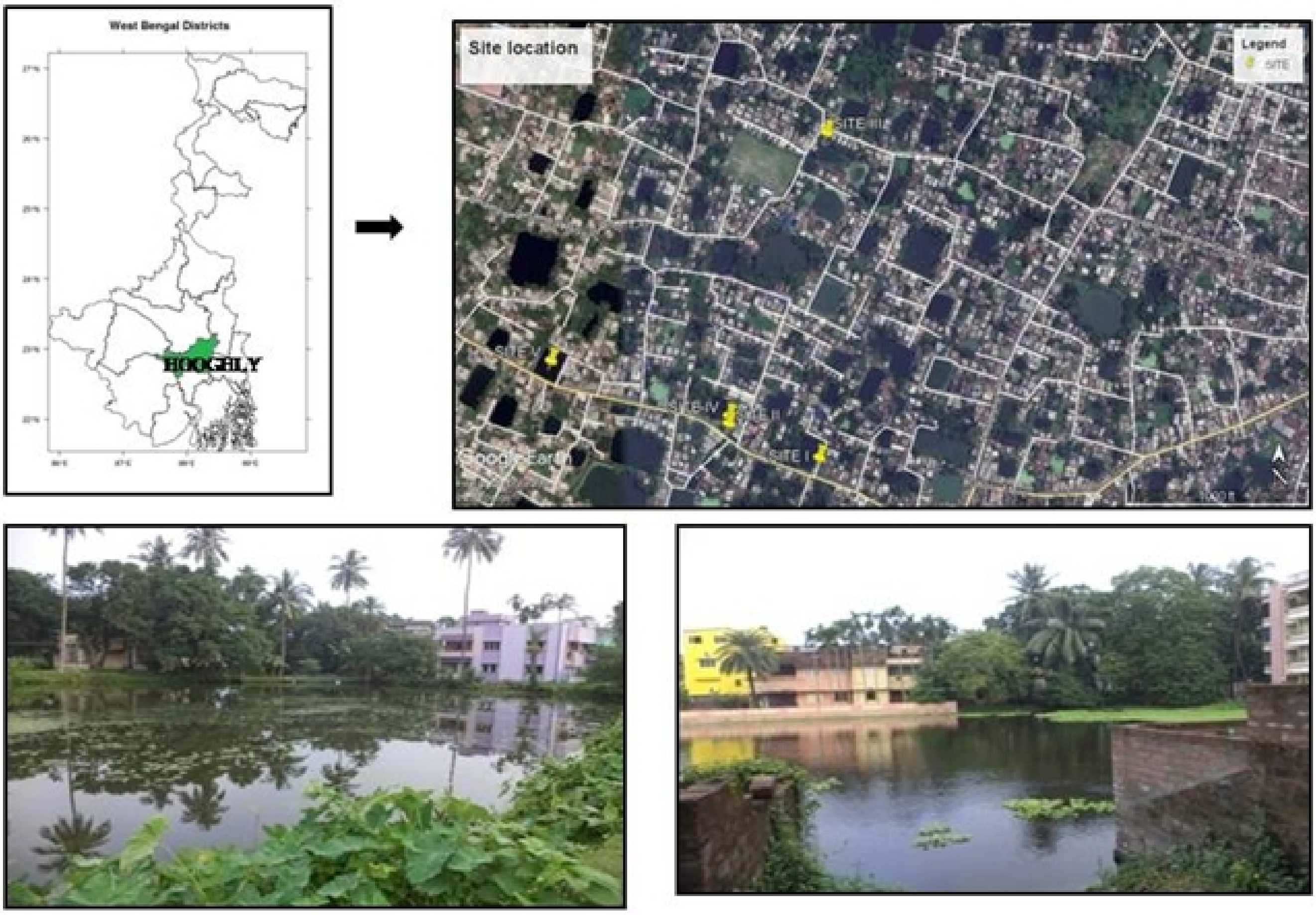

Photo plate 1. Location of study area and sampling sites

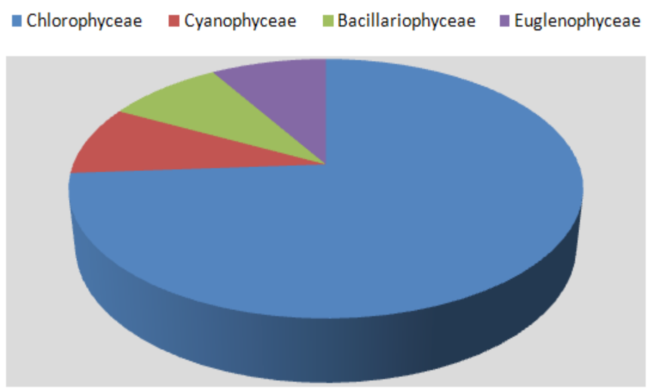

Fig. 1. Distribution percentage of microalgae group within study locations.

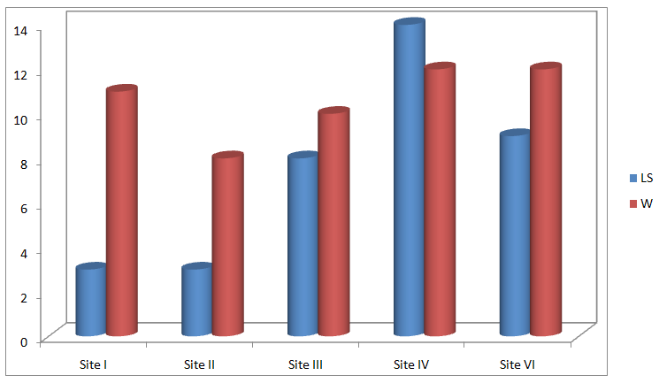

Fig. 2. Occurrence of microalgal genera in late summer (LS) and winter (W) in five sites.

and Euglenophyceae (Table 3). Out of these 23 genera, 17 belong to the class Chlorophyceae followed by Cyanophyceae (2), Bacillariophyceae (2) and Euglenophyceae (2) (Table 2). Microphotographs of some of the organisms were given in Photo plate 2. Out of these genera occurrence of two genera belonging to Chlorophyceae is season specific - while *Chodatella* sp. was recorded in late summer, *Tetrallantos* sp. was recorded in winter season. Two Cyanophycean genera have been recorded namely *Merismopedia* sp. and *Synechocystis* sp. While *Merismopedia* sp. was recorded in both the seasons, *Synechocystis* sp. was recorded only in winter season. Two genera under Bacillariophyceae recorded were *Navicula* sp. and *Pinnularia* sp. Two genera of Euglenophyceae recorded were *Euglena* sp. and *Phacus* sp. According to distribution percentage of microalgal community component, occurrences of Chlorophyceae genera were maximum (74%) followed by members of Cyanophyceae, Bacillariophyceae and Euglenophyceae (8.69% in each) (Fig. 1). A similar result was obtained earlier in another study (29). Relative abundance of microalgal taxa was high in winter season in four sites except site IV. Diversity of taxa has been found to be much more in winter as compared to late summer except Site IV (Fig. 2).

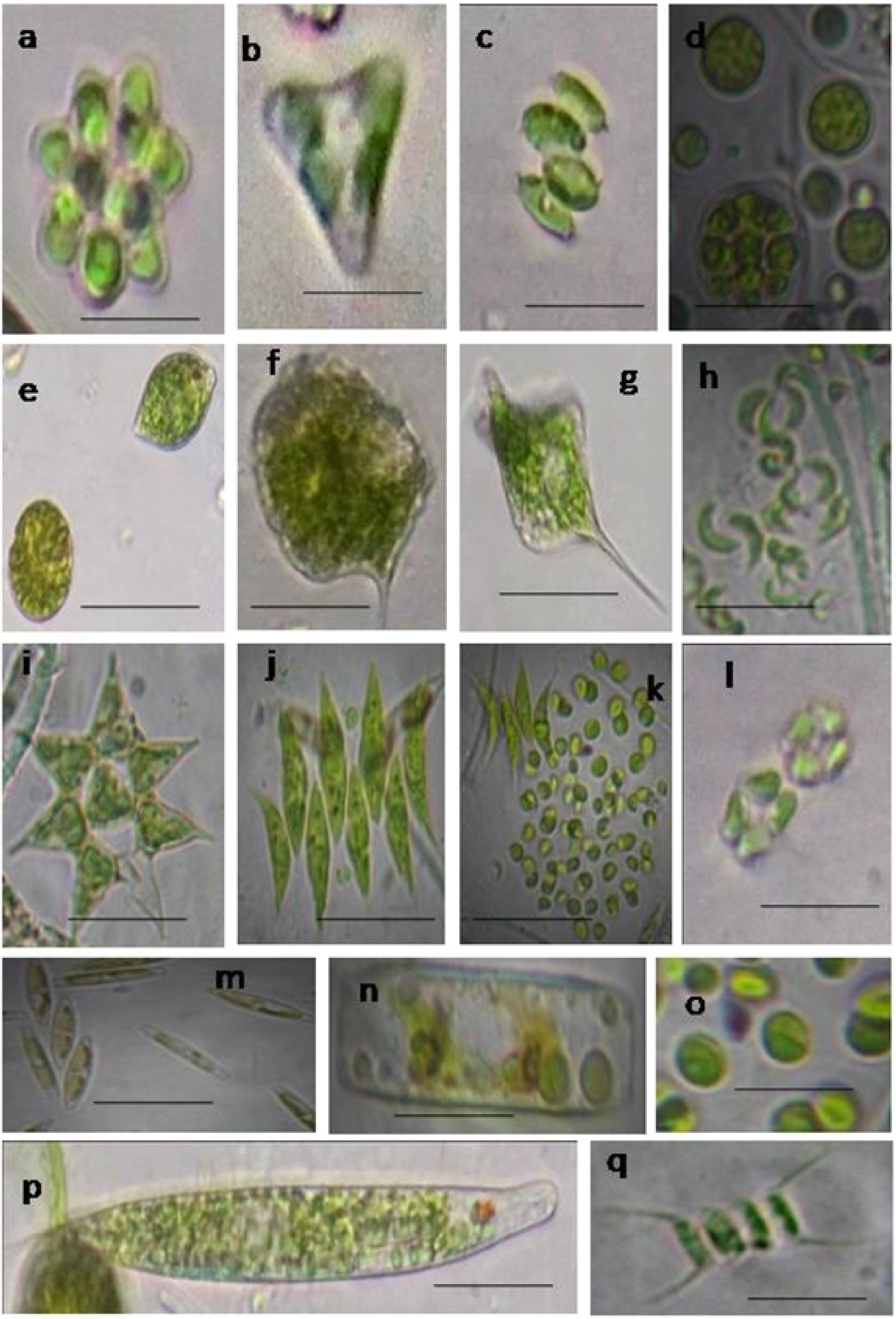

Photo plate 2. (20 µm scale) (a) *Coelastrum* sp., (b) *Tetraedron* sp., (c) *Scenedesmus* sp., (d) *Chlorococcum* sp., (e) *Euglena* sp., (f) *Phacus* sp., (g) *Phacus* sp., (h) *Selenastrum* sp., (i) *Pediastrum* sp., (j) *Scenedesmus* sp., (k) *Scenedesmus* sp., (l) *Crucigenia* sp., (m) *Navicula* sp., (n) *Pinnularia* sp., (o) *Chlorella* sp., (p) *Euglena* sp., (q) *Scenedesmus* sp.

Physico-chemical parameters were recorded both in winter and late summer. Table 3 shows value of physico-chemical parameters in all the sites and in both the seasons. There is significant variation with respect to light intensity and water temperature in these two seasons at all the sites. Though other parameters show very little variations, inorganic phosphorous has higher concentration (1.15mg/l and 0.85mg/l) in site III and site V, electrical conductivity shows high value (0.8ms/cm) in site II except in few cases. In late summer, value of light intensity and water

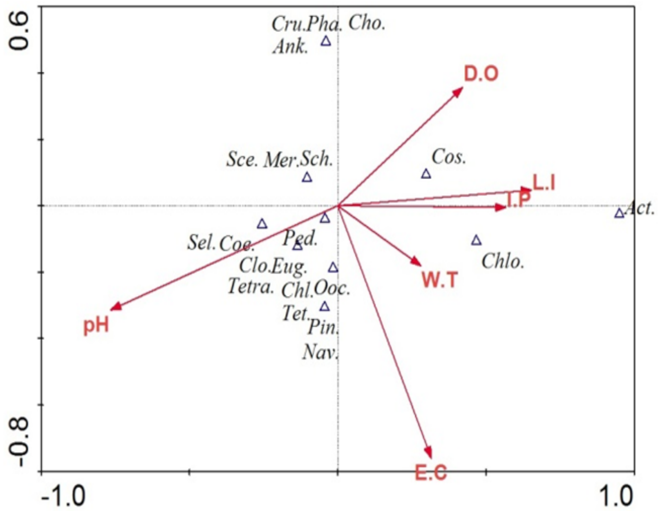

Fig. 3. CCA ordination biplot based on PCA (vide table 4) showing the relative position of species-environment correlation between species occurrence and physico-chemical parameters in late summer season.

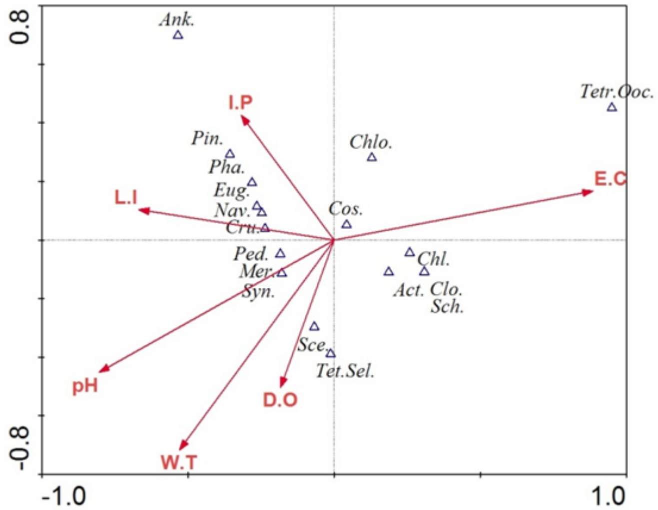

Fig. 4. CCA ordination biplot based on PCA (vide table 5) showing the relative position of species-environment correlation between species occurrence and physico-chemical parameters in winter season.

Table 4. CCA ordination score plot for late summer season based on first two principal components

Axes	1	2	3	4
Eigen values	0.602	0.512	0.490	0.191
Cumulative percentage of variance explained by species	33.5 by PC I	62.1 by PC I and PCII jointly	89.4 by PCI, PCII and PCIII jointly	100.0

Table 5. CCA ordination score plot for winter season based on first two principal components

Axes	1	2	3	4
Eigen values	0.396	0.238	0.197	0.090
Cumulative percentage of variance explained by species	43.0 by PC I	68.9 by PC I and PCII jointly	90.3 by PCI, PCII and PCIII jointly	100.0

temperature are higher than winter season. pH was more alkaline in winter season than late summer and winter except Site II.

Euglenophycean genera were present in Site III, Site IV and Site V in late summer and winter. In Site III both Euglenophycean genera, *Euglena* sp., *Phacus* sp., were present in winter season and also noticed that inorganic phosphorous concentration was high. Same result found in CCA analysis also. Euglenophycean genera plays a role as biological indicator of organic pollution (31). In Site I *Synechocystis* sp. was present in winter season and pH (8) was found to be high when compared to other sites. In Site II *Tetrallantos* sp. was present in winter season and the physico-chemical parameter, electrical conductivity value (0.8) was found to be high than other sites. *Pediastrum* sp. was found in high pH (7.5-8) sites I, sites III, site IV sites V and the same result was observed in CCA analysis. *Chlorococcum* sp., was present in winter season except in Site IV. *Tetraedron* sp., was present in both the seasons in Site IV.

Canonical correspondence analysis showed correlation between species (here species represents taxa) occurrence and environmental parameters both at late summer and winter. In the graph (Fig. 3 and 4) 'Δ' represents occurrence of species and '→' represents environmental variables. The result from the ordination diagram indicates that the relative weight, as denoted by extra fit values, for the environmental variables indicates a narrow range of variation (0.3359 - 0.5126) with a p- value of 0.1160 in late summer sampling (Fig. 3) and (0.1612-0.3467) with a p-value of 0.0580 in winter season, (Fig. 4) respectively. Therefore, result for winter season is statistically more reliable. The eigenvalues for corresponding axes indicate that the first axis is contributing the most in explaining the species environment relationship (0.602) in case of late summer and (0.396) in winter. The second axis is contributing more than remaining two axes in both seasons. In late summer, Axis 1 has the strongest positive correlation with light intensity and weakest positive correlation with water temperature. Axis 2 has the strongest positive correlation with dissolved oxygen and weakest positive correlation with light intensity. Axis 1 shows negative correlation with water pH. Axis 2 shows negative correlation with water

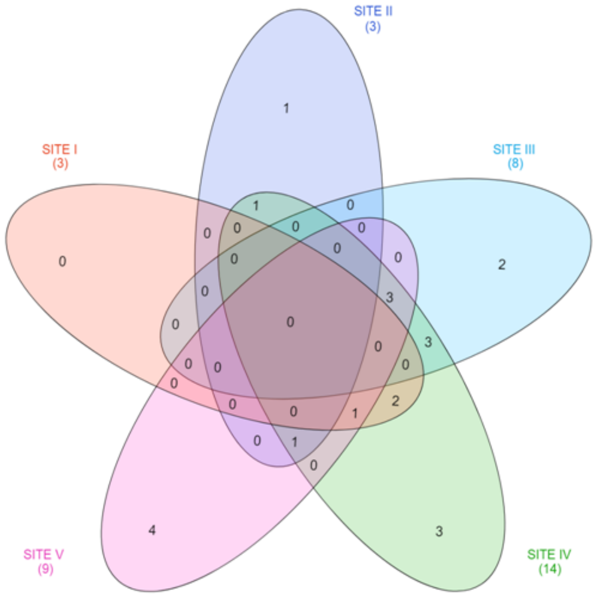

Fig. 5. Graphical presentation of all the microalgal taxa from the five study sites (Site I, II, III, IV & V) in late summer based on commonality of occurrence.

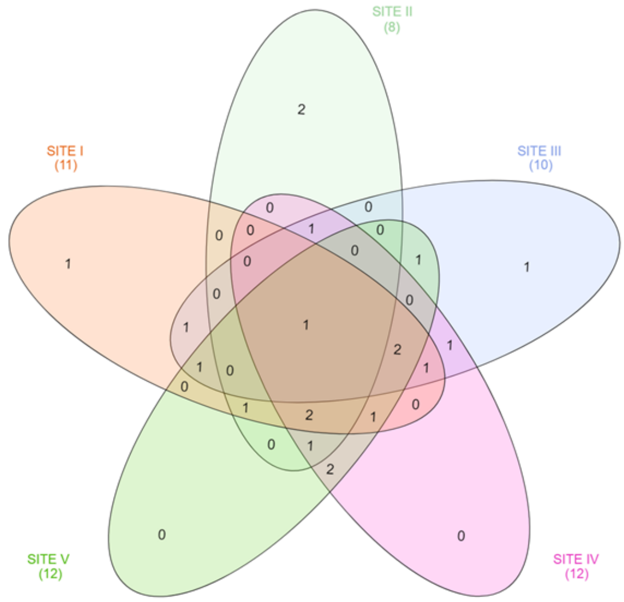

Fig. 6. Graphical presentation of all the microalgal taxa from the five study sites (Site I, II, III, IV & V) in winter based on commonality of occurrence.

Table 6. Diversity indices of microalgal class component in five sites under that investigation (L.S: Late summer, W: Winter)

Diversity indices	Site I		Site II		Site III		Site IV		Site V	
	L.S	W	L.S	W	L.S	W	L.S	W	L.S	W
Taxa (class level)	2	3	1	1	3	4	4	4	3	3
Shannon–Wiener diversity index [$H'(\log_e)$]	0.6365	0.9075	0	0	0.7356	1.221	0.8953	0.837	0.6837	0.5661
Simpson's dominance index (D_{SIPM})	0.4444	0.5289	0	0	0.4063	0.66	0.4592	0.4167	0.3704	0.2917
Pielou's evenness index ($J/$)	0.9449	0.8261	1	1	0.6956	0.8473	0.612	0.5774	0.6604	0.5871
Margalef 's richness index (D_{MARG})	0.9102	0.8341	0	0	0.9618	1.303	1.137	1.207	0.9102	0.8049

temperature, water pH and electrical conductivity, whereas, in winter Axis 1 has the strongest positive correlation with electrical conductivity. Axis 2 has the strongest positive correlation with inorganic phosphorous and weakest positive correlation with light intensity. Axis 1 shows negative correlation with light intensity, water temperature, water pH, dissolved oxygen, inorganic phosphorous. Axis 2 shows negative correlation with water temperature, water pH, dissolved oxygen. All the four eigenvalues reported above are canonical and correspond to axes that are constrained by the environmental variables in both seasons. In late summer (Fig. 3), *Cosmarium* sp. and *Actinochloris* sp. showed affinity with dissolved oxygen, inorganic phosphate and light intensity in axis 1. *Schroederia* sp., *Scenedesmus* sp., *Merismopedia* sp., *Crucigenia* sp., *Phacus* sp., *Chodatella* sp., *Ankistrodesmus* sp. are positive correlated with dissolved oxygen. In axis 2 the species have strong negative correlation with electrical conductivity. *Chlorococcum* sp., *Tetraedron* sp., *Oocystis* sp., *Pinnularia* sp., *Navicula* sp. are aline with electrical conductivity. *Pediastrum* sp., *Euglena* sp., *Closterium* sp., *Tetrastrum* sp., *Selenastrum* sp., *Coelastrum* sp. are aligned with water pH. Water temperature has no significant role in late summer. In winter season (Fig. 4), *Chlorococcum* sp., *Closterium* sp., *Schroederia* sp., are aligned with electrical conductivity in strong relationship. *Phacus* sp., *Euglena* sp., *Pinnularia* sp., *Navicula* sp. are aligned with inorganic phosphorous. *Crucigenia* sp., *Merismopedia* sp., *Pediastrum* sp. are aligned with water pH and light intensity. *Synechocystis* sp. occurs with increasing water temperature. A similar result was found in another study (19). Table 4 and 5 represent the CCA ordination score plot for late summer and winter seasons based on first two principal components.

It is observed from Table 6 that representatives of all the four classes (Chlorophyceae, Cyanophyceae, Bacillariophyceae, Euglenophyceae) were found in both the season in Site IV and in winter season in Site III. Representative of only one class (Chlorophyceae) was found in Site II in both the seasons. Shannon-

Wiener diversity index was highest at winter season of Site III (1.221). It is interesting to note here that Margalef's richness index is also highest here (1.303) and Pielou's evenness index is low here. In Site II, Shannon- Wiener diversity index is 0. Simpson's dominance index value is also 0 as there is representation of only one class was present there. Naturally Pielous's evenness index value is 1 and Margalef's richness index is 0 (29).

In late summer season, unique microalgal taxa observed are as follows (Fig. 5): maximum number was observed in Site V (*Crucigenia* sp., *Chodatella* sp., *Ankistrodesmus* sp., *Phacus* sp.), one in Site II (*Actinochloris* sp.), two in Site III (*Selenastrum* sp. and *Coelastrum* sp.), three in Site IV (*Chlorococcum* sp., *Tetraedron* sp., *Oocystis* sp.).

Following common microalgal taxa were observed in different sites (Fig. 5): one in Site II and Site IV (*Chlorella* sp.), three in Site III, IV and V (*Scenedesmus* sp., *Schroederia* sp., *Merismopedia* sp.). Another three found in Site III and IV (*Tetrastrum* sp., *Closterium* sp., *Euglena* sp.), two in Site I and IV (*Navicula* sp., *Pinnularia* sp.), one in Site I, IV and V (*Pediastrum* sp.) and another one in Site II, IV and V (*Cosmarium* sp.).

In winter season, unique microalgal taxa observed are as follows (Fig. 6): one in Site I (*Synechocystis* sp.) and Site III (*Ankistrodesmus* sp.), two in Site II (*Tetrallantos* sp., *Oocystis* sp.).

Following common microalgal taxa were recorded in different sites (Fig. 6): one in the following sites representing with different taxa: Site II, III and IV (*Chlorella* sp.), Site III and V (*Euglena* sp.), Site III and IV (*Phacus* sp.), Site I, III and IV (*Navicula* sp.), Site I, IV and V *(Scenedesmus* sp.), Site II, IV and V (*Closterium* sp.), Site I, II and V (*Chlorococcum* sp.), Site I, III and V (*Crucigenia* sp.), Site I and III (*Pinnularia* sp.), SITE I -V (*Cosmarium* sp.). Two common taxa were noted in Site I, III, IV and V (*Pediastrum* sp. *Merismopedia* sp.), Site IV and V (*Selenastrum* sp.,*Tetraedron* sp.), Site I, II, IV and V (*Schroederia* sp., *Actinochloris* sp.).

Moreover, these ponds are the sink of all domestic pollution because of through open drainage system from which brings the domestic waste of nearby settlements enters to the specific site. Shannon's diversity values depicted minor fluctuations with respective sites because there was no remarkable difference in the number of taxa composing dominant species at different sites. It has been observed (30,31) that incase of polluted areas, there was a decrease in biomass of the microalgae in phytoplankton associated with increase in species diversity. Diversity index is a good index for assessing and ranking the water quality. Analysis of diversity of microalgal flora of the lake leads to conclusion that the mean values did not differ from each other remarkably at the stations in different seasons studied, which can be directly related to the eutrophication process of aquatic system (32). Wetland ecosystems are the primary shelter for aquatic biodiversity including aquatic flora, fauna and other microorganisms. Phytoplankton is one of the important components of aquatic ecosystems (4). Fresh water ponds play an important role in the social ecology of the region in which they are located. Phytoplankton ecology plays an important role for indicating the eutrophication (33) as well as water quality and further way forward towards ecosystem management.

Major outcome: (i) The ponds located very near to the human residential complexes show a rich microalgal flora dominated by Chlorophycean members belonging to the order Chlorococcales and desmids (Desmidales). (ii) The microalgal genera are more abundant in winter season than in late summer. (iii) Two genera have been found to be season specific *Synechocystis* sp., *Tetrallantos* sp.

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Competing Interests

The authors have no conflict of interests.

Authors' contribution

All authors contributed equally to carry out the research and prepare article.

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