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Diversity and abundance of N_2- fixing cyanobacterial population in rice field soil crusts of Lower Brahmaputra Valley agro-climatic zone

Nijara Bharadwaj and P. P.Baruah

Department of Botany, Gauhati University, Guwahati-781014, Assam, India. E. mail : partha_ghy16@rediffmail.com; nijara_gu@yahoo.in

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Abstarct

Cyanobacteria are one of the pioneer groups of oxygenic, gram negative photosynthetic prokaryotes which flourish in almost all conceivable habitats. Certain representatives have the inherent capacity to fix atmospheric nitrogen for which they have been considered as a potent source of nitrogen in crop fields. The present paper deals with the diversity of nitrogen fixing cyanobacteria in soil crusts of different rice fields in the Lower Brahmaputra Valley flood plains of Assam during June 2011 to May 2012. All together 47 N_2- fixing cyanobacterial taxa belonging to 20 genera under 8 families were isolated. Among them, 12 were heterocystous and 8 were non heterocystous genera. *Nostoc* and *Anabaena* showed the highest number of species. Results also revealed highest abundance *of Anabaena circinalis* followed by *Anabaena oryzae*. A few soil quality parameters showed significantly positive correlation with the cyanobacterial population.

Keywords: Cyanobacteria, diversity, abundance, soil crusts, rice fields.

Introduction

The mighty Brahmaputra river and its tributaries make its valley a suitable alluvium landmass for cultivation of all season crops resulting in development of the agrarian economy of the region. The Lower Brahmaputra Valley Agro-climatic Zone which is located in western Assam is one of the prime hubs for rice production in the state of Assam since historical times. Being a part of Himalayan biodiversity hotspot, the land mass is not only an abode of innumerable number of plants, animals, and insects but also a home of diverse soil microorganisms. Cyanobacteria are one of the main components of the micro biota in different habitats of the region in general and rice fields in particular (Peoples *et al*.1995). They are found abundantly in agricultural soils and rice field ecosystems throughout the world naturally (Whitton 2000). Cyanobacteria are unique amongst the prokaryotic organisms having diverse morphology and biochemistry. Few possess specialized cells called heterocyst which contain nitrogenase enzyme enabling the organisms to fix atmospheric nitrogen (Stewart *et al* 1987; Hamed 2007). They are hence considered as natural biofertilizer (Baftehchi *et al*. 2007). Cyanobacteria also improve soil characteristics by, modifying texture size and subsequent aeration (Ibraheem 2007), increasing phosphorus content (Fuller &Rogers 1952) and enhancing carbon content and water holding capacity(Richert *et al*.2005).

The first account of agronomic potential of cyanobacteria or blue green algae in rice in India was presented by De (1939) and Singh (1942), who attributed the natural fertility of tropical paddy fields to these nitrogen fixing organisms. In Assam, a number of studies have been undertaken on the mere enumeration of blue green algae in different parts of Assam by different workers (Devi 1981, Deka & Bordoloi 1991, Saikia & Bordoloi 1994, Deka 1999, Ahmed 1999, Yashmin 2003, Rout & Borah 2009). However, a little work has so far been done on diversity, distribution and abundance of nitrogen fixing cyanobacteria in relation to different crops (Dihingia & Baruah 2011). The present endeavour is therefore aimed to study the nitrogen fixing cyanobacterial diversity and its distributional pattern in different cultivated fields of the Lower Brahmaputra Valley zone of Assam (India) in general and Nalbari district of Assam in particular.

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Materials and Methods

Description of study site

The study was carried out in the Nalbari district which is located in the eastern part of the Lower Brahmaputra Valley Agro climatic zone of Assam on the north bank of the mighty Brahmaputra river. The maximum temperature may rise up to 38^0C in summer and fall up to 14^0 C in winter. There is heavy rainfall during the months of May to September due to which the area experiences flood. The average annual rainfall and humidity are at the rate of 1500 mm and 80% respectively. Geographical location of the three sampling sites as recorded using GPS (Garmin etrex) were:

Site-1/ SS-1 (Borbhag) N $26^0$22'55.5", E $91^0$28'23,3",

Site-2 /SS-2 (Banikuchi) N $26^0$28'21.4", E $91^0$23'30.9" and

Site-3/SS-3 (Helosa) N $26^0$21'53.5", E $91^0$15'35.6" .

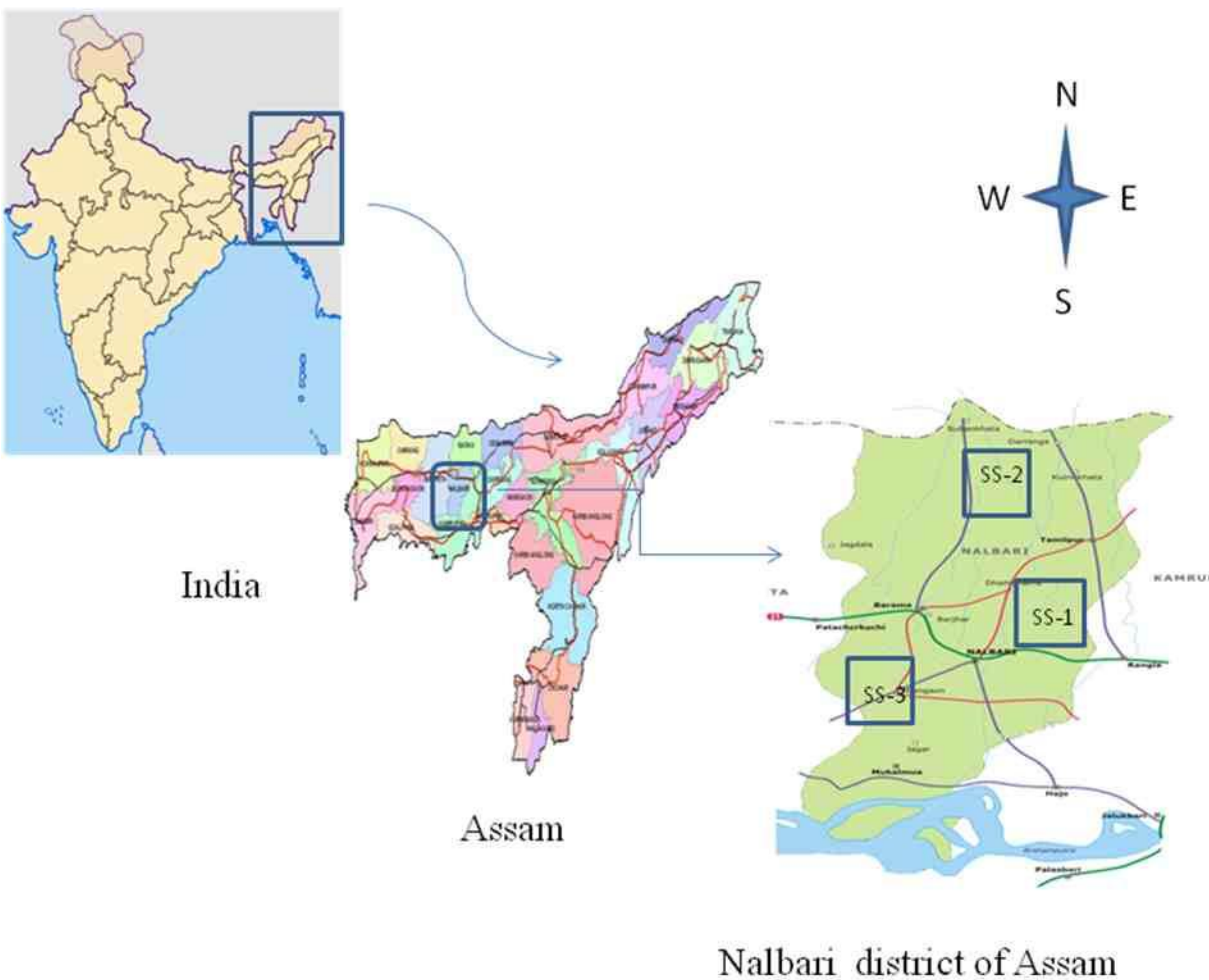

Fig1- Map depicting the Study sites (SS-1, 2, 3) in Nalbari district of the Lower Brahmaputra Valley Zone

Collection, identification, maintenance and preservation of sample

Crust samples were collected from the rice fields at four different seasons, i.e. pre monsoon, monsoon, post monsoon and winter from the upper surfaces of soil. A collection of three samples per site in all the seasons made a total collection of 36 samples from the three study sites. The samples were carried to the laboratory of the Department of Botany, Gauhati University. Field-moist soil was gently broken apart, air-dried and ground to pass through a 2-mm sieve prior to analyses for soil properties. A pinch of crust materials were inoculated in freshly prepared BG_{11} media (Rippka *et al.* 1979) and cultured under aseptic laboratory condition. After three weeks, the number of tubes showing algal growth was counted to enumerate the algal population using MPN values. The isolated strains were identified using standard keys following the monograph of Desikachary (1959).

Physico-chemical properties

The soil temperature, pH and conductivity of the soil samples were determined by using soil thermometer, digital pH meter (Biochem) and conductivity meter (Systronics) respectively. The available soil phosphate, sodium, potassium, total soil nitrogen were estimated in the laboratory as per procedures described by Trivedi and Goel (1986).

Data analysis

The relative abundance of a particular cyanobacteria type was calculated by employing the following formula: Relative abundance $= \frac{Y}{X} \times 100$

Where, X = total number of samples collected

Y=number of samples from which a particular cyanobacteria type was isolated.

The Diversity Index (Shannon- Wiener) was been studied following the formula:

$$Hs = -\sum_{i=l}^{S} (Pi)(lnPi)$$

Where,

Hs - diversity in a sample of S species or kinds

S - the number of species in the sample

Pi - relative abundance of i th species or kinds measures, = ni/N

N - total number of individuals of all kinds

ni - number of individuals of ith species .

ln - log to base 2

Results

A total of 47 species of cyanobacteria (Table-1) were reported from the three different study sites in rice fields of Nalbari District of Lower Brahmaputra valley agro climatic zone. It was comprised of 12 heterocystous forms: *Anabaena, Aulosira, Calothrix, Camphylonemopsis, Cylindospermum, Microchaete, Nostoc, Plectonema* , *Scytonema, Rivularia* , *Tolypothrix* , and *Westiellopsis* and 8 non-heterocystous forms: *Chroococcus, Aphanocapsa, Gloeocapsa, Gloeothica, Gomphosphaerica, Oscillatoria, Lyngbya*, and *Phormidium. Anabaena circinalis* showing the highest percentage of relative abundance (38.88%) was the most abundant species (Table-1) followed by *Anabaena oryzae* (30.55%) ,*Oscillatoria willei* (27.77%) , *Anabaena fertilissima* (25%), *Phormidium tenue* (25%) , *Westiellopsis prolifica* (22%).The lowest abundant species were *Gomphosphaerica sp.*(2.77%) , *Cylindrospermum stagnale* (2.77%) and *Rivularia sp.*(2.77%)(Table-1).

The physico-chemical properties of the soil like pH, conductivity, phosphorus, sodium and potassium and soil nitrogen varies in different sites and seasons (Table 2). The soil temperature was recorded to be between 14.36 ± 0.384 to 34.54 ±0.572 ^{0}C, while the pH of the soil varies from 5.729±0.102 to 7.29±0.015. Conductivity and phosphate varies from 27.4 ± 1.140 to174.6 ± 2.509 and 2.046 ± 0.011 to 8.398 ±0.019 respectively. Similarly, the sodium and potassium content varies from 9.002 ± 0.028 to 16.174±0.099 and 15.13 ± 0.076 to 47.768 ± 0.552 respectively. The nitrogen content of the soil varies from 0.3±0.01 to2.2±0.03.Correlation and regression analysis were carried out between cyanobacterial population and the physical properties of soil (i.e., soil temperature, pH and conductivity) in different study sites Fig 4(a),4(b),4(c).The cyanobacterial population showed positive correlation with soil temperature Fig 4(a),4(c) in site-1(r =0.593, $P<0.05$),and in site -3 (r=0.458, $P<0.05$) , and pH in site-1(r=0.740, $P<0.05$) and site-3(r=0.722, $P<0.05$) respectively. Significant negative correlation was obtained between cyanobacterial population and conductivity (r=0.474, $P<0.05$) in study site-2, but they were positively correlated in study site -3(r=0.705, $P<0.005$).

Among the study sites, site-2 recorded the highest number of species(18) in post monsoon followed by site-1 which had good growth of cyanobacterial strains in pre- monsoon (17) and monsoon (17), respectively (Table-3).The Shannon's diversity index was highest (2.201) in site-1 (Table-3) in pre- monsoon followed by site-2 in the winter season (2.046) and site-1 in the monsoon season (2.002) indicative of the higher number of genera recorded in these soil samples .

Table-1- Occurrence and abundance of cyanobacterial taxa at different study sites:

Sl no	Cyanobacterial taxa	Study sites												Abundance (%)
		SS- 1				SS-2				SS-3				
		Pm	M	Po	W	Pm	M	Po	W	Pm	M	Po	W	
	Chroococcaceae													
1	*Aphanocapsa banaresensis* Bharadwaja	-	1	-	-	1	1	-	-	-	-	2	1	16.66
2.	*Aphanocapsa biformis* A.Br	-	-	1	-	-	-	1	1	-	-	-	-	8.33
3.	*Aphanocapsa crassa* Ghose	-	-	1	1	-	-	-	1	-	-	1	1	13.88
4.	*Aphanocapsa* sp.	1	-	1	-	1	-	-	-	1	-	-	-	11.11

5.	*Chroococcus minor*(Kutz) Nag	-	1	-	-	-	-	1	-	-	-	1	1	11.11
6.	*Gloeocapsa* sp.	1	-	-	-	1	1	-	-	-	-	-	-	8.33
7.	*Gloeothece* sp.	-	-	-	-	-	-	-	-	-	-	1	1	5.55
8.	*Gomphosphaerica* sp.	1	-	-	-	-	-	-	-	-	-	-	-	2.77
	Nostocaceae													
9	*Anabaena aphanizomenoides* Forti	-	1	1	-	1	-	1	-	-	-	-	1	13.88
10.	*Anabaena circinalis* Rabenhorst ex Born.et Flah.	-	-	2	-	2	-	3	1	2	1	2	1	38.88
11.	*Anabaena anomala* Fritsch	-	2	-	-	-	-	-	-	-	1	-	-	8.33
12.	*Anabaena doliolum* Bharadwaja	-	1	-	-	-	-	2	2	-	1	-	-	16.66
13.	*Anabaena fertilissima* Rao,C.B.	-	-	3	-	-	-	2	-	2	-	2	-	25
14.	*Anabaena gelatinicola* Ghose	-	-	1	1	-	-	-	1	-	-	-	-	8.33
15.	*Anabaena iyengarii* Bharadwaja	-	-	-	2	-	-	2	-	-	-	1	-	13.88
16.	*Anabaena oryzae* Fritsch	1	-	3	-	-	-	2	-	2	1	2	-	30.55
17.	*Anabaena virabilis* var.ellipsospora Fritsch	1	-	-	1	-	-	-	-	-	-	-	-	5.55
18.	*Anabaena spiroides* Klebahn	1	1	-	-	-	-	-	-	-	-	-	-	5.55
19.	*Aulosira aenigmatica* Fremy	-	-	-	-	-	1	-	-	-	2	-	-	8.33
20.	*Cylindrospermum muscicola* Kutzing ex Born. et Flah.	-	-	-	-	-	-	-	1	-	-	2	-	8.33
21.	*Cylindrospermum stagnale* (Kutz)Born.et Flah.	-	-	1	-	-	-	-	-	-	-	-	-	2.77
22.	*Nostoc calcicola* Brebisson ex Born .et Flah.	-	-	1	1	1	-	2	-	-	-	-	1	16.66
23.	*Nostoc carneum* Ag. Ex Born. Et Flah.	-	-	1	-	-	2	-	1	-	-	-	-	11.11
24.	*Nostoc ellipsosporum* (Desm.) Rabenh.ex Born.et Flah.	-	-	-	2	-	-	3	-	-	-	-	-	13.88
25.	*Nostoc hatei* Dixit	-	-	-	-	-	-	-	1	-	-	2	-	8.33
26	*Nostoc linckia* (Roth)Bornet ex	-	1	-	-	-	1	-	-	-	1	2	-	13.88

.	Born. et Flah.													
27.	*Nostoc muscorum* Ag.ex Born.et Flah.	-	-	-	1	1	-	-	-	-	-	-	2	11.11
28.	*Nostoc sphaericum* Vaucherex Born.et Flah.	1	-	-	-	-	-	3	2	-	-	-	-	16.66
29.	*Nostoc spongiaeforme* Agardh ex Born.et Flah.	-	1	-	-	-	-	2	2	-	1	-	-	16.66
Oscillatoriaceae														
30.	*Lyngbya contorta* Lemm.	-	1	-	-	-	2	1	1	-	-	-	2	19.44
31	*Lyngbya spilalis* Geitler	1	1	-	-	-	1	1	2	-	1	-	-	19.44
32.	*Phormidium abronema* Skuja	-	-	-	-	-	-	2	-	-	-	-	-	5.55
33.	*Phormidium purpurascens (*Kutz.)Gomont	1	1	-	-	-	-	-	-	-	1	2	-	13.88
34.	*Phormidium tenue* (Menegh.)Gomont	2	1	-	-	-	2	-	-	1	1	1	1	25
35.	*Oscillatoria willei* Gardner em. Drouet	2	-	-	-	3	-	-	2	-	1	1	1	27.77
Scytonemataceae														
36.	*Camphylonemopsis iyengarii* Desikachary	2	2	-	-	-	-	1	1	1	-	-	-	19.44
37.	*Plectonema indica* Dixit	-	-	-	-	-	1	-	-	-	-	-	-	2.77
38.	*Scytonema rivulare* Borzi	1	-	1	-	-	-	-	-	-	1	-	-	8.33
39.	*Tolypothrix distorta* Kutz	-	-	1	-	-	-	1	-	-	-	-	-	5.55
40.	*Tolypothrix robusta* Gardner	-	3	-	-	1	-	-	1	-	1	-	1	19.44
Microchaetaceae														
41.	*Microchaete* sp.	1	-	1	-	-	-	-	1	-	-	-	-	8.33
42.	*Microchaete aequalis (*Fremy)comb.nov.	1	1	-	-	-	-	-	-	-	-	-	-	5.55
43.	*Microchaete loktakensis* Bruhl et Biswas	1	-	-	-	1	-	-	-	-	-	-	2	11.11
44.	*Microchaete uberrima Skuja*	2	1	-	-	-	-	-	-	1	-	-	-	11.11

Rivulariaceae														
45.	*Calothrix braunii* (A.Br.) Bornet et Flahault	-	-	-	-	-	-	1	-	-	-	-	1	5.55
46.	*Rivularia* sp.	-	1	-	-	-	-	-	-	-	-	-	-	2.77
Stigonemataceae														
47.	*Westiellopsis prolifica* Janet	-	-	-	3	-	-	-	-	-	-	2	3	22.22

* Pm - Pre monsoon season, M-Monsoon season, Po-Post monsoon season, W-winter season

Table 2 - Physico- chemical properties of the study sites:

Site	Season	Temp(^{0}C)	pH	Cond (µS)	P(mg/100g)	Na(mg/100g)	K(mg/ 100)g	Soil N(g/kg)
SITE -1	Pre monsoon	24.24 ±0.384	5.729 ± 0.102	174.6 ± 2.509	6.07 ± 0.01	16.174±0.099	26.542 ± 0.144	0.4 ± 0.09
	Monsoon	34.54 ± 0.572	7.29 ± 0.015	168.4 ± 9.126	6.26 ± 0.014	9.742 ± 0.016	21.22 ± 0.060	0.7 ± 0.02
	Post monsoon	32.22 ± 0.813	6.66 ± 0.054	172.4 ± 0.027	6.63± 0.027	9.002 ±0.028	18.466 ± 0.161	0.9 ± 0.01
	Winter	15.16 ± 0.554	6.012 ± 0.168	38.4 ± 2.08	8.398 ±0.019	11.866 ±0.08	23.218 ± 0.10	0.4 ± 0.001
SITE -2	Pre monsoon	29.28 ± 0.228	5.862 ± 0.038	27.4 ± 1.140	5.476± 0.032	13.93 ± 0.085	47.818 ± 0.553	0.8 ± 0.003
	Monsoon	32.6 ± 0.316	5.918 ± 0.106	71.2 ± 1.303	2.046 ± 0.011	9.49 ± 0.021	24.41 ± 0.184	2.2 ± 0.03
	Post mons	31.52 ± 0.216	6.3 ± 0.331	44.4 ± 3.435	2.164± 0.031	9.638 ± 0.023	24.614 ± 0.106	0.3 ± 0.01
	Winter	14.58 ± 0.319	6.046 ± 0.092	37.6 ± 1.140	3.046 ± 0.025	11.48 ± 0.005	42.524 ± 0.482	1.6 ± 0.09
SITE -3	Pre monsoon	27.24 ± 0.260	5.768 ± 0.080	32.84± 3.070	2.728 ± 0.022	10.768±0.008	15.13 ± 0.076	1.9 ± 0.005
	Monsoon	32.32 ± 0.414	6.582 ± 0.020	122 ± 1.581	3.486 ±0.025	9.924 ±0.023	27.184 ± 0.297	1.2 ± 0.07
	Post monsoon	33.34 ± 0.296	6.34 ± 0.219	194 ± 3.2	3.84 4± 0.008	12.572±0.076	47.768 ± 0.552	2.1 ± 0.023
	Winter	14.36 ± 0.384	6.074 ± 0.058	33.6 ± 1.516	2.314 ±0.015	11.38 ± 0.005	19.734 ± 0.134	1.7 ± 0.009

Table 3- Diversity index of rice field cyanobacteria in different seasons at the studied sites:

Location	Season	Total isolate	Species Richness	Diversity Index (Shannon and Weinner)
Site-1	Pre monsoon	21	17	2.201
	Monsoon	21	17	2.002
	Post monsoon	21	14	1.729
	Winter	12	8	1.255
Site-2	Pre monsoon	11	10	1.886
	Monsoon	12	9	1.889
	Post monsoon	31	18	1.908
	Winter	21	16	2.046
Site-3	Pre monsoon	10	7	1.475
	Monsoon	12	13	1.252
	Post monsoon	24	15	1.070
	Winter	20	15	1.074

Fig 2 (a) Correlation between cyanobacterial population(Log MPN x10^4/g soil) and temperature, ph and conductivity respectively of study site -1

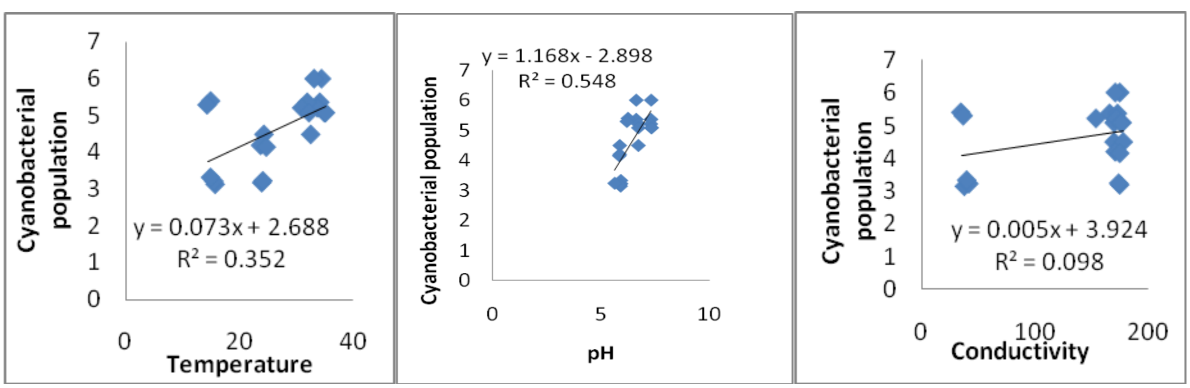

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Fig 2(b) Correlation between cyanobacterial population (Log MPN x10^4/g soil) and temperature, ph and conductivity respectively of study site-2

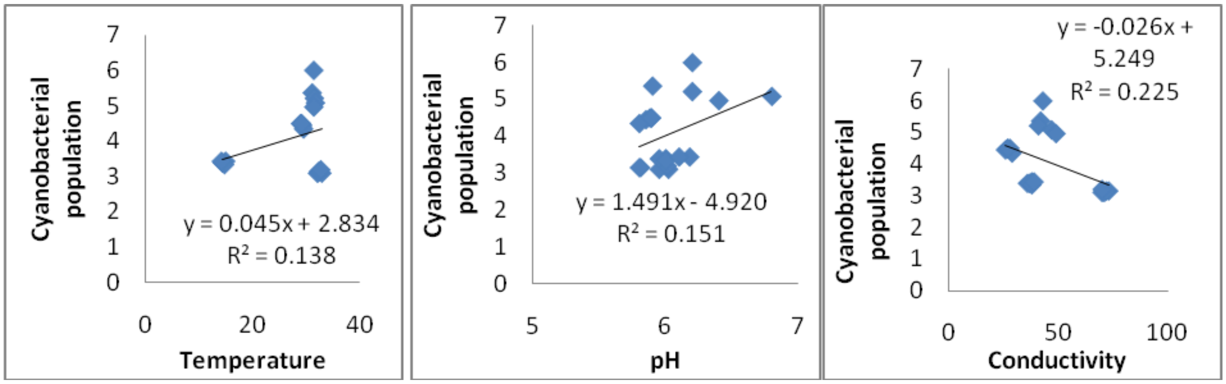
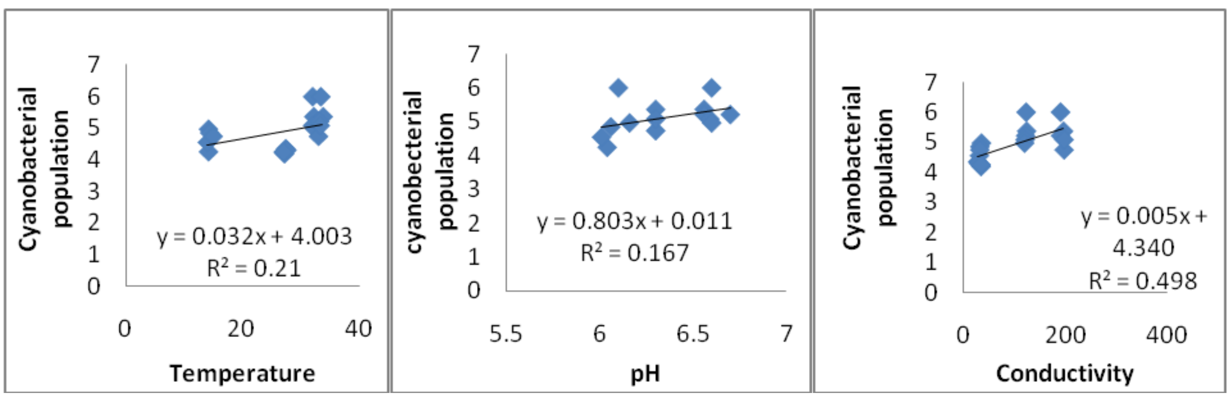


Fig 2(c) Correlation between cyanobacterial population (Log MPN x10^4/g soil) and temperature, pH and conductivity respectively of study site-3



Discussion

The present investigation showed the presence of both heterocystous and non heterocystous forms of cyanobacteria in the Lower Brahmaputra Valley agroclimatic zone. The abundance of heterocystous forms was more as compared to the non heterocystous forms. The results are in conformity with Nayak and Prasanna (2007). The diversity of cyanobacterial genera was higher in the pre monsoon and monsoon seasons in sites-1&3, (Table-3) and in site-2 it was higher in winter and post-monsoon season. The rich diversity of cyanobacteria in the different seasons indicates the ubiquitous distribution of cyanobacteria in the region. Such finding may be attributed to favourable environment in the region with respect to the requirement for light, water, high temperature, and nutrient availability due to use of chemical fertilizers, pesticides, etc. which is in confirmation with the earlier finding of Kondo and Yasuda (2003).

Among different physico-chemical properties, pH is important in determining growth, establishment and diversity of cyanobacterial flora, which is generally been reported to prefer neutral to slightly alkaline pH (Roger and Kulasooriya 1980, Kaushik 1994). In the present study, rice fields showed slightly acidic to alkaline pH and a positive correlation was observed between the soil pH and cyanobacterial population. Higher values of abundance were also recorded concurrently during the seasons having high soil pH. Abundance of more heterocystous cyanobacterial taxa in soil samples having alkaline pH was also in conformity with Nayak and Prasanna (2007) who reported that heterocystous forms were more abundant at alkaline pH in different agro-climatic regions of India. The soil elements like sodium, potassium, available phosphate and mainly the nitrogen content of soil play an important role in the distribution of cyanobacteria. The presence of heterocystous forms,which usually grow during unfavourable conditions and nutrient deficiency may indicate some limiting conditions in the soil. The diversity and abundance of different nitrogen fixing cyanobacterial forms at different study sites are also affected by man made activities like application of fertilizers, agricultural lime etc. as each species react to it differently. Earlier studies by Ibraheem (2007), Hamed (2007), Abdel-Raouf *et al*. (2004) showed that cyanobacteria may help the soil to improve its characteristics such as, carbon – nitrogen ratio, texture, aeration. The magnitude of these improvements is greatly dependent on the physical and chemical characteristics of the soil, including the composition of the algal population. As rice is widely cultivated in the studied region, we can associate its mineral and nitrogen nutrition with the nitrogen fixing blue green algae and hence, emphasis can be given for its proper management for sustainable agriculture and economic development of the region.

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