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Soil-crust Cyanobacteria from Forest Soil of Burdwan, West Bengal: Relating Species Distribution and Physiological Attributes with Soil Parameters

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Abstract: The present study, concerning isolation of cyanobacteria from biological soil crust from forest soil and correlation between their distribution and soil parameters has been conducted on two forests (Garhjangal and 11 miles) located in the district of Burdwan, West Bengal. Five cyanobacterial species were isolated from Garhjangal and three cyanobacterial species were isolated from 11 miles. Soil parameters studied are pH, ammonium, organic carbon, available nitrogen and available iron. Species distribution, physiological parameters and soil parameters were correlated with the help of Canonical Correspondence Analysis (CCA). CCA ordination diagram indicates that high amount of iron helps in occurrence of heterocystous cyanobacteria which augments ammonium content of the soil. Their occurrence is much less in locations with high amount of available nitrogen. Occurrence of thick sheathed cyanobacteria is correlated with high carbon content of the soil. Two populations of one heterocystous cyanobacteria *N. punctiforme* were isolated separately from Garhjangal and 11 miles and characterized physiologically with regard to Chl a, growth, protein, carbohydrate and nitrogenase activity. Macromolecular components of taxa collected from these two sites vary with variation of different soil parameters. High Chl-a content of organisms was found in the organisms isolated from soil with high amount of available iron.

Key words: Burdwan, Cyanobacteria, Forest soil, Soil-crust, Soil parameters.

Introduction

Crusts on soils formed by microorganisms and microphytes are called cryptogamic or crypto-biotic or biological soil-crusts [53]. A consortium of cyanobacteria along with green algae, lichen, fungi, bacteria, mosses and liverworts form biological soil-crusts. Though arid and semi-arid regions are characterized by biological soil-crusts [27], these are found to some degree in most ecosystems [6]. In forest area, the open space between the higher vascular plants is found to be covered by biological soil-crusts [3].

Cyanobacteria, form the primary components of soil-crust in most of the locations including the one with harsh environmental conditions [27,53]. They are key organisms in the long term control of resources and productivity in nitrogen (N) poor environment such as boreal forest. They contribute to soil stability by preventing erosion, soil build up, soil fertility through carbon (C) and N inputs along with several micronutrients and to the soil water regime [10,33,40,49,51]. Nisha *et al.* [27] established that, cyanobacterial application to the organically poor semi-arid soil played a significant role in improving the status of carbon, nitrogen and other nutrients in the soil. It is relevant to note that, the growth and metabolic activity of cyanobacteria in natural environment are also affected by nitrogen, carbon,

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phosphorus and iron content of surrounding substratum [12,20].

Lateritic soils are characterized by acidic pH, low nitrogen, phosphorus and potassium (NPK) and high iron (Fe) content [7,9,31,34,35,36]. However iron content is poor on the top and increases with depth [35]. In West Bengal, lateritic soils are found in Western parts of Burdwan (location of present study), along with Western parts of Birbhum, Midnapur and Bankura district and the whole district of Purulia [16,37]. As the contents of potash, phosphorus and nitrogen are low in lateritic soil, occurrence of soil-crust cyano-bacteria in this type of soil is important to improve the soil quality.

Hence, the present study has been undertaken with the prime objectives of- (i) to know the species composition of soil-crust cyanobacteria in two tropical dry deciduous forests of Burdwan district of West Bengal, Garhjangal and 11 miles, (ii) to correlate the species distribution with soil parameters, (iii) to find out the relation between physiological attributes of soil-crust cyanobacteria with soil parameters.

Materials and methods

Collection site

The two study sites of the district of Burdwan are Garhjangal, Durgapur Range, Burdwan division (23°35’52.3"N latitude, 87°25’42.2"E longitude) and 11miles, Panagarh Range, Burdwan division (23°40’54.4"N latitude, 87°40’20.2"E longitude).

Sample collection and culturing

Crust samples were collected from the upper surface of soil from Garhjangal and 11 miles using a 1ft. /1ft. quadrate and put in pre-sterilized screw cap bottles and brought to the laboratory. In laboratory, each sample was wetted by distilled water or liquid (-N and +N) BG-11 medium in a Petri dish. The Petri dish was placed in culture rack at 25^{o}C under light from cool fluorescent tubes at an intensity 7.5 w/m^2 maintaining 10-14 light dark cycles for up to 10-15 days (depending upon crust type). After 10-15 days, freshly growing cyanobacterial filaments appeared. The newly growing filaments were examined under microscope. Cyanobacterial filaments emerging from crust samples were inoculated at the surface of agar plated (-N and +N) BG-11 medium (depending upon nature of cyanobacteria) and kept at laboratory condition for 20-25 days depending upon crust types. The organisms that appeared in the culture were morphologically and morphometrically studied and identified following Desikachary [11], Anagnostidis and Komárek [1] and microphotographs were taken using Leica trinocular DM 2500 research microscope with digital camera.

Relative abundance of the species

Relative abundance of organisms isolated was calculated following the method of Subrahmanyam and Sambamurty [41].

$$\text{Relative abundance} = \frac{\text{Total number of hits made on the species}}{\text{Total number of hits made}} \times 100$$

Soil analysis

Soil samples for analysis were collected from a depth of few mm. just below the soil-crust within the previously mentioned 1ft. /1ft. quadrate from the above mentioned two sites and were analysed with respect to the following chemical parameters-

a. pH: pH of soil samples were measured following potentiometric method.

b. Organic carbon: Organic Carbon of soil samples were analysed following the rapid titrimetric method [50].

c. Available nitrogen: Available nitrogen of soil samples were measured following alkaline permanganate method [46].

d. Available iron: DTPA-extractable Fe was measured using an atomic absorption spectrophotometer [52].

e. Ammonium: Ammonium content of soil samples was measured using Orion multi-parameter meter [5].

Analysis of correlation between isolated cyanobacterial species and soil parameters

The interspecific association [22]: It was calculated using Dice Index (D. I.). {D.I. = 2*a*/ (2*a* + *b* + *c*)} was calculated with the help of a 2x2 contingency table recording the species presence

or absence in the samples. For each pair of species, 'a' is the number of sampling units (SUs) where both species occur, 'b' is the number of SUs where species A occurs but species B is absent, 'c' is the number of SUs where species B occurs but species A is absent, 'd' is the number of SUs where both the species are absent, and total number of SUs (a + b + c + d) is N.

Canonical Correspondence Analysis (CCA) [42] was performed to determine whether variance in the cyanobacterial community data could be explained by soil parameter variables. CCA ordination was tested for significance with a Monte Carlo test (500 runs) through CANOCO software.

Physiological characterization

Chlorophyll-a (Chl *a*) content was determined following the methodology of Mackinney [24]**.** Carbohydrate content was estimated following the method of Herbert *et al.* [18]. Protein estimation was done following the method of Lowry et al. [23]. Growth was measured at 750 nm. The conversion of acetylene by nitrogenase is assayed by a gas chromatograph (HP) fitted with Porapack N (80-100 mesh) column following the method of Turner and Gibson [47].

Results

Five cyanobacterial species belonging to four genera were isolated from Garhjangal – non-heterocystous filamentous form (*Leptolyngbya tenuis* - Oscillatoriales) and heterocystous, uniseriate, filamentous form (*Nostoc punctiforme, Scytoneama hofmanni, S. guyanense, Calothrix brevissima* - Nostocales) (Fig. 1, Table 1). Three cyanobacterial species were isolated from 11 miles - non-heterocystous filamentous form (*Leptolyngbya tenuis, Limnoraphis hieronymusii* - Oscillatoriales) and heterocystous, uniseriate, filamentous form (*Nostoc punctiforme* - Nostocales). Relative abundance of species is shown in Table 1.

Chemical analysis of soil collected from Garhjangal and 11 miles showed that, pH of both sites were acidic, ammonium content, percentage of organic carbon and available nitrogen of Garhjangal are higher (3.12 mg/l, 0.26 % and 9.1 mg/kg respectively) than 11 miles (1.08 mg/l, 0.195 % and 10.9 mg/kg respectively). Available iron content of 11 miles was very much lower (8.30 mg/kg) than Garhjangal (49.31 mg/kg) (Table 2).

A two-dimensional plexus diagram (Fig. 2) shows close association (D.I.=1) between *C. brevissima* (species code 5) and *S. hofmanni* (species code 3).

Table 1. Relative abundance of species isolated from two sites

Name of isolated taxa	Garhjangal	11 miles
Leptolyngbya tenuis (Gomont) Anagnostidis & Komárek	+ + +	+ + + +
Limnoraphis hieronymusii (Lemmermann) comb. nov.	-	+ + +
Nostoc punctiforme (Kützing ex Hariot) Hariot	+ + + +	+ +
Scytoneama hofmanni Agardh ex Bonret et Flahault	+ + +	-
S. guyanense (Montagne) Bornet et Flahault	+ +	-
Calothrix brevissima G.S. West	+ + +	-

The organisms were identified following Desikachary (1959), Anagnostidis and Komárek (1988)
Relative abundance of organisms isolated was calculated following the method of Subrahmanyam and Sambamurty (2000)
Relative species abundance: + + 5-20 %, + + + 30-50 %, + + + + 50-90 %

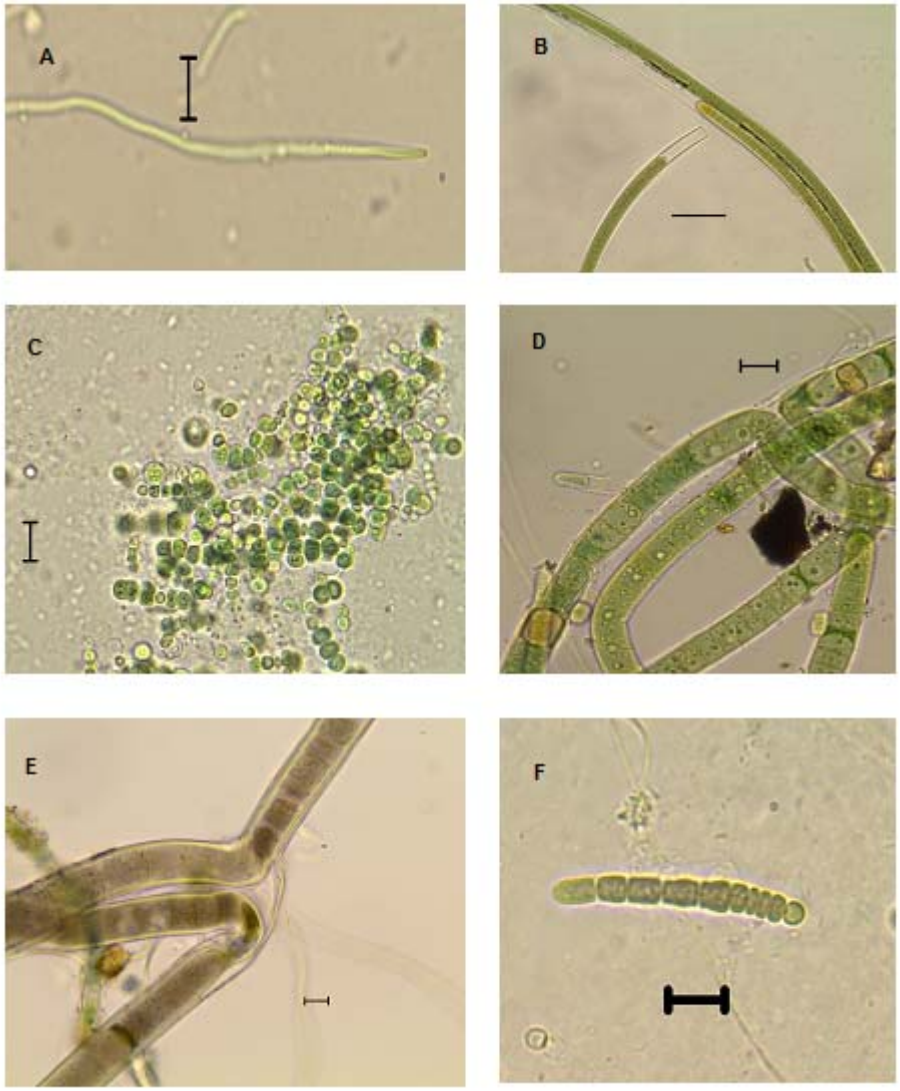

Fig. 1. Microphotographs of soil-crust cyanobacteria A. *Leptolyngbya tenuis*, B. *Limnoraphis hieronymusii*, C. *Nostoc punctiforme*, D. *Scytonema hofmanni*, E. *Calothrix brevissima*

Table 2. Analysis of soil collected from Garhjangal and 11 miles

Location	pH	Ammonium (mg/l)	Organic Carbon (%)	Available Nitrogen (mg/kg)	Available Iron (mg/kg)
Garhjangal	4.9	3.12	0.26	9.10	49.31
11 miles	5.1	1.08	0.195	10.90	8.30

pH of soil samples were measured following potentiometric method, organic carbon of soil samples were analysed following the rapid titrimetric method of Walkey and Black (1934), available nitrogen of soil samples were measured following alkaline permanganate method (Trivedy and Goel, 1984), DTPA-extractable Fe was measured using an atomic absorption spectrophotometer (Whitney, 1998), ammonium content of soil samples was measured using Orion multiparameter meter (Bremmer and Keeney, 1965).

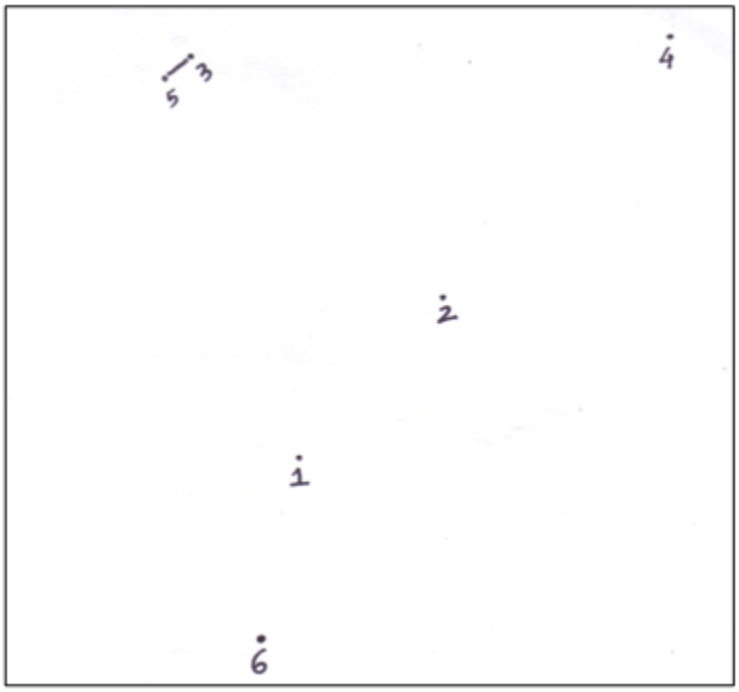

Fig. 2. Plexus diagram for two sites of Burdwan to show interspecific association (species codes: 1 for *Leptolyngbya tenuis*, 2 for *Nostoc punctiforme*, 3 for *Scytonema hofmanni*, 4 for *Scytonema guanense*, 5 for *Calothrix brevissima*, 6 for *Limnoraphis hieronymusii*)

CCA (Fig.3) is applied for species data and environmental variables. The distribution of cyanobacterial species collected from Garhjangal and 11 miles is marked in the ordination diagram by (Δ) and five soil parameters are represented by five arrows (→). The results indicate that the environmental variables may be ranked in terms of their influence, as follows - Available nitrogen, Organic carbon, Ammonium, Available iron and pH. However, the relative weight, as denoted by extra-fit values, for the variables indicates a broad range of variation (0.3535-0.7253) with a p-value of 0.020. The eigenvalues for corresponding axes indicate that first axis is contributing the most in explaining the species-environment relationship. he second axis is contributing more than the remaining two axes.

Axis 1 has the strongest positive correlation with available nitrogen and week but positive correlation with pH. There are negative but strong correlation with organic carbon (- 0.9698) and ammonium (- 0.8244). Axis 2 has positive correlation with ammonium and available iron. There is negative but strong correlation between axis 2 and pH (- 0.5676). Axis 2 has negative correlation with organic carbon and available nitrogen. As the 1st three eigenvalues are canonical, the plotting of species on the diagram can be easily interpreted in terms of the environmental gradient.

P. tenue and *L. hieronymusii* occur with increasing available nitrogen. *C. brevissima* and *S. hofmanii* occur just equidistant from the arrows representing organic carbon and pH. *N. punctiforme* shows affinity towards available iron and ammonium. *S. guanense* presents just opposite to the arrow representing pH. Physiological characteristics of the heterocystous organism *N. punctiforme*, isolated from both Garhjangal and 11 miles are represented in Table 3. Values of physiological parameters varied between populations of some species isolated from different locations

All the graphs (Figs.4-6) establish a fact that macromolecular components of taxa collected from two sites of the district of Burdwan, vary with variation of different soil parameters. Graph (Fig. 4) shows the relationship between carbohydrate content of two different population of *N. punctiforme* and percentage of organic carbon

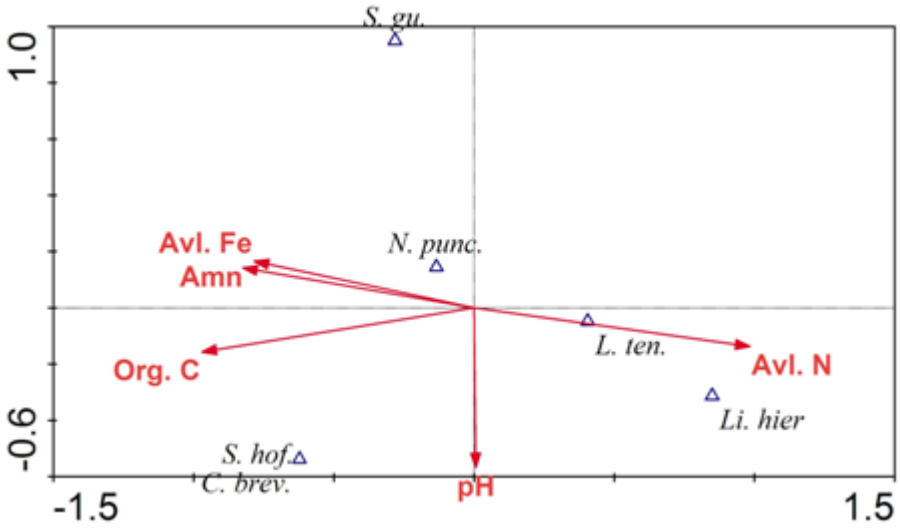

Fig. 3. CCA ordination biplot to relate cyanobacterial species distribution with environmental variables.(Amn. for Ammonium; Avl.N for Available nitrogen; Org.C for Organic carbon; Avl.Fe for Available iron; *L. ten.* for *Leptolyngbya tenuis*; *Li. hier.* for *Limnoraphis hieronimusii*; *N. punc.* for *Nostoc punctiforme*; *S. gu.* for *Scytonema guanense*; *S. hof.* for *Scytonema hofmanni*; *C. brev.* for *Calothrix brevissima)*

Table 3. Physiological characteristics of two populations of *N. commune*

Name of organism	Location	Chl a (µg/g)	Carbohydrate (µg/g)	Protein (µg/g)	Growth (g)	Nitrogenase Activity(n mol ethylene/ (µg Chl a/h)
N. punctiforme	Garhjangal	31.74	93.54	1.610	0.900	0.0026
N. punctiforme	11 miles	29.91	78.00	0.834	0.830	0.0015

Chlorophyll-a (Chl *a*) content was determined following the methodology of Mackinney (1941), carbohydrate content was estimated following the method of Herbert *et al*. (1971), protein estimation was done following the method of Lowry *et al*. (1951). Growth was measured at 750 nm, the conversion of acetylene by nitrogenase is assayed by a gas chromatograph (HP) fitted with Porapack N (80-100 mesh) column following the method of Turner and Gibson (1980)

of the soil from which the organisms were isolated. Organism, isolated from soil with high organic carbon content, shows high carbohydrate content. Graph (Fig. 5) shows the relationship between Chl-a content of two different populations of *N. punctiforme* and amount of available iron of the soil from which the organisms were isolated. High Chl-a content of organisms was found in the organisms isolated from soil with high amount of available iron. Graph (Fig. 6) shows the relationship between nitrogenase activity of two populations of *N. punctiforme* and amount of ammonium of the soil. Cyanobacteria, isolated from soils with high ammonium content show high nitrogenase activity.

Discussion

The cyanobacterial species composition of biological soil-crust has been studied from a particular forest soil of Birbhum district of West Bengal [4]. This study deals with the species composition of soil-crust cyanobacteria from forest soil of adjacent district Burdwan. It is evident that physical and chemical properties of soil such as pH, carbon, nitrogen and available iron influence occurrence and abundance of

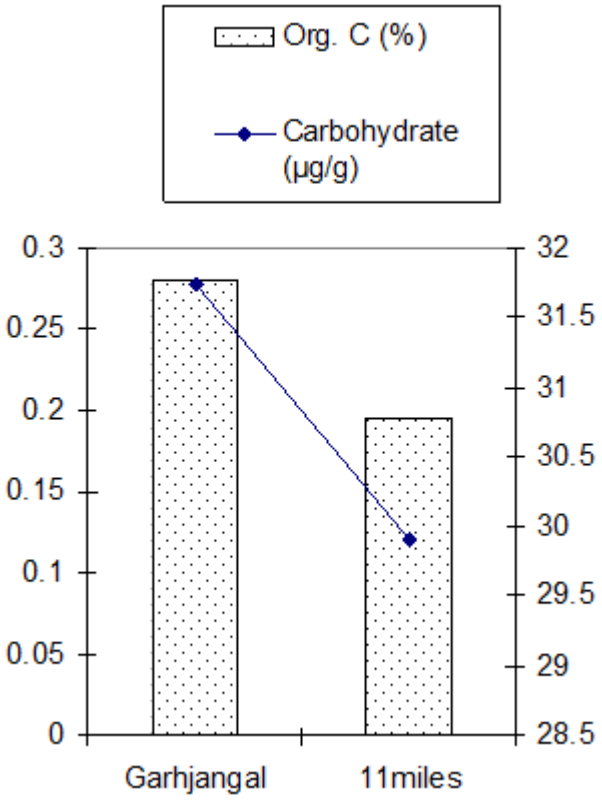

Fig. 4. Graph showing relationship between carbohydrate content of *N. punctiforme* and percentage of organic carbon of the soil from which the organisms were isolated. (Org. C for Organic carbon)

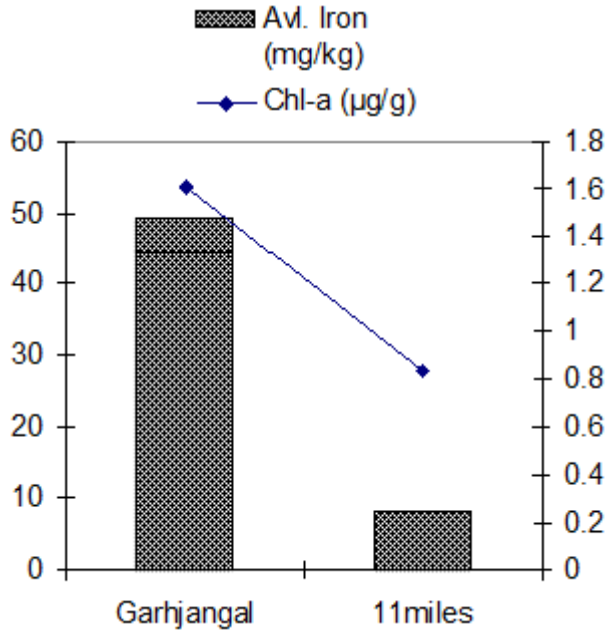

Fig. 5. Graph showing the relationship between Chl *a* content of *N. punctiforme* and amount of available iron of the soil from which the organisms were isolated. (Avl. Iron for Available Iron; Chl-a for Chlorophyll a)

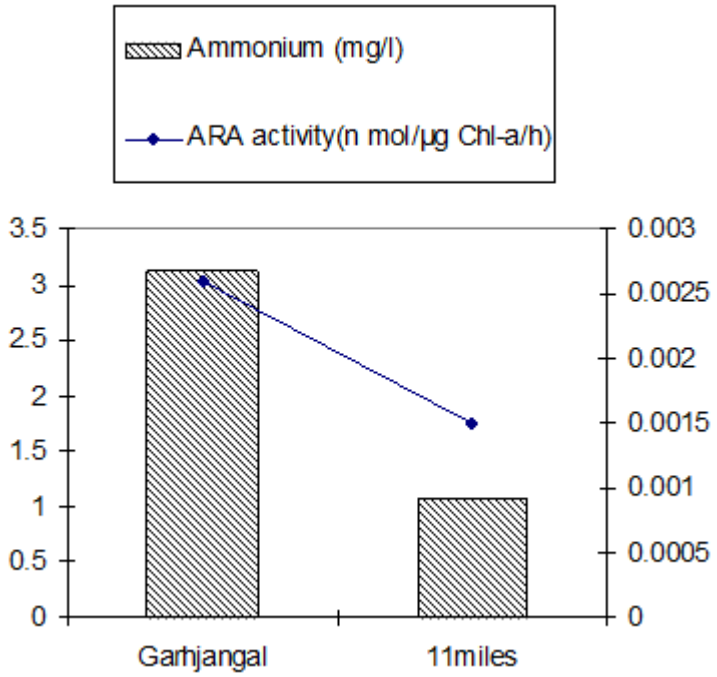

Fig. 6. Graph showing the relationship between nitrogenase activity of *N. punctiforme* and amount of ammonium of the soil from which organisms were isolated. (ARA for Acetylene reduction assay)

cyanobacterial species. Cyanobacteria are efficient iron assimilators and iron imparts ability to compete with the other microflora [28,29,48]. Iron has important role in nitrogen assimilation and cyanobacteria leach ammonium and enhance the amount of ammonium of the soil. Ferredoxin is required as the electron donor for both nitrate and nitrite reductase activities [48] and iron is also a component of Fe-Mo protein of nitrogenase. Four heterocystous species were isolated form soil of Garhjangal where available iron content is high (49.31 mg/kg). Only one heterocystous species has been isolated from soil of the other location, 11 miles where the iron content is much low (8.30 mg/kg). Close occurrence of *N. punctiforme*, a heterocystous cyanobacterium with good ARA activity, in the CCA ordination graph (Fig. 3) with the arrows representing available iron and ammonium supports this. On the other hand, non heterocystous cyanobacteria - *L. tenuis* and *L. hieronymusii* are present opposite to the arrows representing available iron and ammonium.

Certain sheathed cyanobacteria residing in upper layers of arid soils contribute organic matter through carbon fixation [44]. In the present study, thick sheathed cyanobacteria such as *C. brevissima* and *S. guanense* isolated from Garhjangal, with organic carbon which is higher (0.26 %) than 11 miles (0.195 %). Organisms isolated from 11 miles are devoid of thick sheath.

When soil become enriched with available nitrogen, rate of nitrogen fixation of cyanobacterial crust become reduced due to feed back mechanism. After depletion of combined nitrogen forms through mineralization, volatilization or leaching, cyanobacteria begins biological nitrogen fixation [21,26]. In Garhjangal, amount of available nitrogen is 9.1 mg/kg and four heterocystous cyanobacteria were isolated. On the other hand only one heterocystous cyanobacteria was isolated from 11 miles, where amount of available nitrogen in soil is little bit high (10.9 mg/kg). More over, according to CCA, position of all heterocystous cyanobacteria are negative with respect of the arrow representing available nitrogen. On the contrary, two non heterocystous cyanobacteria *L. tenuis* and *L. hieronymusii* occur with increasing available nitrogen.

Putting Plexus diagram (Fig. 2) and CCA ordination graph (Fig. 3) together, it has been

observed that *C. bravissima* and *S. hofmanni* show close association. Both of them were isolated from Garhjangal and show affinity towards sites with high amount of available iron, ammonium and organic carbon.

Variation of pigment content and other biochemical compounds in populations of same species collected from different locations have been documented by Tiwari *et al*. [45]. *Nostoc piscinale*, isolated from Achrol and Pokhran, two arid zones of Rajasthan and characterized them with regards to Chl *a*, soluble protein, carbohydrate, nitrogeanse activity and extra cellular ammonium release. In two different populations of *N. piscinale*, Chl *a* content varied from 1.5 to 7.9 µg/ml, soluble protein varied from 6.1 to 100.9 µg/ml, carbohydrate varied from 41.2 to 134.7 µg/ml and nitrogenase activity varied from 351.7 to 459.7 n mol ethylene/ mg Chl *a*/h. So, it is evident from the above study that macromolecular contents and nitrogenase activity vary with variation in population and the locality from which it is collected. Similar variations in physiological parameters of two populations of *N. punctiforme* are also found in this study (Table 3).

Biological soil-crusts can increase the total surface soil carbon by up to 300 % [2,8,14]. Organic carbon content significantly increased in the crust and its underlying soil with gradual crust development, especially in the first centimeter of soil underneath the cyanobacterial crust, but organic carbon content did not differ among soil layers in physical crusts. The carbohydrate content of two populations of *N. punctiforme* isolated from soil-crusts of two sites under study was compared with organic carbon percentage of soil from which these species were isolated (Fig. 4). It is found that the cyanobacterial population isolated from location with high percentage of organic carbon contained high amount of carbohydrate.

Iron affects the synthesis of the major photosynthetic pigment Chl *a* [32]. In present work, among two populations of soil-crust cyanobacterium, *N. punctiforme*, one with high Chl *a* content was isolated from soil with high amount of available iron (Fig. 5).

In soil with the cyanobacterial soil-crusts, the nitrogen contents increased in the surface soil layer (0-5 cm.) and decreased with soil depth [8,15,43]. Crust organisms enhance soil nitrogen up to 200% by fixing atmospheric nitrogen [13,17,19,39]. They are thought to compensate loss of nitrogen by leaching [25]. In this study, the nitrogenase activity (ARA) of two populations of *N. punctiforme* was compared with ammonium content of the soil from which those organisms were isolated (Fig. 6). It was found that the cyanobacterial population which shows high nitrogenase activity was isolated from soil with high ammonium content.

Hence, with the help of this work, it can be concluded that, individual species as well as associated species, within a location, show affinity towards specific soil-parameters. Moreover, present work can establish relation between physiological attributes of cyanobacteria and parameters of the soil from which the organisms were isolated.

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