Population dynamics of cyanobacteria in alluvial rice grown soils of lower Brahmaputra floodplain

Juthika Dihingia* and P.P. Baruah

Department of Botany, Gauhati University, Assam, India. *Author for correspondence- E.mail: juthikadihingia@gmail.com

Abstract

The present investigation was carried out to study the cyanobacterial diversity and population dynamics during different months in relation to soil physico-chemical parameters in various rice fields in alluvial flood plain of the Brahmaputra River. Altogether 71 species of cyanobacteria under 20 genera and 9 families were recorded. Among the species heterocystous filamentous were dominant (80%) over the other forms and Nostocaceae (54%) was predominant in all the three sites followed by Chroococcaceae (14%) and Rivulariaceae (13%) respectively. In case of population number it ranged in between 0.14x10^5/g to 9.35±9.68x10^5/g of soil. It was recorded highest during July-August (monsoon season) followed by September-October (post monsoon), March-June (pre monsoon) and November-February (winter season) respectively.

Principal component analysis (PCA) justified the seasonal pattern and identified close relation to soil parameters like temperature, moisture, pH, phosphorus and sodium. Pearson’s correlation analysis also revealed significant negative correlation of cyanobacterial number with soil nitrogen, organic carbon and conductivity in the floodplain soils.

Key words: Alluvial soil, Brahmaputra River, cyanobacteria, PCA ordination, population dynamics, rice fields, soil physico-chemical parameters.

Introduction

Cyanobacteria are the successful phototrophic prokaryotes and most widely distributed group among soil microorganisms (Stanier and Cohen-Bazire, 1977). They are found in almost every conceivable environment including vast oceanic areas to freshwater lakes, temperate soils to bare rocks and even extreme habitats like arid deserts, frigid lakes, or hot springs (Hoffmann, 1989). Some of the cyanobacterial taxa are capable of fixing the atmospheric nitrogen and hence maintain the nitrogen-level in the soil in which they grow and flourish (Venkataraman, 1993). Their presence in various ecosystems has also been reported to increase the oxygen concentration and improve the physico-chemical properties of the soil (Mandal et al., 1998). Among the diverse habitats rice fields provide a very congenial condition for abundant growth of N2-fixing cyanobacteria (Nayak et al., 2001; Whitton, 2000). However their diversity, abundance and contribution to the total nitrogen fixed in rice fields are greatly affected by soil, climatic conditions (Pabbi, 2008), different seasons and rice cultivation cycle. So, it becomes utmost important for the algologist to know the factors that allow N2-fixing cyanobacteria to establish and bloom in rice fields. Considerable amount of research have been carried out on diversity and distribution of the cyanobacterial flora of rice fields of India (Kotle and Goyal, 1986; Mishra and Pabbi, 2004; Digambar Rao et al., 2008; Dey et al., 2010; D’souza et al., 2011). A few attempts have also been made in finding the diversity of cyanobacteria in rice fields of Assam (Saikia and Bordoloi, 1994; Ahmed et al., 1999; Dihingia and Baruah, 2011; Bharadwaj and Baruah, 2013; Deb et al., 2013). Though a little information on the soil physico-chemical parameters modulating variation on cyanobacterial population particularly from rice fields were available till date (Chunleuchanon et al., 2003; Song et al., 2005; Thamizh Selvi and Sivakumar, 2011; Das et al., 2011), no such study has been reported from the alluvial soils of Brahmaputra floodplain. Therefore, the present endeavor had been taken an effort to study the diversity and population dynamics of cyanobacteria in relation to seasonal variation and soil physico-chemical parameters in different alluvial rice field’s soils on both the banks of the Brahmaputra River, so that proper management of cyanobacterial population can be maintained for sustainable agro-ecosystem development in the floodplain region.

Materials and methods

Site selection:
A total of nine (9) rice fields were selected from three different sites on both the floodplains banks of the river Brahmaputra in Kamrup district, Assam, which lies between 25°46' to 26°49' N latitudes and 90°38' to 91°50' E longitudes and covers an area of ca. 4345 sq. km. Since the area is blessed with the mighty Brahmaputra River, the valley region is formed with alluvial deposits, and hence is one of the suitable habitat types for growing rice. A group of three rice grown areas located between 26°08' N latitudes and 91°37' E longitudes were selected from the southwest bank of the river; three rice fields (located between 26°12' N
latitudes and 91°34’ E longitudes) from northern bank and three rice fields (located between 26°13’ N latitudes and 91°54’ E longitudes) from the South east bank of the river Brahmaputra.

The climate of Assam in general may be divided into four seasons. The monsoon season which starts from June and continues till September, receives highest rainfall (>79% of annual rainfall) and is characterized by high humidity (80% to 95%) and high temperature (35°C to 39°C). A short post-monsoon with moderate temperature and rainfall falls during the months of October and November. The winter season starts in November and continues till the month of February. It is basically characterized by scanty rainfall with a minimum temperature of 7°C and the maximum of 24°C and with a relative humidity varying from 60% to 90% respectively. Pre-monsoon season starts in the early part of March and extends up to May. Temperature starts rising gradually and at the later part of the season there is occasional thunderstorm, often the weather remains windy and dusty (Chowdhury, 2005).

Sample collection and analysis:
Soil samples were collected in two months intervals from 8-10 randomly selected spots to a depth of 10-15 cm by removing the surface debris. Samples were then mixed, air dried, powdered and sieved and were analyzed for various soil physicochemical parameters following the standard protocols as described by Trivedy et al. (1987).

Cyanobacterial culture and its enumeration:
Soil samples weighed 0.5 g were inoculated in sterilized nitrogen free BG11 medium in pre-sterilized flasks under optimal growth condition for 20-25 days at 30°C ±2°C temperature in 2.3 K lux light intensity. Enumeration of cyanobacterial number was carried out by MPN technique following Pabbi et al. (2010). Identifications of cyanobacteria were done by following the keys given by Desikachary (1959).

Statistical analysis:
Principal component analysis (PCA) and Pearson’s correlation coefficient was carried out between N₂-fixing cyanobacterial population and physico-chemical parameters of the soil at different months using the statistical programs XLSTAT and SPSS.

Results
Species diversity:
A total of 71 species of cyanobacteria belonging to 20 genera under 9 families were identified from the study sites. Out of these 71 species enumerated, 57 species belonging to 14 genera under 7 families were filamentous heterocystous form, 10 species belonging to 5 genera under 1 family were unicellular and 4 species belonging to single genus were filamentous non-heterocystous form. Filamentous heterocystous families reported were Nostocaceae with 4 genera (Anabaena, Anabaenopsis, Aulosira and Nostoc), followed by Rivulariaceae with 3 genera (Calothrix, Gloeotrichia, Rivularia) and Scytonemataceae with 2 genera (Sctyonema, Tolypothrix). Other filamentous heterocystous species represented by only 1 genus each were under families Stigonemataceae (Hapalosiphon), Mastigocladosidaceae (Mastigoclados), Mastigocladosidaceae (Mastigocladosid) and Microchaetaceae (Microchaete). The Oscillatoriaceae was the single recorded filamentous non-heterocystous family with the genus Lyngbya. The Chroococcaceae with 5 genera (Aphanocapsa, Aphanothece, Chroococcus, Gloeocapsa and Synechococcus) was the only recorded unicellular family of cyanobacteria.

Maximum number of species was reported under family Nostocaceae with 38 species which were of the genera Anabaena (22), Anabaenopsis (1), Aulosira (3) and Nostoc (12). It was followed by Chroococcaceae with 10 species comprising of genera Aphanocapsa (4), Aphanothece (2), Chroococcus (1), Gloeocapsa (2) and Synechococcus (1). Family Rivulariaceae followed next with 9 species which were of the genera Calothrix (6), Gloeotrithia (2), Rivularia (1). Under family Scytonemataceae only 5 species were reported which were represented by 2 genus viz. Sctyonema (3) and Tolypothrix (2) and that of family Oscillatoriaceae with 4 species that belonged to a single genus Lyngbya. The family Stigonemataceae was represented by 2 species belonging to genera Hapalosiphon (1) and Westiellopsis (1). Families Mastigocladosidaceae, Mastigocladosidaceae and Microchaetaceae were represented by 1 species each that belongs to genera Mastigoclados, Mastigocladosid and Microchaete respectively.

Population dynamics of cyanobacteria in relation to soil physico-chemical properties:
The population number of cyanobacteria varies in relation to different months in rice fields situated in three different sites (Fig: 1). The highest population number was recorded in the rice fields situated in the SW (4.07±2.98x10⁴/g soil), followed by North (0.94±0.76x10⁴/g soil) and SE bank of the river Brahmaputra (0.52±0.4x10⁴/g soil). In site SW cyanobacterial population number was recorded maximum during the month of September-October (9.35±9.68x10⁴/g soil) and minimum during January-February (0.9±0.56 x10⁴/g soil). In N bank population number recorded maximum during July-August (2.34± x10⁴/g soil) and minimum during November-December (0.15± x10⁴/g soil). In SE bank of the river, maximum number of cyanobacteria was recorded during the months July-August (1.34± x10⁴/g soil) and minimum during January-February (0.14± x10⁴/g soil).
Soil physico-chemical parameters also varied during different months in the three sites. In SW bank of the river Brahmaputra the soil temperature ranged between 17.6±1.34 to 29.4±0.12 °C, moisture between 18.39±0.49 to 29.91±2.02 %, phosphorus between 70.31±1.56 to 125.84±24.62 mg/100gm, sodium between 0.28±0.023 to 0.86±0.16 mg/100gm and calcium ranged in between 2.28±1.32 to 4.13±0.77 mg/100gm. Maximum values were recorded during July-August and minimum were recorded during January-February. The pH value was varied from 6.30±0.10 to 8.05±0.18 and that of potassium from 0.098±0.12 to 0.39±0.1 mg/100gm respectively. July-August was experienced with highest pH and potassium and overall lowest pH was recorded during November-December and Potassium was recorded during May-June. Similarly, conductivity, potassium, magnesium, organic carbon which varied from 62±21.22 to 105.66±16.2 µs, 0.098±0.12 to 0.39±0.1 mg/100gm, 2.386±0.74 to 3.14±0.49 mg/100gm and 1.89±0.66 to 3.39±0.53 % recorded maximum during November-December and minimum during July-August except potassium which recorded minimum during May-June. Nitrogen that ranged between 0.024±0.003 to 0.055±0.001 % recorded maximum level during January-February and minimum during July-August.

In N bank, soil physico-chemical properties like temperature, pH, Phosphorus and calcium which varied from 18.5±0.40 to 30.14±0.028 °C, 5.8±0.29 to 7.31±0.57, 64.93±11.8 to 92.53±4.5 mg/100gm, 1.63±0.44 to 2.77±1.19 mg/100gm respectively and showed similar trend of fluctuation i.e. maximum during July-August and minimum during January-February. Moisture, potassium and sodium that ranged between 13.6±2.01 to 23.46±0.24 %, 0.16±0.05 to 0.81±0.16 mg/100gm and 0.16±0.08 to 0.51±0.20 mg/100gm recorded maximum during July-August while November-December experienced with minimum moisture, September-October experienced with minimum potassium and May-June with sodium. Conductivity,
nitrogen and magnesium that ranged between 50±18.4 to 108.66±34.3 µs, 0.023±0.006 to 0.039±0.009%, and 1.32±0.37 to 2.12±1.51 mg/100gm showed maximum during January-February while conductivity showed minimum during September-October, nitrogen during July-August and magnesium during May-June respectively. Organic carbon ranged between 1.49±0.12 to 2.67±0.67 % recorded maximum during November-December and minimum during March-April.

In SE bank of the river soil parameters, like temperature ranged between 19.2±0.04 to 29.2±0.5 °C, moisture between 13.33±1.54 to 24.95±1.25 %, pH between 5.45±0.07 to 6.76±0.35, phosphorus between 21.05±3.11 to 65.13±26.9 mg/100gm, calcium between 1.44±0.68 to 2.63±0.15 mg/100gm, and sodium ranged between 0.22±0.10 to 0.62±0.18 mg/100gm. Maximum values were recorded during July-August and minimum were recorded during January-February. Potassium and magnesium that varied from 0.31±0.13 to 1.57±0.45 mg/100gm and 1.42±0.50 to 1.72±0.53 mg/100gm recorded maximum during July-August and minimum during November-December. Conductivity varied from 39.66±9.88 to 73.33±14.14 µs and showed maximum during March-April and minimum during May-June. Both nitrogen (0.067±0.003 to 0.038±0.007 %) and organic carbon (2.41±0.63 to 3.97±0.14 %) showed maximum during January-February and minimum during July-August respectively.

In order to understand the direct or indirect influence of season and soil properties on cyanobacterial population dynamics a PCA ordination diagram is presented. The PCA ordination represented the correlation of all the three variables studied. The bi-plot for the axis (X and Y) was maximum in the rice fields of SW bank (91.21%) followed by N bank (89.12%) and SE bank (89.76%) of the river Brahmaputra within Kamrup district. The PCA ordination of SW bank revealed that cyanobacterial number was highest in the months of September-October and was highly related to phosphorus, soil temperature and soil moisture (Fig 1.). Cyanobacterial number in rice fields situated in the North bank was closely related to pH and soil moisture and was highest in the month of July-August and September-October (Fig 2.). Cyanobacterial number was also highest in the months of July-August in rice fields situated in the SE bank and was closely related to pH, soil moisture, phosphorus, sodium and potassium (Fig 3.). So the PCA ordination of the three sites revealed that cyanobacterial number was recorded maximum during July-August (monsoon season) followed during the months September-October (post monsoon). While comparatively less number of cyanobacteria was recorded during March-June (pre monsoon) and November-February (winter season).

Pearson’s correlation coefficient between soil physico-chemical parameters and cyanobacteria revealed significant positive as well as negative correlation. Significant positive correlation was observed between cyanobacterial population and soil physico-chemical properties i.e. soil temperature, soil moisture, pH and phosphorus in all the three sites. Significant positive correlation with potassium (SE bank), magnesium (SW bank) and sodium (SW and SE bank) was also seen. Study revealed a strong negative correlation of population number with nitrogen in all the sites. Conductivity (SW bank) and organic carbon (SW and SE bank) too showed significant negative correlation with the population number of N₂-fixing cyanobacteria (Table: 1).

Table 1: Pearson’s correlation coefficient between population number of N₂-fixing cyanobacteria and soil physico-chemical in each site.

<table>
<thead>
<tr>
<th>Sites/soil parameters</th>
<th>Ts</th>
<th>Ms</th>
<th>pH</th>
<th>Cs</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>Na</th>
<th>OC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Southwest (SW)</td>
<td>0.888*</td>
<td>0.924**</td>
<td>0.855*</td>
<td>-0.894*</td>
<td>0.922**</td>
<td>-0.069</td>
<td>0.698</td>
<td>-0.839*</td>
<td>0.965**</td>
<td>-0.905*</td>
<td></td>
</tr>
<tr>
<td>North bank (N)</td>
<td>0.872**</td>
<td>0.888*</td>
<td>0.935**</td>
<td>-0.673</td>
<td>-0.894*</td>
<td>0.859*</td>
<td>0.658</td>
<td>0.496</td>
<td>-0.686</td>
<td>0.455</td>
<td>-0.715</td>
</tr>
<tr>
<td>Southeast bank (SE)</td>
<td>0.707</td>
<td>0.960**</td>
<td>0.916</td>
<td>-0.483</td>
<td>-0.860*</td>
<td>0.984**</td>
<td>0.915*</td>
<td>0.893*</td>
<td>0.237</td>
<td>0.976</td>
<td>-0.831*</td>
</tr>
</tbody>
</table>

*Significant difference at P<0.05 and P<0.01 respectively. Ts = soil temperature, Ms = soil moisture, Cs = soil conductivity, N = nitrogen, P = phosphorus, K = potassium, Ca = calcium, Mg = magnesium, Na = sodium, OC= organic carbo

Discussion

A rich diversity of 71 species of cyanobacteria under 20 genera and 9 families were recorded in alluvial rice grown soils of lower Brahmaputra floodplain. Among these filamentous heterocystous (80%) cyanobacteria showed clear dominance over the unicellular (14%) and filamentous non-heterocystous (6%) forms. Hazarika (2007) while studying the cyanobacteria of greater Guwahati (Kamrup) reported 20.83% of unicellular/colonial and 30.56% of filamentous non-heterocystous form which was outnumbered by filamentous heterocystous cyanobacteria (48.61%) in rice fields, which was in conformity with the present...
findings. In term of species richness the family Nostocaceae (54%) was found to be dominated in the rice fields situated in all three banks followed by Chroococcaceae (14%) and Rivulariaceae (13%). The family Scytonemataceae, Oscillatoriaceae and Stigonemataceae comprise of only 7%, 6% and 3% of species respectively while Mastigocladiaceae, Mastigocladosiaceae and Microchaetaceae contributed with only 1%. The genera Nostoc and Anabaena are highly competitive and able to colonize whether as floating assemblages or as edaphic forms in rice fields soil (Prasanna and Nayak, 2007). In the present study also genus Anabaena (31%) showed the highest number of species, thereby indicating its dominance in rice growing fields of the district in terms of species richness. Genera Nostoc (17%) and Calothrix (8%) occupied the second and third position followed by unicellular cyanobacteria Aphanocapsa (6%). Similar observations were made in Tripura (Singh et al., 1997a) and Nagaland (Singh et al. 1997b) respectively.

The principal component analysis (PCA) indicated that the cyanobacterial number during the months from July to October (monsoon and post monsoon season) was mainly influenced by soil temperature, soil moisture, phosphorus and pH. Positive significant correlation was also established in between cyanobacterial population number and these soil parameters (Table: 2). Soil moisture induced the cyanobacterial population in the monsoon season where soil contained a relatively higher amount of moisture compared to the months during November to February (winter season). Cold inhibited the growth of cyanobacteria as the optimum temperature range for luxuriant cyanobacterial growth in paddy fields of India is about 30-35°C (Subrahmanyan et al., 1965). Maximum cyanobacterial number occurred in the monsoon season when the temperature was highest that ranged from 29.2°C-30.14°C which was in conformity with the findings of Castenholz and Waterbury (1989). The average high temperature on the other hand increased the rate of decomposition of organic matter which results in a continuous availability of high nutrients supply in rice fields soil. The population number was observed to increase with the increase in pH values. Many evidences are present supporting pH in regulating the increasing number of cyanobacteria which was in conformity with our findings (Mitra, 1951). The amount of phosphorus nutrient which gradually increases during monsoon season and also showed significant positive correlation had a great impact on cyanobacterial population too. De and Sulaiman (1950) and Bisoyi and Singh (1988) and showed much earlier that a good correlation between the level of phosphorus applied and biomass of cyanobacteria. Cyanobacteria were also been reported to excrete organic acids that render phosphorus solubilisation thus contributing available phosphorus to the soil (Fuller and Rogers, 1952; Whitton et al., 1991). Moreover sodium was found to be positively correlated which indicate that this nutrient also played a role in inducing the population number. This was in conformity with the earlier findings of Shubert and Starks (1980) and Issa et al. (2000). Positive correlation with potassium, calcium and magnesium has not been properly reported yet, though we found positive correlation with these soil parameters. However, according to Reynaud (1987) mineral nutrients (calcium, potassium, iron and phosphorus) increase with increased relative and absolute heterocystous biomass. Nitrogen, an important nutrient for plants showed significantly strong negative correlation with cyanobacterial population (Table: 2), thus indicating its establishment in particular soil where the concentration of combined nitrogen is low. This corroborates with the findings of Howarth et al., (1988); Nayak and Prasanna (2007). Heavy rainfall during monsoon season might lead to highly mobile nitrate to leach out which results in overall nitrogen deficit. Negative correlation was also observed between cyanobacterial population and conductivity as well as organic carbon which collaborate with the findings of Reynaud (1987) and Dey et al., (2010).

Later, during winter and pre-monsoon, cyanobacterial patches gradually disappear with the concomitant reduction of soil temperature, moisture, pH and with high accumulation of N and organic carbon due to the decomposition of cyanobacterial biomass. Minimum population number recorded in rice fields situated in the SE bank of the River Brahmaputra (Fig:4L) thus, could be attributed to the presence of high level of average nitrogen and low level of phosphorus and soil moisture (Fig:4E, F and B). Soil pH value towards acidic ranges (Fig:4C) too supports the poor growth of cyanobacterial population in these rice fields. (Nayak and Prasanna, 2007). Higher amount of phosphorus and soil moisture (Fig:4F and B) along with lower amount of nitrogen and alkaline pH (Fig:4E and 4C) could be responsible for maximum population number of cyanobacteria in the rice grown soils in SW bank of the River Brahmaputra.
Fig: 4. Box plot graphics displaying the mean (+ sign), maximum (top dot) and minimum values (bottom dot) of the soil physico-chemical parameters (A-K) and cyanobacterial number (L) of each site. Horizontal lines in each box plot are, from top to bottom: third quartile, median and first quartile.

Acknowledgements

The authors are grateful to the Head, Department of Botany, Gauhati University, Guwahati, Assam for his constant encouragement. Both the authors are thankful to the respective farmers/field owners for allowing them to work on their fields.

References


Diversity and distribution of heterocystous nitrogen fixing cyanobacteria in the rice fields of Kamrup, Assam, India

Juthika Dihingia and P. P. Baruah

Department of Botany, Gauhati University, Guwahati-781014, India
E-mail: juthikadihingia@gmail.com; partha_ghy16@rediffmail.com

ABSTRACT


Heterocystous nitrogen fixing cyanobacterial diversity and distribution were investigated in nine rice fields of Kamrup, which is one of the oldest landmasses of Brahmaputra flood plain of North East India. Rice is the staple crop of the region since times immemorial. The study was conducted from June 2010 to May 2011. A total of 49 heterocyst bearing cyanobacterial stains belonging to 6 families and 12 genera were identified. *Anabaena* with 15 species was the dominant genus followed by *Nostoc* (12 spp.). The other genera recorded were *Anabaenopsis* (1 sp.), *Aulosira* (4 spp.), *Cylindrospermum* (2 spp.), *Scytonema* (2 spp.), *Tolypothrix* (2 spp.), *Câlothrix* (5 spp.), *Rivularia* (1 sp.), *Hapaloniphon* (3 spp.), *Mastigochladius* (1 sp.), *Mastigocladosis* (1 sp.). Sorensen index revealed a little similarity among the fields in terms of cyanobacterial species composition.

Key-words: Nitrogen fixing cyanobacteria, diversity, distribution, rice fields, Kamrup District, Assam, India.

INTRODUCTION

Being situated on the lower Brahmaputra floodplain, the Kamrup region is exposed to recurring inundation during monsoon. The area is best suited for rice cultivation which is the staple food crop of the entire north-eastern India. About 90% of total population of Kamrup lives in rural areas and their potent occupation is agriculture. The consumption of chemical fertilizers in Kamrup is lower than that of the national standard of the country (Gopalakrishna 2000). This indicates dependency of the traditional farmers on natural fertilizer. Since the supplement of chemical nitrogen fertilizers is not a cost effective practice, the marginal farmers are unable to meet this high budgeted process. In addition, utilization of chemical fertilizers has posed a serious threat to soil quality, environment and sustainability of food grain production. It therefore becomes important to understand the soil microflora, with special reference to cyanobacteria, in order to suggest an alternate low cost and renewable source of nitrogen fertilizers for improving the carbon content of the soil to uplift rice production.

Cyanobacteria (BGA) are morphologically diverse and complex group of prokaryotic organisms. A few have the capacity to fix atmospheric nitrogen. Cyanobacteria are considered as natural biofertilizer for many years (Baftehchi et al. 2007). Representatives of the group are found abundantly in crop field ecosystems throughout the world (Whitton 2000). Besides maintaining the nitrogen status and fertility of soils, they are also responsible for improvement of the structure and physico-chemical characteristics of soils by increasing phosphorus content (Fuller & Rogers 1952) by secreting plant growth promoting substances.
(Pandey et al. 2005, Zulpa et al. 2008) and also by enhancing water holding capacity that leads to reduce soil erosion (Richert et al. 2005). They act as potential agents for the biological control of plant pathogenic bacteria and fungi.

The agronomic potential of cyanobacteria, their distribution and their role in maintaining soil fertility have been extensively studied throughout the world (Watanabe 1959, Chuleuchanon et al. 2003, Begum et al. 2008). Although similar studies were undertaken by various workers in different parts of India (Anand 1998, Singh et al. 2002, Choudhary et al. 2010, Prasana & Nayak 2007, Muthukumar 2007), a little study has been carried out on blue green algae (BGA) in crop fields of Brahmaputra floodplain in general (Ahmed 2000) and Kamrup in particular. Almost all studies are confined to enumeration of the BGA and studies on distributional pattern of those microbes are lacking.

The present endeavour, therefore, was aimed to study the heterocystous nitrogen fixing cyanobacterial diversity with their distributional pattern in different rice fields of Kamrup – an area which had been exploited for rice growing since historical times.

**MATERIAL AND METHOD**

**Study area:** The study was conducted in Kamrup (a conglomeration of two administrative districts of Assam) which is located between 25°46' and 26°49' N latitudes and 90°48' and 91°50' E longitudes covering 4345 km² area. It is in the confluence of Himalaya and Indo-Burma hotspots. The area is bounded by foothills of Bhutan in the north, Meghalaya in the south, Nagaon and Darrang districts in the east and Goalpara and Nalbari districts of Assam in the west (Text-figure 1). The climate is sub-tropical with semi-arid summer and cold winter. Average humidity is 75% and the maximum and minimum temperatures range from 37° to 39°C and from 6° to 7°C respectively. The annual average rainfall varies between 1500 and 2600 mm and the area experiences maximum rainfall during June to July.

**Sampling Method:** A total of nine rice fields were selected for sample collection. Sites 1-3 were chosen on the south bank of Brahmaputra from south-west Kamrup. Sites 4-6 were located in the north on the north bank of the river and the sites 7-9 were selected from south-east Kamrup. Soil samples were collected from June 2010 to May 2011 from the depth of 5-15 cm. Each rice field was the composite of five sub-samples and collected soil samples were kept in polythene bags for further processing. Dried and homogenized soil samples were then inoculated in sterilized nitrogen free BG₁₁ medium in pre-sterilized flasks under optimal growth condition for 20-25 days at 30° ±2°C temperature in 2.3 K lux light intensity. Culture samples were studied under the microscope to get the morphological characteristics to identify the cyanobacterial species following the standard literature (Desikachary 1959, Tiwari 1972).
RESULTS AND DISCUSSION

Altogether, 49 heterocystous nitrogen fixing cyanobacteria (Table 1) belonging to 12 genera and 6 families were recorded from nine rice fields of entire Kamrup region. These are: Nostocaceae: (Anabaena, Anabaenopsis, Aulosira, Cylindrospermum, Nostoc), Rivulariaceae (Calothrix, Rivularia), Scytonemataceae: (Scytonema, Tolypothrix),

Table 1. Occurrences of heterocystous Nitrogen fixing cyanobacteria in different rice fields of Kamrup, Assam

<table>
<thead>
<tr>
<th>Species</th>
<th>Study sites</th>
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<tr>
<td></td>
<td>1</td>
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<tr>
<td>Anabaena ambigua</td>
<td>-</td>
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<tr>
<td>Anabaena circinalis v. crassa</td>
<td>+</td>
</tr>
<tr>
<td>Anabaena constricta</td>
<td>-</td>
</tr>
<tr>
<td>Anabaena doliolum</td>
<td>-</td>
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<tr>
<td>Anabaena ferdillisima</td>
<td>-</td>
</tr>
<tr>
<td>Anabaena flos-aqua</td>
<td>+</td>
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<tr>
<td>Anabaena iyengari v. tenuis</td>
<td>-</td>
</tr>
<tr>
<td>Anabaena oryzae</td>
<td>-</td>
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<tr>
<td>Anabaena oscillatoroides</td>
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<tr>
<td>Anabaena smithii</td>
<td>-</td>
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<tr>
<td>Anabaena sphaerica v. tenuis</td>
<td>-</td>
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<tr>
<td>Anabaena spiroides v. crassa</td>
<td>-</td>
</tr>
<tr>
<td>Anabaena torulosa</td>
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<td>Anabaena variabilis</td>
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<td>Anabaenopsis tanganyikae</td>
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<td>Aulosira impexa v. crassa</td>
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<td>Aulosira pseudoramosa</td>
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</tr>
<tr>
<td>Calothrix contarenii</td>
<td>+</td>
</tr>
<tr>
<td>Calothrix marchica</td>
<td>-</td>
</tr>
<tr>
<td>Calothrix membranacea</td>
<td>-</td>
</tr>
<tr>
<td>Calothrix ghosei</td>
<td>-</td>
</tr>
<tr>
<td>Calothrix javanica</td>
<td>+</td>
</tr>
<tr>
<td>Cylindrospermum majus</td>
<td>-</td>
</tr>
<tr>
<td>Cylindrospermum musicola</td>
<td>-</td>
</tr>
<tr>
<td>Hapalosiphon delicatulus</td>
<td>+</td>
</tr>
<tr>
<td>Hapalosiphon fontinalis</td>
<td>+</td>
</tr>
<tr>
<td>Hapalosiphon welwitschii</td>
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</tr>
<tr>
<td>Mastigocladosis jogensis</td>
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<tr>
<td>Mastigocladus laminosus</td>
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</tr>
<tr>
<td>Nostoc calcicola</td>
<td>+</td>
</tr>
<tr>
<td>Nostoc carneum</td>
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</tr>
<tr>
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<tr>
<td>Nostoc haueri</td>
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</tr>
<tr>
<td>Nostoc linckia</td>
<td>+</td>
</tr>
<tr>
<td>Nostoc maculiforme</td>
<td>+</td>
</tr>
<tr>
<td>Nostoc muscorum</td>
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<tr>
<td>Nostoc peludosum</td>
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</tr>
<tr>
<td>Nostoc piscinale</td>
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</tr>
<tr>
<td>Nostoc punctiforme</td>
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</tr>
<tr>
<td>Nostoc spongiaeforme v. varians</td>
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<td>Rivularia hansgirgi</td>
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<td>Scytonema simplex</td>
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<td>Tolypothrix byssoida</td>
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</tr>
<tr>
<td>Tolypothrix tenuis</td>
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</tr>
</tbody>
</table>
Stigmataceae (*Hapalosiphon*), Mastigocladaceae (*Mastigocladus*) and Mastigocladopsidaceae (*Mastigocladopsis*).

The study site nos. six and two showed higher species richness (24 each) out of all the nine studied plots which was followed by study site five (20) and one (19) respectively (Table 1). Of the algal families, Nostocaceae was the dominant with 34 species (*Anabaena* 15 species, *Nostoc* 12 species, *Aulosira* 4 species, *Cylindrospermum* 2 species and *Anabaenopsis* 1 species) followed by Rivulariaceae with 6 species (*Calothrix* 5 species, *Rivularia* 1 species), Scytonemataceae with 4 species (*Scytonema* 2 species, *Tolypothrix* 2 species). The family Stigonemataceae was represented by 3 species belonging to genus *Hapalosiphon*. Mastigocladaceae and Mastigocladopsidaceae were represented by 1 species each belonging to genus *Mastigocladus* and *Mastigocladopsis* (Table 1).

Among the recorded cyanobacterial genera, *Anabaena circinalis* var. *crassa* and *Nostoc spongiaeforme* var. *varians* were the most frequently occurring species which were followed by *Nostoc ellipsosporum* and *Nostoc linckia* (Table 1). The other species under the genus *Anabaena* which occur occasionally were *A. constricta, A. spiroides, A. torulosa, A. variabilis* and those of genus *Nostoc* were *N. carneum, N. hatei, N. peludosum, N. picinale*. The commonly occurring species of the other genera were as follows: *Aulosira: A. aenigmatica, A. implexa; Calothrix: C. marchica, C. ghosei; Cylindrospermum: C. musicola; Hapalosiphon: H. welwitschii; Mastigocladopsis: M. jogensis; Mastigocladus: M. laminosus; Rivularia: R. hansgirgii; Scytonema: S. simplex; Tolypothrix: T. tenius*.

Assam is known to have a conducive agro ecological condition for the growth of the BGA (Deka & Bordoloi 1991, Ahmed 2000), still systematic and quantitative studies were almost lacking. Earlier, Deka and Bordoloi (1991) reported only 11 heterocystous nitrogen fixing cyanobacteria from a rice field of Kamrup district belonging to genera *Anabaena, Cylindrospermum, Nostoc, Scytonema, Tolypothrix, Hapalosiphon* and *Stigonema*. Das et al (2003) reported only 7 species from Deepor Beel, a Ramsar site. The present attempt outnumbered the previous reports with 49 species of nitrogen fixing cyanobacteria. Similarity indices in respect to cyanobacteria inhabiting the nine different rice fields are presented in Table 2. Similarity indices revealed that not even in a single case percentage of similarity were above 80%. The result highlighted not only the differences in cyanobacterial composition in all nine rice grown areas but also indicates heterogeneous nature of the fields. Hence, it needs separate field specific management plans to improve the soil condition and biota. The study revealed that *Anabaena* and *Nostoc*, that belong to the family Nostocaceae, are the two potent nitrogen fixing genera of all the studied rice fields and are also responsible for nitrogen budget therein.

**ACKNOWLEDGEMENT**

The authors are grateful to the Head, Department of Botany, Gauhati University, Guwahati, Assam for providing laboratory facilities and to the cultivators for providing access to their respective crop fields.

<table>
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<th>Study sites</th>
<th>1</th>
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<th>4</th>
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<th>8</th>
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<td>0.41</td>
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<td>0.42</td>
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<td>0.52</td>
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<td>0.20</td>
<td>0.20</td>
<td>0.38</td>
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<td>0.30</td>
<td>0.30</td>
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</tr>
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<td></td>
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<td></td>
<td>1.0</td>
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REFERENCES


Algal Biofertilizers in Rice Fields of Chandrapur

J. Dihingia, N. Bharadwaj, J. Thakuria and P.P. Baruah

Abstract
Five rice fields of Chandrapur block of Kamrup district (Assam) were surveyed for nitrogen fixing Blue Green Algae (BGA). Altogether 21 blue green algal species were reported with having high nitrogen fixing ability. Seasonal variation and physical characteristics of the habitat types were also studied. The paper highlights that yields of rice are highly influenced by the natural BGA fertilizer in those areas.

Keywords: Algal biofertilizers, Nitrogen fixing ability, Rice fields, Chandrapur

Introduction
Algae are photosynthetic autotrophic organism occurring in all types of habitat. Blue green algae, commonly known as BGA are the most ancient group of organisms among algae and some representatives of them were recorded even from pre-cambrian charts (Schopf, 1970). They are morphologically diverse and are gram-negative prokaryotes.

In rice rhizosphere, their presence is inevitable (Ladha and Reddy, 1995). Since the majority of BGA have the capability to fix atmospheric nitrogen in the soil, they are therefore, commonly known as one of the efficient natural biofertilizers (Venkataraman 1981; Anand, 1998; Roger et al., 1987; Saadatnia and Riahi, 2009; Pereira et al., 2009). Venkataraman (1972) reported an average increase in rice yield by 7.29% when 100 kg N/ha fertilizer was applied along with the algae as compared to control where 100kg N/ha fertilizer was applied alone. Srinivasana and Ponnaya (1978) estimated that application of BGA contribute 25-30 kg nitrogen/ha/season to the rice crops.

Soil algalization with N₂-fixing BGA has received a world wide attention now-a-days. BGA act as an alternate source to chemical fertilizers, besides having many other benefits. A large number of workers have been working on algalization traits and their responses on grain yield (Mahapatra and Sharma, 1988; Singh et al., 1985; Dhar et al., 1989; Singh and Singh, 1989). BGA enhance plant growth by synthesizing and liberating growth promoting substances (Karthisikeyan et al., 2007, Zulpa et al., 2008). They excrete organic acids that render phosphorus solubilisation, as a result, phosphorus get available to the crop plants (Fuller and Rogers, 1952). In addition, they add substantial amount of organic matter to the soil too (Goyal, 2002), which act as a storehouse of nutrients like nitrogen, phosphorus and micronutrients.

A little and sporadic study have been undertaken on this aspects of BGA in Assam with special reference to rice grown areas of the region. The present study is therefore, aimed to isolate and identify the potential BGA species that can be used as biofertilizers for rice field soils.

Materials and Methods
**Study area**

The study was conducted in Chandrapur block which is situated in south east part of Kamrup district, Assam on the bank of River Brahmaputra. The soil of Chandrapur is mostly alluvial.

**Sampling method/Collection and culturing of BGA**

Soil samples were collected from five different rice fields in four different seasons during the year 2011. The depth of collection was 5 - 15 cm from the top. Dried and homogenized soil samples were then inoculated in sterilized nitrogen free BG_II medium in pre sterilized flasks under optimal growth condition for 20 - 25 days at 30° ±2 °C temperature in 2.3 K lux light intensity. Culture samples were studied under the microscope to get the morphological characteristics to identify the cyanobacterial species following the standard literatures (Desikachary, 1959; Bharadwaja, 1933).

**Environmental properties**

Soil pH, soil conductivity and soil temperature was determined by digital pH meter, conductivity meter and soil thermometer respectively. Most probable number count for blue green algal population was determined following Pabbi (2008).

**Statistical analysis**

Correlation coefficient (r) between blue green algal population and soil pH, soil conductivity and soil temperature were analyzed by calculating the regression equation and simple linear correlation coefficient.

**Results and Discussion**

During the present investigation altogether, 21 BGA stains belonging to 3 families and 6 genera were identified (Table 1.). These are Nostocaceae: *Anabaena*, *Aulosira*, *Cylindrospermum* and *Nostoc*, Rivulariaceae: *Calothrix* and Mastigocladopsisaceae: *Mastigocladus*. Among all the algal families, Nostocaceae was the dominant one with 19 species (*Anabaena* 6 species, *Nostoc* 10 species, *Aulosira* 2 species and *Cylindrospermum* 1 species) followed by Rivulariaceae and Mastigocladopsisaceae with one species each. Genus *Nostoc* (10 species) and *Anabaena* (6 species) are the most commonly occurring species in all the rice fields.

Table 1: Heterocystous Nitrogen fixing BGA in the rice fields of Chandrapur block:

<table>
<thead>
<tr>
<th>Name of species</th>
<th>Family: Nostocaceae</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Anabaena</em> syengarii v. tenuis</td>
<td><em>Nostoc</em> katei</td>
</tr>
<tr>
<td><em>Anabaena</em> oscillarioides</td>
<td><em>Nostoc</em> linckia</td>
</tr>
<tr>
<td><em>Anabaena</em> sphaerica v. tenuis</td>
<td><em>Nostoc</em> maculiforme</td>
</tr>
<tr>
<td><em>Anabaena</em> spiroides v. crassa</td>
<td><em>Nostoc</em> muscorum</td>
</tr>
<tr>
<td><em>Anabaena</em> torulosa</td>
<td><em>Nostoc</em> peludosum</td>
</tr>
<tr>
<td><em>Anabaena</em> variabilis</td>
<td><em>Nostoc</em> pisinale</td>
</tr>
<tr>
<td><em>Aulosira</em> fritschii</td>
<td><em>Nostoc</em> puntiforme</td>
</tr>
<tr>
<td><em>Aulosira</em> implexa v. crassa</td>
<td><em>Nostoc</em> spongiaformae</td>
</tr>
<tr>
<td><em>Cylindrospermum</em> musicola</td>
<td></td>
</tr>
<tr>
<td><em>Nostoc</em> carneum</td>
<td></td>
</tr>
<tr>
<td><em>Nostoc</em> ellipsosporum</td>
<td></td>
</tr>
</tbody>
</table>

**Family: Rivulariaceae**

*Calothrix* marchica

**Family: Mastigocladopsisaceae**

*Mastigocladus* jogensis
Table 2: Physical properties of the rice field soils of Chandrapur in relation to seasonal variation of BGA density.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Winter</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFN(algae/gm soil)</td>
<td>1.95000</td>
<td>2.19000</td>
<td>23.00000</td>
<td>16.20000</td>
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<tr>
<td>Soil pH</td>
<td>5.68±0.3</td>
<td>6.76±0.5</td>
<td>7.30±0.1</td>
<td>7.06±0.4</td>
</tr>
<tr>
<td>Soil Conductivity (µS)</td>
<td>30.3±0.5</td>
<td>36.6±0.7</td>
<td>52.0±2.0</td>
<td>43.0±3.7</td>
</tr>
<tr>
<td>Soil temperature (°C)</td>
<td>19.3±0.2</td>
<td>26.6±0.1</td>
<td>30.0±4.0</td>
<td>27.5±0.5</td>
</tr>
</tbody>
</table>

Fig.1. Co-relation between blue green algal population and soil pH (r=0.79).

\[ y = 11.59x - 66.818 \]
\[ R^2 = 0.6234 \]

Fig.2. Co-relation between blue green algal population and soil conductivity (r=0.95).

\[ y = 1.0772x - 32.766 \]
\[ R^2 = 0.9051 \]

The physical properties of the rice field soils like pH, conductivity, temperature varied in different seasons (Table 2). The average soil pH over all the five fields in different seasons varies from 5.68±0.03 to 7.30±0.01. Soil conductivity and soil temperature varies from 30.3±0.5 to 52±2.0 and 19.3±0.3 to 30.04 respectively. pH is an important physical factor in determining growth, establishment and diversity of cyanobacterial flora (Roger and Kulasooriya 1980, Kaushik 1994). The present study recorded significant positive correlation between the pH of the rice fields and blue green algal density (Fig. 1). The correlation with soil conductivity (Fig 2) and soil temperature (Fig 3) were also found to be positive.

The blue green algal density was found higher in summer and spring seasons in comparison to autumn and winter seasons. A study on biodiversity and seasonal variation of BGA strains in rice fields of Fujian China showed that diversity was highest in the middle of growth season i.e. in summer and that of the lowest after harvest i.e. in winter (Song et al., 2005), which is in concomitant with the present findings.
Nitrogen fixation by blue green algae balance the soil nitrogen of rice fields, where rice can be grown on the same land even without any addition of fertilizers and without any reduction in yield (Venkatraman, 1972; Nayak et al., 2001; Nayak et al., 2004; Song et al., 2005). Many algologists experimentally proved certain blue green algal species as prominent nitrogen fixers. The algal species *Anabaena* ityengari var. *tenus*, *Nostoc commune* and *Nostoc linckia* proved to be good options for the formulation of biofertilizer (Pereira et al., 2009). Fogg, (1941) studied the nitrogen fixation by *Anabaena cylindrica* and Begum, et al. (2008) studied nitrogen fixation by *Nostoc linckia*, *N. carneum*, *N. ellipsosporum*, *N. piscinale*, *Anabaena oryzae*, *Scytonema mirabile*, *Calothrix marchica* and *Haplotosiphon welwetschii*.

**Conclusion**

It may thus be summarized that Chandrapur rice fields harbour a good number of indigenous BGA species having high nitrogen fixing capacity. Results also throw light that yields of rice are highly influenced by the natural BGA fertilizer in those areas. Therefore efforts need to be focused towards enrichment of this indigenous population of BGA, which are already blessed by the favorable local environment rather than inoculation with foreign stains.

**Acknowledgements**

The authors are grateful to Head, Department of Botany, Gauhati University, Guwahati, Assam for providing laboratory facilities. The authors are also thankful to the cultivators for providing access to their respective crop fields.

**References**


