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ORIGINAL ARTICLE **OPEN ACCESS**

Composition and Seasonality of Phytoplankton in a Crude Oil Effluent Holding Pond of Digboi Oil Field, Assam (India)

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ABSTRACT
The ecology of phytoplankton in an effluent holding pond of Digboi oil field (Assam, India), the oldest oil field in Asia was studied to understand the influence of crude on them. During this endeavor taxonomic composition, abundance and seasonality of phytoplankton along with water quality parameters were studied at monthly interval for one year from January 2013 to January 2014. A total of 36 fresh water phytoplankton species belonging to Cyanophyta (9), Chlorophyta (15), Bacillariophyta (8) and Euglenophyta (4) were recorded. During monsoon along with the pre and post monsoonal months, cyanophycean members were common under the influence of higher free CO_2*, phosphate and nitrate in water. On the contrary, chlorophycean members were common and dominant during winter months and showed positive correlations with DO, TOC, COD and phenol content of the pond. CCA also confirmed the influence of water quality variables on phytoplankton composition and seasonality in this effluent holding pond.*
Keywords: *phytoplankton, oil field, effluent, water quality, Pearson's correlation, CCA.*

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INTRODUCTION
The crude oil industry is truely global, operating in every corner of the world, in all type of habitats from Arctic to desert, from tropical rain forest to temperate woodlands, from onshore to offshore. Being the primary source of energy, crude oil render 36% of total global energy source [1]. In Assam, crude oil exploration activities are the oldest among Asia. In fact, oil exploration in India commenced with the discovery of Digboi oilfield in Upper Assam more than 100 years ago when an oil well was drilled on oil seepage in the exposed anticline associated with the Naga thrust. Now a days, many national and international oil companies are engaged in oil exploration and production in Upper Assam region. The production operations inevitably release effluent in the form of produced waters, storm waters and flushing wastes into the aquatic environment. The effluents are found to contain significant quantities of hydrocarbons and associated pollutants causing harm to the fresh water bodies and their biota in their vicinity.
Ecological investigation of phytoplankton is an important aspect of aquatic ecosystems to understand the pollution stress on them. The species composition and abundance of phytoplankton entirely dependent upon physical and chemical characteristics of water bodies [2].Changes in water quality alter the phytoplanktonic species composition therein [3]. Hence, study on phytoplankton community may be used as a reliable tool to assess the pollution status of water bodies [4].
Phytoplankton of water bodies near the crude oil exploration sites are regularly exposed to hydrocarbon pollutants. The effect of crude oil on phytoplankton both in laboratory as well as in natural environment were studied in various parts of the world [5-7] which showed over all inhibitory results of crude on phytoplankton growth [8-10]. A few studies, on the other hand, showed little significant effect of crude on phytoplankton [11-13]. Since inland water bodies are located near human habitation, crude oil contamination not only degrade the surface as well as ground water of the area but also make it unfit for human consumption and growth of different aquatic organisms [14] including phytoplankton [15]. The present endeavour is therefore aimed to study taxonomy, seasonality and ecology of phytoplankton community in crude oil production waste holding pond at Digboi oil field of Upper Assam.

MATERIALS AND METHODS

The study was conducted in a effluent holding pond located at 27^{0}20'38.4" N latitude and 95^{0} 28'58.6" E longitude at an altitude of 112 msl at Digboi oil field of Tinsukia district (Assam), India. The water samples were collected randomly using Nansen sampler from five locations of the studied pond and combined to get a composite sample. The sampling was done at monthly interval during January 2013 to January 2014. Phytoplankton samples were collected in plastic tubes by filtering 50 L of water using 25 µm mesh size plankton net and fixed immediately on the spot in Lugol's iodine for identification and quantitative analysis. The water temperature, pH, conductivity, turbidity and dissolved oxygen (DO) were measured on the spot using Systronics digital water analyzer 371. Water samples were collected in clean plastic containers and brought to the laboratory of the Department of Botany, Gauhati University for estimating free carbon di oxide (FCO_2), biological oxygen demand (BOD), chemical oxygen demand (COD), phosphate, nitrate, total oil content (TOC) and phenol following APHA [16]. Identification of algal samples were done by consulting literature and monographs [17-23]. Phytoplankton abundance was calculated using Sedgwick rafter plankton counting cell [16].

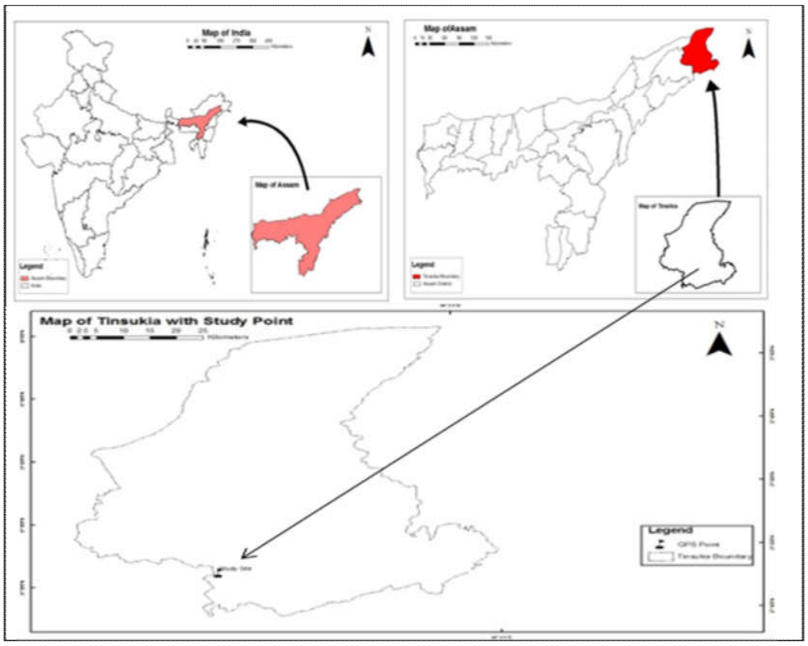

Fig 1: Map showing the studied effluent holding pond of Digboi oil field (Assam, India)

Composition of phytoplankton community was analyzed thoroughly to define dominant forms. The species, having > 75% of the community were grouped under dominant category. The species that constituted 40-75% of the community were designated as common and those <40 % were considered as present. The similarity value indices for phytoplankton communities between different sampling months were calculated with the following formula [24]:

$$QS = \frac{2C}{A + B}$$

Where, QS is the Sorenson's quotient of similarity; C is the number of species common to both the communities; A is the number of species present in one community and B is the number of species present in the other community. The correlation between various water quality variables were analyzed using Pearson's correlation coefficient (significant level $p<0.05$). Canonical Correspondence Analysis (CCA) was further done using statistical software PAST to explain the relationship between phytoplankton and water quality variable.

RESULTS AND DISCUSSION

The monthly mean values as well as the standard error of water temperature, pH, conductivity, turbidity, DO, free CO_2, BOD, COD, phosphate, nitrate, TOC and phenol of the oil field effluent holding pond are given in the Fig. 2 (A to L). Quite significant variations were recorded in physico chemical properties of the pond water in relation to seasons. The water temperature varied with months (Fig. 2-A). The pH of the pond was acidic during pre monsoon and monsoon months (April to August) and alkaline during post monsoon to winter months (September to March) (Fig.2-B). Conductivity was found higher during monsoon months (Fig.2-C). Turbidity of the pond was higher during pre monsoon and monsoon months due to heavy rainfall during the period as compared to other months (Fig.2-D). The overall lower values of DO except during the winter months (Fig.2-E) indicated polluted status of the pond [25-26]. The free CO_2 was recorded higher during pre monsoon and monsoon months (Fig.2-F). BOD was higher in the pre monsoon and monsoon months than the rest of seasons, which was totally opposite for COD (Figs.2 G & H). Phosphate and nitrate showed similar seasonal variation with higher values during the pre monsoon and monsoon months (Figs.2- I& J). In contrast, concentration of both TOC and phenol showed high values during winter months and low from pre to post monsoon months (Figs.2- K & L).

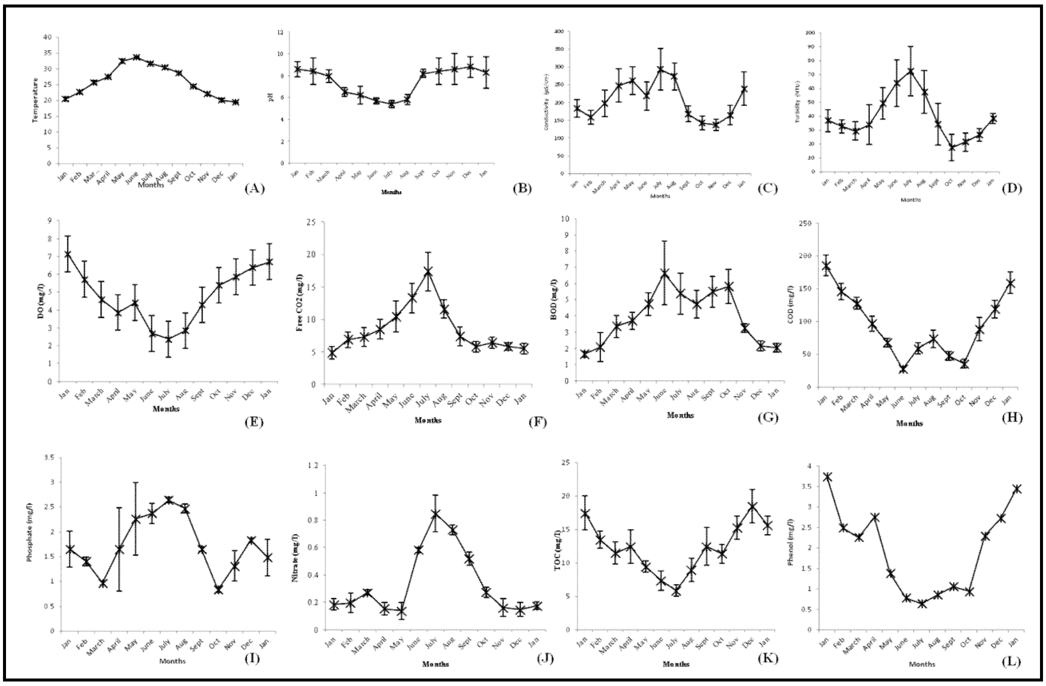

Fig 2: Seasonal variation of water quality parameters from January 2013 to January 2014 in the studied effluent holding pond of Digboi oil field (Assam, India)

The relationship between the water quality variables of the pond was analysed using Pearsons correlation coefficients (Table1). All the variables seem to be linked with each other. Temperature had strong positive correlation with free CO_2 and BOD, but strong negative correlation with pH, DO, TOC and phenol. pH was positively correlated with DO, COD, TOC and phenol and significantly negatively correlated with conductivity, turbidity, free CO_2, BOD, phosphate and nitrate. Conductivity was in positive correlation with turbidity, free CO_2, and phosphate and in negative correlation with DO and TOC. Turbidity was positively correlated with free CO_2, phosphate and nitrate and negatively with DO and TOC. DO had positive correlation with TOC and phenol, but negative with free CO_2, BOD, COD, phosphate and nitrate. Free CO_2 was in positive correlation with BOD, phosphate and nitrate and negative correlation with COD, TOC and phenol. It was found that BOD had strong negative correlation with COD, TOC and phenol and COD had strong positive correlation with TOC. Both phosphate and nitrate were significantly negatively correlated with TOC and phenol. A strong positive correlation was found between TOC and phenol in the pond.

Table 1: Correlation coefficients between variables of water quality

	Temp	pH	Conduct	Turb	DO	Free CO2	BOD	COD	Phosphate	Nitrate	TOC	Phenol
Temp	1.000	-0.890*	0.606*	0.733*	-0.926*	0.839*	0.837*	-0.755*	0.659*	0.665*	-0.914*	-0.817*
pH		1.000	-0.847*	-0.873*	0.896*	-0.916*	-0.631*	0.539*	-0.799*	-0.668*	0.866*	0.620*
Conduct			1.000	0.816*	-0.629*	0.733*	0.263	-0.146	0.740*	0.503	-0.614*	-0.267
Turb				1.000	-0.732*	0.903*	0.454	-0.318	0.902*	0.762*	-0.733*	-0.491
DO					1.000	-0.891*	-0.791*	-0.572*	-0.632*	-0.795*	0.919*	0.808*
Free CO2						1.000	0.643*	-0.572*	0.808*	0.807*	-0.878*	-0.706*
BOD							1.000	-0.955*	0.362	0.650*	-0.820*	-0.925*
COD								1.000	-0.324	0.549*	0.712*	0.918*
Phosphate									1.000	0.639*	-0.535*	-0.433
Nitrate										1.000	-0.743*	-0.766*
TOC											1.000	0.806*
Phenol												1.000

Significant level $p<0.05$

The phytoplankton species composition of the pond during the studied period was given in the table 2. Altogether thirty six phytoplankton species under twenty seven genera were recorded from the studied pond during January 2013 to January 2014. With 15 species, Chlorophyta was the dominant group followed by Cyanophyta (9), Bacillariophyta (8) and Euglenophyta (4), respectively with a proportion of 47%, 28%, 22% and 11% respectively. Joseph and Joseph [27] too recorded highest number of species under Chlorophyta in a crude oil refinery effluent holding pond of Kochi Refinery, Kerela, India.

Table 2: Seasonal variation and abundance of phytoplankton from January 2013 to January 2014 in the studied effluent holding pond of Digboi oil field (Assam, India)

	Codes	Ja	Fe	Ma	Ap	May	Ju	Jl	Au	Se	Oc	No	De	Ja
Cyanophyta														
Aphanocapsa virescens (Hass.)	S1	++	+	-	+	-	-	-	-	+	-	+	++	++
Aulosira fertissima Ghose	S2	-	-	+	++	++	-	+	-	-	-	-	-	-
Chroococcus turgidus (Kutz) Nag	S3	+++	++	-	-	-	-	-	-	-	++	++	+++	++
Gloeocapsa stegophila Rao	S4	-	-	-	+	+	+	+	-	-	-	-	-	-
Cyanosarcina burmensis Skuja	S5	++	+	+	-	-	-	-	-	-	-	+	++	+
Lyngbya contorta Lemm.	S6	-	-	+	-	+	++	+	+	-	++	-	-	-
Oscilatoria princeps Vaucher ex Gemont	S7	-	+	+	+	+	-	++	+	+	-	-	-	+
Phormidium ambiguum Gom	S8	-	-	-	+	++	++	+	-	+	-	-	-	-
Scytonema coactile Montagne ex Born. et Flah	S9	-	-	-	-	+	+	-	-	-	-	-	+	-
Chlorophyta														
Ankistrodesmus spiralis (Turn.)Lemm.	S10	+	++	-	-	-	-	-	-	-	-	+	+	+
Arthrodesmus curvatus Turner	S11	+	+	+	-	+	-	-	-	-	-	-	++	+
Eudorina elegans Ehrenberg	S12	++	+	+	-	-	-	-	-	-	-	-	+++	+
Pandorina morum (Mueller) Bory St. Vincent	S13	+	-	+	+	-	-	-	-	-	-	+++	+++	++
Closterium closteriodies (J. Roy *et* Bisset)	S14	+	-	+	-	+	+	-	-	+	-	-	-	-
Cosmarium moniliforme Turnp.	S15	-	-	-	+	+	-	+	-	+	+	-	-	-
*Oedogonium figuratum*Tiffany	S16	-	-	+	+	+	-	+	-	-	-	-	-	-
Radiococcus nimbatus (De Wild.) Schmid	S17	++	++	-	+	-	-	-	+	-	-	++	+++	++
Scenedesmus acuminatus (Lagerh.)	S18	++	++	-	-	-	-	-	-	-	-	-	++	+
Scenedesmus papillatus Jao	S19	++	+	-	-	-	-	-	-	-	+	-	++	++
Scenedesmus praetervisus Chodat	S20	++	+	+	-	-	-	-	-	-	-	+	+	+
Scenedesmus pseudopoliensis Hortob	S21	+	+	+	-	-	-	-	-	-	-	-	+	+
Selenastrum gracile Reinsch	S22	-	-	-	-	+	-	-	+	-	+	+	-	-
Staurastrum sonthalianum Turner	S23	+	-	+	-	-	-	+	-	+	+	-	+	-
Treubaria setigera (Arch) Smith	S24	-	-	-	-	-	-	-	+	+	-	-	+	-
Bacillariophyta														
Melosira granulate (Ehrenberg) Ralfs	S25	-	+	-	+	-	-	-	-	+	-	+	+	+
Cyclotella glomerata Bachmann	S26	+	+	-	-	+	-	-	-	-	+	-	+	-
*Synedra ulna (*Nitzch) Ehrenberg	S27	+	+	-	+	-	-	-	-	-	-	-	+	+
Amphora elliptica Kütz	S28	-	-	-	-	+	+	+	-	+	+	-	-	-
Cyclotella glomerata Bachm	S29	-	+	+	-	+	+	+	-	-	+	-	-	-
Eunotia camelus Her	S30	+	-	-	-	+	+	-	+	-	-	-	+	-
Navicula viridula Kütz	S31	-	-	+	+	+	-	+	+	+	-	+	-	-
Cymbella gracilis (Ehrnberg) Kützing	S32	-	+	-	+	-	-	-	+	-	-	-	+	+
Euglenophyta														
Euglena gracilis Klebs	S33	-	-	-	+	-	++	++	+	-	-	-	-	-
Euglena viridis Ehrn	S34	-	-	+	-	-	++	+	+	+	-	+	-	+
Phacus curvicauda Swirn.	S35	+	-	-	-	-	-	+	-	+	-	+	+	+
Trachelomonas armata (Ehrenberg)Stein	S36	-	-	+	+	-	-	+	+	-	-	-	+	-

Ja=January, Fe=February, Ma=March, Ap=April, My=May, Ju=June, Jl=July, Au=August, Se=September, Oc=October, No=November, De=December, +++= Dominant (10^4-10^6 Cell/L), ++=common (10^2-10^4 Cell/L), +=present (10^0-10^2 Cell/L), - =absent.

During pre-monsoon months (viz. March, April and May) cyanophycean members *Lyngbya contorta* and *Phormidium ambiguum* were common. They were also common during monsoon months (June, July and August) along with another cyanophycean member *Oscilatoria princeps* and two euglenophycean member *Euglena gracilis* and *Euglena viridis*. Occurrence of *Lyngbya contorta* with another cyanophycean member *Chroococcus turgidus* were common during post-monsoon months (September, October and November).

Chroococcus turgidus, *Eudorina elegans*, *Pandorina morum* and *Radiococcus nimbatus* were the dominant species during winter months (December, January and February). Two cyanophycean members *Aphanocapsa virescens* and *Cyanosarcina burmensis* and five chlorophycean phytoplankton *Ankistrodesmus spiralis, Arthrodesmus curvatus*, *Scenedesmus acuminatus* and *Scenedesmus papillatus* were common during this period. The chlorophycean genus *Scenedesmus* with four species was the dominant genus which is in conformity with the result of Joseph and Joseph [27].
The similarity indices for phytoplankton communities between different sampling months are given in the Table 3. The values ranged between 0.08 to 0.94. The maximum similarity was observed between the months of January and March (0.94) followed by January and December (0.85). The least similarity was found between April and October (0.08).

Table 3: Algal similarity index between different sampling periods from January 2013 to January, 2014

	Jan	Feb	Mar	April	May	June	Jul	Aug	Sep	Oct	Nov	Dec	Jan
Jan	1	0.74	0.94	0.24	0.24	0.21	0.12	0.14	0.27	0.30	0.53	0.85	0.72
Feb		1.00	0.42	0.38	0.24	0.07	0.13	0.21	0.21	0.31	0.48	0.77	0.86
Mar			1.00	0.39	0.50	0.31	0.58	0.37	0.36	0.24	0.36	0.42	0.47
April				1.00	0.45	0.24	0.60	0.46	0.44	0.08	0.37	0.32	0.42
May					1.00	0.62	0.65	0.37	0.43	0.40	0.14	0.21	0.12
June						1.00	0.56	0.38	0.36	0.32	0.09	0.13	0.07
Jul							1.00	0.46	0.59	0.42	0.22	0.16	0.18
Aug								1.00	0.35	0.20	0.35	0.30	0.28
Sep									1.00	0.29	0.42	0.29	0.33
Oct										1.00	0.19	0.19	0.15
Nov											1.00	0.47	0.67
Dec												1.00	0.80
Jan													1.00

The interrelationship of phytoplankton species with water quality variables was represented as CCA triplot (Fig. 3). Phytoplankton – water quality correlation for axis 1 and axis 2 was high with eigen values 0.471 and 0.207 respectively, indicating a strong correlation among phytoplankton distribution and water quality variables used for ordination. The two axis alone explained a large proportion of variance in phytoplankton distribution and water quality (50.9 %), hence axis 1 and axis 2 were considered for construction of the CCA triplot. The water quality variables contributing to this data can be ranked as (1) temperature, (2) BOD, (3) DO, (4) TOC, (5) phenol, (6) COD, (7) pH, (8) free CO_2, (9) nitrate, (10) turbidity, (11) phosphate and (12) conductivity (Table-4). The CCA showed higher correlation between DO and pH; between TOC, COD and phenol and between free CO_2, turbidity and nitrate.

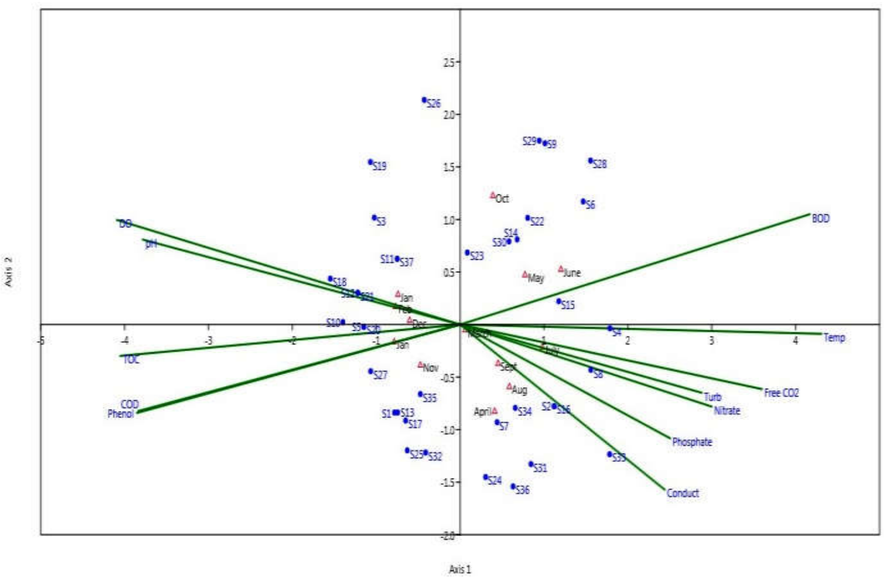

Fig 3: CCA triplot for phytoplankton abundance and water quality variables.

Table 4: Correlation of environmental variables with the axes in CCA

Variables	Axis 1 (λ_1 = 0.471)	Axis 2 (λ_2= 0.207)	Axis 3 (λ_3 = 0.150)	Axis 4 (λ_4 = 0.127)
Temp	0.96	-0.04	0.11	-0.22
pH	-0.84	0.21	-0.30	0.18
Conduct	0.55	-0.38	0.40	-0.04
Turb	0.63	-0.17	0.34	-0.40
DO	-0.90	0.24	-0.07	0.14
Free CO_2	0.79	-0.16	0.21	-0.16
BOD	0.92	0.24	-0.28	-0.13
COD	-0.84	-0.20	0.40	0.10
Phosphate	0.55	-0.25	0.21	-0.51
Nitrate	0.64	-0.18	-0.18	-0.23
TOC	-0.90	-0.04	-0.10	-0.02
Phenol	-0.85	-0.19	0.34	0.06

The length of environmental variables and their orientation on the CCA triplot indicate their importance to each axis. During pre monsoon, monsoon and post monsoon months BOD, temperature, free CO_2, turbidity, nitrate, phosphate, conductivity were positively correlated with axis 1. On the other hand, DO, pH, TOC, COD and phenol were negatively correlated with axis 1 during winter months. The CCA triplot showed that all of the environmental variables are of importance and more or less correlated with phytoplankton community. It was observed that except *Closterium closteriodies* BOD had little influence on phytoplankton distribution. In the month of March *Gloeocapsa stegophila* strongly correlated with temperature. In pre monsoon, monsoon and post monsoon months (March to September) several phytoplankton species like *Phormidium ambiguum*, *Aulosira fertissima*, *Cymbella gracilis*, *Euglena gracilis*, *Oscilatoria princeps*, *Eunotia camelus*, *Phacus curvicauda* and *Staurastrum sonthalianum* strongly correlated with nutrients *viz.* nitrate and phosphate and water quality parameters like FCO_2, turbidity and conductivity. During winter months (November to February) distribution of some phytoplankton species such as *Aulosira fertissima*, *Cyanosarcina burmensis*, *Ankistrodesmus spiralis*, *Arthrodesmus curvatus*, *Radiococcus nimbatus*, *Scenedesmus praetervisus* and *Cyclotella glomerata* were significantly influenced by DO, pH, TOC, COD and phenol. The CCA analysis thus revealed that the distribution of phytoplankton in this effluent holding pond was well adopted to change in water quality as well as seasons.

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