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Dominant cyanobacterial flora of the Religious ponds at Holy Geeta's birthplace, Kurukshetra, India

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ABSTRACT:

The cyanobacterial biodiversity analysis from different fresh water of holy aquatic bodies was conducted during summer (May-June, 2009) and autumn months (Aug.-Sept., 2009) in the village Jyotisar of Kurukshetra, Haryana, India. Similar studies were performed during the same periods of next year (2010). This place is popularly known as the birthplace of holy ***'Bhagwat Geeta'*** (=***'Geeta's Janmsthal'***), which is geographically located at Lat. $29^0$57'53.54"N; Long. $76^0$49'07.56"E. There are mainly two ponds situated there viz. Pond-1 and Pond-2. Both of them were analyzed for their physicochemical and biological properties. Our observations have revealed various noticeable variations recorded in the cyanobacterial flora according to seasonal and environmental variations among the two ancient ponds. Unicellular cyanobacterial strains dominated the Pond-1, where people from all over India take holy dip. Contrary to this, Pond-2 was dominated by mainly filamentous cyanobacteria along with massive growth of higher aquatic plants like *Nelumbo*. The entire microbial community was dominated chiefly by cyanobacteria and diatoms.

Keywords:
Cyanobacterial biodiversity, physicochemical properties, Kurukshetra, 'Geeta' Birthplace'.

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INTRODUCTION

Though, the persistence and stability of various organisms in fresh water ecosystems has been well documented (Scarsbrook, 2002; Soininen, *et al*., 2004) yet, cyanobacteria need particular attention owing to their habit of forming blooms up to extremes throughout the summer in tropical countries like India (Pearl, *et al*., 2001). The taxonomic account of the freshwater algae (mainly Cyanophyta, Bacillariophyta and Chlorophyta) collected from the tank of *'Ekling Ji'* temple at Udaipur; Rajasthan, India has also been well documented (Pandey *et al*., 2004).

The present study site is mainly centered at the northern part of India at the ancient and historical place called *'Jyotisar'* at Dist. Kurukshetra, Haryana, India (**PLATE-I**, actual view & habitat of the study site). It is the birth place of holy *'Geeta'*, where Lord '*Krishna'* preached the lesson '*Geeta'* (the holy epic of Hindus) to *'Arjuna'* during the war of *'Mahabharata'* and later became famous as the *'Geeta Updesh Sthal'*. Its geographical location is $29^0$57'53.54"N Latitude and $76^0$49'07.56"E Longitude (PLATE-I, satellite view). This place is presently a village called '*Jyotisar*', which is about 5 Km from Kurukshetra University. It is believed that the same live holy *'Akshya Vat Wriksha'* (the immortal *Ficus bengalensis* tree) is still present here since the time of *'Mahabharata'*. There are mainly two ponds here. Pond-1 is used mainly for religious purpose, while the 2nd one is from ecological point of view; alternatively it is also used for either recreation purposes, lying idle or occasionally for *Nelumbo* cultivation by Govt. Agencies. Both the ponds are interconnected via a small bridge. This non-religious adjacent pond is the breeding grounds for some higher genera like *Eichhornia, Hydrilla and Nelumbo* etc. The preliminary studies on the biological diversity of cyanobacterial flora in this area have inspired to go for the present investigation (Biban, *et al*., 2010). The cyanobacterial biodiversity of this study site was enumerated during two seasons: summer (May-June 2009) and autumn months (Aug.-Sept. 2009). It was repeated during the same periods of next year, 2010 (**PLATE-II & III**). Similar work has been done on various aquatic habitats of India and in other countries (Pingale, *et al*., 2005; Samad, *et al*., 2008; Macedo, *et al*., 2009), but an ancient place of such historical importance has yet to be touched upon. Therefore, the study site is an interesting religious place to work on the studies of cyanobacterial and other algal flora. The interest towards the study of the cyanobacterial communities present in various water reservoirs of the ancient places at Kurukshetra, fuelled to enumerate the richness in their biodiversity among the holy ponds. Such freshwater habitats are the breeding grounds for various cyanobacterial blooms and diatoms. However their population fluctuates according to the seasonal variations in summer, rainy, autumn and winter seasons. The changes in biomass of N_2-fixing cyanobacteria and density of heterocysts are strongly coupled with the depletion of dissolved inorganic N or N-limitation with high irradiance and high N_2- fixation rates (Phlips, *et al*., 1997). Hence the present work was aimed to understand the biological diversity of cyanobacteria of the two major fresh water ponds at *Jyotisar* and other nearby ancient areas of Kurukshetra for exploring their innate potentials; because such aquatic systems also receive rain water, run off of surrounding agricultural land containing the agrochemicals (like pesticides), affect the algal biodiversity of the pond by ameliorating their ecophysiology (Kalia, *et al*., 2011).

MATERIALS AND METHODS

Study area and sample collection

The observations and sampling was done twice in the calendar year of 2009 and 2010 during summer season, as well as, after the Monsoon rains in autumn season with a regular interval of fortnight. There are several other natural and artificial fresh water ponds distributed in and around Kurukshetra. They are dominated with various seasonal algal blooms. Planktonic cyanobacterial samples were collected from the epilimnion at the two different fresh water ponds of Jyotisar in the month of May-June and Aug-Sept of the year 2009 and 2010 from the two aquatic bodies of *'Jyotisar'* using plastic plankton nets (0.1mm pore size), forceps and knifes. The collected cyanobacterial samples were isolated and transferred to conical flasks with Allen and Arnon's nitrogen free medium (AA^- medium) to grow them in a culture room (24±1^0C) with a16/8 h light/dark cycle and 14.4 Watt/m^2 cool fluorescent light on the surface of the culture vessels for further ecophysiological studies (Allen, *et al*., 1955). The AA^- medium was found to be most suitable to isolate and maintain the strains like *Anabaena, Nostoc* and *Merismopedia* etc. However, *Chlorella, Vaucheria* and *Cladophora* were later maintained in Chu-10 liquid medium (Chu, 1942). The traditional taxonomic criteria based on the morphological characteristics were used for their identification (Desikachary, 1959).

All chemicals used were of analytical reagent grade and prepared in distilled water. A single beam visible spectrophotometer (Electronics India Ltd., India) was used for measuring absorption data.

Parameters studied

a) Temperature measurement: It was measured using a wet thermometer at 30 cm depth of the ponds.

b) pH measurement: The approximate pH was estimated on the spot in the water of both the ponds using pH paper and later verified through a digital pH meter (pH range: 0-14; Electronics India Ltd., India).

c) Transparency estimation: The transparency of aquatic bodies was measured with the help of Secchi disc (20 cm in diameter) pointed alternating black and white.

d) Protein estimation: Protein was quantified as µg protein/ml algal suspension (Lowry, *et al*., 1951; Herbert, *et al*., 1971).

e) Ammonium estimation: Ammonium as NH_4Cl (BDH, U.K.) was estimated calorimetrically (sensitivity range, 0.04-5.0 µg NH_4^+/ml algal suspension) using Nessler's reagent (Burris, *et al*., 1957).

f) Nitrate estimation: NO_3^- concentration in the water of two ponds was measured using conventional colorimetric method (sensitivity range, 10-100 µg NO_3^-/ml algal suspension) using 4% Brucine (SISCO, India) in Chloroform (BDH, India) and concentrated H_2SO_4 (Qualigens, India) (Nicholas, *et al*., 1957).

3. Microphotography: Microphotography was carried out using a microscope (Make: Suswox Optik, India), an electronic camera (Make: Nikon, Model No. Coolpix S4000, Japan) and printed with the help of a laser printer (Make: Canon, model No. LBP-1210, Japan) connected to a computer.

PLATE -I. (i) Satellite view of the study sites:

Geographical location: Lat. $29^0$57'53.54"N; Long. $76^0$49'07.56"E.

(ii) Actual view of the site and their Habitat:

Pond -1 (Religious Pond - Actual worship and pilgrimage area):	**Pond -2 (Non-religious Pond):**

RESULT AND DISCUSSION

The cyanobacterial as well as algal flora were observed as being attached not only to stairs of Pond-I but also in the free floating zones of both the ponds (PLATE-II). Unicellular cyanobacterial strains along with few filamentous genera dominated the Pond-1, where people from all over India take holy dip (PLATE-I, Pond-1). During the summer sampling time of May-June 2009, the dominant cyanobacterial floras were the unicellular forms like *Synechocystis, Merismopedia, Microcystis* and *Chroococcus* and Desmids. In this pond (Pond-1), low diversity of cyanobacteria (May -June 2010) was attributed to a massive bloom of *Microcystis,* which imparted significant ameliorating impacts on other algal populations. During summer season, condensed thick populations of cyanobacterial blooms as observed, were due to the higher concentrations of nutrients like NH_4^+ and NO_3^- getting increased and pH level being reduced, leading to the eutrophication of such aquatic bodies (**Table-1 & 2**). On the other hand, during/after monsoon season when the samples were collected in Aug.-Sept. (autumn season) in both the calendar years, the cyanobacterial blooms revealed less populations among the entire algal community at the study sites. During the sampling period (Aug-Sept 2009), the dominant cyanobacterial flora were the filamentous forms like *Oscillatoria***,** *Anabaena, Lyngbya* etc. and the algae other than the cyanobacteria were predominantly the *Chara, Nitella* and *Coleochaete* on *Eichhornia* growing either submerged or on other higher aquatic plants. Other genera like *Aphanothece, Merismopedia, Oscillatoria* and *Lyngbya* were observed in lesser populations, from Pond-1 which has been attributed to the high pH and lesser bioavailability of the nitrogenous nutrients like NH_4^+ and NO_3^-. Consequently, the protein level of collected cyanobacterial bloom samples also varied

Table No.-1 (POND-1: Religious Pond):

PARAMETERS	Year 2009		Year 2010	
	May-June	August-September	May-June	August-September
Temperature (°C)	30.0±0.8-32.0±0.9	28.0±1.0-30.0±0.9	27.0±0.8-31.0±0.8	27.0±0.9-29.0±0.8
pH range	5.8-7.4	7.5-8.4	5.9-7.4	7.6-8.4
Transparency (cm)	50.2±0.7-52.2±0.8	30.1±0.8-34.0±0.8	51.0±0.9-53.5±0.9	32.2±0.9-35.7±0.8
Protein (µg protein/ml algal suspension)	0.11±0.4-0.14±0.3	0.16±0.5-0.19±0.8	0.10±0.3-0.15±0.7	0.17±0.7-0.20±0.8
NH_4^+ (µg NH_4^+/ml algal suspension)	2.5±0.4-4.0±0.3	1.5±0.5-3.0±0.4	3.5±0.3-4.5±0.4	1.4±0.2-2.8±0.2
NO_3^- (µg NO_3^-/ml algal suspension)	31.0±0.8-35.0±0.7	24.0±0.8-28.0±0.8	32.0±0.8-37.0±0.7	26.0±0.6-29.0±0.8

Values are mean ± 3SD.

Table No.-2 (POND-2: Non-religious Pond):

PARAMETERS	Year 2009		Year 2010	
	May-June	August-September	May-June	August-September
Temperature (°C)	28.0±0.8-31.0±0.9	29.0±0.8-30.0±0.8	29.0±0.7-32.0±0.8	28.0±0.9-29.0±0.8
pH range	6.0-7.0	7.5-9.5	5.5-7.5	7.5-9.0
Transparency (cm)	45.4±0.6-49.2±0.5	25.3±0.7-29.1±0.8	16.2±0.6-19.4±0.2	23.1±0.8-27.3±0.9
Protein (µg protein/ml algal suspension)	0.13±0.1-0.17±0.1	0.18±0.1-0.21±0.1	0.12±0.1-0.16±0.1	0.19±0.1-0.22±0.1
NH_4^+ (µg NH_4^+/ml algal suspension)	2.0±0.3-3.5±0.5	1.4±0.3-2.5±0.4	2.5±0.4-4.0±0.5	1.2±0.2-2.3±0.5
NO_3^- (µg NO_3^- /ml algal suspension)	30.0±0.8-34.0±0.8	23.0±0.5-27.0±0.9	31.0±0.8-36.0±0.6	25.0±0.8-29.0±0.9

Values are mean ± 3SD.

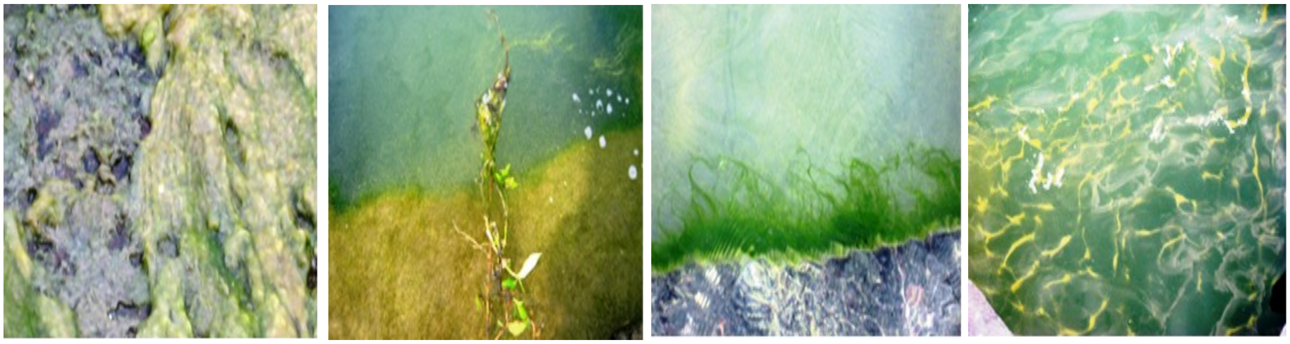


PLATE -II. Cyanobacterial and algal flora attached on the stairs of Pond-1 and in the free floating zone of Pond-1 & 2

according to the seasonal variations (Smith, *et al*., 1999).

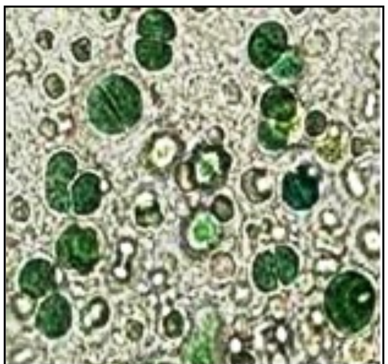
At Pond-1, the high temperatures (30-32^{0}C), low pH (5.8-7.4), high transparency (50.2 to 52.2 cm), lower NH_4^+ (2.5-4.0 µg NH_4^+/ml algal suspension) and NO_3^- levels (31.0-35.0 µg NO_3^-/ml algal suspension) in the year 2009 favoured the growth of unicellular cyanobacteria viz; *Microcystis* blooms, *Merismopedia, Synechocystis, Chroococcus* and *Aphanothece* along with desmids like *Pinnularia, Nitzschia, Nedium, and Navicula* (Table-1; PLATE-III [A]). However, the pattern of cyanobacterial biodiversity was different at Pond-2 where higher aquatic macrophytes like *Eichhornia, Hydrilla, Azolla* and *Nelumbo* etc. dominated the aquatic body. Prominent cyanobacterial blooms of *Microcystis* were recorded during the summer months of both the calendar years (2009 & 2010).

As evident (Table-2) on comparative grounds, during the summer months of 2009, except NO_3^- levels, the temperature, transparency and NH_4^+ were towards lower side. This favoured immense growth of filamentous cyanobacteria like *Oscillatoria, Lyngbya* and *Anabaena* (Table-2; PLATE-III [B]). During the same period in next year 2010, the temperature was comparatively low at Pond-1 with higher pH than that in year 2009, but at Pond-2; it was just a reversed case (Table-1 & 2). It was interesting to note a marked decrease in the transparency from (51.0 - 53.5) to (16.2 - 19.4) in Pond-2 as compared to Pond-1. This favoured to record higher total protein content per ml algal suspension in pond-2 than that of Pond-1, yet, the NH_4^+ and NO_3^- levels of the algal suspensions were towards higher side in Pond-2. A corollary of such observations favoured much better growth of almost all phytoplanktons in the Pond-2. In the post -monsoon season of the year (2009) which was approximately the same for the same period in Pond -2 also. However the pH range and transparency were recorded towards lower sides in both years for both ponds, which favoured higher protein value of algal mass in pond-2 than pond-1. If one looks into the nitrogen balance of pond systems, the NH_4^+ & NO_3^- levels at Pond-1 were lower than that of Pond-2. On comparative grounds in pond-1 & 2, there was an increasing trend observed from the year 2009 to 2010 with respect to NO_3^- levels. While sampling in Aug-Sept 2009, the dominant cyanobacterial flora were the filamentous forms like *Oscillatoria*, *Anabaena, Lyngbya* etc. and the algae other than the cyanobacteria were predominantly the *Chara, Nitella* and *Coleochaete* on *Eichhornia* and other higher aquatic plants. The stairs get slippery due to the growth of organisms like *Aphanothece.* It was also strange to observe the balls of *Anabaena iyengerii* on the leaves of *Nelumbo* in Aug 2010. Six species of the cyanobacteria viz., *Microcystis, Merismopedia, Aphanothece*, *Oscillatoria* and *Lyngbya* were found in the 1st pond (the religious one), whereas 2nd pond (the non-religious one), was mainly dominated by the filamentous cyanobacterial species like *Oscillatoria, Anabaena* and *Lyngbya*. However, in comparison the unicellular forms like *Chroococcus, Chlorella, Synechocystis and Aphanothece* were also detected in smaller strength.

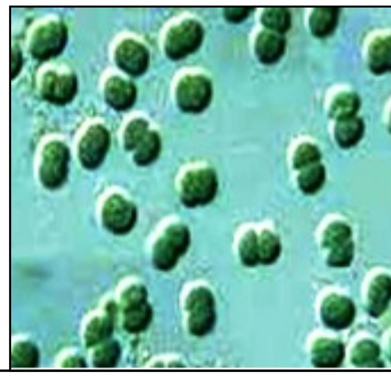
As per the diversity and abundance of cyanobacteria, the members of the family Oscillatoriaceae were dominating in the surveyed ponds. However, the *Diatoms* were in abundance in both ponds together with some other algae like *Chara, Nitella, Coleochaete, Chlorella,* and *Batrachospermum* (data not presented here). Similarly, there was a clear cut variation in other parameters studied (Table-1 and Table-2) for the Pond-1 and Pond-2 respectively. In May-June of both the calendar years (2009 & 2010), the nutrient concentrations get increased at both study sites due to the availability of lesser quantity of water owing to evaporation under hot climatic conditions (temperature ranges, 40-45^{0}C). The transparency of the two ponds was found high during this time period. Such aspects ultimately lead to the eutrophication among the two aquatic bodies.

In any ecosystem, not a single species grows independently and indefinitely, because all the species are interlinked and has cyclic transformation of nutrients according to prevailing environmental conditions. The physicochemical changes in the environment not only induce a particular species but the entire biota. It considerably affects the growth and abundance of other species, which leads to the succession of several species in a course of time. Though, the observations and sample collections were made on a regular basis throughout the year, but the data has been depicted as summer and autumn seasons. It has been observed that the cyanobacterial dominance often occurs when water temperature rises above 20^{0}C, when there is depletion of dissolved inorganic N and free CO_2 from the water (Table-1 & 2). It is reported that low nitrogen to phosphorus ratios favour the dominance and production of high blue green algal biomass in similar aquatic bodies (Smith, 1983). In shallow, mixed water bodies with high total phosphate content organisms like *Oscillatoria* dominates,

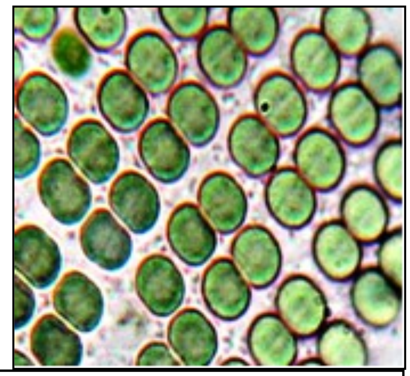
PLATE -III. SAMPLES COLLECTED: [A] Unicellular Cyanobacterial Strains



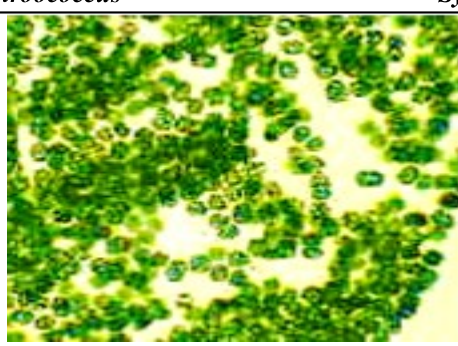
Chroococcus



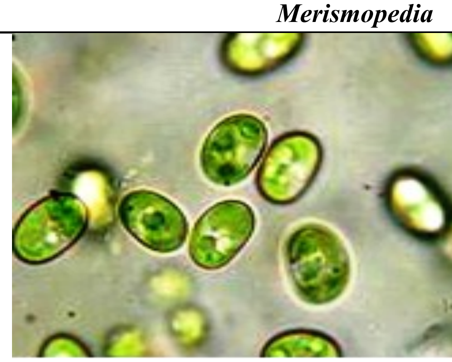
Synechocystis



Merismopedia

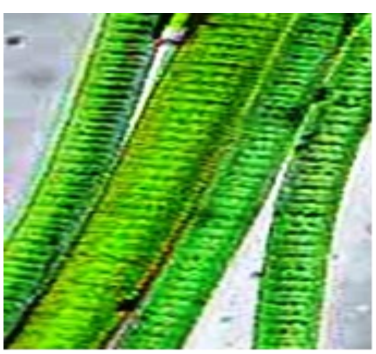


Microcystis

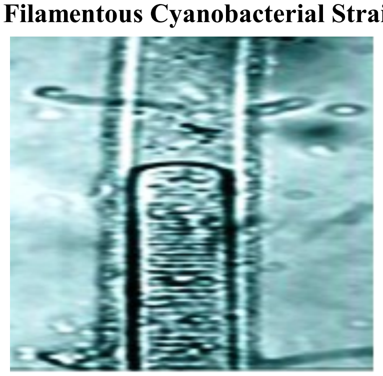


Aphanothece

[B] Filamentous Cyanobacterial Strains:



Oscillatoria



Lyngbya

Anabaena

whereas in stable water columns showing depletion of dissolved inorganic N and high temperature, N_2-fixing organisms like *Anabaena* dominates (Reynolds, *et al*., 1987). Contrary to this, Pond-2 was dominated by mainly filamentous cyanobacteria along with few unicellular ones. However, the adjacent site, Pond-2 was dominated by mainly filamentous cyanobacteria like *Oscillatoria* and *Lyngbya,* along with *Synechocystis, Aphanothece, Chroococcus* and *Anabaena.* The accessory pigments which support the net growth to occur even at low irradiance (Scheffer, *et al*., 1997) and the buoyancy (Walsby, 1994) allowing certain taxa to bloom at the water surface appeared to have played crucial role(s) towards the dominance of cyanobacteria (Reynolds, *et al*., 1987). *Microcystis* is one of the dominant and persistent organisms, invariably associated with the stability of almost permanent blooms in tropical freshwaters that are exposed to constant sunshine, warmth, and nutrients (Scarsbrook, 2002). The development of cyanobacterial blooms in any ecosystem, the siderophore mediated iron uptake is believed to be a contributing factor in their ability to dominate other microalgae (Murphy, *et al*., 1976; Bailey, *et al*., 1980).

A phytoplankton and water quality survey was conducted to evaluate the trophic state and blue -green algal pollution during flood and dry seasons in the year 2000 for the 19 typical reservoirs of *Guangdong* Province in China (Zhao Hui *et al*., 2004). During intense blooms, the photosynthetic activity depletes free CO_2 from the water body and pH is driven up. The phytoplankton community of *Ranital*, India was dominated by the tolerant species of Cyanophyceae, Chlorophyceae and Diatoms (Arjariya, 2003). Their distribution was markedly influenced by the physicochemical as well as biological nature of the aquatic system.

CONCLUSION

Based on our findings, it has been concluded that in the aquatic ecosystems as studied, the seasonal variations in the physicochemical and biological profile of the fresh water aquatic bodies governs the phytoplankton community in general and entire aquatic microbial community in particular. It was dominated mainly by the unicellular cyanobacteria, followed by filamentous ones and other algal organisms.

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