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Taxonomic and Ecological Aspects of Thermophilic Cyanobacteria from Some Geothermal Springs of Jharkhand and Bihar, India

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IN the present research, we have highlighted the taxonomic and ecological aspects of cyanobacteria, identified from some less-explored geothermal springs of Jharkhand and Bihar in India, with respect to water temperature variations and other physicochemical parameters, which is still an understudied topic. In total, twenty six different cyanoprokaryotes were identified. Based on our observations, the Chatra hot spring of Jharkhand area was found to be rich in cyanobacterial diversity where eight different taxa were reported. Among the recorded taxa, *Geitlerinema amphibium*, *Komvophoron* cf. *schmidlei*, *K. jovis* and *Leptolyngbya granulifera* are rare cyanobacterial species with a limited geographical distribution inhabiting the Indian springs based on the previous studies and available literature on the hot springs of India. The correlation of the cyanobacterial species identified and water parameters was studied by redundancy analysis (RDA) under CANOCO 5.0. Moreover, sampling stations were added in the RDA analysis. As a result of the RDA, the total variation was 23,83333, and it is seen that the first two axes explain 47.39% of the total variance. RDA analysis results showed that values of pH, sulfate, and sodium ions were the most effective water factors affecting the distribution of the cyanobacterial taxa identified. Though the water temperature is an important factor controlling the distribution of cyanobacterial taxa inhabiting the thermal water habitats, it was not among the most important abiotic drivers according to the RDA analysis in this work.

Keywords: Cyanobacteria, Diversity, Ecology, Hot springs, India, RDA, Taxonomy.

Introduction

Cyanobacteria are the most important photosynthetic microbes present in almost all extreme environments including geothermal springs. Cyanobacteria, especially mat-forming cyanobacteria, cover the surfaces of the hot springs and function as the primary producers (Castenholz, 1969). Some species of Cyanobacteria living in the hot springs are endemic to a limited geographical distribution and the majority are cosmopolitan in nature and possess an adequate amount of nutrients for their physico-chemical and ecological activities like formation of the community structure and maintaining trophic levels of the food chain. (Yilmaz Cankilic & Arik Berk, 2016). Cyanobacteria are also important for oxygen production and carbon and nitrogen assimilation. The dissolved chemicals and temperature are the two influential factors for the profound growth of cyanobacteria in different thermal springs (Öztürk, 2021).

Thermal springs represent the reservoir of hot water originating from geothermal activities. The primitive environment was found to be similar to the present hot spring environment, maintaining some unique biodiversity of microorganisms including cyanobacteria. Worldwide, many studies have been carried out to explore the cyanobacterial

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diversity and associated ecological activities (Brock, 1978; Castenholz, 1996; Sompong et al., 2005; Dadheech et al., 2013; Öztürk, 2020, 2021). In India, many geothermal springs are marked as heritage sites of ancient culture. The mat-forming cyanobacteria from Bakreswar, West Bengal was reported by Debnath et al. (2009) and many species of the genera *Phormidium*, *Oscillatoria*, *Synechococcus*, *Gloeocapsa*, *Calothrix* and *Fischerella* were recorded. Roy et al. (2014) reported 10 species of mat-forming cyanobacteria including 3 heterocytous and 4 non-heterocytous taxa from the geothermal springs of Panifala, West Bengal. Similarly, the recent study conducted by Singh et al. (2018) reported twenty two different cyanobacterial taxa from nine different hot springs of North-Western Himalayas, India.

The present study aims to determine the diversity of cyanobacteria from different hot springs of Jharkhand and Bihar with special emphasis on morphotaxonomy and species composition in relation to the different physico-chemical parameters. In addition, RDA (CANOCO 5.0 for Windows) has been performed to find a correlation between sampling stations, water parameters and species compositions (Ter Braak & Šmilauer, 2012). The study is also important to understand the rapid environmental changes in relation to geothermal activities and its effect on the diversity of present day microorganisms including cyanobacteria.

Materials and Methods

For sampling, a total of seven hot springs (Fig. 1), three from Jharkhand and four from Bihar districts, were chosen (Table 1). The sampling sites are Chatra (24.140°N, 85.158°E), Palasi (24.377°N, 87.203°E), Nunbel (24.08°N, 87.16°N) from Jharkhand and Rajgir (25.01°N, 85.41°E), Bhimband (25.067°N, 86.393°E) Sitakund (25.36°N, 86.53°E) and Rishikund (25.25°N, 86.52°E) from Bihar. Samples were collected from the 7 sites from November 2018 to December 2019 and species composition was studied. During sampling, both floating and epilithic algal forms were collected from all the thermal outlet areas and core region. Samples were collected in sterile vials and preserved in 4 % formaldehyde solution (v/v) and deposited in the Central National Herbarium, Botanical Survey of India, Howrah (CAL). Light microscopy for morphological observations was carried out with a Nikon microscope Ni-11 fitted with Nikon Digital Camera DS-Ri1-U3 and operated by Nikon Imaging Software NIS-D+EDF. Drawings were made with Camera Lucida. The samples were identified properly with the help of available literature and monographs (Desikachary, 1959; Komárek & Anagnostidis, 1998). For identification, at least 10 different specimens were observed to get the wide range of the cell dimensions for detailed morphotaxonomic study. The cyanobacterial names of the species identified were updated following the AlgaeBase website (Guiry & Guiry, 2022).

Besides sample collection, temperature of water was recorded using a mercuric thermometer and pH of water was also recorded using a portable pH meter. The hot springs' waters collected in sterile Borosil glass containers and physio-chemical analysis of water was executed following American Public Health Association (APHA) protocols (APHA, 2017).

Statistically, correlations between sampling stations, species records, and water parameters were examined with CANOCO 5.0 software for Windows (Ter Braak & Šmilauer, 2012). To find the appropriate correlation method, DCA was first performed on the species records and water parameters and the RDA was ideal. All water parameters were evaluated with a Monte Carlo test (499 permutations) under CANOCO 5.0. The abbreviation of the species to perform RDA is given in Table 2.

Results

Physicochemical analysis

The detailed physicochemical analysis of the water is given in Table 1. From the data it is clearly seen that the temperature was high (65°C) in Bhimband (S5A), Bihar and low (38°C) in Chatra (S1B), Jharkhand. The pH value was found to be moderate, between 7.1 to 7.8 and slightly basic in nature. The level of bicarbonate was high (1176) in Bhimband, Bihar and low (472) in Nunbel, Jharkhand. The value of nitrate, phosphate, sulfate and dissolved oxygen was very close to each other in all sampling stations due to the same microenvironment created by nature. From the table, levels of Na^+, K^+, Ca^{2+} and Mg^{2+} ions were found to be moderate in all the sampling sites to maintain the ecological balance itself.

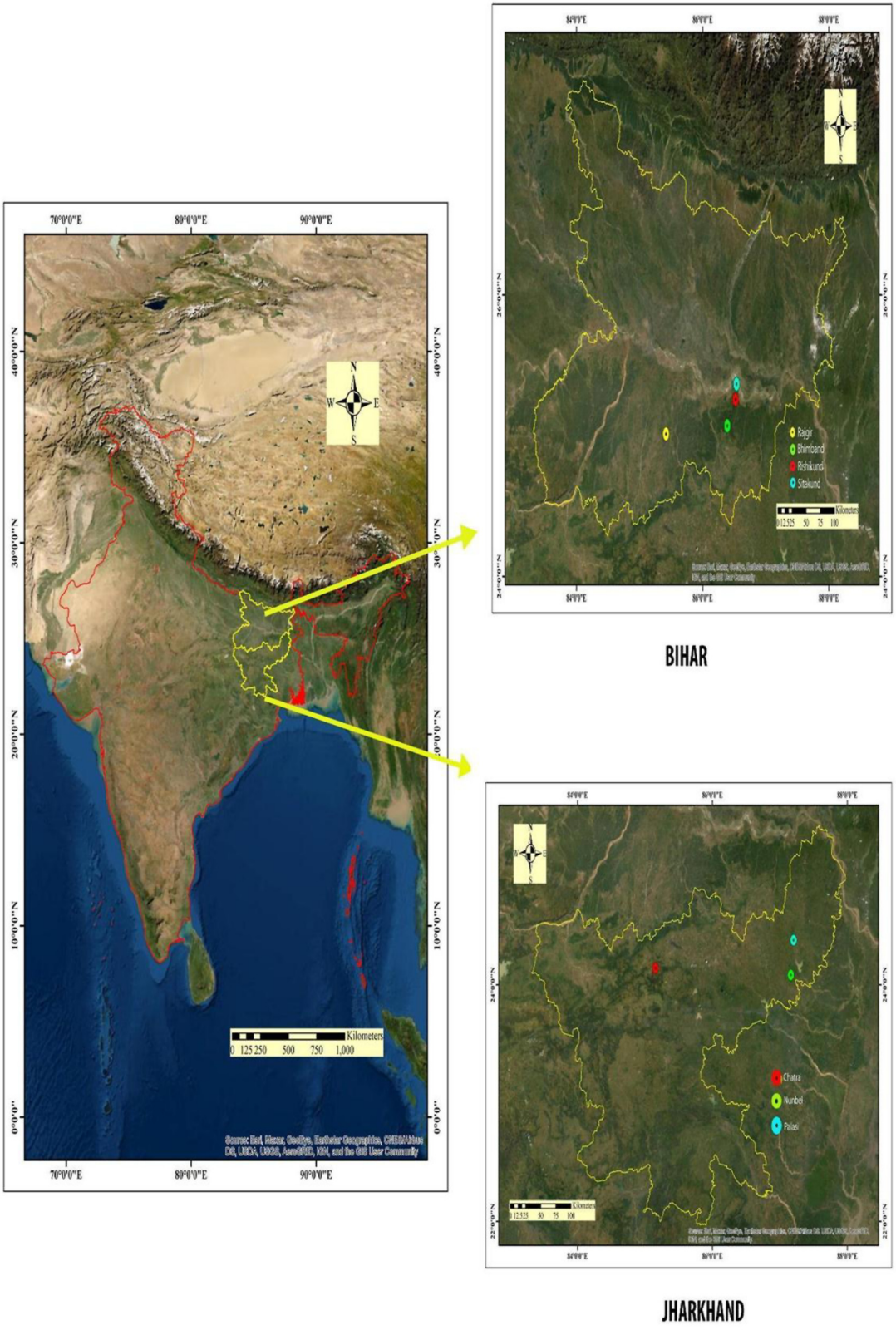

Fig. 1. Satellite map showing sampling sites of the hot springs in Bihar and Jharkhand

TABLE 1. Sampling stations and physicochemical parameters of the water (S-stations, EC-electrical conductivity, Cl^--chlorine, DO-dissolved oxygen, PO_4^{3-}-phosphate, NH_4^+-ammonium, HCO_3^-- bicarbonate, Na^+-sodium, K^+-potassium, Ca^{2+}-calcium, Mg^{2+}-magnesium, SO_4^{2-}-sulfate, S_2^--sulfide, NO_3^--nitrate)

Number of parameters	Water parameters	Sampling station											
		Chatra (Jharkhand) S1		Palasi(Jharkhand) S2				Nunbel (Jharkhand) S3	Rajgir (Bihar) S4	Bhimband (Bihar) S5		Sitakund(Bihar) S6	Rishikund(Bihar) S7
		S1 A	S1 B	S2 A	S2 B	S2 C	S2 D			S5 A	S5 B		
1.	Temperature (°C)	45	38	60	55	50	45	45	50	65	40	60	45
2.	pH	7.3	7.1	7.5	7.4	7.2	7.1	7.6	7.8	7.7	7.5	7.4	7.2
3.	EC ($mS.cm^{-1}$)	530	521	542	535	528	525	581	596	587	545	532	529
4.	Cl^- (mg/L)	76.22	75.59	78.32	79.15	76.73	74.25	82.13	83.21	85.23	84.86	79.51	92.22
5.	DO (mg/L)	7.63	6.94	7.25	7.11	7.15	7.14	7.57	5.83	6.22	6.57	7.12	7.31
6.	PO_4^{3-} (mg/L)	0.05	0.12	0.15	0.11	0.08	0.09	0.10	0.07	0.06	0.05	0.12	0.13
7.	NH_4^+ (mg/L)	31.76	28.77	34.24	33.22	29.54	32.15	27.54	26.68	29.54	23.74	27.21	26.86
8.	HCO_3^- (mg/L)	680	1156	679	589	573	489	472	1058	1176	1182	468	477
9.	Na^+ (mg/L)	97.1	96.5	86.8	86.2	78.4	79.3	82.1	81.5	92.4	91.3	82.3	81.5
10.	K^+ (mg/L)	7.7	7.3	6.8	6.9	7.2	7.1	7.5	6.9	6.8	5.9	7.2	6.5
11.	Ca^{2+} (mg/L)	4.3	4.7	5.2	5.3	6.1	5.8	4.8	4.7	4.4	4.5	4.7	4.3
12.	Mg^{2+} (mg/L)	0.7	0.6	0.5	0.4	0.3	0.4	0.09	0.12	0.3	0.5	0.7	0.6
13.	SO_4^{2-} (mg/L)	47.2	46.8	45.7	44.8	45.2	45.4	48.1	47.2	45.2	46.3	46.3	45.7
14.	S_2^- (mg/L)	14.45	14.42	14.31	13.39	14.42	15.1	13.37	13.52	14.21	13.95	14.21	14.31
15.	NO_3^- (mg/L)	3.78	3.81	3.57	3.72	3.2	3.53	3.63	3.62	3.52	3.29	3.27	3.32

TABLE 2. Species details and the abbrevitions used for RDA analysis

	Sampling station	Sub-station	Temperature	Species recorded	Species code	No. of species
1.	Chatra (Jharkhand) S1	S1 A	45 °C	*Merismopedia thermalis* Kützing 1843	*Mt*	5
				Spirulina subtilissima Kűtzing ex Gomont 1892	*Sps*	
				Komvophoron cf. *schmidlei* (Jaag) Anagnostidis & Komárek 1988	*Ks*	
				Tenebriella curviceps (C.Agardh ex Gomont) Hauerová, Hauer & Kaštovský 2021	*Tc*	
				Mastigocladus laminosus Cohn ex Kirchner 1898	*Ml*	
				Microcystis aeruginosa (Kützing) Kützing 1846	*Ma*	
		S1 B	38 °C	*Pseudanabaena minima* (G.S.An) Anagnostidis 2001	*Psm*	3
				Scytonema hyalinum N.L. Gardner 1927	*Sh*	
2.	Palasi (Jharkhand) S2	S2 A	60 °C	*Leptolyngbya granulifera* (J.J.Copeland) Anagnostidis 1936	*Leg*	2
				Komvophoron jovis (J.J.Copeland) Anagnostidis & Komárek 1988	*Kj*	
		S2 B	55 °C	*Phormidium molle* Gomont 1892	*Pm*	2
				Aulosira implexa Bornet & Flahault 1886	*Ai*	
		S2 C	50 °C	*Chroococcus membraninus* (Meneghini) Nägeli 1849	*Chm*	2
				Planktothrix isothrix (Skuja) Komárek & Komárková 2004	*Pli*	
		S2 D	45 °C	*Oscillatoria princeps* Vaucher ex Gomont 1892	*Op*	1
3.	Nunbel (Jharkhand) S3		45 °C	*Geitlerinema amphibium* (C.Agardh ex Gomont) Anagnostidis 1989	*Ga*	3
				Planktothricoides raciborskii (Wołoszyńska) Suda & Watanabe 2002	*Plr*	
				Oscillatoria simplicissima Gomont 1892	*Os*	
4.	Rajgir (Bihar) S4		50 °C	*Chroococcus subtilissimus* Skuja 1937	*Cs*	4
				Planktolyngbya cf. *brevicellularis* G.Cronberg & Komárek 1994	*Plb*	
				Phormidium molischii (Vouk) Anagnostidis et Komárek 1998	*Phm*	
				Kamptonema jasorvense (Vouk) Strunecký, Komárek & J. Smarda 2014	*Kaj*	
5.	Bhimband (Bihar) S5	S5 A	66 °C	*Chroococcus minor* (Kützing) Nägeli 1849	*Cm*	1
		S5 B	40 °C	*Jaaginema subtilissimum* (Kützing ex Forti) Anagnostidis & Komárek 1988	*Js*	1
6.	Sitakund (Bihar) S6		60 °C	*Pseudanabaena thermalis* Anagnostidis 2001	*Pst*	1
7.	Rishi Kund (Bihar) S7		45 °C	*Jaaginema crassum* (Woronichin) Anagnostidis 2001	*Jc*	1

Taxonomic enumerations

1. *Merismopedia thermalis* Kützing 1843. (Figs. 2A, 4A)

Colonies flat tabular, 24-64 cells. Cells densely-arranged, a little irregular in outline. Mucilage scarcely visible, colorless. Cells spherical, pale blue-green 2-3.5µm in diameter.

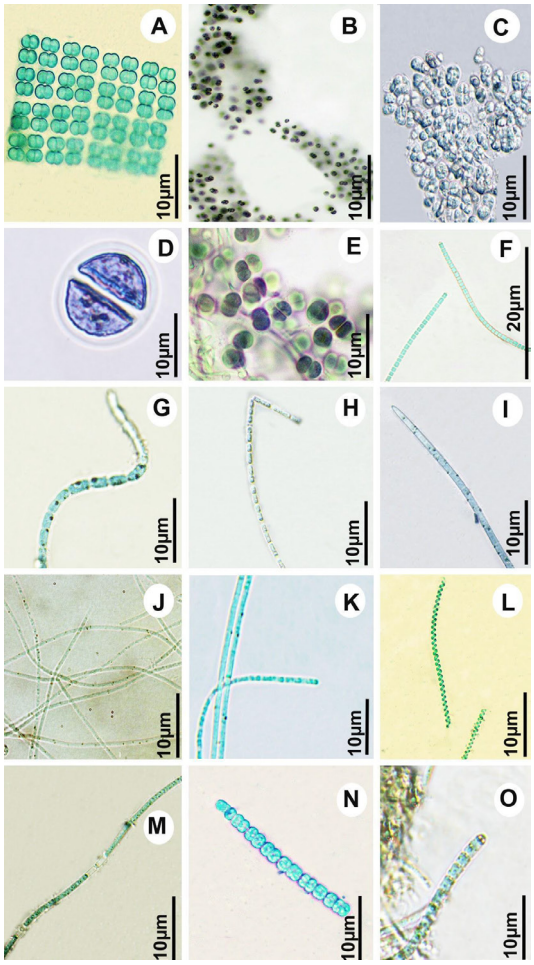

Fig. 2 (A-O). Light microphotographs of the cyanobacterial taxa recorded in the present study; (A) *Merismopedia thermalis*, (B) *Microcystis aeruginosa*, (C) *Chroococcus subtilissimus*, (D) *Chroococcus membraninus,* (E) *Chroococcus minor*, (F) *Pseudanabaena minima*, (G) *Pseudanabaena thermalis*, (H), *Leptolyngbya granulifera*, (I) *Jaaginema crassum*, (J) *Jaaginema subtilissimum*, (K) *Geitlerinema amphibium*, (L) *Spirulina subtilissima,* (M) *Planktolyngbya* cf. *brevicellularis*, (N) *Komvophoron* cf. *chmidlei*, (O) *Komvophoron jovis*

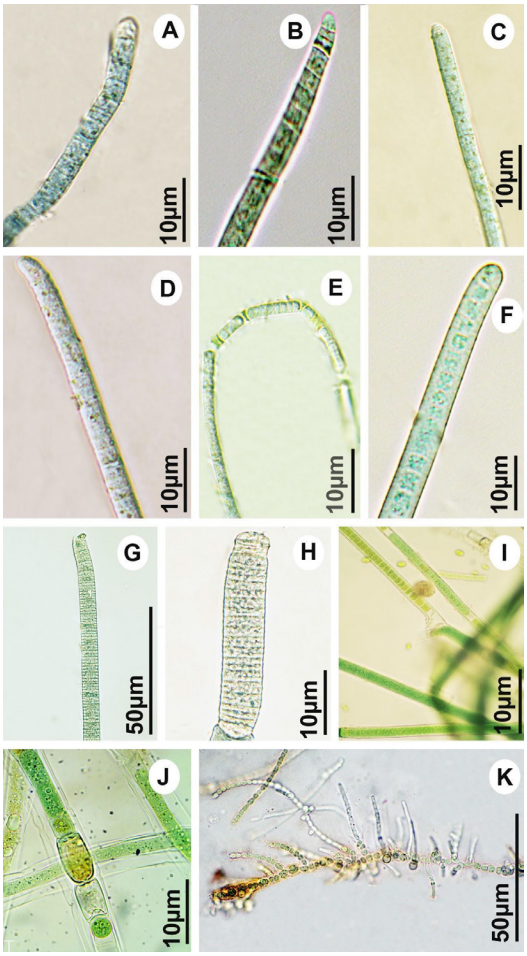

Fig. 3 (A-K). Light microphotographs of the cyanobacterial taxa identified; (A) *Planktothrix isothrix*, (B) *Planktothricoides raciborskii,* (C) *Phormidium molischii*, (D) *Kamptonema jasorvense*, (E) *Phormidium molle*, (F) *Oscillatoria simplicissima,* (G) *Tenebriella curviceps*, (H) *Oscillatoria princeps*, (I) *Scytonema hyalinum*, (J) *Aulosira implexa*, (K) *Mastigocladus laminosus*

Date of collection and accession number: December 21st 2018 and deposited at CAL under the accession number 82103.

Distribution in the present study: Floating on the Chatra hot spring (Jharkhand) at 45°C.

General distribution and ecology: This is the first record of this species from India. We think it was previously identified as *Merismopedia punctata*. This cyanobacterial species has been reported in non-Asian countries like Arctic (Patova et al., 2015), Germany (Stutz & Mattern (eds) et

al., 2018), Virginia (Forest, 1954) , Ghana (Smith et al., 2015), Central Polynesia (Tsuda & Walsh, 2013) and among Asian countries its distribution was limited to only 3 countries Pakistan (Leghari et al., 2005), Singapore (Silva et al., 1996; Pham et al., 2011) and Korea (Lee & Kang, 1986).

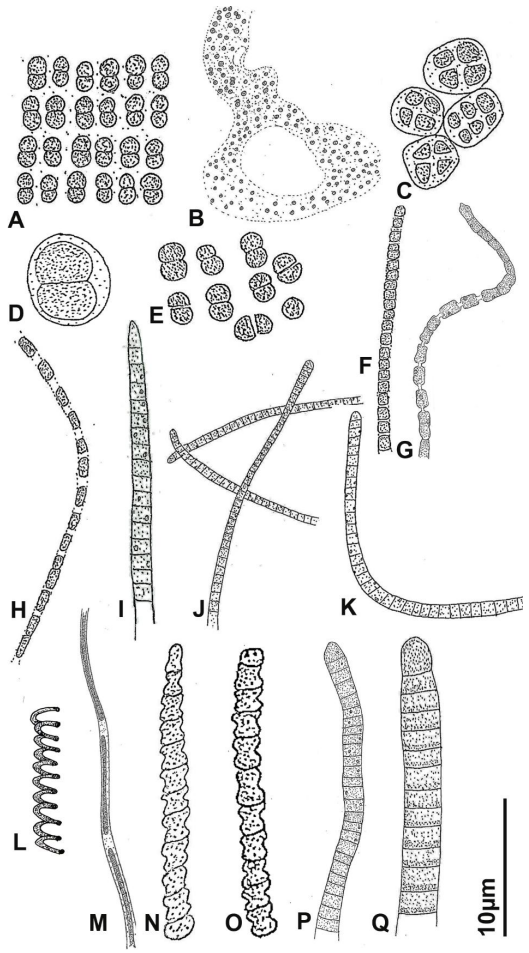

Fig. 4 (A-Q). Line drawings of the cyanobacterial taxa identified; (A) *Merismopedia thermalis*, (B) *Microcystis aeruginosa*, (C) *Chroococcus subtilissimus*, (D) *Chroococcus membraninus*, (E) *Chroococcus minor*, (F) *Pseudanabaena minima*, (G) *Pseudanabaena thermalis*, (H) *Leptolyngbya granulifera,* (I) *Jaaginema crassum*, (J) *Jaaginema subtilissimum*, (K) *Geitlerinema amphibium*, (L) *Spirulina subtilissima,* (M) *Planktolyngbya* cf. *brevicellularis*, (N) *Komvophoron* cf. *chmidlei*, (O) *Komvophoron jovis*, (P) *Planktothrix isothrix,* (Q) *Planktothricoides raciborskii*

2. *Microcystis aeruginosa* (Kützing) Kützing 1846. (Figs. 2B, 4B)

Colonies mucilaginous, microscopic, irregular, lobate with densely packed cells. Mucilage colorless, structureless. Cells spherical, 2.5-3.5µm in diameter. Cell content blue-green with numerous aerotopes.

Date of collection and accession number: December 21st 2018 and deposited at CAL under the accession number 82099.

Distribution in the present study: Floating on the Chatra hot spring (Jharkhand) at 38°C.

General distribution and ecology: Cosmopolitan with exception of polar and subpolar regions. They are mainly present as planktic species in eutrophic water bodies and are sometimes responsible for formation of heavy water blooms.

3. *Chroococcus subtilissimus* Skuja 1937. (Figs. 2C, 4C)

Colonies microscopic, composed of 2-4 celled clusters which are surrounded by colourless envelopes. Cells usually closely packed together, oval or irregular in outline, spherical pale blue-green, 1.87-3µm in diameter.

Date of collection and accession number: December 23rd 2018 and deposited at CAL under the accession number 82040.

Distribution in the present study: Epilithic on wall of the Rajgir hot spring (Bihar) at 50°C.

General distribution and ecology: As reported till date this species is only restricted to thermal springs. It has been reported only from Greece (Komárek & Anagnostidis, 1998). Thus this is the first species reported from any Asian countries.

4. *Chroococcus membraninus* (Meneghini) Nägeli 1849. (Figs. 2D, 4D)

Cells spherical, dark blue-green with finely granular content, irregularly arranged within a non-lamellated mucilage sheath, 8-10µm in diameter.

Date of collection and accession number: December 21st 2018 and deposited at CAL under the accession number 82032.

Distribution in the present study: Epilithic on the outer channel of the Palasi hot spring (Jharkhand) at 50°C.

General distribution and ecology: Reported from Florida (Taylor, 1928), Arkansas (Smith, 2010) Britain (John et al., 2011), Bulgaria (Stoyneva-Gärtner et al., 2015), Slovakia (Hindák & Hindáková, 2016). Kuwait (Silva et al., 1996), Saudi Arabia (Silva et al., 1996). India (Gupta, 2012), China (Liu, 2008; Xia, 2017), Japan (Hirose et al., 1977), Queensland (Bostock & Holland, 2010), French Polynesia (Pari & N'Yeurt, 1997). Present near the thermal springs and usually mixed with other cyanoprokaryotes. (Komárek & Anagnostidis, 1998).

5. *Chroococcus minor* (Kützing) Nägeli 1849 (Figs. 2E, 4E)

Colonies microscopic, irregular, dirty blue-green with irregularly arranged cells. Cells 2-4 celled groups. Mucilage not prominent. Cells spherical or hemispherical, 3-4.8µm in diameter.

Date of collection and accession number: 26th June 2019 and deposited at CAL under accession number 82101

Distribution in the present study: Floating on the main pond of Bhimband (Bihar) at 65°C.

General distribution and ecology: Cosmopolitan. Mainly present on wet stones and wood near the water surface of streams and swamps and also in rice fields.

6. *Pseudanabaena minima* (G.S.An) Anagnostidis 2001 (Figs. 2F, 4F).

Trichomes solitary, straight or sometimes slightly bent, bluish green, 2.5µm wide, constricted at cross-walls. Cells 1.5-2X longer than wide, 4-6.06µm long. Cell content homogeneous and, cells connected by hyaline bridges. Apical cells widely rounded.

Date of collection and accession number: 21st December 2018 and deposited at CAL under accession number 82106

Distribution in the present study: Floating on the Chatra hot spring (Jharkhand) at 38°C.

General distribution and ecology: In India this species was first reported from Punjab (Singh et al., 2022).This species has been reported for the first time from Jharkhand. This species is widely distributed over the temperate zones (Komárek & Anagnostidis, 1998). It occurs on moist soils, in mud, benthic and epipelic in ponds, springs, swamps and salty fields (Komárek & Anagnostidis, 2005).

7. *Pseudanabaena thermalis* Anagnostidis 2001 (Figs. 2G, 4G)

Filaments present in clusters , bright blue-green. Trichomes are variously curved, sometimes parallelly arranged, 2-3.12µm wide, constricted at cross-walls, not attenuated at the ends. Cells cylindrical, longer than wide , 6-9.37µm long, connected with hyaline bridges. Cell content with prominent granules with 1 or 2 polar aerotopes.

Date of collection and accession number: 27th December 2018 and deposited at CAL under accession number 82043

Distribution in the present study: Floating on the main tank of Sita Kund thermal spring (Bihar) at 60°C.

General distribution and ecology: This species was reported from thermal springs of Odisha (Bhakta et al., 2016). This species is restricted to the thermal waters, particularly common in alkaline springs or in other sulfide-containing thermal waters (Komárek & Anagnostidis, 2005)

8. *Leptolyngbya granulifera* (J.J.Copeland) Anagnostidis 1936 (Figs. 2H, 4H)

Thallus free-floating, dark bluish green. Trichomes bright blue-green, straight or curved, 1.87µm wide, clearly constricted at cross-walls, not attenuated at the ends. Cells longer than wide, 2.5µm long. Cell content homogeneous without granules, cells attached with hyaline bridges.

Date of collection and accession number: 21st December 2018 and deposited at CAL under accession number 82027

Distribution in the present study: Epilithic on the main tank of the Palasi thermal spring (Jharkhand) at 60°C.

General distribution and ecology: Rare species. Recorded from North America: Arkansas (Smith, 2010) , South-West Asia: India (Gupta, 2012), Asia: Japan (Hirose et al., 1977).

9. *Jaaginema crassum* (Woronichin) Anagnostidis 2001 (Figs. 2I, 4I)

Trichomes solitary, straight or slightly curved, 3.12µm wide, not or slightly constricted at cross-walls, attenuated at the ends. Cells are two times longer than wide, 6.25µm long. Apical cells conical-rounded.

Date of collection and accession number: 24th December 2018 and deposited at CAL under accession number 82044

Distribution in the present study: Floating on the bathing tank of the Rishikund (Bihar) at 45°C.

General distribution and ecology: Very little is known about the distribution of the species. It was reported from the thermal springs of "Železnovodsk" and "Barjatinskij". Mainly saltwater and planktic species (Komárek & Anagnostidis, 2005).

10. *Jaaginema subtilissimum* (Kützing ex Forti) Anagnostidis & Komárek 1988 (Figs. 2J, 4J)

Trichomes are few in clusters, pale blue-green, straight or slightly bent , not constricted at cross-walls, not attenuated at the ends. Cells longer than wide, 1.2-1.8µm long and 2-3.5µm long. Cell content without arerotopes. Apical cell rounded.

Date of collection and accession number: 26th June 2018 and deposited at CAL under accession number 82105.

Distribution in the present study: Epilithic on the outer channel of the thermal spring of Bhimband (Bihar).

General distribution and ecology: Reported from the thermal springs in France, Yugoslavia, Hungary, Sri Lanka, India, Algeria, Zaire. Apart from the thermal springs, it has been reported from stagnant waters in Europe, also known from the muddy bottom of ponds with water plants in Czech Republic. It grows on H_2S- containing water bodies along with sulfur bacteria. Also, it was reported from pollutant stagnant, fresh, brackish and salt waters (pools, ditches, supralittoral pools) (Komárek & Anagnostidis, 2005).

11. *Geitlerinema amphibium* (C.Agardh ex Gomont) Anagnostidis 1989. (Figs. 2K, 4K)

Thallus deep blue-green forming thin mats. Trichomes straight or sometimes slightly curved, pale blue-green, 1.5 - 2µm wide, not constricted at cross-walls, not attenuated at the ends, not capitate. Cells 2-3 times longer than wide, 3-8µm long. Cell content pale blue-green with 1-2 cyanophycean granules on either side of the cross-walls. Apical cells rounded, hemispherical without calyptra.

Date of collection and accession number: 20th December 2018 and deposited at CAL under accession number 82037.

Distribution in the present study: Floating on the bathing tank of Nunbel hotspring (Jharkhand) at 45°C

General distribution and ecology: Distributed worldwide, cosmopolitan. The species is reported from various water biotopes including thermal springs (Komárek & Anagnostidis, 2005).

12. *Spirulina subtilissima* Kűtzing ex Gomont 1892 (Figs. 2L, 4L)

Trichomes dirty blue-green, 1.5-2µm wide, regularly more or less loosely screw-like coiled, not constricted at the cross.

Date of collection and accession number: 21st November 2018 and deposited at CAL under accession number 82099.

Distribution in the present study: Attached on the outer channel of the Chatra thermal spring (Jharkhand) at 45° C.

General distribution and ecology: Distributed worldwide, cosmopolitan. Reported from thermal and sulfur springs, also in stagnant, polluted, fresh, brackish , salt and mineral-containing water bodies, grows also on stones, shells and algae with other species of *Spirulina* (Komárek & Anagnostidis, 2005).

13. *Planktolyngbya* cf. *brevicellularis* G. Cronberg & Komárek 1994 (Figs. 2M, 4M)

Filaments free-floating , straight or slightly wavy, sheaths thin, firm, colorless.

Trichomes cylindrical, not constricted at cross-walls, not attenuated at the end. Cells cylindrical, shorter than wide or more or less isodiametric, 0.8-1.2µm long and 1.2-1.5µm wide.

Cell content pale blue-green with homogeneous granular content.

Date of collection and accession number: 23rd December 2018 and deposited at CAL under accession number 82042.

Distribution in the present study: Attached on wall of Rajgir hot spring (Bihar) at 50°C.

General distribution and ecology: The species was reported from lakes in Sweden and it is a planktic species (Komárek & Anagnostidis, 2005).

14. *Komvophoron* cf. *schmidlei* (Jaag) Anagnostidis & Komárek 1988 (Figs. 2N, 4N)

Trichomes blue-green or olive-green, solitary, short 15-30 celled, up to 50µm long, straight without sheaths or any mucilaginous envelopes, constricted at cross-walls. Cells barrel shaped, isodiametric or shorter than long, 2-3µm wide and 1.5-2µm long. Cells last longer before division. Apical cells cylindrical with rounded apex.

Date of collection and accession number: 21st December 2018 and deposited at CAL under accession number 82099.

Distribution in the present study: Floating on Chatra hot spring (Jharkhand) at 45°C.

General distribution and ecology: The species is widely distributed but not very common. This is a freshwater species present mainly in stagnant and flowing waters, springs and mineral springs (Komárek & Anagnostidis, 2005)

15. *Komvophoron jovis* (J.J.Copeland) Anagnostidis & Komárek 1988 (Figs. 2O, 4O)

Trichomes solitary, bright blue-green, intermingled with other cyanobacterial taxa, short, straight or slightly bent, constricted at cross-walls, 2.5µm wide without any visible mucilaginous layer. Cells barrel-shaped with rounded ends, usually constricted at the middle, 1.8-3.12µm long. Cell content homogeneous, finely granulated. Apical cell rounded or bluntly conical.

Date of collection and accession number: 20th December 2018 and deposited at CAL under accession number 82027.

Distribution in the present study: Attached to the main tank of Palasi hot spring (Jharkhand) at 60°C.

General distribution and ecology: This species is widely distributed in the thermal springs both in acidic and alkaline thermal waters (31-71°C, pH 5.6 to 9 and the average value 7.5, optimum temperature around 50°C (Komárek & Anagnostidis, 2005). This species has been reported in India (Gupta, 2012), Japan (Hirose et al., 1977).

16. *Planktothrix isothrix* (Skuja) Komárek & Komárková 2004 (Figs. 3A, 4B)

Trichomes pale blue-green, solitary, rarely in clusters, straight or slightly curved at the apical portion, not constricted at cross-wall. Cell isodiametric or shorter than wide, 5.45-7.5µm wide up to 10µm long, with numerous aerotopes. Apical cells slightly conical, rounded, without calyptra or any outer cell wall thickening.

Date of collection and accession number: 20th December 2018 and deposited at CAL under accession number 82029.

Distribution in the present study: Attached to the bathing tank of the Palasi hot spring (Jharkhand) at 60°C.

General distribution and ecology: Distributed worldwide. In India it was reported in Punjab (Singh et al., 2022) and Uttar Pradesh (Singh et al., 2021). It is a freshwater species and present in stagnant waters and lakes.

17. *Planktothricoides raciborskii* (Wołoszyńska) Suda & Watanabe 2002 (Figs. 3B, 4Q)

Trichomes straight or slightly curved towards the end, solitary, pale blue-green with numerous aerotopes scattered all over the cells, not constricted at cross-walls. Cells are isodiametric or shorter than wide, 4.7-7.6µm wide and 4.7µm long. Apical cell 2.8µm wide and 2.3 µm long. Cell content pale blue-green, apical cell conical, rounded without calyptra.

Date of collection and accession number: 20th December 2018 and deposited at CAL under accession number 82037.

Distribution in the present study: Floating

on the bathing tank of the Nunbel hotspring (Jharkhand) at 45°C

General distribution and ecology: Distributed over tropical and subtropical zones, freshwater planktic species, reported from India, Japan, South Africa, Thailand (Komárek & Anagnostidis, 2005).

18. *Phormidium molischii* (Vouk) Anagnostidis et Komárek 1998 (Figs. 3C, 5A)

Thallus dull blue-green forming thin membranous mats. Filaments are straight ; trichome blue-green 4-5µm wide, not constricted at cross-walls with rounded conical ends. Cells more or less isodiametric or longer than wide, 5.5-7µm long; cell content homogeneous , finely granulated. Apical cells conical with rounded ends, longer than other cells without calyptra.

Date of collection and accession number: 23rd December 2018 and deposited at CAL under accession number 82040.

Distribution in the present study: Epilithic on the walls of the Rajgir hot spring (Bihar) at 50°C.

General distribution and ecology: This species is reported only in thermal springs (Komárek & Anagnostidis, 2005).

19. *Kamptonema jasorvense* (Vouk) Strunecký, Komárek & J.Smarda 2014 (Figs. 3D, 5B)

Trichomes olive-green, 4-5.5µm wide, slightly constricted at cross-walls, shortly attenuated with rounded ends, not capitate, sheaths absent. Cells isodiametric or shorter than wide, 3.5-4.5µm long; cell content granular. Apical cells narrow, rounded.

Date of collection and accession number: 23rd December 2018 and deposited at CAL under accession number 82041.

Distribution in the present study: Attached on walls of the Rajgir hot spring (Bihar) at 50°C. General distribution and ecology: Reported from the thermal springs of Croatia, Japan, Russia (Komárek & Anagnostidis, 2005).

20. *Phormidium molle* Gomont 1892 (Figs. 3E, 5C)

Filaments straight, sometimes bent. Sheaths firm, thin, colorless. Trichomes bluish green, 2.5-3.5µm wide, more or less constricted at cross-walls, not attenuated at the ends. Cells isodiametric or longer or shorter than 1.5-4µm long. Apical cells rounded without calyptra.

Date of collection and accession number: 20th December 2018 and deposited at CAL under accession number 82030.

Distribution in the present study: Attached on wall of the bathing tank of the Palasi hot spring (Jharkhand) 55°C.

General distribution and ecology: Distributed worldwide. Freshwater species attached to hydrophytes in stagnant waters, in rice-fields, recorded also on moist soils, in brackish and salty waters and also on the walls of mineral and thermal springs (Komárek & Anagnostidis, 2005).

21. *Oscillatoria simplicissima* Gomont 1892 (Figs. 3F, 5D)

Trichomes blue-green , 8.09µm wide, unconstricted, not attenuated at the ends. Cells isodiametric or shorter than wide, 4.76µm long. Apical cells rounded.

Date of collection and accession number: 20th December 2018 and deposited at CAL under accession number 82035.

Distribution in the present study: floating on the bathing tank of Nunbel hotspring (Jharkhand) at 45°C

General distribution and ecology: Cosmopolitan; species previously reported from Arkansas (Smith, 2010), Slovakia (Hindák & Hindáková, 2016), Egypt (Nassar & Khairy, 2014), Israel (Barinova & Smith, 2022), Japan (Hirose et al., 1977) and India (Singh et al., 2022).

22. *Tenebriella curviceps* (C.Agardh ex Gomont) Hauerová, Hauer & Kaštovský 2021 (Figs. 3G, 5E)

Trichomes solitary, cylindrical, straight, not constricted at cross-walls, blue-green, slightly attenuated at the end. Cells 3.12-4.68µm long, 15.62µm wide. Apical cells flat-rounded.

Date of collection and accession number:

21st December 2018 and deposited at CAL under accession number 82104.

Distribution in the present study: Attached on the wall of the bathing tank of the Chatra thermal (Jharkhand) 45°C

General distribution and ecology: Widely distributed all over the world. Freshwater species present mainly in stagnant or flowing waters, sometimes in brackish and salt waters (Komárek & Anagnostidis, 2005).

23. *Oscillatoria princeps* Vaucher ex Gomont 1892 (Figs. 3H, 5F)

Thallus blackish blue-green, attached or free floating. Trichomes bluish green, 16-20µm wide, straight or sometimes slightly curved, not constricted at cross-walls and with necridic cells, bent and nearly truncated ends. Cells brick-like, shorter than wide, 3.8-4.4µm long, cell content granular. Apical cell rounded and truncated without calyptra.

Date of collection and accession number: 21st December 2018 and deposited at CAL under accession number 82031.

Distribution in the present study: Floating on bathing tank of Palasi (Jharkhand) 45°C

General distribution and ecology: Cosmopolitan, typical species occur mainly in temperate zones, not in marine habitat. Freshwater species attached on mud or rocks in stagnant waters or slow flowing waters, in the thermal springs mostly at low temperatures (Komárek & Anagnostidis, 2005).

24. *Scytonema hyalinum* N. L. Gardner 1927 (Figs. 3I, 5G)

Thallus dirty blue-green, wooly, free floating. Filaments tangled 10-13µm wide with false branches. Branches have the same width as filaments. Sheaths thin and colorless. Trichomes cylindrical, slightly constricted at cross-walls, olive-green, 7-10µm wide. Cells shorter than long or isodiametric, 3.75-6µm wide.

Date of collection and accession number: 21st December 2018 and deposited at CAL under accession number 82099.

Distribution in the present study: Floating on bathing tank of the Chatra hot spring (Jharkhand) at 38°C

General distribution and ecology: This species is widely distributed over tropical regions and also reported from hot springs of China. Mainly remain attached on wet rocks, stones etc. (Komárek, 2013).

25. *Aulosira implexa* Bornet & Flahault 1886 (Figs. 3J, 5H)

Filaments in clusters, rarely solitary. Sheaths thick, colourless. Trichomes cylindrical 8-9.5µm wide, slightly constricted at cross-walls. Cells longer than wide. Heterocysts solitary, cylindrical with rounded or blunt ends, 15-21µm wide, 10µm long.

Date of collection and accession number: 21st December 2018 and deposited at CAL under accession number 82033.

Distribution in the present study: Floating on the outer channel of the Palasi hot spring (Jharkhand) at 55°C.

General distribution and ecology: Distributed worldwide. In India, it was reported from Karnataka (Gupta & Das, 2019). Present mainly as floating mass in stagnant waters (Komárek, 2013).

26. *Mastigocladus laminosus* Cohn ex Kirchner 1898(Figs. 3K, 5I)

Main filament single, 7.6-13.6µm wide. Lateral branches 8-14µm wide, pseudobranches short; sheaths thin. Trichomes of main filament constricted, uniseriate, rarely biseriate. Cells in the main trichome barrel-shaped 3-8µm long, 1-3µm wide, branches 3-4µm wide and up to 93µm long. Heterocytes single and -present in the main filament. Pseudobranches changes to club-shaped hormogonia, 80-250µm long.

Date of collection and accession number: 21st December 2018 and deposited at CAL under accession number 820104.

Distribution in the present study: Floating on the Chatra hot spring (Jharkhand) at 45°C.

General distribution and ecology: Cosmopolitan in thermal springs all over the world. This species is usually recorded between 37-55°C (Komárek, 2013).

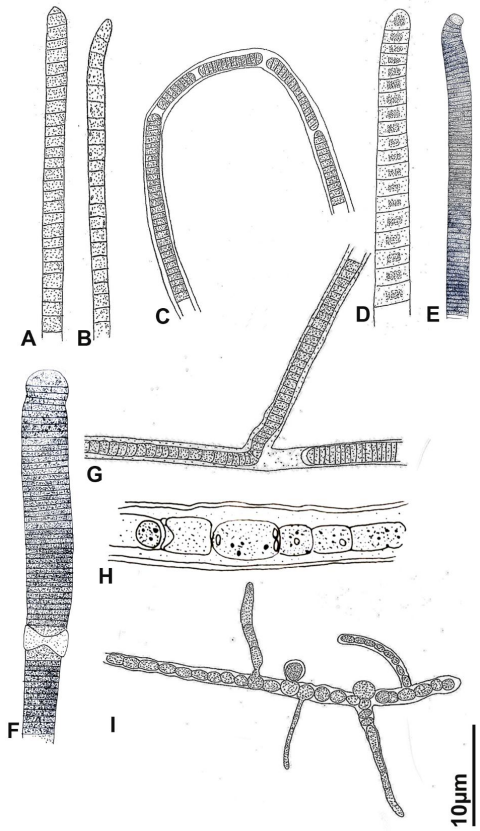

Fig. 5 (A-I). Line drawings of the cyanobacterial taxa identified; (A) *Phormidium molischii,* (B) *Kamptonema jasorvense*, (C) *Phormidium molle*, (D) *Oscillatoria simplicissima,* (E) *Tenebriella curviceps*, (F)*Oscillatoria princeps*, (G) *Scytonema hyalinum,* (H) *Aulosira implexa*, (I) *Mastigocladus laminosus*

Correlation between the hydrochemical variables and the cyanobacterial taxa

Correlations among sampling stations, species recorded, and water parameters were examined with RDA analysis (CANOCO 5.0 for Windows). To find the appropriate correlation method, DCA was first performed on the water parameters and species identified. By evaluating the gradient lengths in the DCA result, it was determined which correlation method was appropriate (Axis 1: 0.00; Axis 2: 0.00). In the DCA analysis, the efficacy values of all parameters were tested individually with Monte Carlo permütasyon under CANOCO 5.0 to determine the most effective water parameters for the distribution of the species. Among the water parameters, eight parameters that were found to be effective in the correlation analysis were included in the RDA analysis with further selection (T-temperature, pH, SO_4^{2-}-Sulfate, NO_3^--Nitrate, K^+-Potassium, Na^+-Sodium, Cl^--Chlorine and PO_4^{3-}-Phosphate). Only the most effective water parameters were included in the RDA analysis as suggested by Ter Braak & Šimilauer (2012). In the RDA analysis, a total of 12 sub-stations, of which 7 were the thermal water sampling stations. Water parameters like temperature (T), pH, SO_4^{2-}, NO_3^-, K^+, Na^+, Cl^-, PO_4^{3-}, and all species records were defined as variables. In particular, the effects of water parameters within these variables were supported by a Monte Carlo permutation test (499 permutations, F ratio = 0.8, P value = 0.95). As per the result of the RDA analysis, the total variation found was 23,83333, and it is seen that the first two axes explain 47.39% of the total variance (Fig. 6).

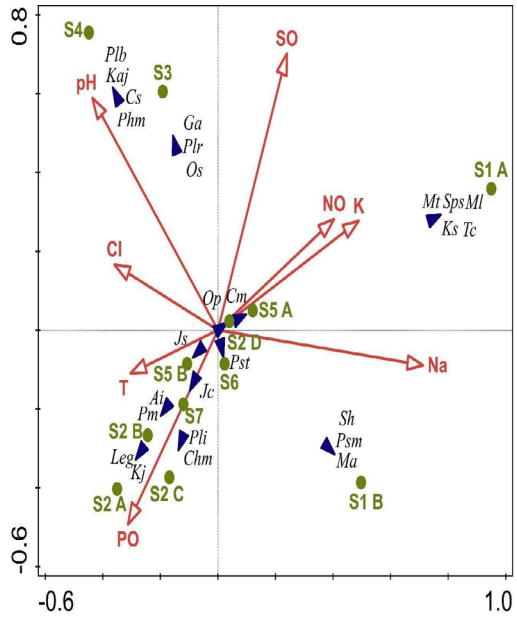

Fig. 6. RDA analysis diagram showing the correlations between sampling stations (with full circle), species records (with full triangle), and water parameters (with arrow). [T-temperature, pH, SO-sulfate (SO_4^{2-}), NO-nitrate (NO^{3-}), K-potassium (K^+), Na-sodium (Na^+), Cl-chlorine (Cl^-) and PO-phosphate ($PO4^{3-}$)].

Discussion

In literature, it is stated that one of the most effective parameters in the distribution of species in thermal water studies is temperature (Ward & Castenholz, 2000). According to the RDA analysis, pH, PO_4^{3-}, Na^+, and SO_4^{2-} were found to be almost equally effective parameters among

themselves in this study. Temperature, on the other hand, has a lower effect on the distribution of species than these parameters. According to the results of the RDA analysis, there are connections between the water parameters selected from these 7 sampling stations and the presence of cyanobacteria species determined (Fig. 6). The RDA diagram showed that Na^+, K^+, NO_3^- were strongly correlated with the first RDA axis, while pH, SO_4^{2-}, PO_4^{3-} were strongly correlated with the second RDA axis.

Based on the RDA analysis, different sampling stations of S1 (Chatra-Jharkhand) differ from each other in terms of water parameters and species recorded. *Merismopedia thermalis*, *Spirulina subtilissima*, *Tenebriella curviceps*, *Komvophoron* cf. *schmidlei*, and *Mastigocladus laminosus* had an affinity with high NO_3^-, K^+, Na^+ and SO_4^{2-} and with low pH, T, CI^-, and PO_4^{3-} in this study (sampling sites S1A). Similar to this study, *Spirulina subtilissima* was not related to temperature in Öztürk (2021), but contrary to these studies, the taxon was detected at higher temperatures in the study by Kanellopoulos et al. (2022). *Mastigocladus laminosus*, which did not show a positive correlation with temperature and pH in this study, on the contrary, has a positive correlation with the temperature and pH in the study by Singh et al. (2018). *Pseudanabaena minima*, *Microcystis aeruginosa*, and *Scytonema hyalinum* showed a negative correlation with pH and were sampled from the S1 station with the lowest pH in this study. Although sampling stations S3 and S4 are two different thermal waters, they contained similar water parameters and species with similar affinities. *Chroococcus subtilissimus*, *Kamptonema jasorvense*, *Phormidium molischii*, *Planktolyngbya cf. brevicellularis* in sampling station S4 and *Geitlerinema amphibium*, *Oscillatoria simplicissima*, *Planktothricoides raciborskii* in sampling station S3 had an affinity with high pH and SO_4^{2-}, and low PO_4^{3-} and Na^+. *Geitlerinema amphibium* was detected in water parameters of 45 °C, 7.6 pH, and 48.1 SO_4^{2-}, and in the hot spring study conducted by Arman et al. (2014), it was reported that it was determined from 53 °C, 6.86 pH, and 1945mg/L SO_4^{2-} water parameters. *Chroococcus minor* (in sampling station S5A), *Oscillatoria princeps* (in sampling station S2D), *Jaaginema subtilissimum* (in sampling station S5B), and *Pseudanabaena thermalis* (in sampling station S6) did not show a correlated distribution with any of the water parameters determined. *Leptolyngbya granulifera*, *Komvophoron jovis* (in sampling station S2A) and *Phormidium molle*, *Aulosira implexa* (in sampling station S2B) correlated with high temperature (T) and PO_4^{3-} water parameters. While *Pseudanabaena thermalis* did not correlate with any of the water parameters determined by the RDA analysis in this study, it was found to be correlated with high pH, total phosphate, low temperature, and other nutrient levels in the CCA analysis in the study by Singh et al. (2018). *Chroococcus membraninus* and *Planktothrix isothrix* (in sampling station S2C) had an affinity with low NO_3^- and K^+ water parameters. Similar to the results of this study, it was reported by Arman et al. (2014) that *Chroococcus membraninus* was detected in thermal water with low NO_3^- value. Likewise, while *Oscillatoria princeps* did not correlate with any of the water parameters determined in the RDA analysis in this study, it only showed a positive correlation with the CO_3 value and a negative correlation with other values in the CCA analysis in the study by Roy et al. (2015).

Conflict of interest: The authors declare no conflict of interests.

Authors' contributions: PB and GGS have designed the experiments and structured the manuscript. SO has critically performed statistical analysis and added to the discussion. GGS and RKG checked the final draft. All authors have mutually approved the final version of the manuscript.

Ethical approval: Not applicable

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