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RESEARCH ARTICLE

CYANOBACTERIAL DIVERSITY OF LOKTAK LAKE, THE LARGEST FRESHWATER WETLAND IN NORTH-EASTERN REGION OF INDIA

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ABSTRACT

Loktak Lake, the largest fresh water lake of North-east India supports a significant population of several species of flora and fauna. In the present study, a survey was conducted to know the cyanobacterial diversity of the lake. A total number of ninety (90) cyanobacterial species belonging to 11 genera were recorded from this wetland. Out of 90 species, 16 were in summer season, 21 in rainy season and 53 in winter season. The number of cyanobacterial strains was more in winter than in summer and rainy seasons. Heterocystous forms showed more frequency of occurrence than non-heterocystous forms. Out of the different habitats, hydrophytes (phumdis) were found to be supporting maximum number of cyanobacterial species whereas waterlogged soil supported the least. Majority of the dominant genera (heterocystous filamentous forms) were observed in winter and summer season. The highest cyanobacterial diversity, Shannon index (H') was observed for *Nostoc* (H'=2.16) and the lowest for *Limnothrix* (H'=0.86). Highest species dominance (Simpson's index, 1-D) was showed by *Nostoc*, 0.89 and the lowest found in *Limnothrix*, 0.5. The diversity of cyanobacteria in the Loktak Lake has not been studied except for a few sporadic reports. The study revealed that the Loktak Lake and its surroundings provide a suitable for diverse groups of cyanobacteria.

INTRODUCTION

Manipur state is famous for its lakes and swamps that are an important part of the geography of the state. Loktak Lake, the largest freshwater lake in the north-eastern region of India, is also called the 'floating lake' because of floating 'phumdis' (local name for floating mats-heterogeneous mass of soil, vegetation and organic matter at various stages of decomposition) in it. The origin and evolution of Loktak Lake may be ascribed to tectonic activity and neotectonism remarkably influenced by a long history of fluviolacustral processes. It is considered to be the 'lifelines for the people of Manipur' due to its importance in their socio-economic and cultural life (Singh and Shyamananda, 1994). It serves not only as the source of water for drinking, power generation and irrigation but also a source of livelihood for the people living in the surrounding villages, islands and on the phumdis.

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Considering, its ecological status and biodiversity values, the lake was initially designated as a 'Wetland of International Importance' under the Ramsar Convention on March 23, 1990. Loktak Lake, being perennial ecosystem with variable cyanobacterial diversity was composed of optimum level of light, water, temperature and nutrient availability that provided a favourable environment for the luxuriant growth of cyanobacteria. Cyanobacteria being ubiquitous in nature possess a high potential of adaptation to diverse environments (Garcia-Pichel *et al*., 2001). In any ecosystem, not a single species grow independently and indefinitely because all the species were interlinked and have cyclic transformation of nutrients. Most commonly, cyanobacteria occur in fresh water, marine or brackish water, however, they can also grow in hot springs, saline lakes, frigid lakes in the Antarctic, oil field ponds, desert areas, and on bare rocks. Many cyanobacteria grow attached on the surface of hygropetric rocks and stones (epilithic forms), on submerged plants (epiphytic forms) or on the bottom sediments (epipelic forms) of lakes. Their ecological tolerance to a broad range of temperatures, high salinity, and adaptations to high and low light conditions contributes to their competitive success as planktonic or benthic organisms in a variety of environments (Sommaruga and Garcia-Pichel, 1999; Badger *et al*., 2006; Cohen and Gurevitz, 2006). Life in the aquatic environment is largely governed by physico-chemical characteristics and their stability. Limnological studies of water bodies also provide information about the trophic status which may help in management and conservation (Marchetto *et al*., 1995). The natural ecosystems such as soil, freshwater bodies-streams, ponds and lakes of this region provide excellent habitats and favourable environments for the luxuriant and diverse growth of cyanobacteria. Literature on cyanobacteria of the lake are very few. The scattered information was available on the diversity of cyanobacteria from the Loktak Lake. Earlier authors namely, Tiwari and Singh (2005); Singh *et al*. (2012); Akoijam and Singh (2011) have also made contributions. However, work and publications on this group of microorganisms from Loktak Lake is sporadic despite the fact that this region falls within Indo-Malayan biodiversity hotspots (Myres *et al*., 2000). Exploration of cyanobacterial diversity of Loktak Lake, Manipur part of the Indo-Myanmar hotspot-holds promise for isolation of biotechnologically significant strains of cyanobacteria and even novel species. The present study was undertaken to study the limnology parameters and cyanobacterial diversity of Loktak Lake to determine a relationship between cyanobacterial diversity and limnology of this lake. In addition, the influence of abiotic parameters on richness and abundance of cyanobacteria were analyzed.

MATERIALS AND METHODS

Study sites and sampling

Loktak lake (Fig 1) is the largest freshwater wetland in the North-Eastern region of India located between 24º25′ to 24º42′ N latitudes and 93º46′ to 93º55′ E longitudes and is situated 38 km away in south direction from Imphal city, the capital of Manipur, India. It is a shallow wetland with depth varying from 0.5 m to 10 m. The lake is oval in shape with its long axis running north to south. In order to get maximum number of cyanobacterial species, samples from the lake were collected in summer, rainy and winter seasons. Samples of visually conspicuous cyanobacterial growth on submerged plus exposed soils, water plants and stones (Fig 2) were collected in polythene bags containing native water. The exposed surface of roots and stems of some angiospermic plants were collected for cyanobacterial isolation. In some cases, small stones covered with cyanobacterial growth were considered as samples. Geographical details were also recorded using Global Positioning System, GPS (Garmin eTrex Vista). The samples were processed within 48 h of their collection.

Physico-chemical characteristics

Water samples were examined for different physico-chemical parameters such as temperature, pH, nutrients (nitrate and phosphate) and total dissolved solids (APHA, 1989; Trivedi and Goel, 1986). Water temperature and pH were measured in the field conditions at the time of sample collection using a mercury thermometer and pen type pH meter (Hanna Instruments). Analysis for the remaining physico-chemical parameters was carried out in the laboratory. Total dissolved solid (TDS) was determined as the residue left after evaporation of filtered sample (Gravimetric method). Nitrate and phosphate were determined spectrophotometrically.

Isolation and purification of cyanobacteria

Samples were initially examined under a light microscope for the presence of cyanobacteria, and a small portion of each sample was inoculated to sterile 100 ml of BG-11 medium (Stanier *et al*., 1971). After 10-15 days of incubation, when visible algal growth appeared 2-3 wet mounts from each flask were prepared and observed microscopically using trinocular research microscope (NIKON Eclipse 80*i*). After the microscopic observation, it was streaked on BG-11 agar plate medium with N_2 source for non-heterocystous forms and without N_2 source agar plate for heterocystous forms. Inoculated plates were incubated in the culture room which was maintained at 28±2°C illuminated with cool white 40W fluorescent tubes at an irradiance of 54-67 µmol photons $m^{-2}s^{-1}$ with photoperiodic automatic model timer to provide alternative light and dark phases. The plates were observed regularly for algal growth and isolated filaments of cyanobacteria. Isolation of the cyanobacterial strains was done by randomly picking different types of colonies developed on the petridishes and then examined under microscope. If growth of heterotrophic bacteria or any other mixed culture was observed under any of the condition after incubation, cyanobacterial colonies were continuously cultured onto fresh plate medium until unialgal cultures were obtained.

Identification of cyanobacteria

Morphological identification

Slides of the unialga was prepared on a clean glass slide along with cover slip and examined. Photomicrography was carried out using trinocular research microscope (NIKON Eclipse 80*i*) and Carl Zeiss fluorescence microscope, Axio Scope A1 coupled with Carl Zeiss Imaging Systems 32 software AxioVision 4.7.2 followed by taxonomical characterization of referring to monographs and books (Desikachary, 1959; Komarek and Anagnostidis, 2005). Filament/trichome structure, constrictions, sheath, shape, presence or absence of heterocyst and akinete were the major points considered for taxonomical characterization. The identified cyanobacterial isolates were deposited to Freshwater Cyanobacterial and Microalgal Repository of IBSD, Imphal, Manipur, India (National facility created by Department of Biotechnology, Government of India with reference No. BT/PR 11323/PBD/26/171/2008 dated 31-03-2009), Institute of Bioresources and Sustainable Development (IBSD), Imphal, Manipur, India.

Cyanobacterial diversity analysis

The cyanobacterial diversity in terms of relative abundance and Shannon index (Shannon, 1948) and Simpson index (Simpson, 1949) was analyzed by using PAST software with portable IBM SPSS statistics version 19.

Relative abundance

The relative abundance of a particular cyanobacterium was calculated by using the following formula:

$$\text{Relative abundance} = \frac{X}{Y} \times 100$$

Where,
X = total number of samples collected
Y = number of samples from which a particular cyanobacteria type was isolated.

RESULTS

Isolation of cyanobacteria

Seasonal collection of cyanobacterial and water samples was carried out in summer, rainy and winter seasons. A total of 90 cyanobacterial strains were isolated belonging to 11 genera (Table 1). Cyanobacterial strains were purified as unialgal and classically identified upto genus/species level along with their taxonomical and morphological characteristics (Table 2).

Table 1. Generic representation of cyanobacterial strains in Loktak Lake

Name of the cyanobacterial genera	No. of strains
Anabaena Bory	24
Nostoc Vaucher	26
Calothrix Ag.	08
Microchaete Thuret	05
Cylindrospermum Kutz.	01
Westiellopsis Janet	04
Hapalosiphon Nag.	01
Plectonema Thuret	02
Phormidium Kutz.	14
Lyngbya Ag.	03
Limnothrix Meffert	02

Total no. of genera=11; Total no. of strains= 90

Photomicrographs were shown in the photoplates which were captured in 40x and 100x objectives (Fig. 3-4). During summer season, a total of 16 strains, namely; *Anabaena* (4), *Nostoc* (4), *Calothrix* (2), *Microchaete* (2), *Westiellopsis* (1), *Phormidium* (2) and *Lyngbya* (1) were encountered. During rainy season a total of 21 strains, namely, *Anabaena* (1), *Nostoc* (2), *Calothrix* (1), *Plectonema* (2), *Phormidium* (12), *Lyngbya* (2) and *Limnothrix* (1) and during winter season a total of 53 strains, namely; *Anabaena* (19), *Nostoc* (20), *Calothrix* (5), *Microchaete* (3), *Cylindrospermum* (1), *Westiellopsis* (4) and *Hapalosiphon* (1) were established (Table 3). The number of cyanobacterial strains was more in winter than in summer and rainy seasons. Heterocystous forms showed more frequency of occurrence than non-heterocystous forms. Filament/trichome structure, constrictions, sheath, shape, presence or absence of heterocyst and akinete were the major points considered for taxonomical characterization. Branching pattern, row of cells in filament were main features considered for taxonomical identification of *Plectonema*, *Hapalosiphon* and *Westiellopsis*. Sheath pattern was recorded as thin, hyaline, diffluent or firm in different sheath bearing cyanobacteria. Different shape of cell was recorded as quadrate, acute/ conical end cell, cells longer than broad (*Phormidium*), barrel to cylindrical, quadratic (*Nostoc*, *Anabaena*), long and broad (*Calothrix*, *Microchaete*). Heterocyst shape varies from spherical to sub-spherical, oval to oblong (*Nostoc*, *Anabaena*, *Cylindrospermum*, *Calothrix* and *Microchaete*), oblong-cylindrical (*Westiellopsis*). Different position of heterocyst was observed as intercalary (*Anabaena*), both intercalary and terminal (*Nostoc*), basal (*Calothrix, Microchaete*). Akinete shape varies from oval (*Cylindrospermum*), oval to oblong (*Nostoc*, *Anabaena*), sub-spherical to ellipsoidal (*Nostoc*), cylindrical and next to heterocyst (*Calothrix*, *Cylindrospermum*), spherical (*Westiellopsis*) and oblong (*Hapalosiphon*).

Table 2. Taxonomic assignment and morphological characteristics of cyanobacterial species isolated from Loktak Lake

Taxonomic assignment	BTA code	Filament/Trichome/Cell description
Nostocales		
Nostocaceae	56	
Nostoc paludosum		Filament entangled with distinct and colourless sheath. Trichome blackish green, cells barrel shaped. Spherical heterocyst broader than vegetative cells and oval akinete
Nostoc commune	67	Filaments densely entangled, flexuous with distinct sheath. Trichome blue-green, barrel cells and very compact in thick sheaths with a spherical heterocyst and oblong akinete
Nostoc muscorum	950	Filaments densely entangled, flexuous or highly coiled with diffluent and colourless sheath. Trichome pale blue-green, cells barrel shaped. Spherical heterocysts and akinetes oblong
Nostoc calcicola	984	Filaments loosely entangled with hyaline and colourless sheath. Trichome pale blue-green, cells barrel shaped. Sub-spherical heterocysts and akinetes
Nostoc spongiaeforme	1018	Filaments loosely entangled, flexuous with diffluent sheath. Trichome brownish, cells subcylindrical. Heterocysts spherical and akinetes sub-spherical

Taxonomic assignment	BTA code	Filament/Trichome/Cell description
Anabaena circinalis	945	Filament flexuous and entangled with hyaline and indistinct sheath. Trichome light green, barrel shaped cells. Heterocyst sub-spherical with cylindrical akinetes
Anabaena ambigua	983	Filament bent, enclosed in a mucilaginous, thin and hyaline sheath. Trichome blackish green with barrel shaped cells with constriction at the joints. Heterocyst spherical, broader than cells with akinetes ellipsoidal
Anabaena ballyganglii	1009	Filament circinate with hyaline sheath. Trichome pale brown with barrel shaped cells which is long and broad and granular contents. Heterocyst sub-spherical and akinetes ellipsoidal
Anabaena oryzae	1026	Filament straight and aggregated with colourless sheath. Trichome pale blue-green and barrel shaped cells which is longer than broad. Heterocyst oval, broader than vegetative cells; and akinetes ellipsoidal
Anabaena iyengarii	1058	Filament curved; end cell conical and diffluent sheath. Trchome blue-green and barrel shaped cells. Spherical heterocyst and ellipsoidal akinetes
Anabaena vaginicola	1074	Filament straight and parallel with distinct. Trichome pale blue-green and sub-quadrate to cylindrical shaped cells with conical apical cell. Heterocyst cylindrical and akinetes oblong or cylindrical
Anabaena variabilis	1075	Filament bent with hyaline sheath. Trichome blue-green and barrel shaped cells. Heterocyst spherical or oval and akinetes oblong
Cylindrospermum muscicola	963	Filament broad with constrictions at cross-walls and hyaline sheath. Trichome faint blue-green with quadrate to cylindrical shaped cells. Heterocyst oblong and akinetes oval
Rivulariaceae		
Calothrix ghosei	57	Filament slightly bent with hyaline and distinct sheath. Trichome pale blue-green with quadratic shaped cells, slightly longer than broad. Heterocyst basal and spherical with cylindrical akinetes next to heterocyst
Calothrix geitonos	998	Filament bent, constricted at cross-walls and colourless sheath. Trichome light brown with long and broad shaped cells. Heterocyst basal and spherical

Taxonomic assignment	BTA code	Filament/Trichome/Cell description
Calothrix clavata	1002	Filament slightly bent and colourless sheath. Trichome pale blue-green with long and broad shaped cells, constrictions at the cross walls. Heterocyst basal and hemispherical
Calothrix marchica	1014	Filament straight with thin and colourless sheath. Trichome brown with long and broad cells. Heterocyst basal and spherical
Microchaetaceae		
Microchaete uberrima	1043	Filament straight and thin sheath. Trichome pale blue-green with long and broad cells. Heterocyst basal and spherical
Stigonematales		
Stigonemataceae	58	
Hapalosiphon welwitschii		Filament flexuous and lateral branches short as broad as the main filament with hyaline sheath. Trichome pale blue-green with elongate cells. Heterocyst quadratic and oblong akinete
Westiellopsis prolifica	51	Filament erect, thin and elongate with true branching. Trichome pale blue-green and short barrel shaped cells. Heterocyst oblong and spherical akinete
Nostocales		
Oscillatoriaceae	1042	
Phormidium fragile		Filament flexuous and constricted at cross-walls with diffluent sheath. Trichome blue-green with quadrate cell shape and conical end cell
Phormidium tenue	1045	Filament straight with thin and diffluent sheath. Trichome pale blue-green with cell shaped longer than broad
Phormidium corium	1065	Filament long, straight ends with thin sheath. Trichome blue-green with quadrate shaped cells
Lyngbya aestuarii	66	Filament straight with flat end cells, - firm and lamellated sheath. Trichome blue-green with short and granulated shaped cell
Lyngbya putealis	1013	Filament curved and thin sheath. Trichome pale blue-green with constricted at cross-walls and granulated
Lyngbya birgei	1080	Filament straight with firm and colourless sheath. Trichome blue-green with short and rounded ends
Oscillatoriales		
Pseudanabaenaceae	987	
Limnothrix redekei		Filament solitary, slightly curved. Trichomes pale blue-green, without sheath, unconstructed at cross wall, with two small polar aerotopes at the septa. Apical cell rounded

Taxonomic assignment	BTA code	Filament/Trichome/Cell description
Limnothrix vacuolifera	1051	Straight, thin and long filaments and sometimes arranged in parallel. Thin and hyaline sheath observed. Trichomes pale blue green. Granules scattered and later aerotypes at cross-walls

Table 3. Seasonal variation of cyanobacterial strains in Loktak Lake

Differentseasons	Genera										
	Ana	*Nos*	*Cal*	*Micro*	*Cylin*	*Wes*	*Hap*	*Plec*	*Phor*	*Lyn*	*Limno*
Summer	4	4	2	2	0	1	0	0	2	1	0
Rainy	1	2	1	0	0	0	0	2	12	2	1
Winter	19	20	5	3	1	4	1	0	0	0	0

Ana: *Anabaena*; ***Nos***: *Nostoc*; ***Cal***: *Calothrix*; ***Micro***: *Microchaete*; ***Cylin***: *Cylindrospermum*; ***Wes***: *Westiellopsis*; ***Hap***: *Hapalosiphon*; ***Plec***: *Plectonema*; ***Phor***: *Phormidium*; ***Lyn***: *Lyngbya*; ***Limno***: *Limnothrix*

Table 4. Physico-chemical properties of water samples of Loktak Lake

Different seasons	pH	Temperature (°C)	Nitrate (mgl^{-1})	Phosphate (mgl^{-1})	Total dissolved solids (mgl^{-1})
Summer	6.94-7.05	18.7-26.7	0.20-0.88	0.19-0.62	24.62-70.00
Rainy	6.97-7.24	21.4-29.5	0.24-0.97	0.24-0.86	40.51-110.23
Winter	6.88-6.98	9.80-19.7	0.21-0.90	0.21-0.70	35.72-89.32

Table 5. Cyanobacterial strains relative abundance (in %) of Loktak Lake

Genera	*Ana*	*Nos*	*Cal*	*Micro*	*Cylin*	*Wes*	*Hap*	*Plec*	*Phor*	*Lyn*	*Limno*
Relative abundance	40.00	43.33	13.33	8.33	1.67	6.67	1.67	3.33	23.33	5.00	3.33

Ana: *Anabaena*; ***Nos***: *Nostoc*; ***Cal***: *Calothrix*; ***Micro***: *Microchaete*; ***Cylin***: *Cyloindrospermum*; ***Wes***: *Westiellopsis*; ***Hap***: *Hapalosiphon*; ***Plec***: *Plectonema*; ***Phor***: *Phormidium*; ***Lyn***: *Lyngbya*; ***Limno***: *Limnothrix*

Table 6. Diversity indices of cyanobacterial strains of Loktak Lake

Name of genus	Shannon index, H (species diversity)	Simpson's index, 1-D (species dominance)
Anabaena	2.13	0.86
Nostoc	2.16	0.86
Calothrix	1.32	0.72
Microchaete	1.05	0.64
Cylindrospermum	0	0
Westiellopsis	1.38	0.75
Hapalosiphon	0	0
Plectonema	0.69	0.5
Phormidium	1.44	0.72
Lyngbya	1.09	0.66
Limnothrix	0.69	0.5

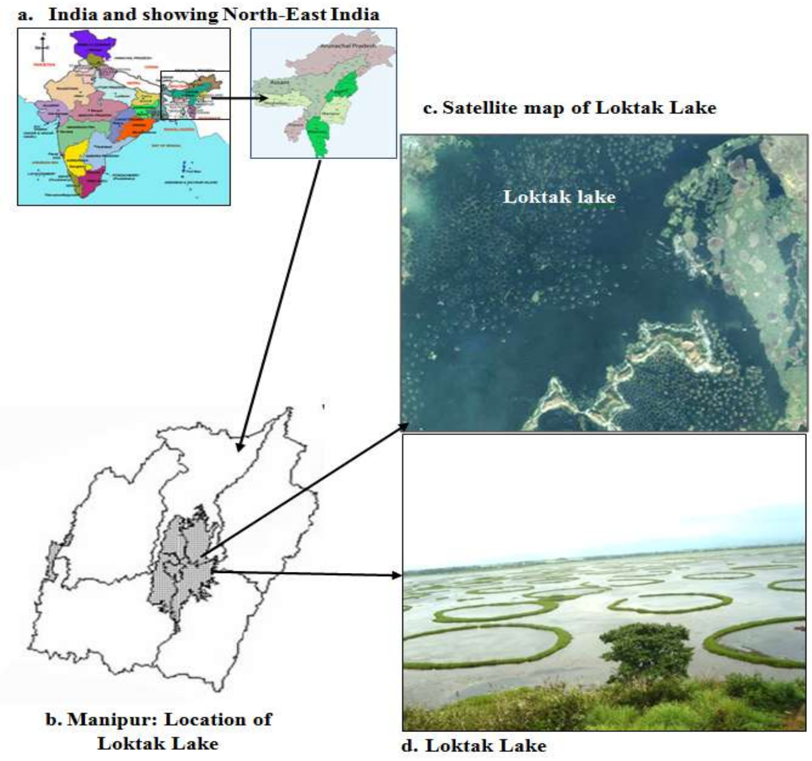

Fig. 1(a)(b)(c)(d). Location of Loktak Lake

Fig 2. Growth of cyanobacteria in natural habitats of Loktak Lake

A. moist soil **B.** stagnant water **C and H.** attached on hydrophytes **D.** attached on stem of submerged hydrophytes **E.** attached on wooden surface of boat **F.** attached on stones **G.** Rhizosphere **I.** attached on soil

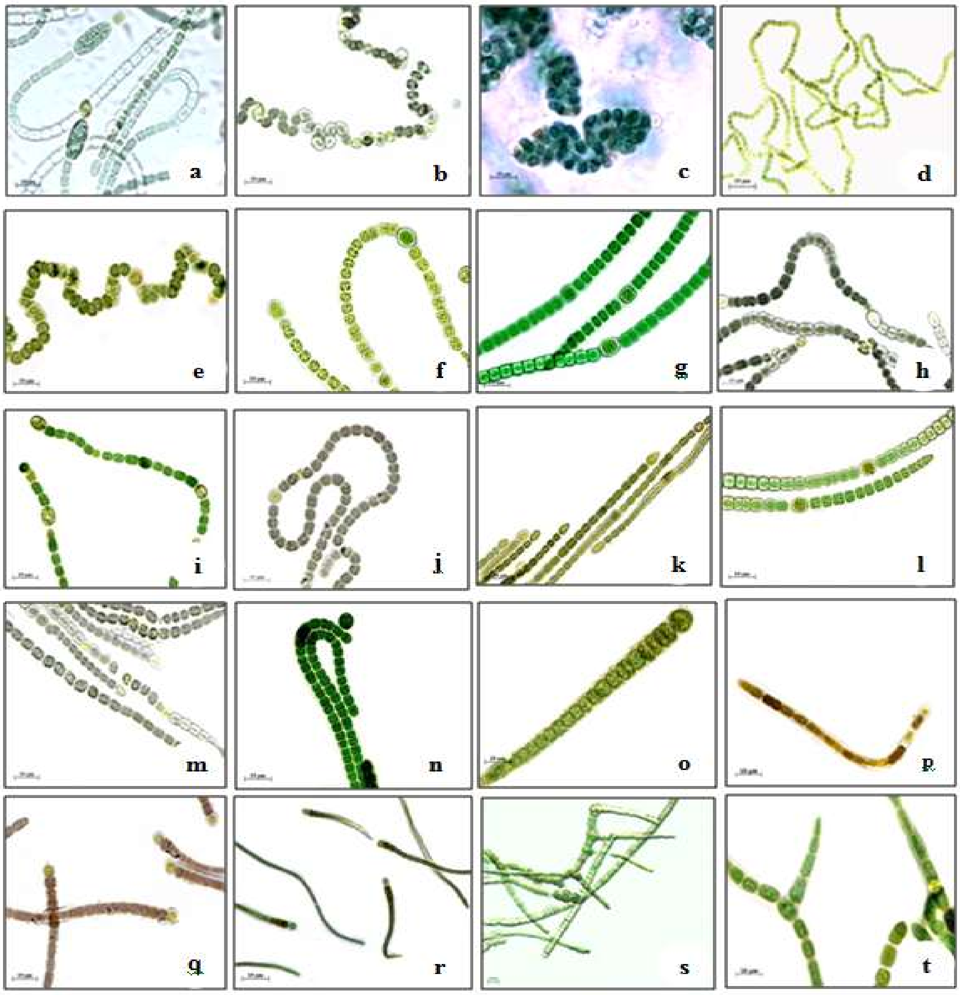

Fig 3. Microphotographs of cyanobacterial species isolated from Loktak Lake

(a) *Cylindrospermum muscicola* **(b)** *Nostoc paludosum* **(c)** *Nostoc commune* **(d)** *Nostoc muscorum* **(e)** *Nostoc calcicola* **(f)** *Anabaena circinalis* **(g)** *Anabaena iyengarii* **(h)** *Anabaena ambigua* **(i)** *Nostoc spongiaeforme* **(j)** *Anabaena ballyganglii* **(k)** *Anabaena oryzae* **(l)** *Anabaena iyengarii* **(m)** *Anabaena vaginicola* **(n)** *Anabaena variabilis* **(o)** *Microchaete uberrima* **(p)** *Calothrix ghosei* **(q)** *Calothrix marchica* **(r)** *Calothrix clavata* **(s)** *Westiellopsis prolifica* **(t)** *Hapalosiphon welwitschii* (Scale bar: 10 µm)

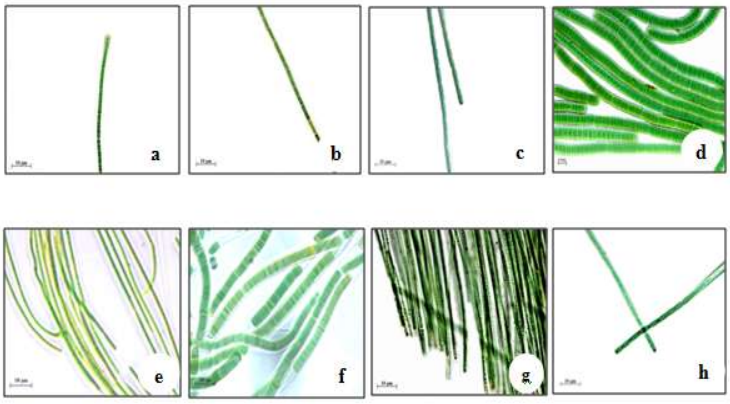

Fig 4. Microphotographs of cyanobacterial species isolated from Loktak Lake

(a) *Phormidium fragile* **(b)** *Phormidium tenue* **(c)** *Phormidium corium* **(d)** *Lyngbya aestuarii* **(e)** *Lyngbya putealis* **(f)** *Lyngbya birgei* **(g)** *Limnothrix redekei* **(h)** *Limnothrix vacuolifera* (Scale bar: 10 µm)

False branching was observed in *Plectonema*, however, *Hapalosiphon* and *Westiellopsis* showed true branching. *Hapalosiphon* always bear single row of cells in filament while *Westiellopsis* having two or more rows of cell in filament during later stage of growth.

Physico-chemical parameters of water samples

Seasonal variations in physico-chemical parameters of water samples of Loktak Lake were presented (Table 4).

Diversity indices

Data for relative abundance and diversity index (Shannon index, H and Simpson's index, 1-D) were presented (Table 5 and 6). The highest cyanobacterial diversity, Shannon index (H') was observed for *Nostoc* (H'=2.16) and the lowest for *Limnothrix* (H'=0.86). Highest species dominance (Simpson's index, 1-D) was showed by *Nostoc*, 0.89 and the lowest found in *Limnothrix*, 0.5.

DISCUSSION

The North-eastern region of India has been described as biodiversity hotspots harbouring different kinds of flora and fauna unique to this region. The natural ecosystems such as soil, freshwater bodies-streams, ponds and lakes of this region provide excellent habitats and favourable environments for the luxuriant and diverse growth of cyanobacteria. The present study described the distributional pattern and diversity of filamentous cyanobacteria strains of Loktak Lake. Out of the different habitats, hydrophytes (phumdis) were found to be supporting maximum number of cyanobacterial species whereas waterlogged soil supported the least. This might be because of nutrients availability in phumdis of the lake. *Nostoc* was found to be dominant in all the samples followed by *Anabaena* strains emphasizing their ability to adapt to a wide range of ecological niches. The other genera were relatively infrequent in their occurrence (*Phormidium*, *Calothrix*, *Westiellopsis*, *Plectonema*, *Microchaete*, *Lyngbya*, *Limnothrix*, *Hapalosiphon* and *Cylindrospermum*) pointing to their limited ability to adapt to changes from their optimum growth conditions. In the present observation, it was observed that majority of *Nostoc* spp. were associated with phumdis occurred mainly as epiphytic on the surfaces of the plant. *Calothrix* species were found to occur near the shore of the lake on benthic habitat or as free floated near the shore. *Calothrix* strains formed brown patches on soil or rock surfaces. Alternatively, they were deep blue-green in colour in their free floating forms. While growing on the soil surface it forms patches which remain dark blue-green in colour. As nutrient load increases, species that can utilize increased nutrients efficiently took advantage and multiplied rapidly at the expense of the less efficient species, which eventually were reduced numerically (Valsaraj and Rao, 1999). In the present study, the presence of diverse forms of cyanobacteria indicates a good balancing of the ecosystem. Hence, the present study concluded in spite of the fact that the cyanobacteria were ubiquitous; their population dynamics are often influenced by the available nutrients and the physico-chemical conditions of the ecosystem. Low levels of nitrogen source in the environment were also eliminating non-heterocystous forms, since nitrogen free medium was commonly used for the isolation and purification of heterocystous forms. In the present study, the distributional pattern showed that heterocystous filamentous forms dominated in all the sampling sites. In general, slightly acidic habitats harboured more of heterocystous filamentous forms than unicellular or non-heterocystous filamentous forms.

The interplay among the other environmental parameters such as temperature, moisture content, availability of nutrients, etc. that are found in lake ecosystem also contributed towards shaping the cyanobacterial diversity. Among the different environmental parameters, pH is one of the most important factors responsible for proliferation of cyanobacteria (Nayak and Prasanna, 2007). Since physico-geographically, Manipur consists of hilly terrains and during heavy rainfall, these components get dissolved in the soil and water augmenting their acidic properties. Majority of the dominant genera (heterocystous filamentous forms) were observed in winter and summer season. It was followed by a drastic fall immediately in the next season i.e. rainy season for non-heterocystous filamentous forms. *Anabaena*, *Nostoc* and *Calothrix* were observed to be consistent in all the seasons. Chellappa *et al*. (2004) reported the collective dominance by the species of cyanobacteria was due to their capacity to grow in turbid water and low light intensity to maintain buoyancy. Diversity indices take into account both species richness and the relative abundance of each species to quantify how well species are represented within a community. In the present study, it was observed that cyanobacteria especially, *Nostoc*, *Anabaena* and *Calothrix* were observed as dominant and co-dominant isolates. *Nostoc* sp. showed high Shannon (2.16) and Simpson's (0.86) indices which emphasizing the resilience and strong ability of *Nostoc* to adapt to variations in their surroundings. *Cylindrospermum* and *Hapalosiphon* showed no Shannon and Simpson's indices which implies that they showed no diversity and species richness. Generally, cyanobacteria increased the frequency of heterocysts during unfavourable environment and nutrient deficiency. The abundance of heterocystous forms indicates suitable environmental conditions for their growth. Nevertheless, good numbers of heterocystous strains were observed during winter season which suggest the presence of some limiting factors in heterocyst development. In Manipur, winter is characterized by low temperature, water and light deficiency which may acts as limiting factors. Similar assumptions were made by several workers and reported predominance of heterocystous form during dry periods (Roger and Reynaud, 1976; Roger and Kulasooriya, 1980). Nutrient accumulation may have different forces on the ecosystem at different periods (Glibert *et al*., 2007). In the present study, the results on physico-chemical composition of sampling sites revealed that they were nutrient rich environments in rainy season; in particular, total phosphorous and nitrate concentrations which may have greatly supported the abundance of different cyanobacterial morphotypes. Species diversity responds to changes in environmental gradients and may characterize many interactions that can establish the intricate pattern of community structure. Normally, it was found that any slight alteration in environmental status can change diversity until there is no adaptation or gene flow from non-adaptive sources. Rainy season characterized by high temperature and light intensity, supported maximum growth of non-heterocystous forms. Low quantities of nitrates, phosphates, total dissolved solids, acidity of water bodies in seasons enhance the growth of heterocystous forms. Winter season has shown maximum number of *Nostoc* isolates. In this study, pH of 6.88-6.98 during winter season and the moist conditions just after rainy season might have favoured the growth of *Nostoc*. There was a significant relation between pH and heterocystous cyanobacterial dominance in winter season. This may be due to the fact that some essential elements were bio available at certain required pH. A similar phenomenon was found in lake Doirani (Temponeras *et al*., 2000) and lake Taihu (Chen *et al*., 2003). It has been suggested that cyanobacteria are favoured in high pH environments, possibly because of their ability to use bicarbonate ion as a carbon source when nitrogen or phosphorus depletion occurs in summer (Shapiro, 1984; Dokulil and Teubner, 2000). Changes in the pH of water may be the result of various biological activities (Gupta *et al*., 1996). The lake water was acidic to alkaline and lower value of pH was observed during winter periods. The pH was recorded highest (pH 7.24) during rainy and lowest (pH 6.88) during winter season. Variations of pH over a higher range are often observed in the lakes due to several factors such as interactions with suspended matter, influence of freshwater inputs, pollution and photosynthesis. The highest pH value was recorded during rainy season, this might be attributed high photosynthetic due to the abundance of the algal population and increase of carbonate (Reid, 1961). The lower pH during other season was evidently due to the increased decomposition under low water depth. The high pH value is probably due to the addition of hydroxyl, bicarbonate and carbonate anions (Zutshi *et al*., 1980). A large amount of fertilizers residues are washed down the lake from the lake periphery paddy fields during the rainy season and accelerate pollution in the lake. In the present study, non-heterocystous cyanobacterial growth was observed during rainy season. The warm water and high trophic levels favoured non-heterocystous cyanobacterial growth also reported by the previous workers (Dokulil and Teubner, 2000; Lei *et al*., 2005). Dominance of cyanobacteria in water bodies depended not only on factors such as light and temperature but also on the nutrient load which affect the species composition (Riegman *et al*., 1990). In this study, it was found that the availability of nitrate and phosphate in the rainy and winter seasons were greater in comparison to the summer season. A high level of nitrogen source in the environment was also eliminating heterocystous forms, since nitrogen free medium was commonly used for the isolation and purification of heterocystous forms. This availability supported the abundant growth of cyanobacteria. The water of Loktak Lake is utilized for agriculture, fish culture and domestic purpose. Water temperature ranged from 18.7°C-26.7°C and 9.80°C-19.7°C which was highest during summer season and lowest during winter. Higher temperature obtained from the lake could be due to increase in rate of chemical reaction and nature of biological activities, since temperature is one of the factors that govern the assimilative capacity of the aquatic system. It is also because of the shallowness of the lake and consequently the volume of water in contact with air, a close relationship exists between atmospheric temperature and water temperature and as such the water is warmer during summer and rainy, colder during winter also supported by previous investigators (Jawale and Patil, 2009; Narayana *et al*., 2008; Anita *et al*., 2005). Total dissolved solids were recorded in the range of 40.51-110.23 mgl^{-1} during the rainy season and this may be attributed to influx of soil particles and the organic materials from the catchment area due to rain. This variation may be due to the size of the water body, inflow of water, consumption of salt by algae and other aquatic plants, and the rate of evaporation (Lashari *et al*., 2009). Total dissolved solids had a cyclic of seasonal changes and maximum during rainy season and minimum in summer. Similar results from Loktak Lake was also reported by Sharma and Sharma (2011). This indicated that the dissolved materials were of allochthonous origin, which was brought into the lake system with surface runoff. Similarly, Jawale and Patil (2009) and Salve and Hiware (2006) reported seasonal analysis and stated that least total dissolved solids recorded in summer season while maximum value in rainy season. Lake water quality is extremely influenced by the relative abundance of nutrients. High concentration of nutrients in the water may be attributed to the fact that the lake receives huge amount of domestic and municipal sewage and solid waste from the surface run-off and effluents discharging from the catchment area and surrounding agricultural fields. The main source of nitrate and phosphate is the run-off and decomposition of organic matter. The higher inflow of water and consequent land drainage cause high value of nitrate (Thilanga *et al*., 2005). The seasonal variations of nitrates showed that there is a relative increase in the nitrates during rainy season. Nitrates were maximum during rainy (0.24-0.97 mgl^{-1}) and minimum during summer season (0.20-0.88 mgl^{-1}). This is mainly attributed to the oxidation of existing ammonia, yielding nitrate as intermediate state especially in abundant oxygen during winter (Wetzel, 1983). During summer season, nitrates could be due to algal assimilation and other the reduction in biochemical mechanism. Similar results have been reported by Gohram (1961); Rajashekhar *et al*. (2007). Phosphate-phosphorus concentrations fluctuate between 0.19 mgl^{-1} and 0.86 mgl^{-1} at various sites of the lake. Overall high amount was observed during rainy season. This may be attributed to the inflow of nutrients from the catchment area where fertilizers are extremely used for agriculture. Also due to rain draining onto the lake with the nutrient rich soil deposited from the catchment areas of the lake by its feeder streams and rivers. Phosphate might be contaminated by the fertilizer used in nearby agricultural field and detergent that are widely used. Similar results have been reported by the previous workers (Chary, 2003; Rao, 2004). In conclusion, Loktak Lake shows a high level of cyanobacterial diversity dominated by *Anabaena* and *Nostoc*. This preliminary study shows that further work for cyanobacterial diversity and the lake is important to protect public health where surface water is used for recreational activities. From the above survey and related studies carried out so far, we can come to the conclusion that Loktak Lake and its surroundings support diverse cyanobacteria species. Our studies will establish the rich cyanobacterial diversity of the region, especially the various niche habitats of the lake and also help conserve and utilize them in bioindustry. Further intensive studies on the cyanobacterial diversity of unique biotopes in Loktak Lake should form an important input into Indian biotech industry.

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Conflict of interest

The authors declare that they have no conflict of interest.

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