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Composition and Diversity of Phytoplankton Community of a Fish Pond in the Central Himalayan Region of Uttarakhand, India

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Abstract Phytoplankton community of a fish pond in Central Himalayan region of Uttarakhand was studied January to April 2016. Samples were collected in 15 days interval. The quantiative and qualitative analysis of the phytoplankton was done along with measurement of selected physico-chemical parameters. A total of 38 genera of belonging to 22 families of 11 orders in 07 classes of algae were recorded. Chlorophyceae was the most dominant group followed by Bacillariophyceae, Cyanophyceae, Euglenophyceae, Xanthophyceae, Chrysophyceae, and Rodophyceae. Total average density was observed 1164.92 ±105.56 unit/ml during the present study. The mean percentage composition of phytoplankton constituted of 52.8% of Chlorophyceae, 32.23% of Bacillariophyceae, 6.68% of Myxophyceae and 1.5% of Euglenophyceae, 6.26% of Xanthophyceae, 0.29% of Rhodophyceae and 0.24% of Chrysophyceae. In canonical corresponding analysis, among the 10 determinants, pH, conductivity, water temperature, depth dissolved oxygen phosphate and nitrate were the most significant. The present study revealed that the phytoplankton density greatly varied in the studied fish pond and it showed tendency towards eutrophic level due to the high proportion of green algae.

Keywords Phytoplankton, Fish pond, Community structure, Canonical corresponding analysis.

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Introduction

Among the various biotic elements occurring in aquatic habitats the phytoplankter are integral components. They are microscopic, free floating unicellular and colonial autotrophic organisms which grow in aquatic ecosystem and their movement depends upon water currents (Millman et al. 2005). For maintenance of equilibrium between the biotic and abiotic components of the aqatic ecosystem they play a key role by synthesizing organic matter in aquatic (Pandey et al. 2004). Phytoplankton is a major source of dissolved oxygen in fish ponds.

Fish ponds are among the most common type of standing water habitat. The productive status of a water body is well indicated by quantitative and qualitative abundance of phytoplankton. Therefore, detailed information about abundance of phytoplankton and its spatial and temporal quality in relation to environmental conditions is quite important for fish production (Siddika et al. 2012). The study of phytoplankton provides an appropriate and handy point of focus for understanding the nutrient status and its effects on the aquatic ecosystem like fish pond. Maintenance of healthy aquatic ecosystem is dependent on the abiotic properties of water and the biological diversity of the ecosystem. The aim of the present study was to investigate major group of the phytoplankton and the water quality in a small fish pond built for fish rearing at Garhwal Himalayan region of Uttarakhand.

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Materials and Methods

Study area

The present study was conducted in a single pond of Fish Hatchery consisting of 5 rearing ponds built in the Department of Zoology and Biotechnology, Chauras, HNB Garhwal University. It is located at 30°13´36.43´´ N latitude and 78°48´08.79´´ E longitude at an altitude of 535 m asl in central Himalayan region of the state of Uttarakhand. The average length and width of the pond is about 15 m and 10 m while depth is about 1.25 m and has been stocked with *Barlius bendelisis.*

Sample collection

Selected physico-chemical parameters namely temperature, mean depth, conductivity, total dissolved solids (TDS), pH, dissolved oxygen (DO), free CO_2, total alkalinity (TA), phenolphthalein alkalinity (PA), nitrate and phosphate content of water were measured and analyzed at all the sampling spots following standard methods outlined in Trivedy and Goel (1984), Welch (2003), and APHA (2005) during January 2016 to April 2016. For different physico-chemical analysis the water samples were taken from the surface of the pond, which was well mixed due to low depth.

Sampling for the phytoplankton was done at selected five different spots in the fish pond at every 15 days interval by using plankton net of mesh size 200 µm (APHA 2005). Hundred liters of water from different spots was filtered through plankton net and the samples were collected in a glass bottle, and preserved in 4% formalin. Identification of different taxa of phytoplankton was carried out to the lowest recognizable level usually genera with the help of different keys by Ward and Whipple (1959), Sarode and Kamat (1984) and APHA (2005). Identification was done with the help of Olympus Stereo zoom binocular microscope (SZ-series). The details of each specimen for identifying characters were noted and subsequently captured as picture using Magnus MIPS USB 2.0, Capture and Display software. The quantitative analyses of plankters were observed and calculated with the help of Sedwick-Rafter counting cell and calculated according to Welch (2003). Density counts were performed at the desired taxonomic resolution on the samples obtained from each quadrate. The density was then computed as :

$$n = \frac{(a \times 1000)\, c}{1}$$

Where, n = number of plankters per liter of original water (unit liter^{-1}) , a = average number of plankters in all counts in counting unit of 1 cu.mm.capacity , c = volume of original concentrate in counting cell, 1 = volume of original water expressed n liters.

Data analysis

The data collected on phytoplankton counts were further analyzed by calculating different ecological indices viz., Shannon-Wiener Species Diversity Index (Shannon and Wiener 1963), Species Richness Index (Margalef 1957, Odum 1971) and Evenness. In all the indices natural log ($\log_e$) was used. Correlation among the different physico-chemical parameters were analyzed by using SPSS 20.0. Relationship between physico-chemical and phytoplankton density was calculated by canonical correspondence analysis (CCA) by using PAST. Cluster analysis of phytoplankton density was calculated by using PAST with Jaccard mode.

Table 1. Physico-chemical parameters recorded in fish pond (January—April 2016).

Physico-chemical parameters	Samples 20 Jan	04 Feb	19 Feb	08 Mar	23 Mar	07 Apr	Range Min	Max
pH	8.6	8.9	9.36	9.46	8.73	9.16	8.6	9.46
Water temperature (°C)	19.6	16.9	23.7	29.7	25.3	24.6	16.9	29.7
Depth (cm)	28.5	30.0	30.8	34.4	34.2	34.2	28.5	34.4
Conductivity (µs/cm)	183.0	219.0	243.0	239.0	284.0	226.0	183.0	284.0
Total dissolved solids (mg/l)	158.81	108.87	108.87	118.86	140.83	94.89	94.89	158.81
Dissolved oxygen (mg/l)	9.9	9.85	9.04	7.26	7.62	8.58	7.26	9.9
Free CO_2 (mg/l)	-	-	-	-	-	-	-	-
Phenolphthalein alkalinity (mg/l)	15.0	13.5	9.5	9.3	14.0	15.0	9.3	15.0
Total alkalinity (mg/l)	150.0	145.0	119.0	112.0	135.0	150.0	112.0	150.0
Phosphate (mg/l)	0.012	0.016	0.013	0.023	0.021	0.028	0.012	0.028
Nitrate (mg/l)	0.06	0.08	0.07	0.1	0.12	0.14	0.06	0.14

Results and Discussion

Regular sampling of phytoplankton per 15 days during January 2016 to April 2016 was done along with the physico-chemical parameters at the five sampling sites (S_1-S_5). The samples were carried to the laboratory and analyzed.

Physico-chemical characteristics of fish pond

During the present study period the water temperature varied up to 13°C (16.9-29.7°C), while pH, conductivity, TDS, TA and PA, also shows high variation in fish pond. Nitrate and phosphate concentration increases during March-April. Dissolved oxygen remains high (7.26 to 9.9 mg/L), however the free CO_2 was absent during the entire study period (Table 1). Among the many factors that control the presence of phytoplankton in the aquatic system temperature is an important one. The optimal temperature for fish culture is 26.06-31.97°C (Boyd 1982). However, along with temperature other factors such as conductivity, nutrients and high pH are also accountable for phytoplankton variation and organic production (Pulle and Khan 2003). Generally, the high DO values reflect the oxygen saturation at low temperatures through upwelling, tidal currents and wind forces and consequently the waters are well-oxygenated in winter (El-Sherif et al. 2010). The alkalinity was highest in April and minimum in February. Earlier, similar trend was also reported by Kadam et al. (2007), Narayana

Table 2. Correlation between various physico-chemical parameters in fish pond. *Correlation is significant at the 0.05 level (2-tailed), ** Correlation is significant at the 0.01 level (2-tailed).

Physico-chemical parameters	pH	Water temperature	Conductivity	Total dissolved solids	Dissolved oxygen	Phenolphthalein alkalinity	Total alkalinity	Depth	Phosphate	Nitrate
	Pearson correlation coefficient r									
pH	1	0.624	0.226	-0.706	-0.450	-0.810	-0.740	0.454	0.343	0.173
Water temperature	0.624	1	0.550	-0.141	-0.930**	-0.524	-0.689	0.826*	0.597	0.513
Conductivity	0226	0.550	1	-0.168	-0.739	-0.294	-0.478	0.709	0.382	0.529
Total dissolved solids	-0.706	-0.141	-0.168	1	0.092	0.286	0.153	-0.370	-.0.486	-0.419
Dissolved oxygen	-0.450	-0.930**	-0.739	0.092	1	0.398	0.612	-0.912*	-0.662	-0.629
Phenolphthalein alkalinity	-0.810	-0.524	-0.294	0.286	0.398	1	0.959**	-0.178	0.127	0.262
Total alkalinity	-0.740	-0.689	-0.478	0.153	0.612	0.959**	1	-0.359	0.008	0.113
Depth	0.454	0.826*	0.709	-0.370	-0.912*	-0.178	-0.359	1	0.890*	0.879*
Phosphate	0.343	0.597	0.382	-0.486	-0.662	0.127	0.008	0.890*	1	0.951**
Nitrate	0.173	0.513	0.529	-0.419	-0.629	0.262	0.113	0.879*	0.951**	1

et al. (2008) and Reddy et al. (2009). The pH of fish pond remained alkaline throughout the study period as it varied between 8.6 and 9.46. It seems to favor the growth of blue-green algae. Similar, observations were also reported by King (1970), Parikh and Mankodi (2012) and Jyotsna et al. (2015).

Relationships among the various physico-chemical parameters

Various physico-chemical parameters recorded during study period correlate with each other. The dissolved oxygen showed highly significant negative correlation with water temperature (r = -0.930, p>0.01) and depth (r= -0.912, p>0.05), while phosphate (r=0.890, p>0.05) and nitrate (r= 0.879, p>0.05) showed significant positive correlation with depth. Nitrate showed highly significant positive correlation (r=0.951, p>0.01) with phosphate (Table 2). Lower and higher concentration of dissolved oxygen during dry and wet season respectively could be attributed to changes in water temperatures and increased aeration due to agitation by wind in the wet season (Akpan and Offem 1993, Ochang et al. 2005).

Phytoplankton communities

A total of 38 genera belonging to 22 families of 11 orders in 07 classes of algae from the five sampling spots of fish pond during the present study were observed (Table 3). Six classes of phytoplankton were recorded during study period Myxophyceae or Cyanophyceae (blue green algae), Euglenophyceae, Chlorophyceae (green algae), Xanthophyceae, Chrysophyceae, Rhodophyceae and Bacillariophyceae (diatoms). In which, 12 genera of Bacillariophyceae, 11 genera of Chlorophyceae, genera, 4 genera each of Xanthophyceae and Myxophyceae, 3 genera of Euglenophyceae and 2 genera each of Chrysophyceae and Rodophyceae were recorded . Among the

Table 3. Check list of phytoplankton occurring in fish pond.

Phylum	Class	Order	Family	Genus
Algae	Myxophyceae	Nostocales	Rivulariaceae	*Calothrix*
				Gloeotrichia
		Oscillatoriales	Oscillatoriceae	*Phormidum*
				Oscillatoria
	Euglenophyceae	Euglenales	Euglenaceae	*Euglena*
				Leptocinclis
				Peranema
	Chlorophyceae	Tetrasporales	Palmellaceae	*Gloeocystis*
			Coccomyxaceae	*Dactylothece*
			Characiaceae	*Dicyosphaerium*
			Hydrodictyaceae	*Pediastrum*
			Oocystaceae	*Chlorella*
				Oocystis
				Westella
			Desmidiaceae	*Closterium*
				Staurastrum
				Cosmarium
			Zygnemataceae	*Spirogyra*
	Xanthophyceae	Heteroccocales	Gloeobotrydiaceae	*Gloeobotrys*
				Chlorobotrys
		Heterotrichales	Tribonemataceae	*Tribonema*
		Heterosiphonales	Botrydiaceae	*Botryococcus*
	Chrysophyceae	Chrysomonadales	Chromulinaceae	*Chrysococcus*
		Chrysocapsales	Chrysocapsaceae	*Chrysocapsa*
	Rhodophyceae	Bangiales	Erythrotrichiaceae	*Campsopogon*
			Lemaneaceae	*Lemanea*
	Bacillariophyceae	Bacillariales	Fragilariaceae	*Diatoma*
				Meridion
				Fragilaria
				Synedra

Table 3. Continued.

Phylum	Class	Order	Family	Genus
			Achnanthoideae	*Achnanthes*
			Nitzschaiaceae	*Nitzschia*
			Naviculaceae	*Amphipleura*
				Frustulia
				Pinularia
				Stauroneis
				Navicula
			Cymbellaceae	*Cymbella*

various genera occurring in the pond, *Cosmarium* was dominant at all the spots in the present study followed by *Chlorella.* The ascendancy of Bacillariophyceae among other phytoplankton could be credited to their ability to grow under the surroundings of low temperature and weak light, which are less favorable for other algae (Zutshi 1991).

Total average density observed in all the spots during study period was 1164.92 ± 105.56. The average density during the study ranged between 3.00 ± 1.25 (Chrysophyceae) and 636.38 ± 88.96 (Chlorophyceae) (Table 4). The density of phytoplankton was observed to be lowest in the begining of sampling (January) and there after it increased up to the month of March. Earlier, Prakash et al. (2002) also observed the same. The mean percentage composition of different groups were Chlorophyceae (52.8%), Bacillariophyceae (32.23%), Myxophyceae (6.68%) and Euglenophyceae (1.50%), Xanthophyceae (6.26%), Rhodophyceae (0.29%) and Chrysophyceae (0.24%) (Fig.1). The occurrence of Chlorophyceae is known to prefer inorganic nutrients providing moderately high temperature and alkaline pH (Rao 1995, Krishnan 2008).

The Shannon-Wiener's diversity ($\overline{H}$) varied between 2.94 and 2.10; the richness (d') was recorded between 3.26 and 5.22, while the evenness (e) ranged from 0.36 to 0.59. The maximum Shannon-Wiener's diversity was recorded in class Bacillariophyceae ($\overline{H}$ =2.05) whereas it was minimum ($\overline{H}$=0) in Chrysophyceae and Rodophyceae. In Myxophyceae, it ranged from 0.74 to 1.36, while in Euglenophyceae it varied from 0 to 0.91. It was recorded between 1.00 to 1.75 in Chlorophyceae and 0.13 to 1.38 in Xanthophyceae. The lower $\overline{H}$ value indicates lower phytoplankton diversity and this condition highly influences the stability of living environment of water body, but it frequently changes with small environmental influence. The richness was recorded maximum (1.93) in Bacillariophyceae. In Myxophyceae it ranged from 0.62 to 1.00, while in Euglenophyceae it ranged from 0.33 to 1.44. It was recorded between 0.84 to 1.64 in Chlorophyceae and 0.19 to 1.44 in Xanthophyceae. The values of Evenness Index (e) showed minimum

Table 4. Phytoplankton density (units/ml) calculated for different classes in fish pond.

Class	S_1	S_2	S_3	S_4	S_5	Average density (units/ml)
Myxophyceae	78.3 ± 1.32	85.03 ± 3.46	101.7 ± 3.86	76.7 ± 1.02	50.02 ± 3.67	78.35 ± 10.78
Euglenophyceae	16.6 ± 1.26	29.67 ± 1.94	21.6 ± 1.321	20.01 ± 1.13	10.004 ± 1.05	19.57 ± 4.03
Chlorophyceae	701.02 ± 35.7	456.8 ± 19.7	813.6 ± 38.3	728.6 ± 37.1	481.9 ± 32.5	636.38±88.96
Xanthophyceae	75.03 ± 8.72	65.7725 ± 8.48	106.7 ± 13.8	88.37 ± 11.12	56.6 ± 3.81	78.49 ± 11.29
Chrysophyceae	0 ± 0	0 ± 0	5.0025 ± 0.56	5.0025 ± 0.83	5.002 ± 0.83	3.00 ± 1.25
Rhodophyceae	6.67 ± 0.62	3.335 ± 0.55	3.335 ± 0	1.6675 ± 0.27	1.66 ± 0.27	3.335 ± 0.48
Bacillariophyceae	438.5 ± 7.5	300.1 ± 8.00	433.5 ± 11.14	348.4 ± 11.33	208.4 ± 12.1	345.78 ± 47.00
Total (units/ml)	1296.2 ± 8.48	940.8 ± 59.8	1485.6 ± 115.1	1268.9 ± 101.9	813.7 ± 66.7	11.64.92±105.56

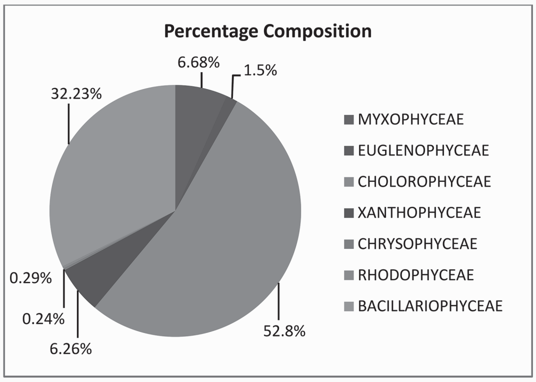

Fig. 1. Percentage composition of different groups of phytoplankton in fish pond.

(0.39) in Chlorophyceae and maximum (01) in Chrysophyceae and Rodophyceae. It was recorded between 0.41 to 0.70 in Bacillariophyceae and 0.63 to 0.98 in Myxophyceae. It varied between 0.7 to 1.00 in Euglenophyceae and 0.57 to 1.00 in Xanthophyceae (Table 5). If evenness index approaches to zero, the species evenness in the community is low and inversely if the Evenness Index approaches to 1 the species in the community is the same (Pirzan et al. 2017).

Relationships between physico-chemical parameters and phytoplankton communities

The canonical correspondence analysis (CCA) of phytoplankton density was observed with 10 physico-chemical parameters. The first two statistically significant canonical axes explained together 66.44% (λl=0.27108, $\lambda 2 = 0.22992$) of the total inertia in the phytoplankton assemblages at 5 sites in fish pond during 06 times viz., January, February (Feb A and B), March (Mar A and B), and April (Fig.2).

Among the 10 determinants, pH, conductivity, water temperature, depth, dissolved oxygen, phosphate and nitrate were the most significant. In the right upper side quadrant, the variables namely depth, nitrate, phosphate, and total alkalinity were interrelated and accounted for distribution of *Gloeotrichia, Dactylothece, Oocystis* and *Lemanea* were recorded.

Table 5. Diversity ($\overline{H}$), richness (d') and evenness (e) indices calculated for different classes of phytoplankton in fish pond.

Class	Indices	20 Jan	04 Feb	19 Feb	08 Mar	23 Mar	07 Apr
Myxophyceae	$\overline{H}$	1.03	0.74	1.07	0.92	1.16	1.36
	d'	0.84	0.53	0.62	0.69	1.00	0.75
	E	0.70	0.70	0.97	0.63	0.79	0.98
Euglenophyceae	$\overline{H}$	0.91	0.33	0.68	0.69	0.69	0.0
	d'	0.63	0.33	0.45	1.44	1.44	0.0
	E	0.83	0.70	0.99	1.0	1.0	1.0
Cholorophyceae	$\overline{H}$	1.0	1.06	1.75	1.47	1.72	1.72
	d'	1.04	0.84	1.97	1.64	1.37	1.38
	E	0.45	0.48	0.52	0.39	0.69	0.55
Xanthophyceae	$\overline{H}$	0.59	0.69	1.38	0.59	0.13	0.26
	d'	0.37	1.44	1.13	0.51	0.19	0.30
	E	0.90	1.0	0.79	0.90	0.57	0.65
Chrysophyceae	$\overline{H}$	0.0	0.0	0.0	0.0	0.0	0.0
	d'	0.0	0.0	0.0	0.0	0.0	0.0
	E	1.0	1.0	1.0	1.0	1.0	1.0
Rhodophyceae	$\overline{H}$	0.0	0.0	0.0	0.0	0.0	0.0
	d'	0.0	0.0	0.0	0.0	0.0	0.0
	E	1.0	1.0	1.0	1.0	1.0	1.0
Bacillariophyceae	$\overline{H}$	2.05	1.41	1.90	1.42	1.41	1.22
	d'	1.93	1.75	1.80	1.56	1.05	0.90
	E	0.70	0.41	0.66	0.41	0.58	0.68
Total	$\overline{H}$	2.74	2.10	2.94	2.38	2.33	2.31
	d'	4.39	3.58	5.22	4.31	3.40	3.26
	E	0.57	0.34	0.59	0.36	0.45	0.44

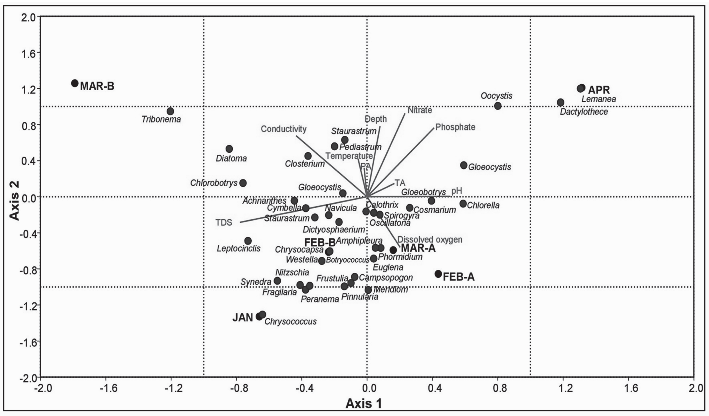

Fig. 2. Plot showing relationship between physico-chemical parameters and phytoplankton density in fish pond.

February A and March A plotted in the right lower quadrant of the diagram (Fig. 2). The phytoplankton assemblages at this quadrant were subjected to dissolved oxygen, while pH was transitional being plotted between the right upper and lower quadrant. The taxa accounted for this quadrant were *Calothrix, Phormidium, Oscillatoria, Euglena, Chlorella, Cosmarium, Spirogyra, Gloeobotrys and Amphipleua.* March B plotted in the left upper quadrant, the variables namely temperature, conductivity and phenolphthalein alkalinity interrelated with *Closterium, Staurastrum, Gloeocystis, Pediastrum, Chlorobotrys, Tribonema* and *Diatoma*. January and February B are plotted in the left lower quardrat of the diagram, this quadrant shows total dissolved solid correlates with *Leptocinclis, Peranema, Westella, Chrysococcus, Chrysocapsa, Campsopogon, Synedra, Achnanthes, Nitzschia, Amphipleua, Frustulia, Pinnularia, Stauroneis, Navicula and Cymbella.*

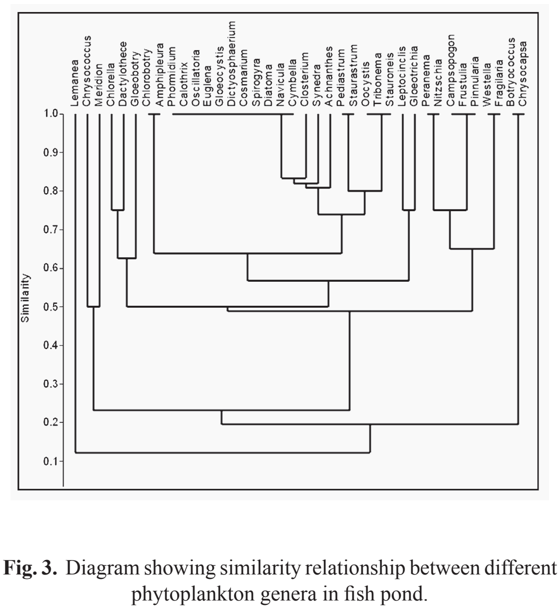

Fig. 3. Diagram showing similarity relationship between different phytoplankton genera in fish pond.

In the present study dissolved oxygen was observed to vary reciprocally to the phytoplankton density. This could be attributed to physical conditions in which the water temperature and transparency had a direct relationship with abundance of phytoplankton (Devika et al. 2006). Bacillariophyceae (Diatoms) were higher at high temperature and they showed significant correlation with temperature. Earlier, it was also observed that the relative contribution of small sized phytoplankton, cyanobacteria and diatoms is higher at high temperature and low nutrients condition (Prakash 2001, Tundisi et al. 2002, Chitra and Meera 2004). It was observed that if, the phytoplankton were at minima, there may not be enough oxygen produced. However, excessive algal bloom often has negative impacts on the dissolved oxygen and animal life in a pond. A common impact of an excessive bloom on a pond is sudden death of the phytoplankton population (Conte and Cubbage 2001). Also, the nitrate and phosphate are nutrients for phytoplankton growth, when the phosphate and nitrate concentrations were highest, phytoplankton density was also observed high.

The dominance of cyanobacteria in phytoplankton biomass was raised by high phosphorus content and the stock of zooplanktonivorus fish species (Watson et al.1997, Pechar 1995, 2000).

Cluster analysis showed great similarity between *Phormidium, Oscillatoria, Euglena, Calothrix,Gloeocystis, Dictyosphaerium, Cosmarium, Spirogyra*, *Navicula, Diatoma* and *Cymbella* on the basis of their density (Fig. 3). In general, the phytoplankto of eutrophic fish ponds is characterized by high percentage of littoral species of green algae (Chlorophyceae) and dominance of golden-brown algae is representative of oligotrophic fish ponds (Pouličková 2011).

The diversity of phytoplankton was observed to show temporal variation during the entire study period and was maximum during the month of March. The class Chlorophyceae constitutes the highest average density and the percentage composition in the fish pond.Canonical correspondence analysis (CCA) reveals that pH, conductivity, water temperature, depth, dissolved oxygen, phosphate and nitrate were mostly correlated with phytoplankton assemblages. The present study concludes that the phytoplankton density greatly varied in the fish pond and showed tendency towards eutrophic level due to the high proportion of green algae.

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