

Seasonal biodiversity of cyanobacteria in besmirched habitats

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ABSTRACT

Cyanobacteria inhabit a diverse range of ecosystems, a number of features often contribute to their success. Growth of these organisms in many ecosystems is limited by the availability of nutrients. High load of solids, carbon and nutrients indicate proliferation of cyanobacteria, while low nutrient condition diminishes cyanobacterial growth. This study examines cyanobacterial diversity in domestic and hospital sewage of Sagar, Madhya Pradesh (M.P), India, from January 2013 to December 2013. Cyanobacterial biodiversity was higher during study period and dominated by *Aphanocapsa*, *Chroococcus*, *Phormidium* and *Nostoc* species. The present investigation exhibits a baseline of information on cyanobacterial diversity associated with wastewater under the influence of urbanization. Massive urbanizations in developing countries have polluted fresh water bodies and terrestrial areas nearby. This information can be utilized to identify cyanobacterial species for bioremediation of sewage. There are a number of Cyanophyceae members which are tolerant to organic pollution and resist environmental stress by pollutants. These species may be further used as pollution indicators for such habitats. Cyanobacterial species can constrain future pollution and can play a key role to accomplish the dream of pollution-free environment.

KEY WORDS

Biodiversity; bioremediation; cyanobacteria; urbanization.

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INTRODUCTION

Nowadays, water pollution is a serious concern; due to unplanned urbanization and industrialization most of the resources have reached to a point of crisis. Dumping of different waste materials in different drainage systems pollutes aquatic bodies and surrounding terrestrial environment, thus affecting the growth of vegetation and aquatic life.

Cyanobacteria are common components of phytoplanktonic community in most aquatic ecosystems. The ecophysiology of cyanobacteria can provide them with a substantial advantage over other phytoplanktons. The recent studies on cy-

anobacteria have emphasized their important role in ecosystems. The abundance and composition of cyanobacterial population in surface waters of ponds and lakes have been discussed by many studies. Cyanobacteria flourish well either in nutrients-rich warm water or, at times, in water with apparently low temperature and bright light conditions (Philipose, 1960; Seenayya, 1972; Fogg, 1975). The number of water bodies suffering from eutrophication is increasing around the world. Such an eutrophication primarily comes from municipal wastewater, agricultural runoff, domestic sewage, stability of water column and increased light exposure.

Cyanobacteria are pioneer oxygenic, gram negative, photosynthetic prokaryotes and are widely distributed. The cyanobacterial diversity of sewage can be used as biomonitor of organic pollution load in other water habitats and surroundings. Cyanobacterial community structure was found to be influenced by anthropogenic activities. The use of cyanobacteria as an indicator of water quality and pollution has been emphasized by Venkateswarlu (1981). Only a few researchers (Manoharan & Subramanian, 1992a, b; Boominathan, 2005; Vijayakumar, 2005) have investigated the effect of effluents on the physiology and biochemistry of the cyanobacterial systems.

To develop suitable and an efficient wastewater treatment system, it is obligatory to understand the mutual influence and interactions between the effluents and the organisms, so that manipulations to improve the treatment system may become feasible and hence the future scenario must select suitable species of cyanobacteria which would be minimally influenced by the adverse conditions in the effluent, but would help removing pollutants maximally (Singh & Saxena, 1969; Rai & Kumar, 1979; Sahai et al., 1985; De la Noue & Proulx, 1988; Wilkinson et al., 1989).

Sagar, located in Bundelkhand region of Madhya Pradesh, has exhibited urbanization rapidly in last few years. It has a lake, Lakha Banjara, lying in the middle of the city, which has become a besmirched aquatic habitat. During the past few decades, partially treated and untreated wastewaters were discharged into the lake and surrounding croplands and used for agriculture, pisciculture and other domestic purposes. Keeping the above facts in view, the present study was aimed at the analysis of physico-chemical properties of wastewater in relation to cyanobacterial diversity.

MATERIAL AND METHODS

Sampling sites

District Sagar is situated in the north central region of Madhya Pradesh, India, and lies between the north latitude 23°10' to 24°27' and east longitude 78°4' to 79°21' at an altitude of 1758 feet above the sea level. A number of temporary and residential water bodies are present in this region.

The city harbours a shallow rainfed fresh water lake, Lakha Banjara (23°49'N and 78°44'E) with small catchment. A hot summer and general dryness characterize the climate of the area. The climate of Sagar can be categorized as "monsoon type" and commences from mid June and continues till September. This period is distinguished by heavy rains, high temperatures and relatively high humidity. About 90% of the annual rainfall is received during this period. The monsoon is followed by a brief post-monsoon period October to November, when temperature remains high and the humidity decreases considerably; only a nominal precipitation occurs and wind velocity is also lower. Winter starts from late November and continues up to February. It is characterized by low temperature, low irradiant and moderate relative humidity. The average annual rainfall varies from 565 mm to 1680 mm. The maximum temperature recorded was 44.8 °C in the month of May and the minimum temperature was 5 °C in January (IMD, 2013). Keeping in mind inflow sources of wastewater, present study was carried out at the besmirched sites of Lakha Banjara Lake viz. Site 1, Site 2 and Site 3.

Sites are subjected to human interferences and receive discharges from the surrounding localities which make the water highly polluted and pollutants like domestic sewage, straw, hospital discharge and industrial effluent etc. get accumulated in large quantities.

Collection of sample

The wastewater samples were collected in triplicates (2 liters each) from each of the three sites in sterilized colored plastic bottles (Tarsons Products Pvt. Ltd., New Delhi, India) from January 2013 to December 2013 in every month of all seasons Winter (W), Summer (S) and Rainy season (R). Samples were taken in the mid of the each month in bottles thoroughly cleaned with diluted HCl (AR grade, 99.9% Merck Pvt. Ltd., Mumbai, India) and rinsed with distilled water twice, dried in an oven (Yarco) and then analyzed for various physico-chemical parameters.

Physico-chemical study

Physico-chemical analysis of waste water was

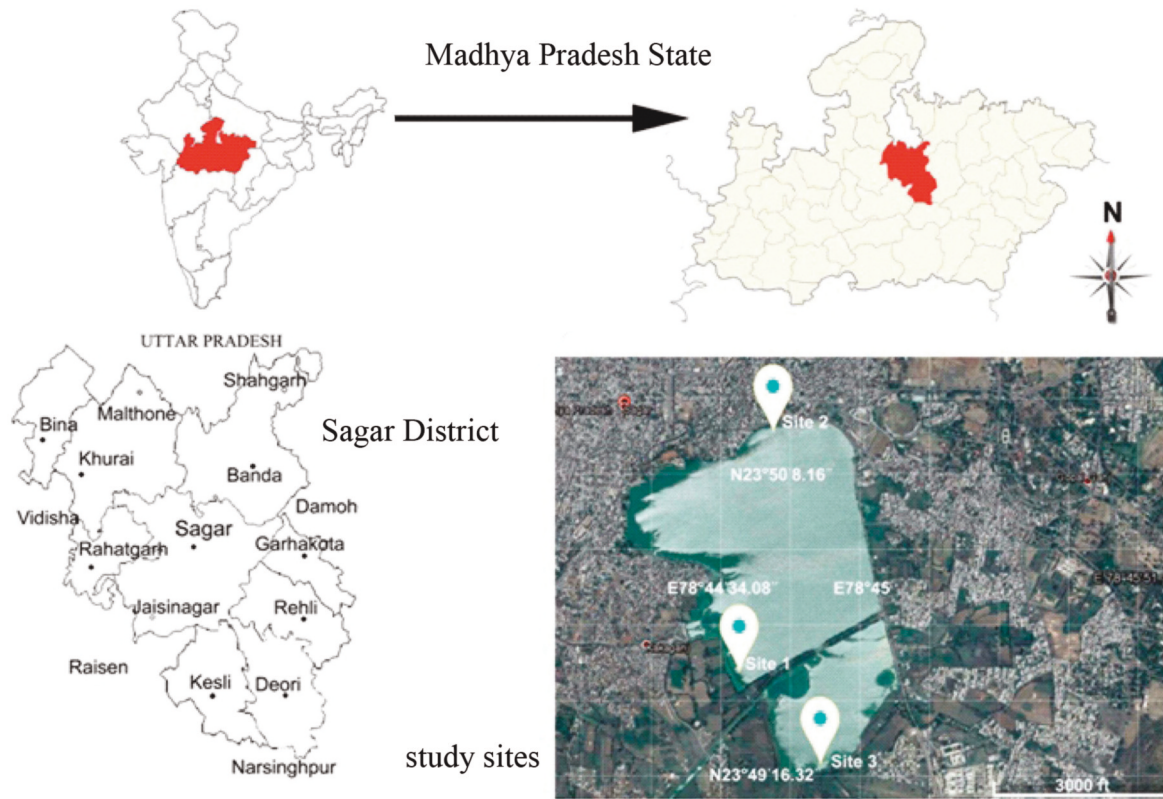


Figure 1. Map showing the locations of three different sampling sites of Sagar District, Madhya Pradesh State in India.

performed as per the standard methods of Adoni (1985) and APHA (2005). Turbidity (Turb), Dissolved Oxygen (DO), pH and temperature (Temp) were recorded onsite during collection of samples. pH, Temp and Total Dissolved Solids (TDS) were recorded with the help of digital meters. Turbidity was measured by Secchi disk method (Cialdi & Secchi, 1865). Water samples were taken directly from the sites into Biological Oxygen Demand (BOD) bottles for BOD and DO, and fixed instantly with manganese sulphate and alkaline iodide azide. They were analyzed immediately for DO and after five days for BOD as per Winkler's Modified method (APHA, 2005). Chemical Oxygen Demand (COD) was estimated by Close reflux method. Alkalinity (Alk) and Hardness (HN) were determined by Titrimetric method as per APHA (2005). Phosphate (Ph) and nitrate (N) were carried out by the Molybdophosphoric acid method and Brucine method respectively (APHA, 2005).

Cyanobacterial quantification

Samples were collected from each of three experimental sites and fixed with 1% Lugol's iodine solution (AR grade, 99.9% Merck) for cyanobacterial quantification. Serial dilutions were prepared for enumeration of Most Probable Number (MPN) of cyanobacteria (Buchanan & Fulmer, 1928) and tabulated.

Isolation of cyanobacteria

One ml of sample was added to agar plates made with 25 ml of sterilized BG-11 media (Rippka et al., 1979) and Chu No.-10 (Chu, 1942) in petri dishes and simultaneously one ml of sample was inoculated in 50 ml of sterilized BG-11 and Chu No.-10 broth media in flask. After inoculation samples were incubated for 45 days at 2500 lux light intensity for 16 hours and 8 hours of dark

interval at temperature 25 ± 2 °C. After 12 days of incubation, cyanobacterial colonies appeared on the agar plates and on broth media in flasks. Isolated species further spread on to fresh agar plates. After the development, colonies appearing in agar plates were examined microscopically and transferred to agar slants. This process was repeated until axenic cultures were obtained.

Microscopic analysis

Cyanobacterial species were observed under microscope for morphometric analyses. Camera lucida drawings were prepared and taxonomically important data such as trichome shape, filament color, akinetes and heterocyst shape, size, position and number were recorded. Identification of cyanobacteria was done using the keys given by Desikachary (1959) and Komarek & Anagnostidis. (1986; 1989).

Data analysis

Following formulae were applied for data analysis

Frequency of occurrence (FO)

$$FO = \frac{\text{Number of samples containing the species}}{\text{Total number of samples examined}} \times 100$$

Relative Frequency (RF)

$$RF = \frac{\text{Number of samples containing a species}}{\text{Total number of occurrence of all the species}} \times 100$$

Relative Density (RD)

$$RD = \frac{\text{Number of CFU of a species in all samples}}{\text{Total number of CFU all the species in all the samples}} \times 100$$

Relative Abundance (RA)

$$RA = \frac{\text{Number of samples containing the species}}{\text{Total number of occurrence of all the species}} \times 100$$

Diversity index- Shannon-Wiener diversity index (Shannon, 1948)

$$H = - \sum_{i=1}^S (P_i)(\ln P_i)$$

Where,

H - Shannon-Wiener diversity index

S - The number of species in the sample

P_i - The relative abundance of each group of organisms

N - Total number of individuals of all kinds

n_i - Number of individuals of ith species

Statistical analysis

The samples were analyzed in triplicates and a computer statistical software was used to calculate minimum and maximum mean with standard error. To understand the influence of seasonal physico-chemical properties of sampling sites on cyanobacterial diversity, correlation analyses and comparisons among them were performed using IBM SPSS-16.0 with level of significance maintained at 95% for each operation.

RESULTS

During the present investigation water samples were collected in three seasons i.e. Winter, Summer and Rainy season from three sewage sites associated with lentic water body. Cyanobacterial species were observed microscopically and further illustrated with the help of camera lucida. Taxonomical characteristics such as presence of heterocysts, akinetes, hormogonia and size of vegetative cells etc. were studied. During the study period, a total of 45 species from 24 different cyanobacterial genera were isolated (Table 1). Of these 45 species, 9 were unicellular, 4 non-heterocytous filamentous and 32 heterocytous filamentous forms.

Genera belonging to orders Chroococcales and Nostocales showed the highest relative abundance in all three sites. Relative abundance of *Hydrococcus rivularis* Kützinger, 1833 was exceptionally high at Site 3. Relative abundances of the two species of *Haplosiphon* were high at Site 1 and Site 2. The presence of *Chroococcus indicus* Zeller, 1873 was observed in all seasons at all three sites. *Aphanocapsa* spp., *Gleocapsa* spp. and *Phormidium* spp. were recorded at all sites in all seasons.

The pH is one of major characteristics which determine the growth of cyanobacteria (see Verma & Mohanty, 1995; Prasanna & Nayak, 2007). In all the study, pH of water was in the alkaline mean range of 7.50 to 8.50 in all seasons at all the sites shown in figure 1.

Generally speaking, water temperature plays an important role either in controlling the occurrence and abundance of phytoplankton (Nazneen, 1980) or in regulating the periodicity of cyanobacteria (Mahar et al., 2009). In this study, temperature values were minimum in rainy season at Site 1 with an

average of 19.7°C and maximum with an average of 29.7°C in summer season (Fig 2).

Maximum Turbidity of 39.8 NTU was recorded in rainy season at site 1 and minimum, 16.9 NTU, in summer at site 2. Turbidity is also a limiting factor of productivity because it affects light penetration (Semila Pushpam et al., 2014). Maximum TDS was recorded in pre-monsoon season with an average of 414 mg L⁻¹ at site 2 and minimum of 289 mg L⁻¹ at Site 1 (Fig 2). According to Goher (2002) TDS is a chemical constituent of water and contributes to productivity within water body. Due to high load of nutrients, an enhanced growth of cyanobacterial flora was noticed during pre and post-monsoon period. The high amount of TDS during pre-monsoon season might be due to the increase in the rate of evaporation. High concentration of TDS is an indication of nutrients enrichment leading to eutrophication (Gonzalves et al., 1946). Besides it, high level of alkalinity indicates the pollution level of surrounding of lentic water body. Among all sites, maximum alkalinity (461 mg L⁻¹) was recorded at Site 1 in winter season and minimum values (289 mg L⁻¹) at Site 2 in rainy season (Fig. 2). According to Solanki et al. (2010), decomposition of sewage materials coupled with mixing of garbage and industrial effluent increase the level of alkalinity in waste water bodies.

DO was lowest (3.30 mg L⁻¹) at Site 1 in winter season and highest (4.70 mg L⁻¹) in rainy season at Site 3 (Fig. 3). With an increase in water temperature, the DO was reduced in summer, whereas the DO was maximum during monsoon due to low temperature and increased mixing of waters. As per Central Pollution Control Board (CPCB), India (CPCB, 2010) threshold level of DO is 4.0 mg L⁻¹ for supporting aquatic lives. Very low DO indicates limited growth of aquatic flora, irrespectively of heavy load of nutrients.

The maximum value of BOD (22.28 mg L⁻¹) was recorded during summer at Site 3 and the minimum one (12.75 mg L⁻¹) at Site 2 in rainy season (Fig. 3). High BOD in summer could be due to high evaporation and elevated temperature coupled with effluent of organic pollution load and reduced water inflow.

Discharge of treated and untreated sewage and other waste into the water body led maximum COD value up to 49.22 mg L⁻¹ at Site 3 in winter and min-

imum, 28.35 mg L⁻¹, at Site 2 in summer (Fig. 3). According to Tiwari (2001) hardness of water, mainly due to presence of calcium and magnesium content, indicates water quality. Maximum hardness (178.44 mg L⁻¹) was recorded at Site 1 in summer season and minimum (64.9 mg L⁻¹) at Site 2 in rainy season.

According to Gupta & Dubey (2014) phosphate gets accumulated in sewage due to excessive use of detergent. Maximum of phosphate (0.40 mg L⁻¹) was estimated at Site 1 in rainy season and minimum (0.15 mg L⁻¹) at Site 3 in winter. The maximum nitrate value, 20.8 mg L⁻¹ at Site 3 in summer, can be attributed to effluent; whereas the minimum value (7.9 mg L⁻¹ at Site 1 in rainy season) might be due either to mixing of waters or biological nitrogen fixation by cyanobacteria. At site 1 a maximum mean value of cyanobacterial count (6650.3) in summer, and a minimum (1826.8) at site 2 in rainy season, were recorded. The minimum TN/TP ratio (8.1) was recorded in winter at site 2 and the maximum (20) at site 3 in summer. TN/TP ratio plays an important role in cyanobacterial diversity.

The correlation between the different physico-chemical parameters of Site 1, Site 2 and Site 3 is given in Tables 2–4. pH is positively correlated with TCC at all three sites. pH is statistically ($p < 0.01$) higher during summer season and no significant difference was noted among the sites. Temperature is the main factor influencing the species richness and diversity of phytoplankton. Temperature values showed variation among sampling sites. Statistically ($p < 0.01$), Site 1 temperature was higher than Site 2 and Site 3. Tables 2 to 4 show an inverse relationship between DO and temperature. Alkalinity showed significantly difference ($p < 0.01$) in pH within Site 1 and slightly differences were noted among the sites. BOD and nitrate show significant differences (Anova test, $p < 0.05$) with respect to the TCC during the seasons within sites. There was no significant difference ($p < 0.05$) in hardness considering the seasons and sampling sites. Anova at $p < 0.05$ shows significant differences in BOD and COD values during the seasons within the sites. COD shows significantly differences in the COD during the seasons within the sites. TCC shows significant differences at $p < 0.05$ within the sites during the seasons and shows significant differences with DO and COD during seasons within sites.

Agarkar (1998) and Nair (1999) reported variation in correlation of the physico-chemical parameters and phytoplankton. In our present study both heterocystous and nonheterocystous forms are found in wastewater, while Rai & Kumar (1976) did not find heterocystous cyanobacteria in polluted water. Our observations of presence of nonheterocystous genera such as *Oscillatoria*, *Phormidium*, *Gleocapsa* and *Chroococcus* are in line with previous results (Palmer, 1969; Ghadai

et al., 2010).

Diversity indices of different genera of cyanobacterial populations were calculated with the aid of Shannon Wiener index (see Table 4). Genus *Chroococcus* had the highest diversity index (8.30) at Site 3, while *Aphanothece* and *Anabaenopsis* had the same lowest diversity index (0.03) at Site 3. *Aphanocapsa*, *Anabaena*, *Anabaenopsis*, *Aulosira*, *Haplosiphon* and *Phormidium* showed diversity indices between 2 to 3.

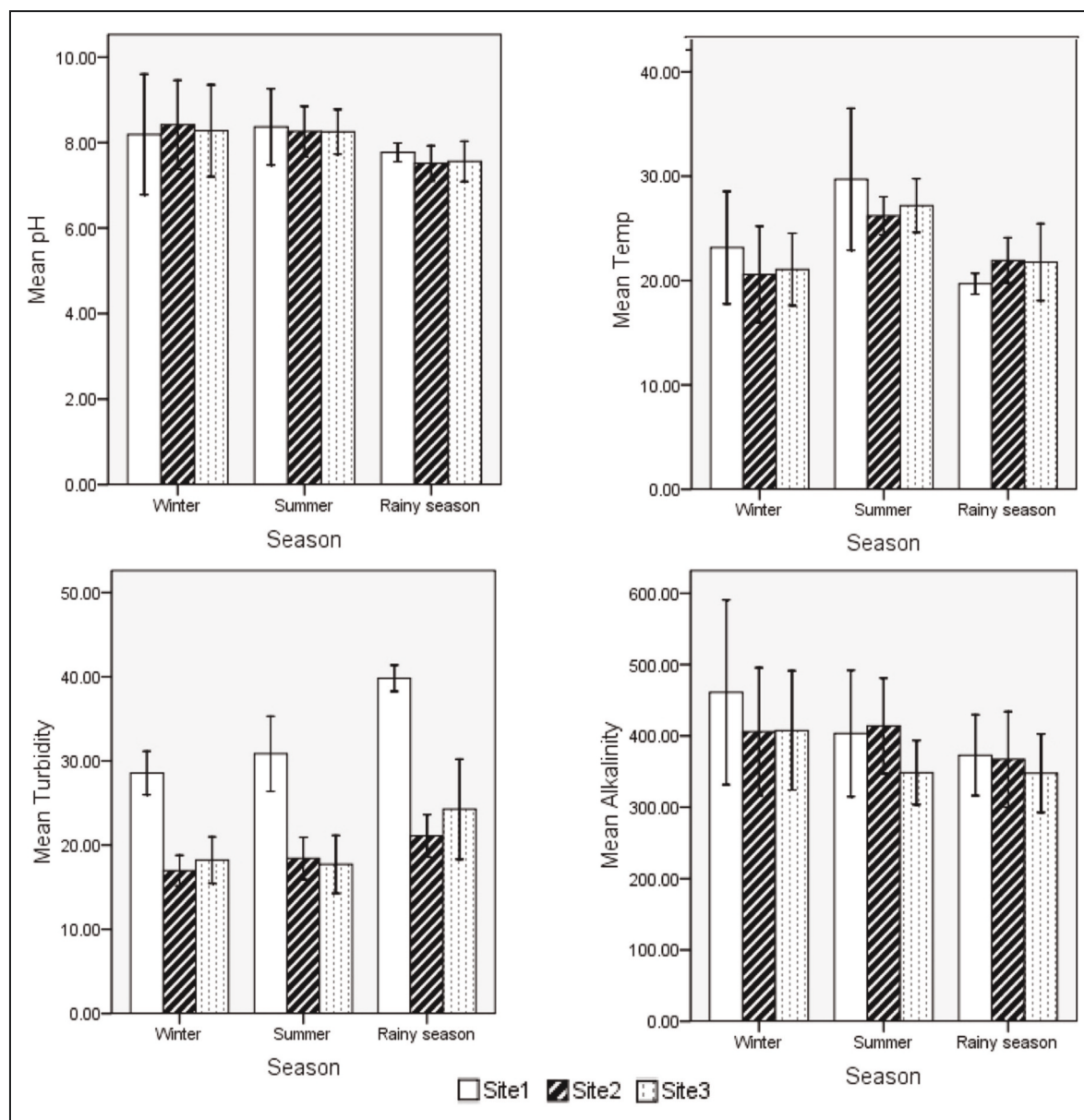


Figure 2. Seasonal variation of mean pH, Temperature, Turbidity and Alkalinity of three waste watersites.

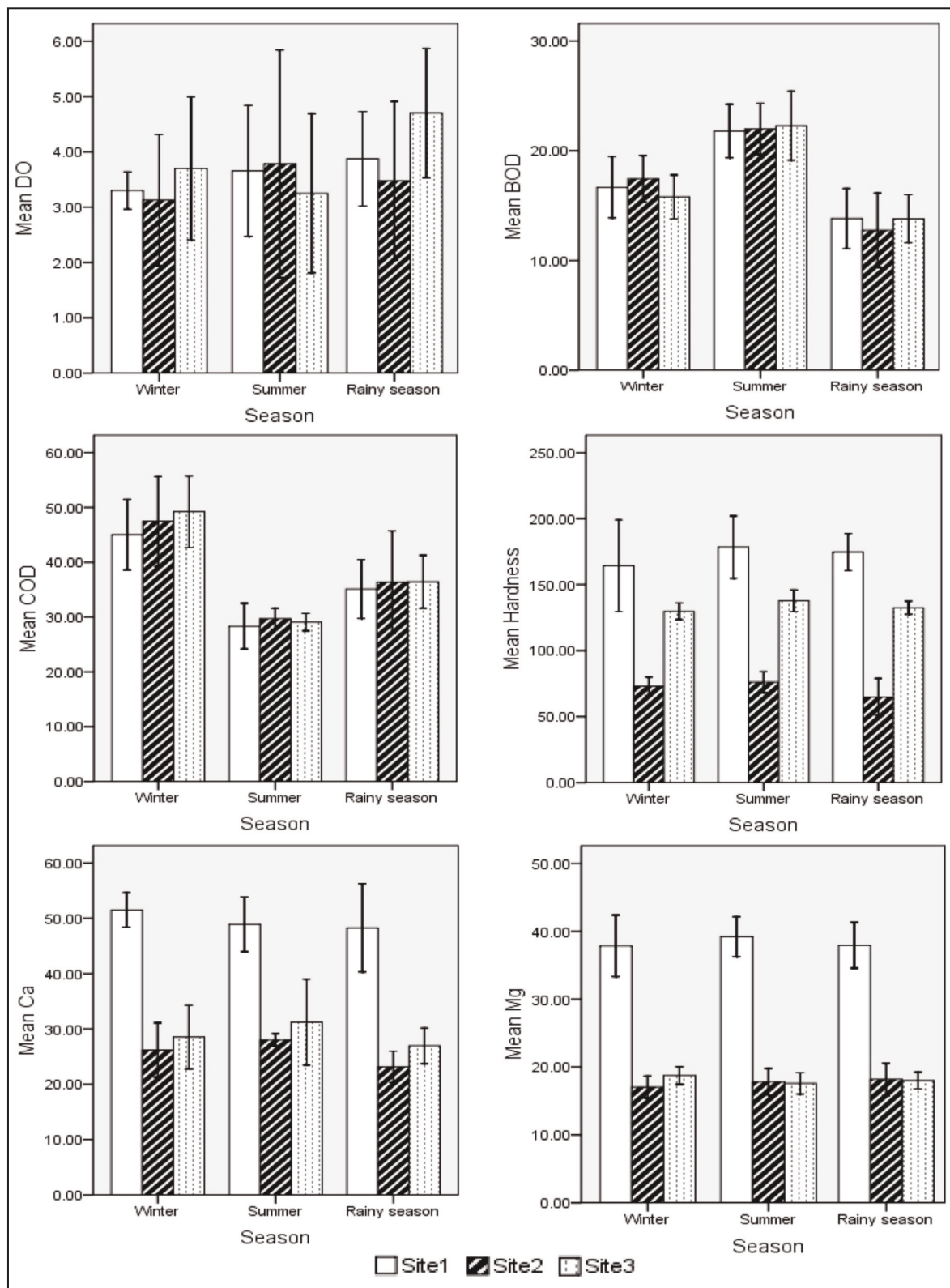


Figure 3. Seasonal variation of mean DO, BOD, COD, Total Hardness with Ca and Mg of three wastewater sites.

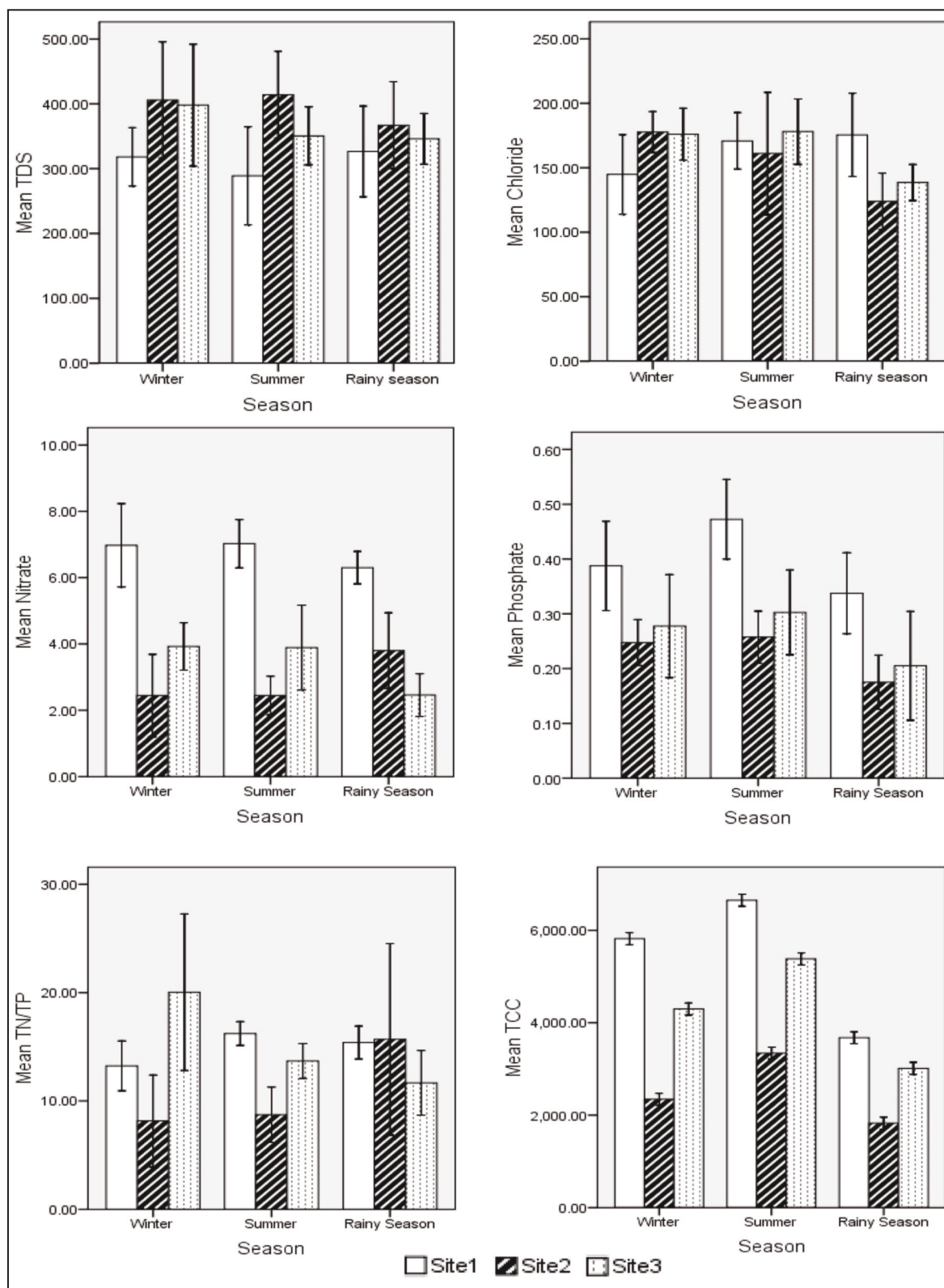


Figure 4. Seasonal variation of mean TDS, Chloride, Phosphate, Nitrate, TN/TP and Total cyanobacterial count (TCC) of three wastewater sites.

		SITE 1			SITE 2			SITE 3			SITE 1				SITE 2				SITE 3			
	Species	W	S	R	W	S	R	W	S	R	F	RF	RD	RA	F	RF	RD	RA	F	RF	RD	RA
1	<i>Anabaena azollae</i>	+	+	+	+	+	+	-	+	+	96.30	3.99	1.74	1.05	55.56	1.20	0.31	0.54	0	0.00	0.00	0.00
2	<i>Anabaenopsis arnoldii</i>	-	+	+	-	-	+	-	-	+	88.89	3.69	2.45	1.61	51.85	1.45	0.39	0.55	7	0.56	1.02	3.09
3	<i>Aphanocapsa biformis</i>	+	+	+	+	+	-	+	-	-	92.59	3.84	0.63	0.40	85.19	6.02	1.16	0.43	81	6.16	1.06	0.29
4	<i>Aphanocapsa koordersi</i>	+	+	+	+	+	-	+	-	-	25.93	1.08	0.32	0.72	18.52	1.69	0.85	1.04	30	2.24	0.42	0.32
5	<i>Aphanocapsa littoralis</i>	-	+	+	-	+	-	-	-	-	18.52	0.77	0.43	1.34	22.22	3.61	1.37	0.78	33	2.52	2.04	1.37
6	<i>Aphanothece microscopic</i>	+	+	-	+	+	-	+	-	-	29.63	1.23	0.47	0.93	25.93	3.37	2.59	1.59	26	1.96	3.69	3.18
7	<i>Aulosira fertilissima</i>	+	+	-	-	+	-	+	-	-	62.96	2.61	0.39	0.36	29.63	1.93	1.24	1.33	7	0.56	0.10	0.31
8	<i>Calothrix castellii</i>	+	+	+	-	+	-	-	+	-	66.67	2.76	0.80	0.70	7.41	0.48	0.38	1.64	4	0.28	0.48	2.88
9	<i>Calothrix marchica</i>	+	+	+	-	-	-	-	+	-	55.56	2.30	1.33	1.39	0.00	0.00	0.00	0.00	11	0.84	0.16	0.32
10	<i>Calothrix parietina</i>	+	+	-	-	-	+	-	+	-	62.96	2.61	0.41	0.38	3.70	0.24	0.35	3.03	11	0.84	0.44	0.88
11	<i>Chloroglea fritschii</i>	-	+	-	-	+	-	-	+	-	22.22	0.92	0.44	1.15	3.70	0.24	0.26	2.20	4	0.28	0.06	0.37
12	<i>Chroococcus disperses</i>	+	+	+	+	+	+	+	+	+	51.85	2.15	0.35	0.39	55.56	6.27	5.45	1.80	89	6.72	4.40	1.11
13	<i>Chroococcus indicus</i>	+	+	+	+	+	+	-	-	+	77.78	3.23	6.27	4.68	96.30	5.30	6.99	2.72	30	2.24	4.17	3.15
14	<i>Chroococcus micrococcus</i>	+	+	+	+	+	+	+	+	-	70.37	2.92	4.70	3.89	51.85	4.82	5.85	2.51	33	2.52	4.71	3.16
15	<i>Chroococcus minor</i>	+	+	+	-	+	+	+	-	-	70.37	2.92	5.50	4.54	59.26	3.37	5.81	3.56	26	1.96	5.96	5.15
16	<i>Chroococcus tenax</i>	+	+	+	-	+	+	-	+	+	96.30	3.99	5.14	3.10	59.26	3.86	2.48	1.33	30	2.24	6.97	5.26
17	<i>Chroococcus turgidus</i>	+	+	+	-	+	+	-	-	+	88.89	3.69	4.70	3.36	81.48	3.86	3.95	2.11	22	1.68	3.14	3.16
18	<i>Chroococcus varius</i>	+	+	+	-	+	+	+	+	+	92.59	3.84	12.53	7.87	74.07	2.89	7.44	5.32	63	4.76	1.88	0.67
19	<i>Gleocapsa atrata</i>	+	+	-	-	+	+	-	+	-	77.78	3.23	5.00	3.74	44.44	3.61	4.25	2.43	11	0.84	0.44	0.89
20	<i>Gleocapsa calcarea</i>	+	+	+	+	-	-	+	+	-	55.56	2.30	4.67	4.88	3.70	0.24	0.29	2.48	15	1.12	5.92	8.94
21	<i>Haplosiphon flagelliformis</i>	+	+	-	+	-	+	+	+	-	66.67	2.76	2.82	2.46	44.44	2.89	4.66	3.33	30	2.24	3.57	2.70
22	<i>Haplosiphon luteolus</i>	+	+	+	-	+	-	+	+	-	48.15	2.00	4.86	5.86	25.93	1.69	3.83	4.69	33	2.52	6.16	4.13
23	<i>Homoeothrix juliana</i>	+	+	+	-	+	-	+	+	-	59.26	2.46	2.51	2.46	33.33	0.24	0.39	3.31	15	1.12	2.38	3.60

Table 1. Diversity of cyanobacteria in three different wastewater sites.
For the explanation of the abbreviations see in the text.

		SITE 1			SITE 2			SITE 3			SITE 1				SITE 2				SITE 3			
	Species	W	S	R	W	S	R	W	S	R	F	RF	RD	RA	F	RF	RD	RA	F	RF	RD	RA
24	<i>Hydrococcus rivularis</i>	+	+	+	-	+	-	+	+	-	44.44	1.84	1.88	2.46	3.70	2.17	4.09	3.90	4	0.28	3.18	19.20
25	<i>Johannesbaptisia pellucida</i>	+	+	+	-	+	-	+	+	-	25.93	1.54	1.10	2.46	3.70	0.24	0.15	1.27	7	0.56	1.39	4.20
26	<i>Lyngbya aerugineo-coerulea</i>	+	+	+	+	-	+	+	+	-	33.33	1.38	1.41	2.46	66.67	4.34	0.72	0.34	33	2.52	1.79	1.20
27	<i>Lyngbya palmarum</i>	+	-	+	+	-	+	+	-	-	7.41	0.31	0.17	1.35	3.70	4.34	1.63	0.78	26	1.96	0.41	0.35
28	<i>Mastigocladus laminosus</i>	+	-	+	+	-	-	+	-	-	7.41	0.31	0.19	1.48	22.22	0.24	0.18	1.55	15	1.12	0.22	0.33
29	<i>Merismopedia glauca</i>	+	+	-	+	-	+	-	-	+	18.52	0.77	0.32	1.01	66.67	1.20	0.53	0.90	7	0.56	0.21	0.64
30	<i>Microcoleus chthonoplastes</i>	+	-	+	+	-	+	-	-	+	11.11	0.46	0.17	0.87	18.52	0.96	0.90	1.92	7	0.56	0.26	0.78
31	<i>Microspora tumidula</i>	+	+	-	-	-	+	-	-	+	7.41	0.31	0.20	1.59	14.81	1.45	0.30	0.43	7	0.56	0.24	0.72
32	<i>Myxosarcina burmensis</i>	+	-	-	-	-	+	-	-	-	3.70	0.15	0.15	2.35	3.70	0.24	0.37	3.15	0	0.00	0.00	0.00
33	<i>Nodularia spumigena</i>	+	+	+	+	-	-	-	-	+	40.74	1.69	1.57	2.24	14.81	0.96	0.78	1.67	30	2.24	1.99	1.50
34	<i>Nostoc calcicola</i>	+	+	+	+	-	-	-	+	+	62.96	2.61	2.66	2.46	25.93	1.69	2.84	3.47	63	4.76	3.37	1.20
35	<i>Nostoc carneum</i>	+	+	+	+	-	+	-	+	+	59.26	2.46	2.51	2.46	48.15	3.13	4.29	2.83	67	5.04	3.18	1.07
36	<i>Nostoc linckia</i>	+	+	+	+	-	+	-	+	+	51.85	2.15	2.19	2.46	55.56	3.61	4.54	2.60	52	3.92	2.78	1.20
37	<i>Nostoc paludosum</i>	+	+	+	-	-	+	-	+	-	40.74	1.69	1.72	2.46	44.44	2.89	1.03	0.74	33	2.52	2.18	1.47
38	<i>Nostoc spongiaeformae</i>	+	+	+	-	+	-	+	+	-	66.67	2.76	3.29	2.87	18.52	1.20	0.81	1.39	59	4.48	4.17	1.57
39	<i>Oscillatoria angusta</i>	+	+	+	+	-	-	+	+	-	62.96	2.61	2.04	0.19	3.70	0.24	0.18	1.52	30	2.24	2.58	1.95
40	<i>Oscillatoria tenuis</i>	+	+	-	-	-	+	+	+	-	48.15	2.00	1.88	2.27	11.11	1.45	2.62	3.75	30	2.24	1.59	1.20
41	<i>Oscillatoria willei</i>	+	+	-	-	+	-	-	+	-	55.56	2.30	1.25	1.31	22.22	0.72	1.69	4.84	26	1.96	2.38	2.06
42	<i>Phormidium dimorphum</i>	+	+	-	+	+	-	-	+	-	66.67	2.76	1.74	1.52	40.74	2.65	2.69	2.10	33	2.52	2.20	1.48
43	<i>Phormidium jenkelianum</i>	+	+	+	-	+	-	+	+	+	96.30	3.99	2.33	1.41	33.33	2.17	1.44	1.38	96	7.28	2.96	0.69
44	<i>Phormidium molle</i>	+	+	+	+	+	+	-	+	-	66.67	2.76	2.02	1.76	40.74	0.72	1.82	5.19	56	4.20	0.79	0.32
45	<i>Phormidium purpurascens</i>	+	+	+	-	+	-	+	+	+	40.74	1.69	0.63	0.89	11.11	2.65	4.86	3.79	30	2.24	2.56	1.93

Table 1. Diversity of cyanobacteria in three different waste watersites.
For the explanation of the abbreviations see in the text.

	pH	Temp	Turb	Alk	TDS	DO	BOD	COD	HN	Ca	Mg	N	Chl	Ph	TCC	TNIP
pH	1															
Temp	0.783**	1														
Turb	-0.129	-0.279	1													
Alk	0.749**	0.453	-0.369	1												
TDS	-0.226	-0.413	0.070	-0.034	1											
DO	-0.346	-0.416	0.155	-0.115	0.333	1										
BOD	0.250	0.669*	-0.550	0.165	-0.306	-0.429	1									
COD	-0.236	-0.358	-0.139	-0.069	0.238	0.073	-0.519**	1								
HN	0.192	0.077	0.146	-0.193	0.194	-0.285	0.169	-0.399**	1							
Ca	-0.293	-0.188	-0.173	-0.099	0.503	0.331	-0.207	0.564	-0.494	1						
Mg	0.171	0.259	-0.111	0.206	-0.238	-0.171	0.314	-0.111	-0.395	0.306	1					
N	0.533	0.724**	-0.710**	0.450	-0.215	-0.492	0.791**	-0.176	0.076	0.083	0.480	1				
Chl	-0.134	0.081	0.181	-0.431	0.185	0.257	0.230	-0.444	0.341	-0.159	-0.094	-0.108	1			
Ph	-0.580*	-0.574	0.217	-0.502	0.194	0.193	-0.426	0.442	-0.057	0.074	-0.443	-0.508	0.177	1		
TCC	0.296	0.644*	-0.839**	0.231	-0.242	-0.233	0.749**	-0.130	-0.018	0.121	0.134	0.826**	0.038	-0.365	1	
TNIP	-0.018	-0.476	0.260	0.284	0.380	0.253	-0.582*	0.494	-0.110	0.071	-0.069	-0.391	-0.300	0.333	-0.647*	1

Table 2. Correlation between the physico-chemical parameters of Site 1. For abbreviations see in the text.

	pH	Temp	Turb	Alk	TDS	DO	BOD	COD	HN	Ca	Mg	N	Chl	Ph	TCC	TNIP
pH	1															
Temp	0.434	1														
Turb	-0.428	0.125	1													
Alk	0.696*	0.262	-0.399	1												
TDS	0.129	-0.394	0.047	-0.047	1											
DO	0.099	0.206	-0.465	0.457	-0.258	1										
BOD	0.657*	0.551	-0.437	0.489	-0.239	0.037	1									
COD	-0.177	-0.785**	-0.356	-0.194	0.160	-0.138	-0.342	1								
HN	0.156	0.189	-0.294	0.341	-0.158	-0.030	0.434	-0.051	1							
Ca	0.381	-0.058	-0.592*	0.386	0.021	0.171	0.500	0.167	0.069	1						
Mg	-0.358	-0.251	0.110	0.079	-0.464	-0.180	0.009	0.226	0.180	0.046	1					
N	0.773**	0.453	0.535	0.267	0.002	0.009	0.779**	-0.128	0.204	0.409	-0.483	1				
Chl	0.644*	0.088	0.458	0.394	0.024	-0.015	0.337	0.247	0.145	0.397	-0.207	0.635*	1			
Ph	-0.194	-0.159	0.578*	-0.223	0.408	-0.633*	-0.393	-0.132	0.062	-0.313	0.091	-0.458	-0.331	1		
TCC	0.326	0.526	-0.412	0.241	-0.426	0.138	0.801**	-0.391	0.409	0.547	-0.032	0.650*	0.359	-0.455	1	
TNIP	-0.033	-0.344	0.122	0.270	-0.152	0.105	-0.322	0.276	-0.368	-0.276	0.474	-0.402	0.070	-0.083	-0.499	1

Table 2. Correlation between the physico-chemical parameters of Site 2. For abbreviations see in the text.

	pH	Temp	Turb	Alk	TDS	DO	BOD	COD	HN	Ca	Mg	N	Chl	Ph	TCC	TNIP
pH	1															
Temp	0.503	1														
Turb	-0.339	-0.145	1													
Alk	0.556	-0.110	-0.292	1												
TDS	0.077	-0.027	0.161	-0.213	1											
DO	-0.376	-0.429	0.459	-0.377	0.026	1										
BOD	0.489	0.572	-0.567	0.090	0.032	-0.294	1									
COD	-0.089	-0.652*	-0.090	0.322	-0.403	0.076	-0.578*	1								
HN	0.109	0.737**	-0.080	-0.251	-0.087	-0.377	0.256	-0.581*	1							
Ca	0.635*	0.684*	-0.258	0.316	0.077	-0.790**	0.321	-0.283	0.625*	1						
Mg	-0.432	-0.468	-0.048	0.065	-0.618*	0.420	-0.294	0.546	-0.331	-0.643*	1					
N	0.675*	0.499	-0.691*	0.314	-0.148	-0.534	0.838**	-0.172	0.177	0.453	-0.235	1				
Chl	0.698*	0.258	-0.590*	0.503	0.110	-0.609*	0.648*	-0.046	-0.070	0.462	-0.424	0.879**	1			
Ph	0.332	0.576*	0.252	0.082	0.344	-0.182	0.176	-0.553	0.464	0.592*	-0.502	-0.077	-0.075	1		
TCC	0.405	0.533	-0.710**	0.015	-0.309	-0.401	0.814**	-0.280	0.298	-0.111	0.884**	0.624	-0.235	-0.132*	1	
TNIP	0.085	-0.309	0.144	0.057	0.486	0.194	0.019	-0.199	-0.201	-0.113	-0.276	-0.164	-0.007	-0.180	0.402	1

Table 2. Correlation between the physico-chemical parameters of Site 2. For abbreviations see in the text.

DISCUSSION AND CONCLUSIONS

Sagar lake had become hypertrophic due to unbalanced physical and chemical factors (Vaishya & Adoni, 1993) which raised the trophic level of water body. Cyanobacteria are important primary producers in food web in many aquatic environments. The present study reveals that the physico-chemical characteristics of wastewater determine the growth and diversity of cyanobacteria. Species belonging to the genera *Chroococcus*, *Gleocapsa*, *Haploisiphon* and *Phormidium* were dominant at all sites. These taxa are adapted to flourish under stress environment and are able to utilize high load of nutrients and immobilize pollutants. Our observation on presence of *Anabaena* spp., *Oscillatoria* spp. and *Nostoc* spp. in wastewater is in line with the findings of Deep et al. (2013). *Aphanocapsa* and *Anabaena* were found to be very frequent, which suggests their potential to exploit sewage waste. Availability of nitrate and phosphorus nutrients at Site 3 can justify the highest diversity of species. Increased nutrients such as nitrate, phosphate, chloride and temperature accelerated the growth of cy-

anobacteria. In fact, different physico-chemical properties effect relative frequency, relative density, relative abundance and occurrence of cyanobacteria. Species can tolerate fluctuation of available resources, predation and high load of chemical contaminants. Cyanobacterial flora of wastewaters should be defined genotypically and metabolically in their natural microbial community and anthropogenic stressed environment. High pH values accelerate the pollution rate in lake. pH 6-8.5 is ideal for planktonic growth (Veerendra et al., 2008). In present study pH 7-8 increased the growth of cyanobacteria in all 3 sites. Trophic level of water rises due to high alkalinity (Kumar & Sharma, 1991) and it favours abundance of cyanobacteria (Nandan et al., 2002). Alkalinity was high in summer at site 3 which induced the growth of cyanobacteria (Tiwari & Shukla, 2007); but other nutrients limited the growth as compared to the other two sites. High turbidity influences primary productivity because it affects the penetration of light in water body, moreover, causing particles to absorb phosphate, nitrogen and potassium in ionic form, turbidity limit the growth of phytoplankton

(Pandey et al., 1999). Phosphorus and nitrogen are limiting factors for the growth of cyanobacteria (Lapointe, 1989; Larned, 1998; Russ & McCook, 1999). Increasing nutrients availability at Site 1 in summer season resulted in a better growth of cyanobacteria (Miller et al., 1999). Agricultural runoff and domestic sewage from catchment area increase the phosphate level in Site 1, 2 and 3 during summer. These results imply that biodiversity of cyanobacteria was driven by local environmental factors such as temperature, pH, DO, nitrate and phosphorus contents.

Physico-chemical parameters and biological monitoring together provided evidence of evolution of microbes of polluted habitats. These species are stress tolerant, so they easily grow on these environments and can be further deployed for bioremediation and carbon sequestration purposes. Bloom forming species were also encountered near cultivated land and freshwater lake, thereby it is to be considered a threat for aquatic flora and fauna. The discharge of untreated wastewater nearby the lake area and agricultural land should be immediately stopped.

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