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Influence of extreme field's soil ecology on cyanobacteria diversity and abundance around Bhopal

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ABSTRACT

Cyanobacteria flourish in rice fields, and play a major role in sustaining the fertility of this agro-ecosystem. The present study is an attempt to decipher the abundance and diversity of cyanobacteria in various extreme fields around Bhopal, Madhya Pradesh. A total of 87 cyanobacterial morphotypes belonging to sixteen different genera were isolated from the Raisen, Bhopal, Sehore, and Dhar blocks. The genus *Anabaena* was found to be the most abundant followed by genus *Anabaena*, *Nostoc*, and *Calothrix*. These genuses were widely distributed in all the sites emphasizing their adaptability and resilience under diverse environmental conditions. Raisen had mainly acidic pH (5.6), and low nitrogen (155 kg/hec), and showed higher diversity index (Shannon-Weiner's index 2.339), and lowest evenness (0.7391) than Sehore having alkaline pH (7.9), medium nitrogen (307 kg/hec) showing lowest diversity index Shannon- Weiner's index (1.906), and highest evenness (0.961). These findings revealed that in Sehore physico-chemical parameters were normal for cyanobacterial growth and abundance but had lowest diversity index whereas Raisen block had highest diversity index where physcio-chemical parameters were extreme. Results further suggested the practical utilization of these nitrogen fixing cyanobacteria towards the development of region-specific inocula capable of performing better and provide maximum benefits to the crop.

KEYWORDS: Cyanobacteria, Diversity, Extreme fields, Nitrogen fixation, Biofertilizer.

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INTRODUCTION

Blue-green algae constitute the largest, extremely diverse, widely distributed, gram negative group of prokaryotes, capable of performing oxygenic photosynthesis and are amongst oldest life forms present [1]. Moreover, cyanobacteria can live in some of the most extreme habitats on earth like use of huge amount of pesticides and chemical fertilizers in irrigation of agriculture fields, high temperature and low pH, hot springs, hyper-saline waters, freezing environments, and arid deserts [2, 3]. During their long evolutionary history, cyanobacteria had undergone several structural and functional modifications responsible for their versatile physiology and wide ecological tolerance. Their abilities to tolerate high temperature, UV radiation, desiccation, water and saline stresses contribute to their competitive success in a wide range of environments [4].

An ever- increasing amount and number of pesticides are used for high yield production in agriculture. Pesticide use is effective for the protection of plants from pest but the extensive use of pesticide over the past four decades has resulted in the disturbance of natural biological system and ecological condition [5, 6]. Pesticides interact with soil organisms and their metabolic activities and may alter the physiological and biochemical behaviour of soil microbes, through rice fields provide suitable environment for the growth of cyanobacteria by providing optimum temperature, nutrient and water [7]. In return, cyanobacteria provide nitrogen and phosphorus, the most required nutrients at the time of rice cultivation. Cyanobacteria produce a wide array of compounds like amino acids, auxins, gibberellins, cytokinins [8]. All these compounds increase the availability of nutrients and help the plants in taking up nutrients [9].They also excrete several organic acids that increase and maintain soil fertility, nutrient availability and water holding capacity [10, 11] and soil productivity both directly and indirectly[12, 13].

Madhya Pradesh lies between latitude $23^0$47N and longitude 77^0 94 E. The state is endowed with rich natural resources, salubrious climate and fertile agro climatic conditions. Soils of Madhya Pradesh vary as per the structure, colour, texture and composition in the different regions. The shallow and medium black, deep medium black, alluvial, and mixed reds black soil is found in Madhya Pradesh (forest and climate change ,Govt of india, 2017).This soil is highly fertile for the production of wheat, rice, oilseed and juwar crops because it has the capacity to hold moisture for long time but these soils develop deep cracks in summer, which helps in the aeration of the soil [14]. These soil properties and climate condition are favourable for cyanobacterial growth and abundance, therefore maximum cyanobacterial diversity is found in different blocks of Madhya Pradesh. The present investigation dealt with the collection of cyanobacterial morphotypes from different fields of

Madhya Pradesh and analysis of physicochemical properties of soil, along with their impact on the diversity and morphology of the cyanobacterial occurrence in selected blocks.

MATERIALS AND METHODS

Study site

Four different blocks were selected from different cultivated lands, namely, Raisen, Bhopal, Sehore, and Dhar. The areas were selected on the basis of different texture of soils and different water resources and pesticides by which they irrigated.

The fields were divided in to 4 blocks as:-

1. Raised district (Mandideep)
2. Bhopal district(Govindpura)
3. Sehore district(Pilukhedi)
4. Dhar district(Pithampur)

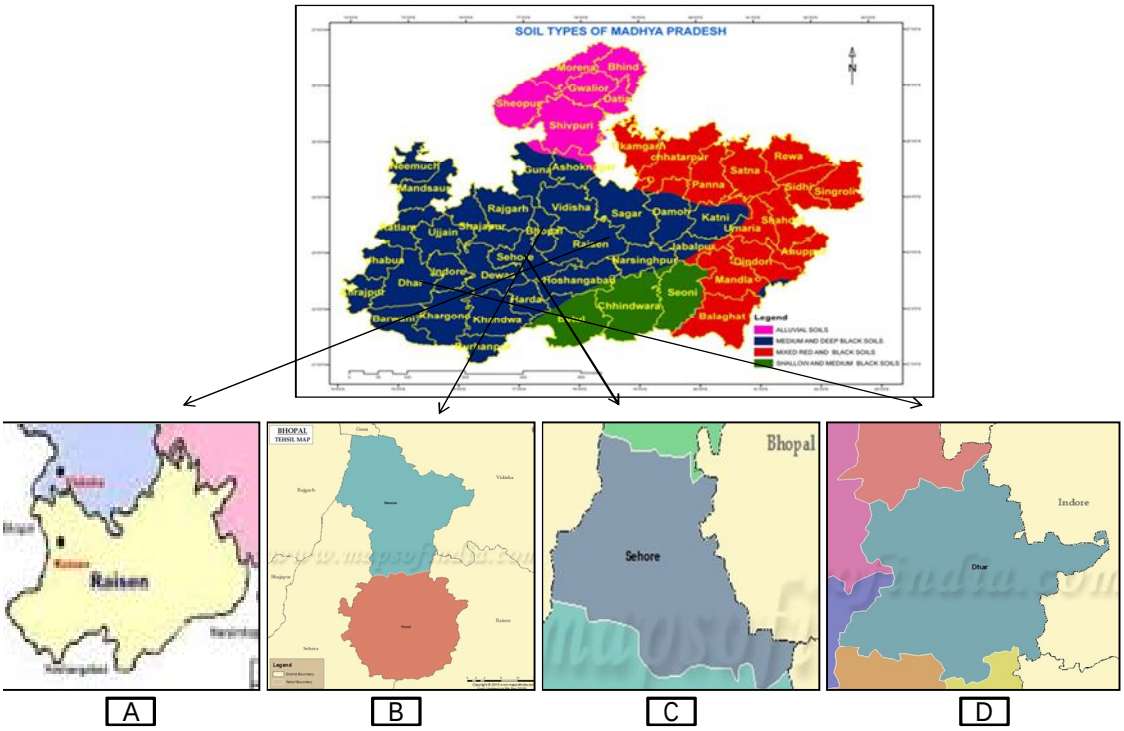

Fig. 1- Map showing the study site and prevalence of soil types (Centre of Madhya Pradesh state of environment, forest & climate change ,Govt of india, 2017) (A) Raisen (B) Bhopal (C) Sehore (D) Dhar.

Samples collection

Soil samples were collected in the month of August to November 2016. Representative randomized [15] waterlogged and moist soil surface samples collected from 8-10 spots of upper soil

crust 0.5 cm from study areas. The samples were collected in a sterile polythene bags and bottles and transferred to the laboratory for further use for the isolation and analysis purpose.

Physico-chemical analysis of soil

A number of physico-chemical properties of soil such as pH, electrical conductivity, total organic carbon, nitrogen, phosphorus and potassium were analyzed from different blocks of extreme fields. Total sixteen soil sample were analyzed in laboratory as follow-

(a) Soil pH

pH of the soil samples were determined by pH meter [16].

(b) Electrical conductivity

The electrical conductivity of soil solution was measured by electrical conductivity meter [16].

(c) Total organic carbon

The most easily oxidisable carbon determination method is modified walkey-black method use for determination of total organic carbon [17].

(d) Total nitrogen

Total nitrogen content in soil was determined by Semi-Micro Kjeldahl method [18]. The % N in soil was calculated by the following formula:-

$$\% N = [\{(R-B) \times 1.4\}/ W] \times S$$

Where, R= Reading of the sample in burette (H_2S0_4), S= Strength of H_2S0_4, B= Value of blank reading, W = Weight of the soil in mg, 1.4= Correction factor.

(e) Available phosphorus

Available phosphorus present in soil was determined by Olsen's method colorimetrically, where $SnCl_2$ was used as a reductant. Extracted with 0.5 M sodium bicarbonate solution [19] and developed blue color by $SnCl_2$ reduction and measured the color colorimetrically [18], with the help of spectrophotometer at 660 nm wave length [20].

(f) Available potassium

Available potassium was determined by ammonium acetate extraction method [20].

Isolation, purification and identification of cyanobacteria

BG-11 and modified Hughes's [21] media were utilized as enrichment medium for isolation of cyanobacterial morphotypes. BG-11 agar medium was taken in petri plates and green soil scrape from agriculture fields were inoculated on the surface of solid medium. Plates were incubated under light intensity of 3000 lux (16h light and 8h dark period) at $25^0C \pm 1^0C$ for 15 days. After 7 days of growth the individual cyanobacterial colonies were picked and transferred to respective liquid medium. Further purification of the culture was done by centrifugation and sub-culturing [22].Streptomycin and Nystatin (100µg/ml) were used to inhibit the growth of contaminants like bacteria and fungus [21, 23]. For identification of the morphotypes, the plates were inoculated with purified cyanobacteria and observed under digital microscope (Leica analytical system, Mumbai) after one week of growth. The morphotypes were identified based on their morphological features and cell structure following the monograph of Desikachary [24, 25].

Diversity Analysis

The isolated cyanobacterial morphotypes under each genus were identified and counted for estimating diversity and evenness of each study area. The diversity was calculated using Shannon-Weiner Index (H) (Shannon and Weaver, 1949), Simpson's diversity (1-D) (Simpson, 1949) and Hill's Index (Hill, 1973) using following formulae -

Shannon-Weiner Index, $H = -\sum Pi\ (lnPi)$

Simpson's Dominance, $D = \sum(pi)^2$

Simpson's Diversity Index, $1\text{-}D = 1\text{-}\sum(pi)$

(Where, pi = total no. of strains of genus i/total number of all strains)

Mclntosh Evenness Index

McE = [N-√ (Σni)] / [N-(N/√S], (McIntosh, 1967)

(Where, ni= No. of strains of genus, i and S= Total no. of genera and, N= Total no. of strains)

The Percent abundance was calculated as follows:

Percent Abundance $= Y/X \times 100$

(Where, X = Total number of isolates, Y = Number of isolates belonging to a particular)

RESULTS

Physico-chemical parameters

Total sixteen soil samples collected were analysed for physico-chemical parameters (Table 1) such as soil type, latitude, longitude, pH, electric conductivity, total organic carbon, nitrogen, potassium, and phosphorus.

Table No. 1: "Physico-chemical parameters of the sampling blocks"

Physico-chemical parameters	Sampling blocks			
	Raisen	**Bhopal**	**Sehore**	**Dhar**
Soil type	Deep black soil	Medium black and deep soil	Deep black soil	Medium black soil
Latitude/longitude	23.22N 78.20 E	23.25N 77.41E	23.20N 77.08E	22.60N 75.30E
pH	5.60 ± 0.40	6.42 ±0.46	7.9±0.5	7.1±0.8
Electric conductivity(dS/m)	2.56±0.45	0.95±0.33	1.0±0.25	1.5±0.20
Organic carbon (%)	1.33±0.22	0.75±0.8	0.65±0.5	1.12±0.2
Nitrogen (Kg/hec)	155±0.45	301±0.78	307±0.40	250±0.50
Phosphorus (Kg/hec)	22±0.8	12±0.5	15±0.4	20±0.5
Potassium (Kg/hec)	185±0.35	303±0.50	298±0.50	250±0.30

Sample collection, isolation and identification of cyanobacteria

A total of 87 cyanobacteria were isolated from four different randomly chosen blocks of Madhya Pradesh. These isolates morphologically belonged to heterocystous filamentous, non-heterocystous filamentous and unicellular forms. Microscopic observation showed presence of 16 different cyanobacterial genera which are listed in Table 2. Seven heterocystous filamentous morphotypes such as *Anabaena, Nostoc, Calothrix, Scytonema, Tolypothrix, Aulosira, Cylindrospermum,* four non- heterocystous filamentous morphotypes, *Oscillatoria, Lyngbya, Phormidium, Plectonema,* and five unicellular morphptypes, *Gloeocapsa, Synechococcus, Chroococcaceae. Aphanothece, Microcystis.*

Table No. 2: "Genera wise abundance of cyanobacterial morphotype"

S. No.	Genus	Cyanobacterial morphotype		
		Heterocystous morphotype	Non-heterocystous morphotype	Unicellular morphotype
1	*Anabaena*	20	-	-
2	*Nostoc*	16	-	-
3	*Calothrix*	12	-	-
4	*Gloeocapsa*	-	-	5
5	*Synechococcus*	-	-	3
6	*Scytonema*	4	-	-
7	*Oscillatoria*	-	6	-
8	*Chroococcaceae*	-	-	2
9	*Aphanothece*	-	-	1
10	*Lyngbya*	-	6	-
11	*Microcystis*	-	-	2
12	*Tolypothrix*	2	-	-
13	*Phormidium*	-	2	-
14	*Aulosira*	2	-	-
15	*Plectonema*	-	2	-
16	*Cylindrospermum*	2	-	-
	Total	58	16	13

Cyanobacterial abundance

Percent abundance of cyanobacterial morphotype is presented in the Fig 2. Heterocystous filamentous morphotype recorded highest abundance of cyanobacterial genera followed by non-heterocystous filamentous and unicellular morphotype, which are summarized in Table 2. The most abundant morphotype among all isolates were found to be heterocystous filamentous (67%), *Anabaena, Nostoc, Calothrix, Scytonema, Tolypothrix, Aulosira Cylindrospermum* followed by non-heterocystos filamentous (18%), *Oscillatoria, Lyngbya, Phormidium, Plectonema* and unicellular (15%), *Gloeocapsa, Synechococcus, Chroococcaceae, Aphanothece, Microcystis.*

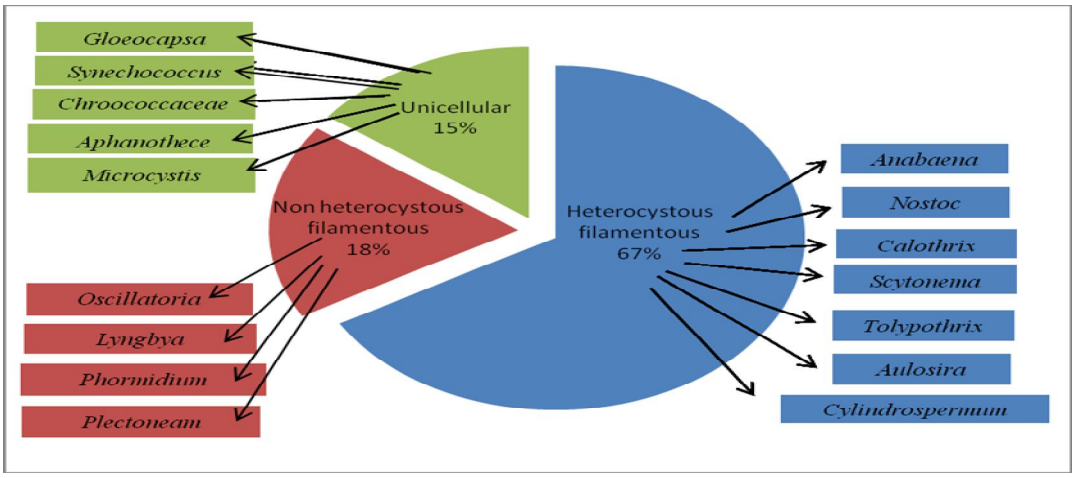

Fig. 2 - Total percent abundance of cyanobacteria morphotypes of around Bhopal.

Diversity analysis

Diversity and richness in blocks is measured by the number of species present in a specific area. More the number, higher is the richness. Diversity indices take into accounts both relative abundance and richness to predict how well species are distributed within a community. Four different statistical analyses were used to encompass various diversity parameters in predicting the diversity indices of the individual blocks as well as the overall diversity of all the study blocks undertaken for analyses. *Anabaena, Nostoc,* and *Calothrix* genus were widely distributed in all the blocks emphasizing their adaptability and resilience under diverse environmental conditions. Calculations based on Shannon-Wiener diversity index showed Raisen has the highest diversity (2.337) followed by Bhopal (2.245), Dhar (2.210) and the lowest was found in Sehore (1.906) Similarly, Simpson's Diversity index was highest Raisen (0.8766) followed by Bhopal (0.8765), Dhar (0.8611), and the lowest was found in Sehore (0.8438). However, Evenness index was highest for Sehore (0.961) followed by Bhopal (0.8584), Dhar (0.7598), and lowest in Raisen (0.7391). Simpson's dominance index was maximum in Sehore (0.1563) and minimum in Raisen (0.1234). All these diversity indices for the different four blocks are graphically represented in the Fig. 3.

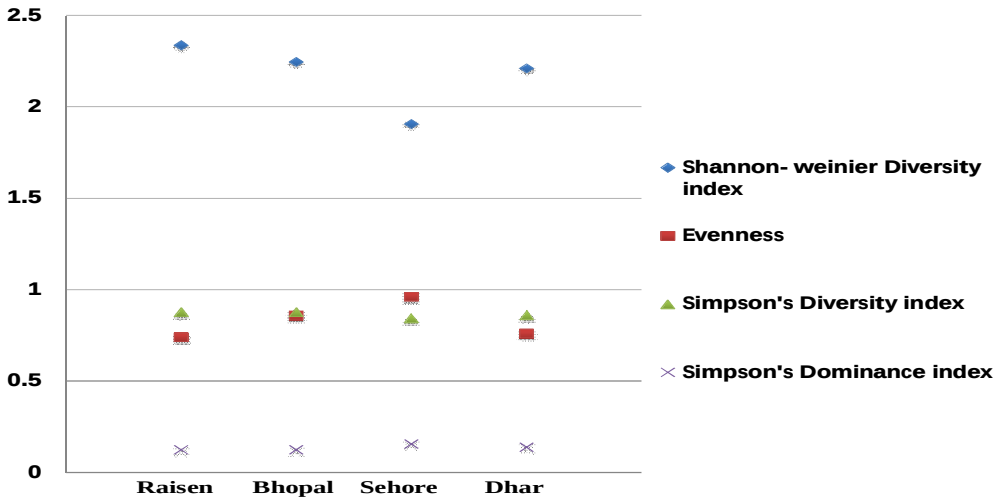

Fig. 3 - Diversity indices for the various locations sampled, as a measure of Shannon's-weinier index, Evenness, Simpson's diversity and Simpson's dominance of cyanobacterial populations.

DISCUSSION

The total of 87 cyanobacterial morphotypes were purified from the four different blocks selected around Bhopal. Sixteen different genera including heterocystous and non-heterocystous, unicellular cyanobacterial morphotypes were found in these blocks. The number of heterocystous morphotypes were far more in number than the non-heterocystous types. The results highlight the fact that growth and distribution of cyanobacteria in any ecosystem are not affected by the different physico-chemical parameters that constitute their surrounding environment. The presence of higher number of heterocystous forms further emphasizes the fact that heterocystous forms are more adjustable than the non-heterocystous forms in any environment [30]. Many researchers have already shown importance of physico-chemical parameters on the distribution and diversity of the cyanobacteria [31, 32, 33]. Among soil properties pH and nitrogen is a very important factors in growth establishment and diversity of cyanobacteria, which have generally been reported to prefer neutral to slightly alkaline pH for optimum growth [34]. However, in our study, Sehore recorded alkaline pH of 7.9 and medium nitrogen 307 kg/hec but showing lowest diversity index like Shannon- wiener's index 1.906, Simpson's diversity index 0.8438 and highest Simpson's dominance index 15.63 and evenness 0.961. Sehore block showing lowest diversity index whereas physico-chemical parameters ranges are normal for cyanobacteria growth and abundance but number of morphotypes are very less compare to other blocks. Raisen block showing higher cyanobacterial diversity index like Shannon-Weiner's index 2.339, Simpson's diversity index 0.8766 and lowest Simpsons dominance index 0.1234 and evenness 0.7391 whereas physcio-chemical parameters range are extreme such as acidic

pH of 5.6 and low nitrogen 155 kg/hec. Raisen recorded maximum number of heterocytous filamentous cyanobacteria but the most abundant are *Anabaena and Nostoc*. This finding revealed that cyanobacteria survive in extreme condition and may enhance the understanding of the nutrient status of the extreme field because cyanobacteria are capable of both carbon assimilation and nitrogen fixation thereby enhancing productivity in a variety of environments. Some strains are growing in acidic pH because they maintained a high intracellular pH. This is indicative of an efficient internal pH regulating mechanism in these rather rare strains. Cyanobacterial cell known to develop a certain electrical surface charge expressed as zeta potential, depending upon the pH of the surrounding medium, the size of which affect the permeability of the cell wall. Therefore the physiological adaptation of these cyanobacteria strains towards the H^+ needs thorough investigation at the micro-environment level.

Many cyanobacteria have the ability to fix atmospheric nitrogen. Nitrogen fixation of cyanobacteria is catalyzed by the nitrogenase which is extremely sensitive to free oxygen and function only under anaerobic condition. Some taxa have evolves heterocyst's (special thick-walled cells that protect nitrogenase against damage from oxygen) however there appears to be no universal system to protect the enzyme complex from both atmospheric and intracellular sources of oxygen in non-heterocystous cyanobacteria. It is clear that environment perturbations and their associated stress induction have acted as a major stimulus to the evolution of stress. Heterocystous cyanobacteria are the site for nitrogen fixation. Heterocysts produce and secrete certain vital substances which stimulate growth and cell division in adjacent vegetative cells keeping them in active physiological state. In addition cyanobacteria had a special task to protect their nitrogenase from the oxygen liberated as a result of their own photosynthetic activities. For the most effective adaptation for the protection of nitrogenase in cyanobacteria was the acquisition of a highly specialized nitrogen fixation cell, the heterocyst. Heterocystous cyanobacteria are able to fix nitrogen efficiently under ambient atmospheric conditions.

CONCLUSION

Cyanobacteria are potentially important in managing nutrients to reduce agrochemical use with modern crop technologies. This study revealed that the cyanobacterial diversity of a location is determined by interaction among various prevailing environmental factors such as pH, conductivity and pollutants etc. The genus *Anabaena* and *Nostoc* showed highest resilience and members of this genus were most abundant in all four blocks. They also recorded higher heterocyst frequency and nitrogenase activity. The growth of these stable isolates under extreme conditions paves the way for

their agronomic importance as biofertilizers due to their N_2-fixing ability that helps them to grow successfully in habitats where little or no combined nitrogen is available. The technology can be easily adopted by farmers for multiplication at their own level.

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