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Original article

Diversity and distribution pattern analysis of cyanobacteria isolated from paddy fields of Chhattisgarh, India

Satya Shila Singh*, Kikku Kunui, Robin Anigo Minj, Prashant Singh

Department of Botany, Guru Ghasidas Vishwavidyalaya, Bilaspur, Chhattisgarh, India

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ABSTRACT

Cyanobacteria have received much attention in soil due to their nitrogen-fixing ability and significant contribution in primary production. The diversity and distribution of unexplored cyanobacteria of Chhattisgarh has been investigated. We attempted to isolate, identify, and characterize the different cyanobacterial strains from different unexplored sites of Chhattisgarh. Twenty-nine strains of cyanobacteria, comprising two unicellular, four colonial, nine unbranched nonheterocystous, 12 unbranched heterocystous, and two pseudobranched cyanobacteria were phenotypically characterized on the basis of microscopic observation, that is, cell width, cell length, average filament length, colonial diameter, and position, shape and dimensions of heterocysts and akinetes. Results suggested that the phenotypic attributes were strain specific. Principle component analysis of heterocystous and nonheterocystous strains proved that environmental factors and physicochemical properties cumulatively decided the structure and distribution of cyanobacteria. These approaches also uncovered cyanobacterial diversity, which help in studying molecular diversity and documentation of unexplored cyanobacteria of Chhattisgarh, India.

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Introduction

Cyanobacteria are oxygen-evolving photoautotrophic prokaryotes. They have an incipient type of nucleus, loosely arranged thylakoids, hexagonal carboxysomes, gas vesicles, phycobilisomes, ribosomes, and a variety of storage granules consisting of glycogen, cyanophycin lipids or polyphosphate (Mishra et al., 2013; Singh et al., 2013). Some cyanobacterial species have a gas vesicle that helps in buoyancy and also maintains a certain vertical position in water in response to physical and chemical properties (Mishra et al., 2013). In spite of having typical prokaryotic cell structure, they contain chlorophyll a as the main photosynthetic pigment and several accessory pigments such as phycocyanin, allophycocyanin and phycoerythin, along with oxygenic photosynthetic ability. Cytological characters of cyanobacteria are similar but their ecological, biological and morphological characters are varied (Flores et al., 2006; Flores and Harrero, 2010; Kalaitzis et al., 2009; Komárek, 2010). They are one of the significant primary producers in the natural ecosystems (Field et al., 1998; Ohkouchi et al., 2006). Cyanobacteria are commonly found in diverse habitats (Whitton, 2000). They are found in free-living as well as in symbiotic association with plants, fungi and animals (Adams, 2000). The ability of these organisms is not only restricted to the nitrogen fixation and phosphorus fixation but also to metabolism of CO_2, H_2 and O_2 (Haven et al., 2003; Saadantia and Riahi, 2009; Whitton, 2000; Wilson, 2006).

Cyanobacteria are ancient organisms that inhabit the crust of the Earth and have some specialized features to accommodate the changing or different environmental conditions. Light, pH, temperature, and nutrient availability have received much attention because they participate in making the cyanobacteria withstand almost all the ecosystems (Kirrolia et al., 2012; Saadantia and Riahi, 2009).

Paddy fields are a suitable environment for the growth of diazotrophic, oxygenic cyanobacteria, by providing suitable temperature, nutrient and water facilities. In return, cyanobacteria provide a large amount of nitrogen and phosphorus, which are the most required nutrients at the time of rice cultivation. Association and importance of cyanobacteria with paddy fields have been known from ancient times. Appearance of cyanobacteria in paddy fields is observed during the early stage of sowing due to continuous supply of water, rich nutrient availability, and high level of CO_2

* Corresponding author. Tel.: +91 7587231571; fax: +91 7752 260154.
E-mail address: satyashila@rediffmail.com (S.S. Singh).
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that favours the growth of cyanobacteria. Apart from nitrogen and phosphorous fixation, they also excrete several organic acids that increase and maintain soil fertility, nutrient availability and water holding capacity (Roger and Reynaud, 1982; Saadantia and Riahi, 2009; Wilson, 2006).

Chhattisgarh is situated at 17° 46′ N to 24° 5′ N latitude and 80° 15′ E to 84° 20′ E longitude, which is a hot torrid zone possessing a tropical type of climate that varies among districts. Chhattisgarh is known for the presence of two types of soil, black clay and less fertile sandy soil. During the summer season, entrapped moisture from the soil is evaporated. These soils contain around 62% clay, 10% alumina, 9–10% iron oxide, and 6–8% lime and magnesium carbonate. Soils of Chhattisgarh are fertile, which makes them suitable for production of various crops including rice.

Despite their importance in paddy fields, few reports are available about cyanobacteria in the paddy fields of Chhattisgarh (Bajpai, 2013). However, these reports are confined to identification of cyanobacteria in the paddy fields. To date, no comprehensive research or data are available to establish a list of cyanobacteria in paddy fields or detailed microscopic observations of different cyanobacterial communities at different locations in Chhattisgarh. The present investigation dealt with the collection of cyanobacterial strains from different paddy fields in Chhattisgarh and analysis of physicochemical properties of soil, along with their impact on the diversity and morphology of the cyanobacterial strains in different areas of Chhattisgarh.

Materials and methods

Collection of the cyanobacterial strains

Cyanobacterial strains were collected from 13 different sites of Chhattisgarh: Mopka, Chillhati, Koni, Turkadih, campus of Guru Ghasidas Vishwavidyalaya, Sirgiti and Gatoura area belonging to Bilaspur district; Rasmada and Borsi belonging to Durg-Bhilai

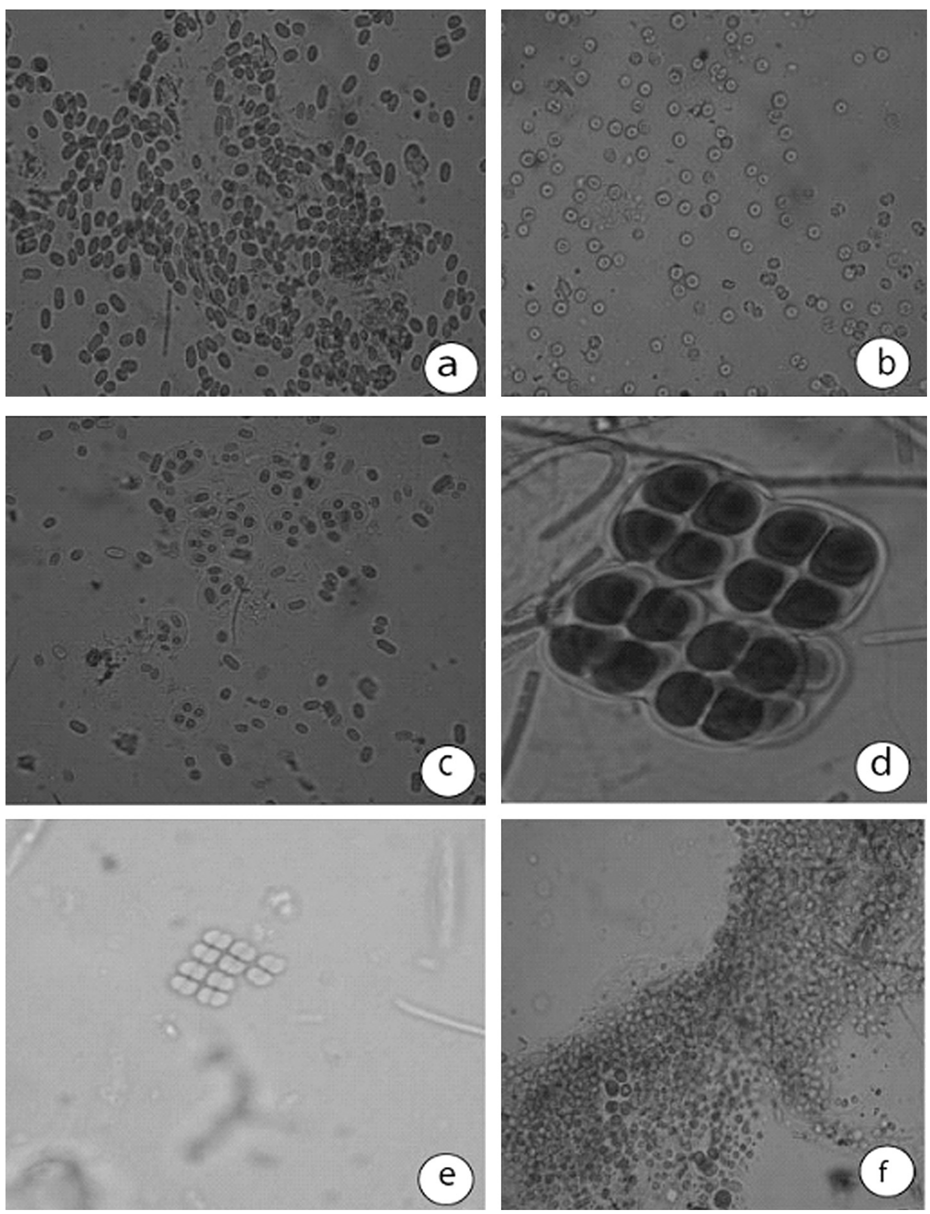

Figure 1. Photographs of cyanobacteria belonging to order Chroococcales: (a) *Aphanothece* sp.; (b) *Gloeothece* sp.; (c) *Gloeocapsa* sp.; (d) *Chroococcus* sp.; (e) *Merismopedia* sp.; (f) *Microcystis* sp.

district; Baramkela and Sarangarh of Raigarh district; and Mandir Hasaud and Karga area of Raipur district.

Isolation and maintenance of cultures

The isolated cyanobacterial strains were brought in the Department of Botany, Guru Ghasidas Vishwavidyalaya and maintained under specific culture conditions. Strains were transferred in liquid nutrient BG 11 medium (Rippka et al., 1979). All the collected cyanobacterial strains were maintained at 7.2 pH, 28 ± 2°C and light intensity of 50–55 μE/m^2/s with a 14/10-hour light/dark cycle photoperiod (Singh et al., 2013).

Morphological analyses

Morphological observations [presence and absence of sheath, shape and size of the vegetative cells, heterocysts, akinetes (if present), position, and branching pattern] of the axenic cultures of cyanobacterial strains were made using an Olympus KIC22809 microscope fitted with a digital camera, as described by Desikachary (1959) and Komarek and Anagnostidis (1998, 2005). Cyanobacterial images were captured at 100× magnification (Figures 1–5).

Determination of physicochemical properties of soil samples

The physicochemical properties of soil such as pH, temperature, conductivity, total dissolved solids, salinity, and dissolved oxygen were measured as per the following standard protocol:

Temperature and pH

The temperature of the soil of the paddy fields was measured with the help of a deluxe laboratory thermometer and pH of the soil was measured with the help of a Water and Soil Analysis Kit Model 161(EI Products, Panchkula, Haryana, India). The pH rod supplied with the kit worked on the principle of determination of the negative logarithm of [H^+] ion concentration. Before measuring [H^+] ion concentration of the soil, the electrode contained KCl and was properly calibrated by using pH buffer of 4.0 and 9.2. The soil samples were prepared by dissolving 2.5 g of soil sample in 10 mL double distilled water. The readings were measured in triplicate. The average of three observations was taken (Table 3).

Determination of total dissolved solids and salinity

Bicarbonates, carbonates, magnesium salts, phosphates, chlorides, potassium salts, iron salts, and manganese salts are some of the common salts found in dissolved states in natural water. Total dissolved solids were measured in mg/L, whereas salinity range was measured in g/L.

Determination of conductivity

Conductance of the collected samples from different paddy fields of Chhattisgarh was measured according to the value being provided in the manual with respect to the temperature of the sample. Conductivity was measured in μS/cm.

Determination of dissolved oxygen

Before measuring dissolved oxygen in the soil, the temperature of the samples was evaluated. According to the temperature, oxygen solubility was determined in ppm after proper calibration of the dissolved oxygen (probe).

Principle component analysis

The profound effect of various physicochemical factors on the morphology of cyanobacteria was found to be both on individual

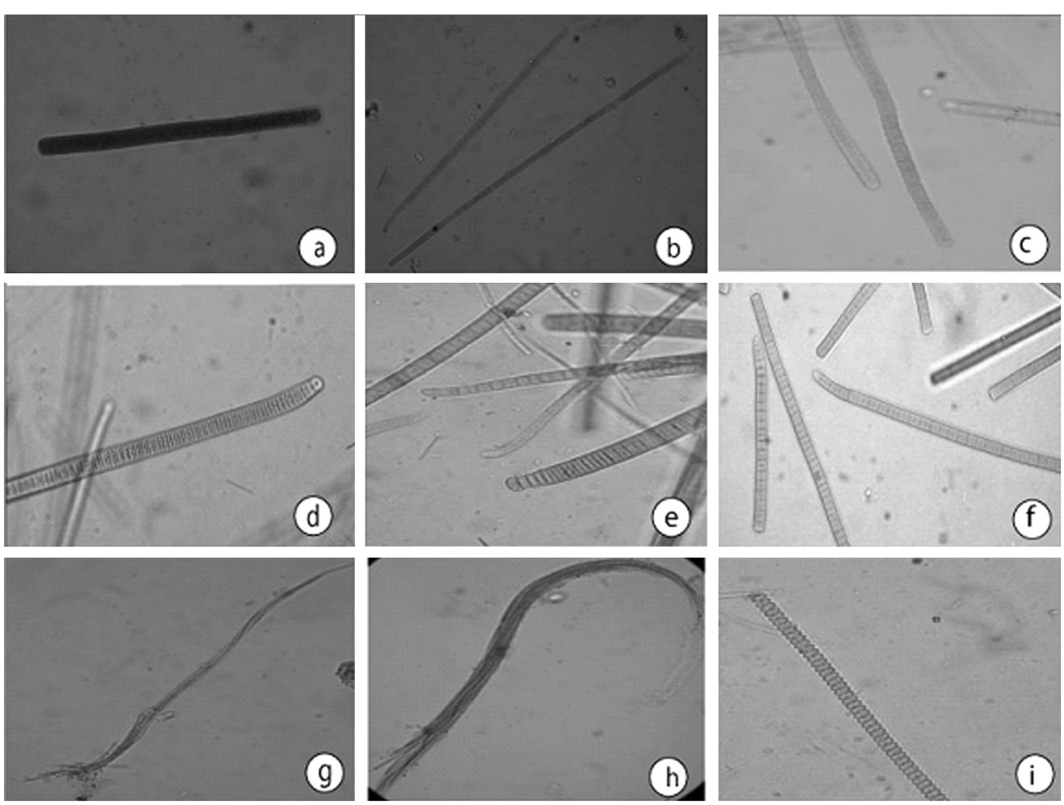

Figure 2. Photographs of nonheterocystous cyanobacteria belonging to order Nostocales: (a) *Oscillatoria princeps*; (b) *Oscillatoria corntiana*; (c) *Oscillatoria quadrapanculata*; (d) *Oscillatoria curviceps*; (e) *Oscillatoria acuta*; (f) *Oscillatoria proboscidea*; (g) *Microcoleus major*; (h) *Microcoleus chthonoplastes*; (i) *Arthrospira spirulinoides*.

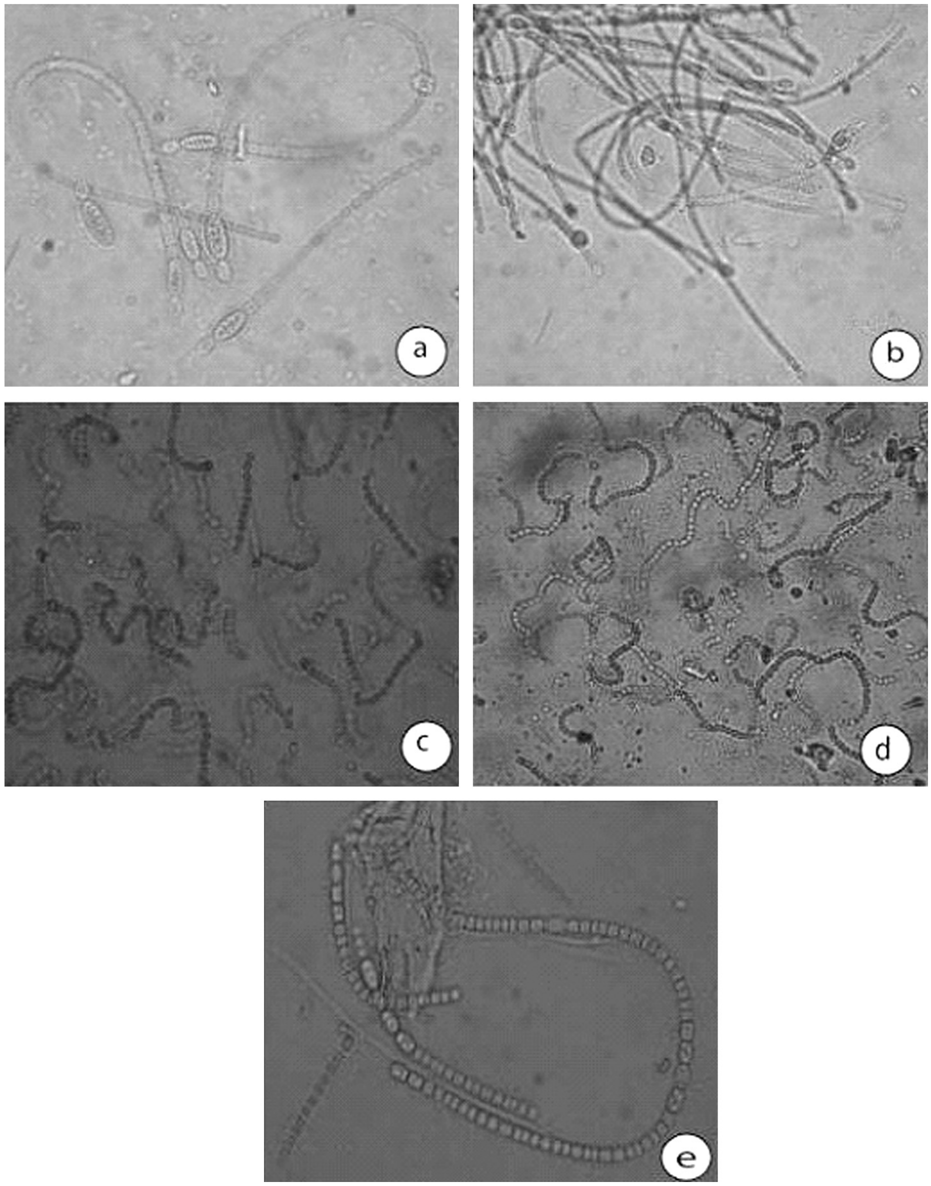

Figure 3. Photographs of heterocystous cyanobacteria belonging to order Nostocales: (a) *Cylindrospermum michailovskoense*; (b) *Cylindrospermum indicum*; (c) *Nostoc* sp.; (d) *Nostoc commune*; (e) *Anabaenopsis* sp.

and cumulative scale with the morphology being influenced significantly. The environmental factors acted in synchrony with each other, along with having both synergistic and antagonistic effects on single trait morphology. All the factors acted cumulatively to show their impact. In order to understand these types of effects, principal component analysis is an excellent tool for studying the combined holistic effects (Figure 6).

Results and discussion

Structure and distribution pattern of cyanobacterial strains

Two unicellular, four colonial, nine unbranched nonheterocystous, 12 unbranched heterocystous, and two pseudobranched cyanobacteria were identified and characterized on the basis of microscopic observations. All the nonheterocystous cyanobacterial strains were found to vary in their cell width, cell length and average length of filament (Table 1). Minimum filament was observed in *Arthrospira spirulinoides* (35.7 ± 3.12 μm), which was collected from Baramkela Farm, Raigarh, whereas the largest filament length was reported in *Oscillatoria quadrapanculata* (58.9 ± 3.68 μm). Different nonheterocystous cyanobacterial strains from different sites in Chhattisgarh showed various sizes of vegetative cells. Various types of unicellular and colonial cyanobacterial strains were characterized on the basis of cell width, cell length and colonial diameter (Table 1). Maximum colonial diameter was observed in *Microcystis* sp. (124 ± 8.46 μm), whereas it was highest in unicellular *Aphanothece* sp. (3.5 ± 0.42 μm) (Figure 1).

On consideration of heterocystous cyanobacterial strains, 14 species were identified on the basis of average filament length, shape and size of the vegetative cell; and shape, size and position of heterocyst and akinete (Table 2). Eight strains, *Anabaena constricta*, *Anabaena* sp., *Anabaena khannae*, *Scytonema* sp., *Tolypothrix* sp., *Rivularia* sp., *Nostoc commune* and *Calothrix marchia* did not posses akinetes, whereas the rest showed the presence of intercalary or terminal akinetes. The shape and size of the akinetes also varied

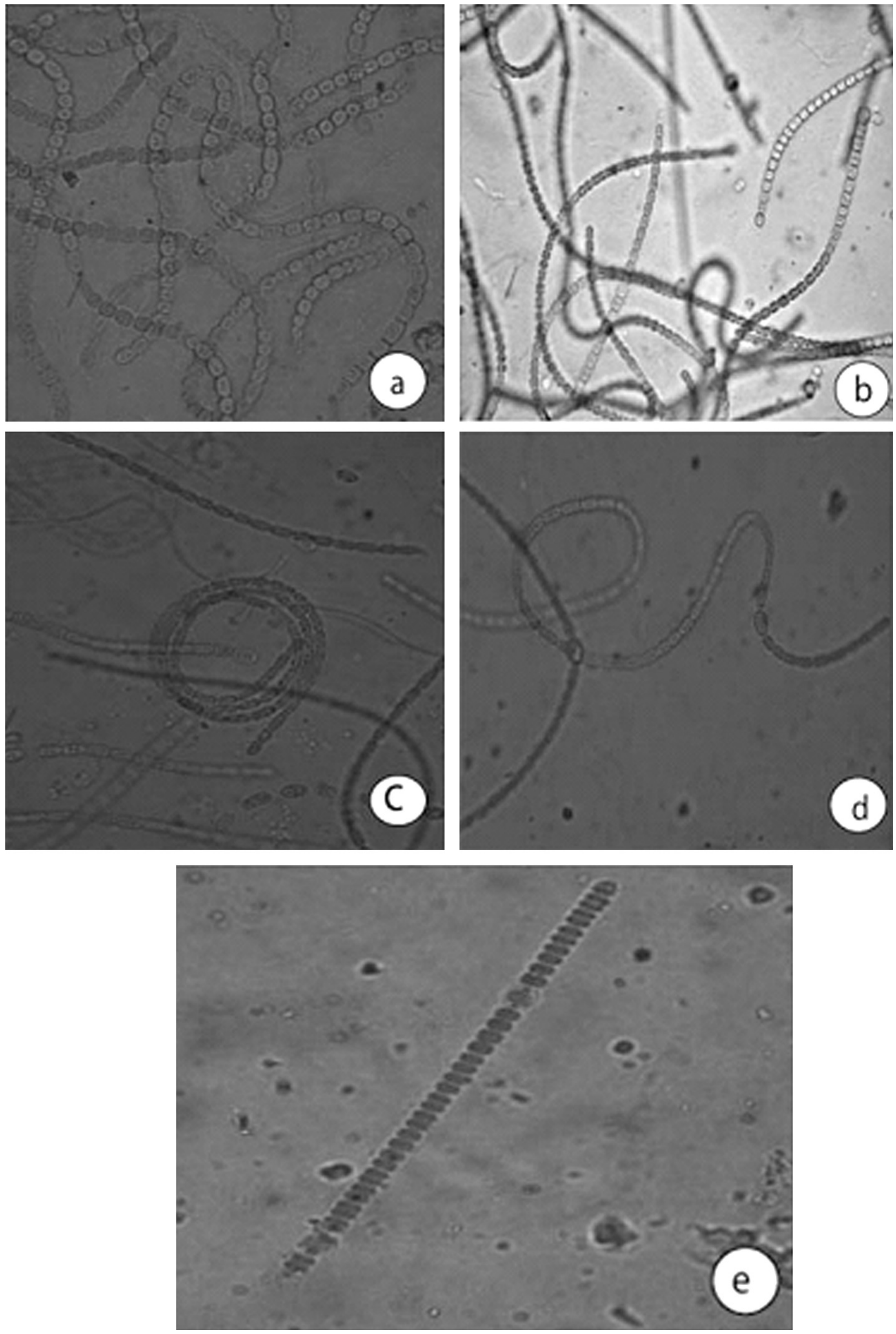

Figure 4. Photographs of cyanobacteria belonging to order Nostocales: (a) *Anabaena variabilis*; (b) *Anabaena constricta*; (c) *Anabaena* sp.; (d) *Anabaena khannae*; (e) *Anabaena inequalis*.

among the different heterocystous cyanobacteria. The largest filament length (57 ± 1.65 μm) was reported in *Anabaena khannae*, whereas *Calothrix marchia* possessed the smallest filament size (24.4 ± 1.05 μm). Different shapes of vegetative cell (elliptical, spherical, barrel, cylindrical, rectangular and quadrate) and heterocyst (elliptical, barrel, spherical and oval) were observed among the cyanobacteria. The size of the vegetative cell and heterocyst ranges from 0.05 ± 0.002 μm to 1 ± 0.25 μm (length) × 0.02 ± 0.001 μm to 0.5 ± 0.033 μm (width) and 0.05 ± 0.03 μm to 2.25 ± 0.15 μm (length) × 0.05 ± 0.005 μm to 1.15 ± 0.11 μm (width). The heterocyst and akinetes were found to be terminal and intercalary differentiated. After consideration of structural diversity among the cyanobacteria of the different types of soils of Chhattisgarh, there was great heterogeneity in the cyanobacterial community at different sites (Tables 2 and 3).

Effect of physicochemical properties on cyanobacterial community

In the collected soil samples, the highest pH was 8.53 (Chillhati Agricultural Farm, Bilaspur) whereas the lowest pH was 6.9 (Mandir Hasaud Farm, Raipur), and the rest of the paddy fields of Chhattisgarh had pH 6.9–8.53 (Table 3). It was clear from our

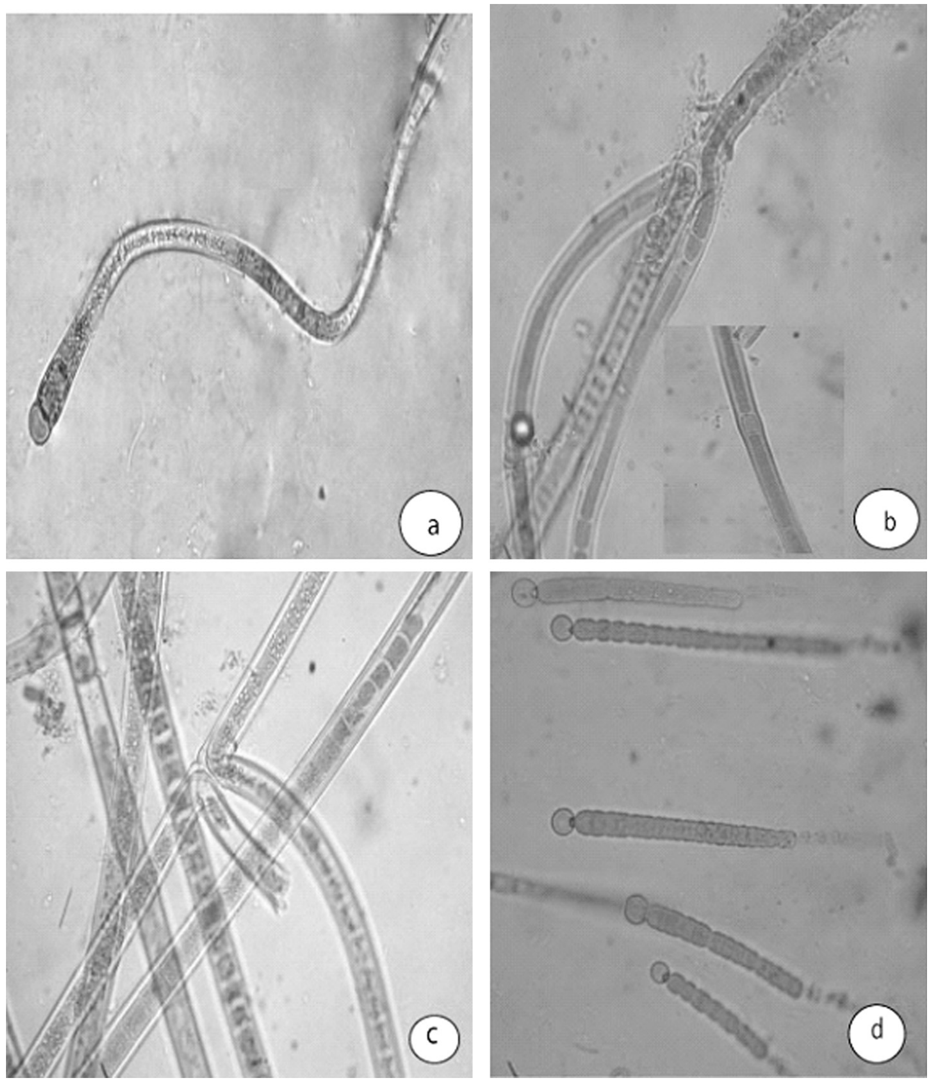

Figure 5. Photographs of cyanobacteria belonging to order Nostocales: (a) *Calothrix marchia*; (b) *Scytonema* sp.; (c) *Tolypothrix* sp.; (d) *Rivularia* sp.

Table 1
List of unicellular, colonial and nonheterocystous cyanobacterial strains and their collection sites.

S. No.	Cyanobacterial strains	Cell dimension (μm)		Average colony diameter/average filament length (μm)	Collection sites
		L	W		
Unicellular cyanobacteria					
1.	*Aphanothece* sp.	1.25 ± 0.22	0.75 ± 0.04	3.5 ± 0.42	Turkadih Agricultural Farm, Bilaspur
2.	*Gloeothece* sp.	1.50 ± 0.11	0.72 ± 0.03	2.0 ± 0.14	Chillhati Agricultural Farm, Bilaspur
Colonial cyanobacteria					
3.	*Gloeocapsa* sp.	1.24 ± 0.10	0.5 ± 0.02	5.6 ± 0.41	Mandir Hasaud, Raipur
4.	*Chroococcus* sp.	18.2 ± 0.14	20.0 ± 1.6	108.0 ± 5.23	Turkadih Agricultural Farm, Bilaspur
5.	*Merismopedia* sp.	5.2 ± 0.38	0.4 ± 0.01	3.0 ± 0.15	GGV Campus, Bilaspur
6.	*Microcystis* sp.	1.08 ± 0.06	0.91 ± 0.04	124 ± 8.64	Mopka Farm, Bilaspur
Filamentous nonheterocystous cyanobacteria					
7.	*Oscillatoria princeps*	1.5 ± 0.24	1.75 ± 0.14	40 ± 0.28	Sarangarh Agricultural Farm, Raigarh
8.	*Oscillatoria corntiana*	0.50 ± 0.02	0.85 ± 0.03	47.5 ± 2.46	Rasmada Paddy Field, Durg
9.	*Oscillatoria quadrapanculata*	1.6 ± 0.02	0.72 ± 0.01	58.9 ± 3.68	Karga Farm, Raipur; Koni, Bilaspur
10.	*Oscillatoria curviceps*	2.6 ± 0.25	0.76 ± 0.03	37.3 ± 2.48	Sarangarh Agricultural Farm, Raigarh
11.	*Oscillatoria acuta*	1.62 ± 0.14	0.64 ± 0.03	42.5 ± 2.86	Sirgiti Paddy Field, Bilaspur
12.	*Oscillatoria proboscidea*	1.46 ± 0.05	0.48 ± 0.02	36.4 ± 1.64	Gatoura Paddy Field, Bilaspur
13.	*Microcoleus major*	1.2 ± 0.06	1.75 ± 0.08	39.5 ± 2.24	Chillhati Agricultural Farm, Bilaspur
14.	*Microcoleus chthonoplastes*	1.76 ± 0.07	0.80 ± 0.02	46.20 ± 3.42	Sirgiti Paddy Field, Bilaspur
15.	*Arthrospira spirulinoides*	1.1 ± 0.03	0.5 ± 0.02	35.7 ± 3.12	Baramkela Farm, Raigarh

L = length; W = width; S. No = serial number.

Table 2
General characteristics of heterocystous filamentous cyanobacterial species collected from different paddy fields of Chhattisgarh.

S. No.	Cyanobacterial strains	Vegetative cells			Heterocysts				Akinetes				Average filament length (μM)	Collection sites
		Shape	Dimension (μM)		Shape	Dimension (μM)		Position	Shape	Dimension (μM)		Position		
			L	W		L	W			L	W			
1.	*Cylindrospermum michailovskoense*	Elliptical	0.05 ± 0.002	0.02 ± 0.001	Elliptical	1.25 ± 0.09	0.09 ± 0.003	Terminal	Cylindrical	0.75 ± 0.05	0.25 ± 0.019	Terminal	39.26 ± 0.29	Mandir Hasaud, Raipur
2.	*Cylindrospermum indicum*	Elliptical	1 ± 0.25	0.40 ± 0.03	Barrel	2.25 ± 0.15	1.5 ± 0.11	Terminal	Oval	0.75 ± 0.04	0.20 ± 0.009	Terminal	35.2 ± 2.06	Borsi Farm, Durg
3.	*Nostoc* sp.	Spherical	0.25 ± 0.019	0.10 ± 0.005	Spherical	0. 5 ± 0.03	0.25 ± 0.013	Intercalary	Oval	0.75 ± 0.05	0.50 ± 0.09	Intercalary	50 ± 4.44	Borsi Farm, Durg
4.	*Nostoc commune*	Barrel	0.50 ± 0.023	0.50 ± 0.033	Spherical	0.75 ± 0.50	0.50 ± 0.040	Intercalary	–	–	–	–	48.5 ± 2.36	Rasmada Paddy, Field, Durg
5.	*Anabaenopsis* sp.	Cylindrical	0.55 ± 0.03	0.2 ± 0.001	Oval	0.75 ± 0.050	0.25 ± 0.015	Intercalary	Oval	0.5 ± 0.02	0.5 ± 0.02	Terminal	25.8 ± 1.91	GGV Campus, Bilaspur
6.	*Anabaena variabilis*	Cylindrical	0.05 ± 0.003	0.09 ± 0.001	Oval	0.75 ± 0.025	0.1 ± 0.009	Intercalary	Spherical	0.75 ± 0.4	0.25 ± 0.019	Intercalary	47.1 ± 3.52	Turkadih Agricultural Farm, Bilaspur
7.	*Anabaena constricta*	Elliptical	0.25 ± 0.018	0.09 ± 0.007	Oval	0.5 ± 0.035	0.09 ± 0.006	Intercalary	–	–	–	–	46.1 ± 3.92	Mopka Farm, Bilaspur
8.	*Anabaena* sp.	Barrel	0.75 ± 0.048	0.25 ± 0.016	Barrel	1 ± 0.35	0.50 ± 0.039	Intercalary	–	–	–	–	53 ± 2.36	Chillhati Agricultural Farm, Bilaspur
9.	*Anabaena khannae*	Rectangular	0.50 ± 0.035	0.15 ± 0.014	Oval	0.75 ± 0.042	0.25 ± 0.019	Intercalary	–	–	–	–	57 ± 1.65	Turkadih Agricultural Farm, Bilaspur.
10.	*Anabaena inequalis*	Cylindrical	0.50 ± 0.002	0.25 ± 0.002	Spherical	0.75 ± 0.005	0.25 ± 0.01	Intercalary	Elliptical	0.75 ± 0.003	0.5 ± 0.004	Intercalary	55 ± 3.33	Borsi Farm, Durg.
11.	*Calothrix marchia*	Quadrate	0.50 ± 0.03	0.25 ± 0.015	Spherical	0.80 ± 0.05	0.50 ± 0.03	Terminal	–	–	–	–	24.4 ± 1.05	GGV Campus, Bilaspur.
12.	*Scytonema* sp.	Spherical	0.40 ± 0.02	0.25 ± 0.010	Barrel	0.45 ± 0.02	0.75 ± 0.05	Intercalary	–	–	–	–	47.5 ± 2.33	Sarangarh, Raigarh.
13.	*Tolypothrix* sp.	Quadrate	0.75 ± 0.05	0.50 ± 0.03	–	–	–	–	–	–	–	–	50 ± 2.25	Baramkela, Raigarh.
14.	*Rivularia* sp.	Spherical	0.50 ± 0.002	0.040 ± 0.002	Spherical	0.75 ± 0.005	0.50 ± 0.005	Terminal	–	–	–	–	49 ± 3.39	Chillhati Agricultural Farm, Bilaspur.

L = length; W = width; S. No = serial number.

investigations that alkaline pH supported the growth of all the reported cyanobacteria except *Cylindrospermum michailovskoense* and *Gloeocapsa* sp. (Tables 1–3). Similar findings were reported in some cyanobacteria of shallow lakes of Pantanal of Nhecolándia, which is one of the extreme environments (alkaline) for various life forms (Kristjánsson and Hreggvidsson, 1995; Valient and Quesada, 2004). The data proved that *Gloeocapsa* sp. and *Cylindrospermum michailovskoense* showed luxuriant growth at slightly acidic pH ($<$ 7.0).

In all the tested soil samples of Chhattisgarh, the temperature varied from 26.0°C (Gatoura paddy field, Bilaspur) to 38°C (Rasmada paddy field, Durg) (Table 3). *Oscillatoria corntiana* and *Nostoc commune* were reported at higher temperature of 38°C, followed by *Arthrospira spirulinoides* and *Tolypothrix* sp. at 34°C, whereas *Oscillatoria proboscidea* was reported at the lowest temperature (26°C). Present investigations also proved that there was a fluctuation (26–38°C) in the temperature of soils from different sites. Collection of cyanobacteria was done during August to November, which is why temperature was not so high and it was suitable for cyanobacterial growth.

Turkadih paddy field belonging to Bilaspur district showed the highest salinity 148.9 ± 12.9 g/L, whereas the lowest soil salinity of 32.0 ± 1.01 g/L was observed in Mandir Hasaud area, Raipur district. The reported genera from Mandir Hasaud, Raipur were *Cylindrospermum michailovskoense* and *Gloeocapsa* sp. at lowest salinity. *Aphanothece* sp., *Chroococcus* sp., *Anabaena variabilis* and *Anabaena khannae* were grown at highest salinity. Our results were contradictory to the findings of Nielsen et al. (2003) who reported that salinity $>$ 1 g/L may cause adverse effect on aquatic biota. In our case, salinity ranged from 32 ± 1.01 g/L to 148.9 ± 12.9 g/L, and it supported the growth of the cyanobacteria.

Total dissolved solids in different soil samples ranged from 100 ± 3.9 mg/L to 600.5 ± 52.3 mg/L. Turkadih was the only area of Bilaspur that had the highest total dissolved solids content, namely, 600.5 ± 52.3 mg/L.

Although the total dissolved solids were around 100 mg/L at all the investigated sites, that is, Raipur, Raigarh and Durg, the cyanobacterial community was variable. This meant that total dissolved solids alone were not the deciding factor for the growth of the cyanobacteria at the specific site.

Oxygen has a diffusible tendency, due to which it rapidly diffuses in the surrounding water. Dissolved oxygen has a link with temperature (Ugwu et al., 2006). If the temperature is increased up to 20–38°C, the dissolved oxygen level is also increased to a greater extent, and this increased dissolved oxygen level is responsible for increased photosynthetic activity. The highest level of dissolved oxygen was 70.0 ± 3.1 ppm reported from the soil samples collected from the paddy field of Karga, Raipur, followed by Rasmada paddy field (68 ± 4.94 ppm). *Oscillatoria quadrapanculata* was reported from Karga Farm, Raipur, and *Oscillatoria corntiana* was reported from Rasmada paddy field, Durg. The lowest dissolved oxygen level of 12.3 ± 0.9 ppm was reported from Turkadih Agricultural Farm, Bilaspur. *Aphanothece* sp., *Chroococcus* sp., *Anabaena khannae* and *Anabaena variabilis* were present at the lowest dissolved oxygen level. Our results also agree with the above findings (Table 3). *Oscillatoria corntiana* and *Nostoc commune* were reported at the higher temperature and had the highest dissolved oxygen level. The lowest dissolved oxygen level supported the growth of *Aphanothece* sp., *Chroococcus* sp., *Anabaena khannae* and *Anabaena variabilis*.

Normal water is a good conductor of electricity due to the presence of acid, base, salts and other dissolved components that acts as electrolytes. Water acts as the basic carrier of many important minerals and salts from cell to cell. The theory of non-equilibrium thermodynamics proves coupling between water and

Table 3
Analysis of physicochemical properties of soil sample of different paddy fields of Chhattisgarh.

Area	pH	Temperature (°C)	Salinity (g/L)	Total dissolved solid (mg/L)	Dissolved oxygen (ppm)	Conductivity (µS/cm)
GGV Campus Bilaspur	8.28	28.2	135.2 ± 11.05	401 ± 39.2	18.1 ± 1.61	416.6 ± 25.3
Koni, Bilaspur	7.3	31.6	97.1 ± 7.9	153.25 ± 6.9	67.35 ± 4.2	87.85 ± 6.41
Mopka Farm Bilaspur	8.21	28.2	83.6 ± 5.43	210 ± 19.2	57.7 ± 3.61	801.8 ± 52.8
Gatoura Paddy Field, Bilaspur	7.56	26.0	86.9 ± 6.32	222 ± 15.4	28.0 ± 0.19	36.4 ± 1.14
Sirgiti Paddy Field, Bilaspur	7.87	32.4	74.3 ± 5.41	200 ± 16.4	63.3 ± 2.42	66.17 ± 4.56
Chillhati Agricultural Farm, Bilaspur	8.53	32.3	58.7 ± 3.36	120 ± 10.4	33.4 ± 1.18	57.38 ± 3.32
Turkadih Agricultural Farm, Bilaspur	7.49	32.2	148.9 ± 12.9	600.5 ± 52.3	12.3 ± 0.09	128.6 ± 11.64
Sarangarh Agricultural Farm, Raigarh	8.1	33	41 ± 3.02	104 ± 5.49	40.9 ± 1.16	34.87 ± 2.14
Baramkela Farm Raigarh	7.9	34	39 ± 1.12	100 ± 3.9	52 ± 2.09	93.1 ± 7.61
Mandir Hasaud	6.9	32	32 ± 1.01	105.2 ± 9.5	36 ± 2.15	52.08 ± 3.20
Karga Farm Raipur	8.2	32	40 ± 1.15	105 ± 6.1	70 ± 3.1	84.81 ± 6.25
Borsi Farm Durg	7.8	33	56 ± 2.63	108 ± 8.9	65 ± 2.73	59.59 ± 4.51
Rasmada Paddy Field, Durg	8.3	38	60 ± 4.00	105 ± 6.1	68 ± 4.94	61.67 ± 4.44

solute flow from one cell to another (Katchalsky and Curran, 1965). Time-dependent water transport shows that the rate constant of water exchange between the cell and its surroundings is determined by the geometric parameters such as hydraulic conductivity of the cell (Dainty, 1963; Wan, 2010). Hydraulic conductivity of the soil is maintained due to coarse texture of soil which is implemented and maintained by microbiotic activity. If the microbiotic community is disturbed, the flow rate of water through soil is increased. Microbiota including cyanobacteria maintains soil structure (Williams et al., 1999).

Different conductivity was observed in different soil samples and the highest was reported to be 801.8 ± 52.8 µS/cm from Mopka, Bilaspur, followed by Guru Ghasidas Vishwavidyalaya campus, Bilaspur, whereas the lowest conductivity of 34.87 ± 2.14 µS/cm was found at Sarangarh Agricultural Farm, Raigarh. *Anabaena constricta* and *Microcystis* sp. were reported at the highest conductivity and *Oscillatoria curviceps*, *Oscillatoria princeps* and *Scytonema* sp. at the lowest conductivity.

Principle component analysis was performed among heterocystous and nonheterocystous cyanobacterial strains collected from different paddy fields of Chhattisgarh. The analysis focussed on assessing the effect of environmental factors as well as physicochemical properties cumulatively on the average filament length, vegetative cell size, heterocyst size, akinete size of heterocystous cyanobacteria, and cell width, cell length, and average filament length/average diameter of the colony of nonheterocystous cyanobacteria.

In heterocystous cyanobacteria, the microscopic observations of sp. *Calothrix marchia* and *Anabaenopsis* sp. belonging to Order Nostocales showed almost similar and minimum cumulative effect by ecological and physicochemical properties (Figure 6A). Although *Cylindrospermum indicum* and *Cylindrospermum michailovskoense* belong to the same order of Nostocales, they were influenced differently by physicochemical properties. At the same time, *Anabaena inequalis* and *Nostoc* sp. were highly influenced by physicochemical properties, whereas highest impact was reported in *Anabaena khanna*.

A lower impact of physicochemical properties of soil was observed in *Gloeothece* sp., followed by *Aphanothece* sp., *Gloeocapsa* sp. and *Merismopedia* sp. (Figure 6B). The morphology of *Oscillatoria proboscidea*, *Arthrospira spirulinoides*, *Oscillatoria curviceps*, *Microcoleus major*, *Oscillatoria princeps* and *Oscillatoria acuta* was influenced similarly by physicochemical properties of soil. Although *Oscillatoria corntiana* and *Oscillatoria quadrapanculata* belong to the same order and family, the latter was influenced/behaved differently due to the physicochemical properties of the soil. Optimum impact of physicochemical properties was observed in *Microcystis* sp. followed by *Chroococcus* sp.

We concluded that 29 strains of cyanobacteria with detailed microscopic analysis were reported from different sites of Chhattisgarh. Presence and absence of different cyanobacterial strains at different sites were decided by the cumulative effect of physicochemical properties of soils, as well as various environmental factors. It can be extrapolated that combination of different

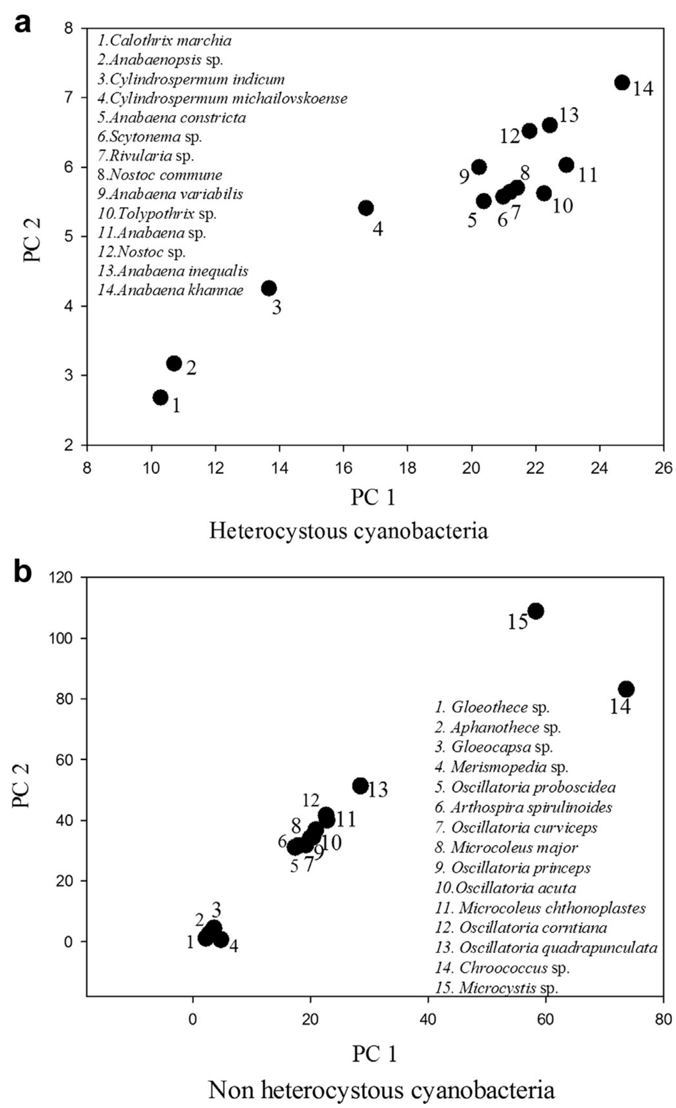

Figure 6. (a) Representation of principal component analysis showing effect of environmental factors and physicochemical properties on the structure of non-heterocystous cyanobacteria. (b) Representation of principal component analysis showing effect of environmental factors and physicochemical properties on the structure of heterocystous cyanobacteria.

physiological characteristics provides a basis for different combinations of cyanobacterial strains in different sites of Chhattisgarh, India.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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References

Adams DG. 2000. Symbiotic interactions. In: Whitton BA, Potts M, editors. *The ecology of cyanobacteria: their diversity in time and space*. Alphen aan den Rijn: Kluwer Academic. pp. 523–561.
Bajpai P. 2013. Phylogenetical characterization of cyanobacteria from paddy field of Chhattisgarh (Part I). *International Journal of Pharmaceutical Science Research* 4: 1110–1115.
Dainty J. 1963. Water relation of plant cells. *Advances in Botany Research* 1:279–326.
Desikachary TV. 1959. *Cyanophyta*. New Delhi: Indian Council of Agricultural Research.
Field CB, Behrenfeld MJ, Randerson JT, Falkowski P. 1998. Primary production of the biosphere: integrating terrestrial and oceanic components. *Science* 281: 237–240.
Flores E, Harrero A, Wolk CP, Maldener I. 2006. Is the periplasm continuous in filamentous multicellular cyanobacteria? *Trends in Microbiology* 14:439–443.
Flores E, Herrero A. 2010. Compartmentalized function through cell differentiation in filamentous cyanobacteria. *Nature Reviews in Microbiology* 8:39–50.
Haven KE, James RT, East TL, Smith VH. 2003. N: P ratios, light limitation, and Cyanobacterial dominance in a subtropical lake impacted by non-point source Nutrient pollution. *Environmental Pollution* 122:379–390.
Kalaitzis JA, Cheng Q, Thomas PM, et al. 2009. *In vitro* biosynthesis of unnatural enterocin and wailupemycin polyketides. *Journal of Natural Products* 72: 469–472.
Katchalsky A, Curran PF. 1965. *Nonequilibrium thermodynamics in biophysics*. Cambridge: Harvard University Press. pp. 113–132.
Kirrolia A, Bishnoi NR, Singh R. 2012. Effect of shaking, incubation temperature, salinity and media composition on growth traits of green algae *Chlorococcum* sp. *Journal of Algal Biomass Utilization* 3:46–53.
Komárek J, Anagnostidis K. 1998. Cyanoprokaryota 1. In: Ettl H, Gartner G, Heynig H, Mollenhauer D, editors. *Teil: Chroococcales. Jena-Stuttgart-Lübeck-Ulm: Süswasserflora von Mitteleuropa 19/1*. Gustav Fischer. p. 548.
Komarek J, Anagnostidis K. 2005. Cyanoprokaryota 2. Teil/ 2nd Part: Oscillatoriales. In: Budel B, Krienitz L, Gartner G, Schagerl M, editors. *Süsswasserflora von Mitteleuropa 19/2*. Heidelberg: Elsevier/Spektrum. p. 759.
Komárek J. 2010. Recent changes (2008) in cyanobacteria taxonomy based on a combination of molecular background with phenotype and ecological consequences (genus and species concept). *Hydrobiology* 639:245–259.
Kristjánsson JK, Hreggvidsson GO. 1995. Ecology and habitats of extremophiles. *World Journal of Microbiol Biotechnology* 11:17–25.
Mishra AK, Shukla E, Singh SS. 2013. Phylogenetic comparison among the heterocystous cyanobacteria based on a polyphasic approach. *Protoplasma* 250:77–94.
Nielsen DL, Brock MA, Rees GN, Baldwin DS. 2003. Effects of increasing salinity on freshwater ecosystems in Australia. *Australian Journal of Botany* 51:655–665.
Ohkouchi N, Kashiyama Y, Kuroda J, et al. 2006. The importance of diazotrophic cyanobacteria as primary producer during cretaceous oceanic anoxic event. *Biogeoscience* 3:467–478.
Rippka R, Deruelles J, Waterbury JB, et al. 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Journal of General Microbiology* 111:1–61.
Roger PA, Reynaud PA. 1982. *Free-living blue-green algae in tropical soils*. La Hague: Martinus Nijhoff.
Saadantia H, Riahi H. 2009. Cyanobacteria from paddy fields in Iran as a biofertilizer in rice plants. *Plant Soil and Environment* 55:207–212.
Singh P, Singh SS, Elster J, Mishra AK. 2013. Molecular phylogeny, population genetics and evolution of heterocystous cyanobacteria using *nif*H gene sequences. *Protoplasma* 250:751–764.
Ugwu CU, Aoyagi H, Uchiyama H. 2006. Influence of irradiance, dissolved oxygen concentration, and temperature on the growth of *Chlorella sorokiniana*. *Photosynthetica* 45:309–311.
Valient EF, Quesada A. 2004. A Shallow water ecosystem: rice-fields. The relevance of cyanobacteria in the ecosystem. *Limnetica* 23:95–108.
Wan X. 2010. Osmotic effects of NaCl on cell hydraulic conductivity of corn roots. *Acta Biochimica et Biophysica Sinica* 42:351–357.
Whitton BA. 2000. Soils and rice–fields. In: Whitton BA, Potts M, editors. *The ecology of cyanobacteria :their diversity in time and space*. Alphen aan den Rijn: Kluwer Academic. pp. 233–255.
Williams JD, Dobrowolski JP, West NE. 1999. Microbiotic crust influence on unsaturated hydraulic conductivity. *Arid Soil Research and Rehabilitation* 13:145–154.
Wilson LT. 2006. Cyanobacteria: a potential nitrogen source in the rice fields. *Texas Rice* 6:9–10.