



SEASONAL FLUCTUATION IN CYANOBACTERIAL FLORA OF ANTHROPOGENIC WATER RESERVOIR OF KAILASHAHAR, UNAKOTI, TRIPURA, INDIA

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Abstract

Present paper deals with the biodiversity of cyanobacterial flora of an anthropogenic water reservoir of Kailashahar situated near Indo-Bangla International border, Kailashahar, Unakoti (Tripura), India. Study revealed the occurrence of total 13 strains of 11 genera belonging to three major groups of cyanoprokaryotes viz., *Aphanocapsa nubilum*, *Chroococcus minutus*, *C. turgidus*, *Coelosphaerium kuetzingianum*, *Cyanobacterium minervae*, *Merismopedia glauca*, *Microcystis aeruginosa*, *Heteroleibleinia kuetzingii*, *Leibleinia nordgaardii*, *Phormidium pavlovskoense*, *Planktolyngbya circumcreta*, *P. tallingii* and *Microchaete uberrima*. Study also revealed that dominant forms of cyanobacterial strains may vary from season to season round the year and the ratio of nonheterocystous and heterocystous cyanobacteria was 91:09. However, ration of Nonheterocystous coccoid, Nonheterocystous filamentous and Heterocystous filamentous genera was 55:36:09 respectively.

Key words : Anthropogenic, Cyanobacteria, Diversity, Reservoirs, Tripura.

Introduction

North Eastern Region of India is considered as one of the Mega Hot spot of the world for its biodiversity richness of fauna and flora including microalgae and Cyanobacteria (Blue-green Algae). The Cyanobacteria is the largest group of gram negative photosynthetic prokaryotes (Stanier and Cohen-Bazire, 1977; Fogg *et al.*, 1973). They are ubiquitous in occurrence and widely distributed in the planet Earth. Cyanobacteria are well known primary colonizer and contribute significantly to the ecosystem and capable to grow almost in all types of known environment wherever a little light and moisture is available but aquatic environments (natural and anthropogenic water reservoirs) are more suitable to flourish them. They are also abundant in large natural as well as anthropogenic water reservoirs and many other extreme environments such as arid deserts, hot springs, and in parts of Antarctica and particularly in the moist tropics, free or in symbiotic association. Cyanobacteria exhibit a wide range of morphological diversity ranging from unicellular to branched heterocystous forms.

Tripura is a landlocked state of North-East India and the state is surrounded by Bangladesh from three sides west, north and south. Details are given in modified Google geographic map (Fig. 1). The climate of Tripura is divided into four main seasons pre-monsoon or summer (March to April); monsoon (May to September) and post-monsoon (October to November) and winter (December to February). Tripura enjoy highest rainfall during monsoon season (June to August) and lowest rainfall during winter season (November-January). Tripura face highest temperature (32-36°C) during pre-monsoon season (March-May) and lowest temperature (10-12°C) during winter season (December-January).

In India, cyanobacteria (blue-green algae) have been explored by a large number of researchers (Mitra, 1950, 1951; Pandey, 1965; Tiwari, 1972; Tiwari and Pandey, 1976; Tiwari *et al.*, 2007, 2009; Laloraya and Mitra, 1973; Anand, 1989; Singh and Bisoyi, 1989; Nayak *et al.*, 1996; Sahu *et al.*, 1996; Kant *et al.*, 2004, 2005, 2006, 2008; Dwivedi *et al.*, 2010) due to their diazotrophic potential (De, 1939), but the investigations were limited to the rice fields of several states of the country including A.P., Bihar, Chhattishgarh, H.P., J & K, M.P., Maharashtra,

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Rajasthan, U.P. and some parts of Assam, India or it was confined to the easily approachable areas of North Eastern regions of few state except Tripura because of poor connectivity with rest part of the India due to lack proper transportation facilities to the Tripura with main parts of India. Few researchers (Das *et al* 2010., Adhikary *et al.*, 2010, Bhakta *et al.*, 2010, Kant, 2012) tried to explore the Cyanobacterial diversity of Tripura but their work was limited to nearby Agartala or upto few archeological and religious sites of Tripura including but none could explore the remote areas of rest part of the Tripura, and very scanty information is available in literatures about cyanobacterial diversity of Tripura. The main objectives of the present study were to explore the diversity of cyanobacterial flora and to study the seasonal fluctuation in cyanobacterial flora round the year according to the seasonal variations in water reservoir located near to Indo-Bangla International Border, Kailashahar, Tripura, India.

Materials and Methods

Study site

The study site of the present study was an anthropogenic water reservoir (pond) situated very adjacent to the New Kali Temple (Nutan Kali Bary) and approximately 200 meter from Indo-Bangla International borderline of Kailashahar, Unakoti, Tripura, India. The study area lies between 24° 3260' and 24° 3252' N latitude and between 91° 9989' and 91° 9993 E longitude. Details are given in Map (Fig. 1).

Sampling

Total 200 water samples containing algal growth were collected during four seasons from the Nutun Kalibari pond during the study period from July 2015-June 2017.

Enrichment culturing of cyanobacterial

From collected water samples, 50 ml was homogenized by homogenizer (REMI RQT-127AD) and were transferred into petridishes (Borosil) filled by liquid and semisolid BG-11 culture medium (Stainer *et al.*, 1971) and their enrichment culture were raised as per described methods (Kaushik, 1987; Kant *et al.*, 2005).

Microscopic analysis of cyanobacteria

One week old colonies from enrichment culture of cyanobacterial growth were observed with the help of Trinocular Research Microscope (Olympus, CH20i microscope) and digital camera (Magnus, Magcam DC 10) and morphological observation were recorded. Morphological details are given photoplates 1-2.

Identification of cyanobacterial genera

Appeared growth / colonies of blue-green algal strains

were observed and genera were identified with help of available literatures (Geitler, 1939; Desikachary, 1959; Komárek and Anagnostidis, 1998; Komárek, 2013).

Results

Present investigation revealed the occurrence of total 13 strains of 11 genera belong to three major groups of cyanoprokaryotes *viz.* *Aphanocapsa nubilum*, *Chroococcus minutes*, *C. turgidus*, *Coelosphaerium kuetzingianum*, *Cyanobacterium minervae*, *Merismopedia glauca*, *Microcystis aeruginosa* (Nonheterocystous coccoid cyanobacteria), *Heteroleibleinia kuetzingii*, *Leibleinia nordgaardii*, *Phormidium pavlovskoense*, *Planktolyngbya circumcreta*, *P. tallingii* (Nonheterocystous filamentous cyanobacteria) and *Microchaete uberrima* (Heterocystous filamentous cyanobacteria). Morphological descriptions of all the 13 strains of 11 genera are given in redrawn figureplate (Figures 5A-Q) and photoplate (Figs. 6A-T). Statistical analysis of data of results of field observation of the present study revealed that dominant forms of cyanobacterial strains may vary from season to season round the year (Table 1). The Heterocystous cyanobacterium *Microchaete uberrima* was dominant during post monsoon season (September-December). However, *Microcystis aeruginosa* was dominant during post winter season (January-February) of the study period. By considering total cyanobacterial genera 100%, the ratio between nonheterocystous and heterocystous groups of cyanobacteria was 91:9. However, the ratio of Nonheterocystous coccoid, Nonheterocystous filamentous and Heterocystous filamentous genera was 55:36:9. Details of cyanobacterial diversity are given in pie diagrams (Figs. 2-4).

Morphological Description of Cyanobacteria

Order- Chroococcales, Family- Merismopediaceae, Sub-family- Merismopedioideae

***Aphanocapsa nubilum* Komárek & Kling (Figs. 5A-5C and 6A-B, E)**

Unicellular and colonial cyanobacterium, colonies small, microscopic, free floating, spherical or irregular mucilaginous with numerous cells, upto 56 µm in diameter. Colonial mucilage colourless and homogeneous. Cells more or less spherical, without individual envelopes, light blue-green, 1.2-2µm in diameter. Cell division by binary fission in two at right angle planes in successive generations.

***Merismopedia glauca* (Ehrenberg) Kützing (Figs.5D and 6C)**

Colonies microscopic, free floating, tabular, consist

Table 1: Showing seasonal fluctuation in the appearance and growth of cyanobacterial genera in the anthropogenic water reservoir of Kailashahar, Unakoti, Tripura.

| Cyanobacteria genera | Summer (March to Apr) | Rainy (May-Sept) | Post Monsoon (Oct-Nov) | Winter (Dec-Feb) |
|-------------------------------------|--------------------------|---------------------|---------------------------|---------------------|
| <i>Aphanocapsa nubilum</i> | R | A | R | R |
| <i>Chroococcus minutus</i> | R | R | C | C |
| <i>C. turgidus</i> | R | R | C | C |
| <i>Coelosphaerium kuetzingianum</i> | C | A | C | R |
| <i>Cyanobacterium minervae</i> | R | R | C | R |
| <i>Merismopedia glauca</i> | C | R | C | C |
| <i>Microcystis aeruginosa</i> | C | R | C | D |
| <i>Heteroleibleinia kuetzingii</i> | C | C | C | C |
| <i>Leibleinia nordgaardii</i> | C | R | C | R |
| <i>Phormidium pavlovskense</i> | C | R | C | C |
| <i>Planktolyngbya circumcreta</i> | C | R | C | C |
| <i>P. tallingii</i> | R | R | C | C |
| <i>Microchaete uberrima</i> | C | C | D | C |

A=Absent, C=Common, D=Dominant, R=Rare

of few to numerous cells, usually 64 celled (rarely more), regularly and densely arranged cells, sometimes form sub-colonies, colonial mucilage amorphous and colourless; Cells spherical or oval or broadly ellipsoidal, cells regularly arranged in single layer in perpendicular rows. Cell division in two planes at right angle to each other in a regular pattern.

Sub-family- Gomphosphaerioideae

Coelosphaerium kuetzingianum Nägeli (Figs. 5I and 6F, 6K)

Colonies micro to macroscopic, free floating, spherical or oval mucilaginous colonies upto 100 µm in diameter but sometimes consists of 2 sub colonies. The cells are arranged irregularly in one marginal layer (but sometimes slightly shifted to one another), or near the surface of the sphere. Cells spherical or hemispherical after division, pale or bright blue-green and 1.8-3(4) µm in diameter without gas vesicles. Cell division occurs in two planes in successive generations, perpendicular to one another and to the colony surface. Reproduction is by disintegration of a colony and by liberation of sub-colonies.

Family-Chroococcaceae

Chroococcus minutus (Kützing) Nägeli (Figs. 5E and 6I)

Cells single or in groups of 2-4 (-8), cells individually surrounded by lamellated colourless or coloured mucilaginous envelopes. Cells spherical or after division hemispherical or quadrant, gray, pale blue-green, yellowish, orange, reddish or violet homogeneous, 4-12 µm in diameter. Cells division in three planes at right angle to each other in successive generations, later divisions

irregular.

C. turgidus (Kützing) Nägeli (Figs. 5F and 6J)

Colonial, colonies microscopic, usually 2-8 (-32) celled, without colonial mucilage. Cells bright blue-green, sometimes coarsely granulated, 8-32 µm. (without sheath), sheaths 2-6 µm in thickness. Colony of 2-4 cells, covered with layered gelatinous sheath; cell body (6) 8-32 (45) µm in diameter excluding sheath, 13-40 µm (including sheath). Cells spherical or widely oval but later during successive binary fissions hemispherical or in the form of segment of a sphere, individual cells with hyaline, lamellated sheath.

Family-Synechococcaceae, Aphanothecoideae

Sub-family-

Cyanobacterium minervae (Copeland) Komárek, Kopeck & Cepák (Figs. 5J and 6D)

Cells solitary or in pairs during division but always separating before next fission. Cells almost spherical / oval or cylindrical, pale blue green or olive green with visible longitudinally striated chromatoplasma, 2-10 µm long and 2.2-5.2 µm wide. Cell division is symmetric but sometimes asymmetric in olive green or blue green masses.

Family-Microcystaceae

Microcystis aeruginosa Kützing (Figs. 5G-5H and 6G-6H)

Colonies micro or macroscopic, free floating, mucilaginous or oval or irregular, chlathrate or composed of sub-colonies, 40-1000 µm, rarely upto 8 mm long, mucilage fine colourless, homogeneous diffluent or with distinct margin. Cells spherical filled with gas vesicles,



Fig. 1: Location map showing the study area, Kailashahar, Unakoti (Tripura).

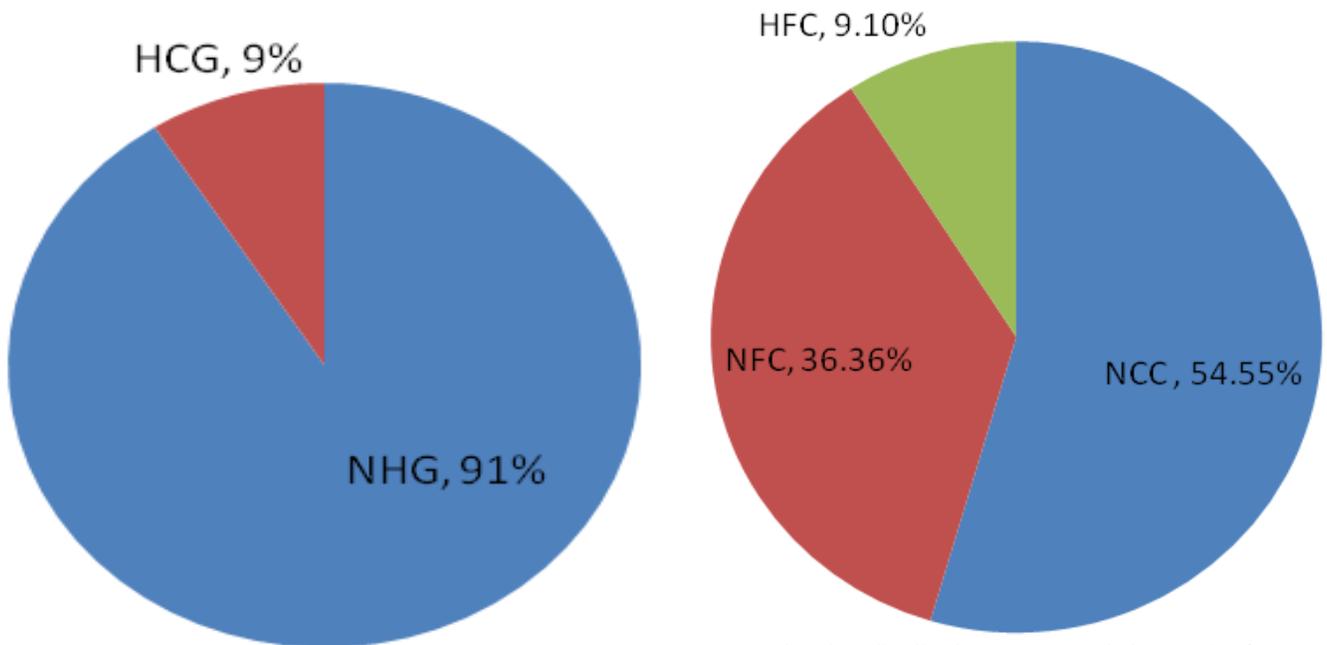


Fig. 2: Showing occurrence and share (%) of Nonheterocystosus (NHG) and Heterocystosus groups of Cyanobacteria in anthropogenic water reservoir of Kailashahar, Tripura.

Fig. 3: Showing distribution pattern and share (%) of genera of Nonheterocystosus Coccoid Cyanobacteria (NCC), Nonheterocystosus Filamentous Cyanobacteria (NFC) and Heterocystosus Filamentous Cyanobacteria (HFC) in water reservoir of Kailashahar, Tripura.

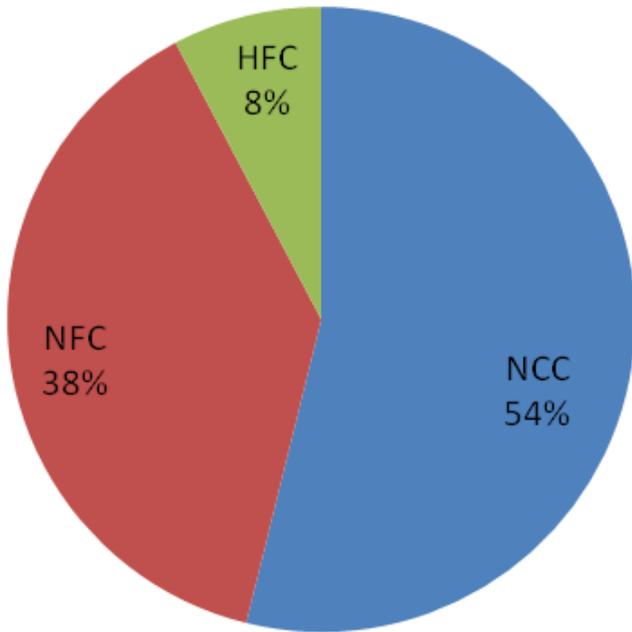


Fig. 4: Showing distribuion pattern and share (%) of species of three different groups of cyanobacteria water reservoir of Kailashahar, Tripura.

light blue green, 3-9.4 µm in diameter. Cell division in three planes, at right angle to each other in successive generations.

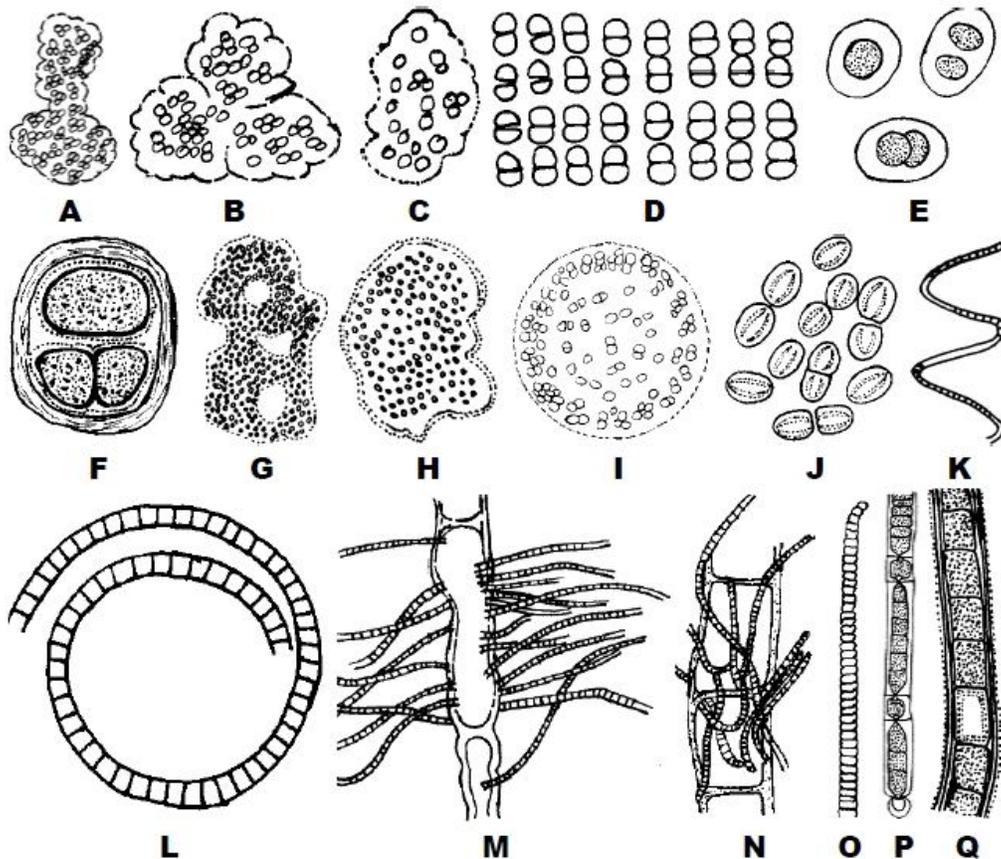
Order- Oscillatoriales, Family-Pseudanabaenaceae, Sub-family-Heteroleibleinioideae

***Heteroleibleinia kuetzingii* (Schmidle) Compère (Figs. 5M and 6S)**

Thallus filamentous, filaments erect, straight or slightly curved rigid and attached to one end of the substrate. Sheath thin, colourless extending beyond the trichome end, trichomes light blue-green, 0.5-2 µm wide, 30-80 µm long. Cells 0.5-1.0 µm wide and 0.5-1.5 µm long. Apical cells rounded without calyptra or thickened outer cell wall. Reproduce by motile hormogonia liberating from the apical part of the trichomes with the help of necridic cells. Hormogonia join by one end to the substrate and germinate at the opposite end.

Sub-family- Leptolyngbyoideae

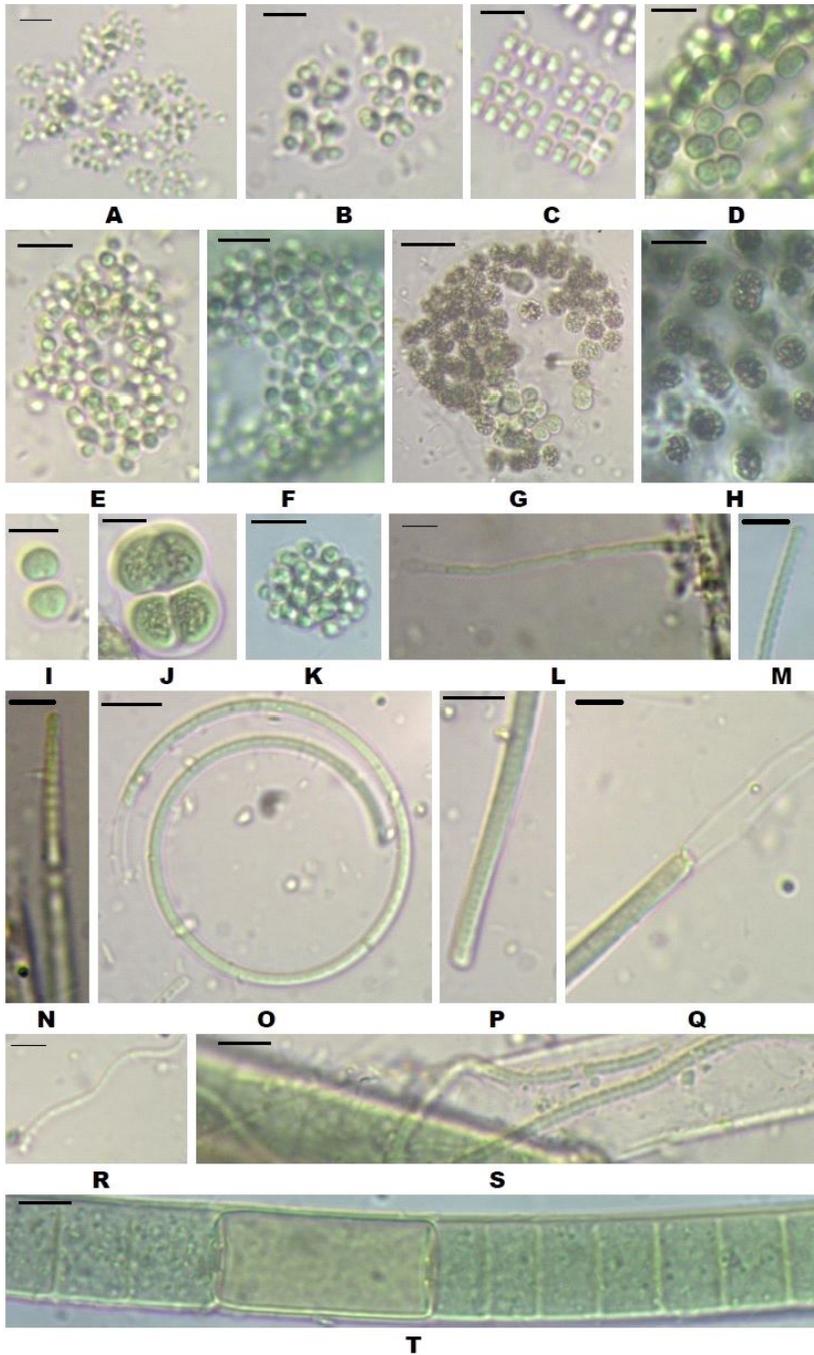
***Leibleinia nordgaardii* Anagnostidis &**



Legends: PHOTOPLATE-1 (Figs-A-Q)

A-C. *Aphanocapsa nubilum*, D. *Merismopedia galuca*, E. *Chroococcus minutes*,

F. *C. turgidus*, G.-H. *Microcystis aeruginosa*, I. *Coelosphaerium kuetzingianum*, J. *Cyanobacterium minervae*, K. *Planktolyngbya tallingii*, L. *P. circumcreta*, M. *Heteroleibleinia kuetzingii*, N. *Leibleinia nordgaardii*, O. *Phormidium pavlovskoense*, P-Q. *Microchaete uberima*



Legends: PPHOTOPLATE-2

A-B, E. *Aphanocapsa nubilum*, **C.** *Merismopedia glauca*, **D.** *Cyanobacterium minervae*, **E.** *Chroococcus minutus*, **F-K.** *Coelosphaerium kuetzingianum*, **G-H.** *Microcystis aeruginosa*, **I.** *Chroococcus minutus*, **J.** *C. turgidus*, **L.** *Planktolyngbya circumcreta*, **M-N.** *Phormidium pavlovskoense*, **O-Q.** *Planktolyngbya circumcreta*, **R.** *Leibleinia nordgaardii*, **S.** *Heteroleibleinia kuetzingii*, **T.** *Microchaete uberrima* (Scale bar- A, L,R=20 μ m; M,N,S,T=5 μ m; Rest-10 μ m).

Komárek (Figs. 5N and 6S)

Filamentous cyanobacterium, solitary or aggregated in small clusters, filaments are attached to the substrate usually by their middle part with one or both the free

ends, erect or straight or \pm curved, moderately long, 150-300 μ m, forming grey violet or grey brown thallus, 1.5-3 μ m wide. Sheaths very delicate, smooth and colourless. Trichomes light blue green or grayish green, 1.5-2 μ m wide, 5-7 μ m long, distinctly constricted at cross walls but not attenuated at the ends. Cells isodiametric. Apical cells rounded without calyptra or thickened cell wall.

Family- Pseudanabaenaceae, Sub-family- Leptolyngbyoideae

***Planktolyngbya circumcreta* (West) Anagnostidis & Komárek (Figs. 5L and 6O-6Q)**

Thallus filamentous, filaments solitary, straight or spirally coiled, 2.4 μ m wide, coils broad (35-47 μ m) circular in outline with 2-9 (mostly 2-2.5) turns, with thin, simple and colourless consisting of firm sheaths. Trichomes light blue-green, 1.8-2.1 μ m wide, unstricted at the ungranulated cross walls. Cells cylindrical or nearly quadrate, rarely shorter than wide 1-2 (4) μ m long. Apical cells rounded without calyptra.

***Planktolyngbya tallingii* Komárek & Kling (Figs. 5K and 6R)**

Thallus filamentous, filaments free floating irregularly coiled to 1.3 μ m wide. Trichomes 0.8-1.2 μ m wide constricted at cross walls and pale grayish blue green; cells 3.5-9.8 μ m long.

Family- Phormidiaceae, Sub-family- Phormidioideae

***Phormidium pavlovskoense* Elenkin (Figs. 5O and 6M-6N)**

Thallus filamentous, filaments 4.2-6 μ m, mats greenish or yellowish. Trichomes straight or slightly coiled, greenish-yellow 4-5.8 μ m wide, slightly constricted or unconstructed at ungranulated cross-walls, slightly narrowed towards the ends which are slightly curved. Sheaths are fine, colourless and diffluent. Cells \pm isodiametric or shorter than wide. The apical cells rounded or conical without calyptra.

Order- Family-Microchaetaceae

***Microchaete uberrima* Carter (Figs. 5P-5Q and**

6T)

Colonies macroscopic, free floating, Blue-green or brown in colour. Thallus filamentous, filaments cylindrical along the whole length and straight or slightly flexuous at the base and sometimes bent upto 5 mm long and 16-18(25.5) μm wide. Sheaths rigid and brown. Trichomes cylindrical and constricted at cross-walls (6.5) 10-14 μm wide. Cells are isodiametric, barrel shaped or cylindrical or slightly longer or shorter than wide. Heterocysts basal (\pm spherical) or intercalary (barrel shaped to cylindrical) \pm of the same width as vegetative cell. Akinetes thick walled, cylindrical or quadrate in long rows.

Discussion

Cyanobacteria (Blue-green Algae) have been most fascinating group of microorganisms since long back because of their contribution in the ecosystem as primary colonizer due to diazotrophic potential. The importance of Cyanobacteria in enriching tropical soils, particularly paddy fields has been emphasized after the original suggestion of De (1939). Mitra (1950, 1951) made an extensive study of Cyanobacteria from enrichment cultures of Indian soils except North East region of the country. Although Tiwari *et al.*, (2007, 2009) made an exhaustive survey on occurrence and distribution of cyanobacteria in Indian habitats and they surveyed all type of habitats, but ignored Tripura and reported total 1538 taxa of Indian cyanobacteria. Kant *et al.*, (2003, 2004) studied occurrence and distribution pattern of unicellular and colonial cyanobacteria of Uttar Pradesh and reported total 32 genera and 325 taxa. Only very scanty reports are available on cyanobacteria from Tripura. Das *et al.*, (2010) reported total 9 species of 6 genera from Tripura, but they explored only habitats of Agartala and adjoining area but ignored rest part of the state. Kant (2013) reported 41 strains of five genera of Nostocacean cyanobacteria from Tripura, but ignored the Non-heterocystous form of cyanobacteria. Occurrence of total 13 strains of 11 genera of three major groups of cyanoprokaryotes Nonheterocystous coccoid cyanobacteria, Nonheterocystous filamentous cyanobacteria and Heterocystous filamentous cyanobacteria. Out of total 11 genera 13 species, four genera and five species *viz.* *Heteroleibleinia kuetzingii*, *Leibleinia nordgaardii*, *Planktolyngbya circumcreta*, *P. tallingii* *Microchaete uberrima* are being reported first time from Tripura, India. As well as the seasonal fluctuation in cyanobacterial flora is concerned, it clearly indicates that seasonal variations heavily influence the dominancy of particular cyanobacteria in anthropogenic water reservoirs of Tripura. *Microchaete uberrima*, a

heterocystous cyanobacterium was dominant during post monsoon season (September-December) and *Microcystis aeruginosa*, a toxic strain was dominant during post winter season (January-February). Absence or rare occurrence of visual growth of certain strains *viz.* *Aphanocapsa nubilum* and *Coelosphaerium kuetzingianum* may be due muddiness during rainy season and due to dilution of water by heavy rainfall.

Conclusion

On the basis of present study on cyanobacterial diversity of anthropogenic water reservoir of Indo-Bangla International Border of India, Kailashahar, Unakoti (Tripura) India, it is concluded that Tripura state is unexplored and have very good possibility of new strains, which may be new to the science and new flora for Tripura. It needs more extensive study to develop comprehensive information on such important group of microorganisms with tremendous potential. The appearance and growth of dominants forms of cyanobacteria *Microchaete uberrima* and *Microcystis aeruginosa* are greatly influenced by seasonal changes, which also need more study to understand the reasons of occurrence of dominant forms during different seasons.

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