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ISSN: 2455-4855 **Original Article**

ABUNDANCE AND DIVERSITY OF UNICELLULAR, FILAMENTOUS CYANOBACTERIA (BGA) AND OTHER HIGHER ALGAL GENERA IN DIFFERENT RICE FIELDS OF NORTH 24 PARGANAS DISTRICT IN WEST BENGAL, INDIA

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Abstract

The distribution of unicellular and filamentous blue green algae (BGA) in relation to seasonal variation in rice fields in three major rice growing different blocks of North 24 parganas in West Bengal has been under taken. Present research paper deals with continued three successive years (2011-2014) studied for the first time. The different physicochemical parameters were chosen for this study. The occurrence of Cyanobacteria and altogether 48 species of nitrogen fixing cyanobacteria samples belonging to 25 genera and various orders were isolated in different rice growing fields. The increasing range of soil pH was certainly the important factor for the development of BGA up to certain limits. Comparatively more number of filamentous and heterocystous forms were developed in July to October (kharif season) and less number of unicellular forms were in November to June (Rabi season). When water level was low, starting the crop season the rice growing fields developed mainly Unicellular, colonial and Non-Heterocystous filamentous cyanobacteria and slight developed water level in the middle of rice growing time, Non-Heterocystous and Heterocystous Cyanobacteria were observed. When water level was more and at the end of rice crop season mostly heterocystous and filamentous cyanobacteria (BGA) were developed.

Key words: Blue green algae, Nitrogen fixation, Physic-chemical, Distribution pattern.

Introduction

Cyanobacteria are prokaryotic, but have oxygenic photosynthesis. They are conspicuous in their occurrence and also have a wide range of thallus organization. Cyanobacteria are interesting organisms as they have affinities with both the prokaryotic bacteria as well as the eukaryotic algae.

The nitrogen fixing potential of diazotrophic microorganisms (cyanobacteria) is of great significance for enriching of nitrogen level in soil. The cyanobacterial biofertilizer technology is well proven but still it is facing problem of availability of proper strains as starter cultures. North 24-Parganas District zone is the most important and Potential zones for the rice growing area of the state of West Bengal mainly due to their pattern of rainfall, temperature, and soil class and weather variability. The Ganga, mostly under Indo Gangetic basins which are intersected with so many canals, beels, jheels and low-lying areas. Generally these have developed a semi-saucer and saucer - shaped wetland ecosystem bounded by different short and long villages and lands which are some waterlogged during the year, either permanently, semi permanently or temporarily.

Cyanobacteria exhibit a great morphological diversity and their broad spectrum of physiological properties reflects their widespread distribution and tolerance to environmental stress (Tandeau and Howard, 1993). It is a nitrogen fixing potential of diazotrophic microorganism, which is of great significance for enriching of nitrogen level in soil. Debnath et al., 2009; Keshri and Chatterjee, 2010; Zhang et al., 2010 observed some problem for the cyanobacterial distribution and its peculiarity to proper identification. The cyanobacterial biofertilizer technology is well proven but still it is facing problem of availability of proper strains as starter cultures. Therefore, it is very essential to undertake extensive survey to rice fields to explore the status of cyanobacterial flora especially nitrogen fixing species along with ecology and physiochemical properties of different rice growing fields. In respect to their role in increasing the fertility of rice soils cyanobacteria are of the special academic and applied interest. We survey and studied the cyanobacteria biodiversity of three different major rice growing places i.e., Amdanga, Habra and Gaighata and their adjoining areas of North 24-parganas district in West Bengal.

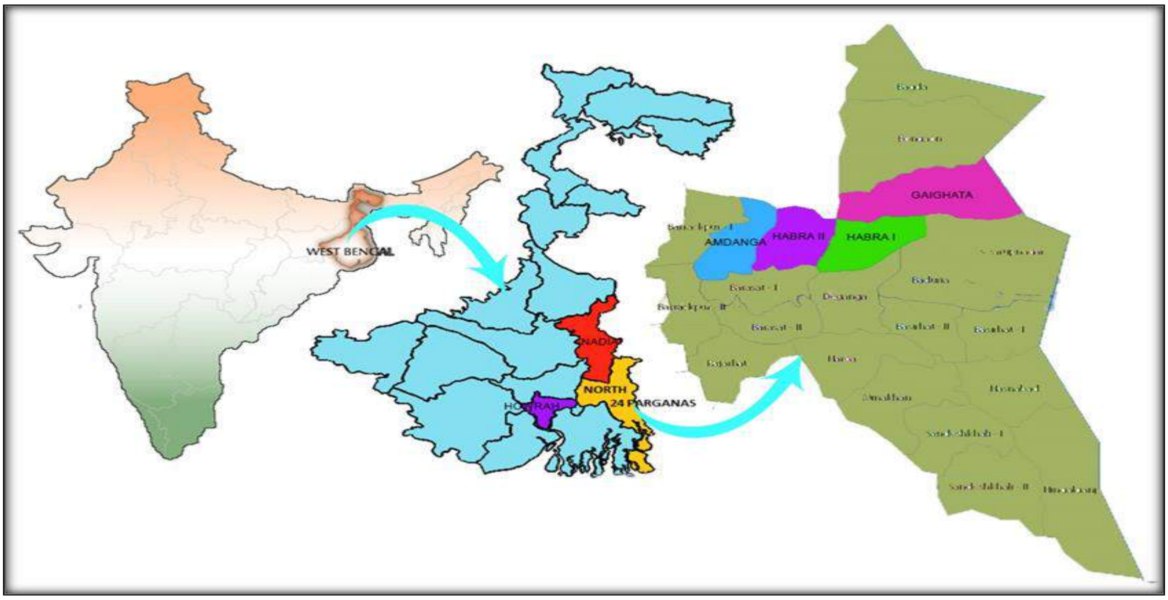

Figure 1. Map showing different sample collection site of North 24 Parganas, West Bengal, India.

Materials and Methods

1. Isolation of the culture

Cyanobacteria samples along with soil and water were collected from major rice growing three major places i.e., Amdanga, Habra and Gaighata in North 24-parganas and their adjoining areas of North 24-parganas district. Collection were made during Kharif crop season i.e., July to October and in Rabi i.e., November to June from 2011- 2014 and samples were isolated under investigation.

During the collection, prominently visible growths of blue green algae were collected in plastic and polythene containers for direct observation with the help of MOTIC Trino-oculer microscope imaging system and the heterogeneous suspension was diluted with the liquid culture BG11 Medium (Stanier et al., 1971). The samples were first studied under stereomicroscope for their general morphological features like nature of colony, colour and general appearance of thallus, branching, sheath, apical cell etc., spores in details.

2. Selection of media

In the beginning Bold's Basal Medium (Bischoff and Bold, 1963), Chu No 10 (CHU, 1942) and BG11 Medium (Stanier et al., 1971) have been used. Among these, BG11 medium supported the better growth of the cultures. The final pH was adjusted at 7.5. During the study, all the experiments performed in BG11 medium (liquid and solid, with or without Nitrate nitrogen) and strains were maintained in stock cultures in this medium with in sterile soils and incubated for 25-30 days at $30^{0}C \pm 2$ and 4000-5000 Lux light intensity under 14/10 LD cycle.

3. Maintain of Cyanobacterial strains

All the isolated strains of Cyanobacteria are being maintained in culture collection in the Department of Botany, Uluberia College (University of Calcutta), Howrah, West Bengal.

4. Physico-chemical Properties of Soil

Different parameters i.e., Soil temperature, pH, EC (Electric Conductivity), N, P, K and Organic carbon were taken into consideration for the measurement of fertility levels in soils. The available soil phosphate, potassium, total soil nitrogen and organic carbon were estimated as per procedures described by Trivedi and Goel, 1986 in the laboratory. The soil temperature, conductivity and pH were determined by using soil thermometer, conductivity meter and digital pH meter respectively.

5. Identification of the isolates

The identification of the selected isolates for morphological and physiological studies have been made using standard monographs of Geitler, 1932; Desikachary, 1959; Komarek and Anagnostidis, 1986, 1988 and our present observations. The observations made on all the isolates are described below only with the occurrence, nature of the thallus, sheath, cells, heterocytes, development of hormogons, benching pattern, akinete, spores.

Results

Table 1. Detailed survey have been made and collected dataare given below:

<table>
<tr><th colspan="2">Total Area</th><th colspan="2">CHARACTER OF SOIL</th><th colspan="3">WEATHER RECORD</th></tr>
<tr><td colspan="2">3927 km²</td><td>Soil Color</td><td>Grayish to light blackish</td><td>Average Rainfall</td><td>June to September</td><td>275mm ±5</td></tr>
<tr><td colspan="2">Geographical Location</td><td rowspan="2">Soil Type</td><td rowspan="2">Sandy to loamy and clay</td><td rowspan="2">Average Temperature</td><td>Summer</td><td>25⁰C ±2 to 42⁰C ±2</td></tr>
<tr><td>Latitude</td><td>20⁰ 53′ to 24⁰ 11′ N</td><td>Winter</td><td>9⁰C±2 to 25⁰C ±2</td></tr>
<tr><td rowspan="3">Longitude</td><td rowspan="3">88⁰09′ to 88⁰48′ E</td><td colspan="5">Average Humidity % of Range</td></tr>
<tr><td colspan="2">July to September</td><td colspan="2">Summer</td><td>Winter</td></tr>
<tr><td colspan="2">69to 92 %± 5</td><td colspan="2">51-69 %± 5</td><td>41-60 %± 5</td></tr>
</table>

Table 3. Physicochemical status of 3 Blocks i.e., I. Amdanga II. Habra and III. Gaighata in North 24-parganas district.

Blocks	Year	OC (%)	pH range		Total Nitrogen (%)	Soil texture	Range of Phosphorus (ppm)
			Kharif (July to Oct.)	Rabi (Nov. to June)			
Amdanga	2011	1.14±0.20	8.73±0.50	5.8±0.50	0.86±0.20	Loam & Sandy	6.72±0.20
	2012	0.99±0.20	7.07±0.50	6.7±0.50	0.87±0.20	Clay & Loam	5.58±0.20
	2013	0.97±0.20	6.01±0.50	7.4±0.50	0.82±0.20	Clay & Sandy	6.23±0.20
Habra	2011	0.96±0.20	7.67±0.50	6.82±0.50	0.55±0.20	Sandy & Loam	5.50±0.20
	2012	0.82±0.20	7.5±0.50	5.70±0.50	0.63±0.20	Clay & Sandy	5.50±0.20
	2013	0.83±0.20	6.8±0.50	6.88±0.50	0.72±0.20	Loam & Sandy	4.93±0.20
Gaighata	2011	0.98±0.20	6.98±0.50	6.69±0.50	0.78±0.20	Loam& Sandy	5.90±0.20
	2012	0.96±0.20	7.10±0.50	6.59±0.50	0.82±0.20	Clay & Sandy	6.38±0.20
	2013	0.91±0.20	5.25±0.50	6.62±0.50	0.18±0.20	Loam & Sandy	2.89±0.20

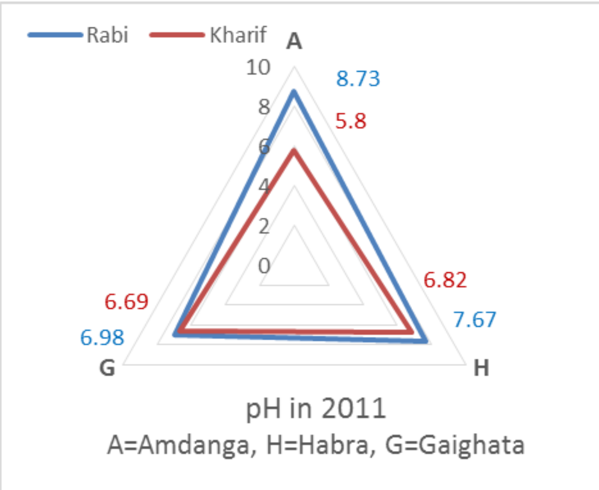

Figure 2. Range of pH in 2011

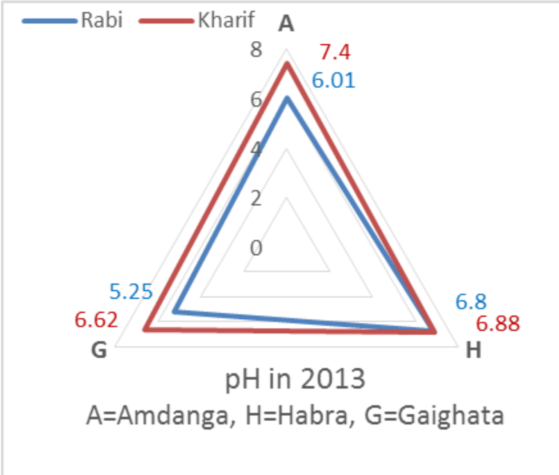

Figure 4. Range of pH in 2013.

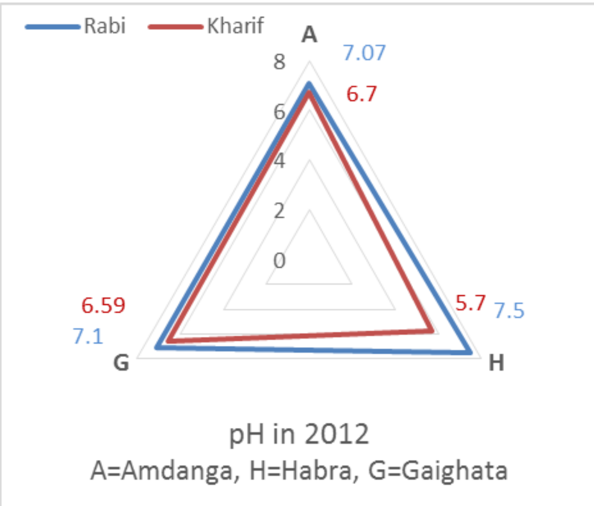

Figure 3. Range of pH in 2012.

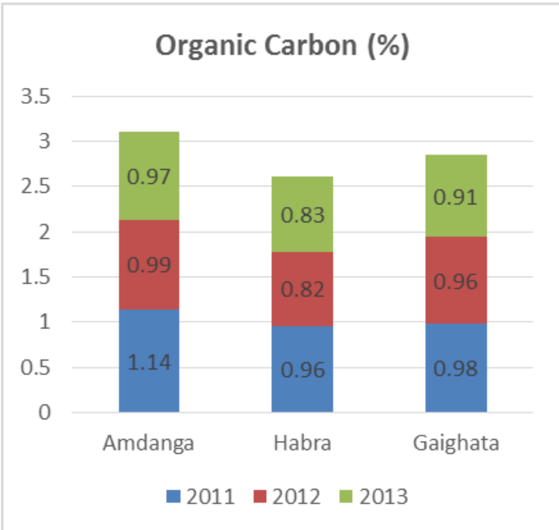

Figure 5. Percentage of Organic Carbon.

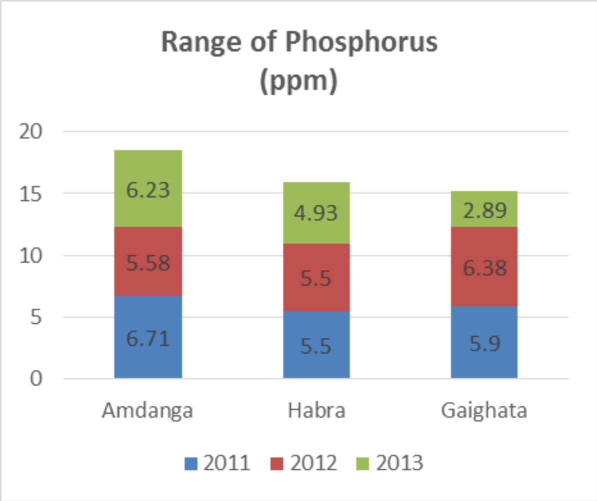

Figure 6. Range of Phosphorus (ppm)

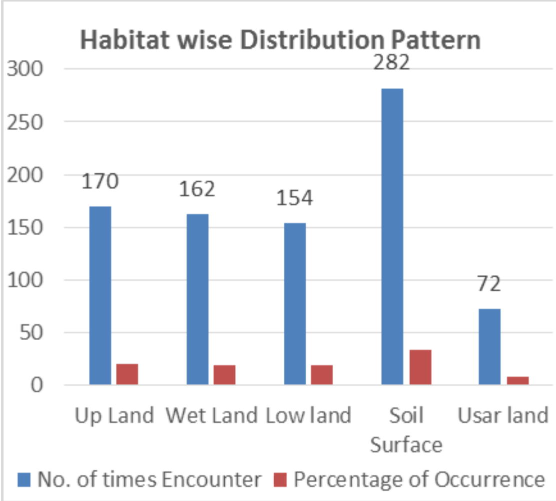

Figure 8. Habitat wise distribution Pattern.

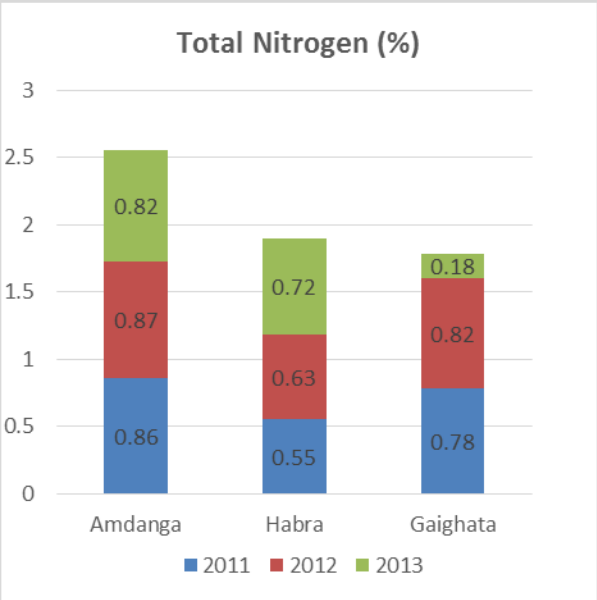

Figure 7. Percentage of Total Nitrogen.

Table 4. Habitat wise Distribution Pattern of Cyanobacteria genera in different blocks of North 24 Parganas District :

Sl No	Nature for the fields/Habitat	No. of times encountered	% occurrence
1	Up land	170	20.24%
2.	Wet land	162	19.15%
3.	Low land	154	18.33%
4.	Soil Surface	282	33.71%
5.	Usar land	72	08.57%
	TOTAL	**840**	**100%**

Table 6. Occurrence of different groups of Cyanobacteria in rice fields of North 24 Parganas District:

Cyanobacteria	Genera	Strains	Total (%)
Unicellular and Colonial	8	11	23.42%
Non-Heterocystous filamentous	6	14	29.78%
Heterocystous	12	22	46.80%
TOTAL	**26**	**47**	**100%**

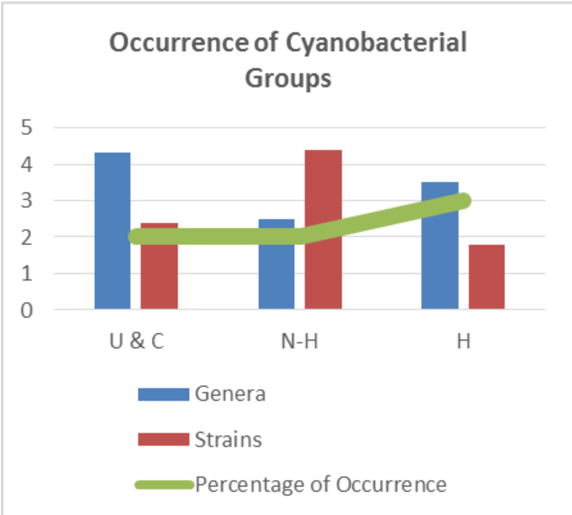

Figure 9: Occurrence of different Cyanobacterial Groups; U&C = Unicellular and Colonial, N-H=Non-Heterocystous, H= Heterocysyous

Table 5. Agronomic practices and application of agrochemicals in rice fields in 24-PARGANAS (N) district:

Main Crops	Boro	Kharif
Rice	IIT-4786, WGL-20471, IIT-17430, GONTRA-3, IIT-723	PANKOSE, IIT-8002, IIT-7041, IIT-14105, BN-20, PBT-5204
Accessory Crops	Jute, vegetables, sugarcane, potato, flowers, Sun flower, Mustard, pea, mung etc	
Chemical fertilizers used	Urea, DAP, NPK (60:30:30)	
Green manures	Green manuring with Dhaincha/kalai/mung/cowpea etc.	
Compost	Urban(N:P:K::1:0.5:1.5) , Rural(N:P:K::0.5:0.5:1)	
Sulphur	@20 kg/ha at normal land preparation	
Zn	Zinc.sulphate@25 kg/ha at normal land preparation	
FYM	@5t/ha(N:P:K::1.22:0.62:1.2)	
Biofertilizers used	Very few	
Pesticides used	Malathion, Butachlor, Dimecron; 2,4-D etc.(FORATOX, FUIRIDUM, HONOSIN etc)	

Table 7. Showing occurrence of other algae in rice field of West Bengal during cropping season these are very common:

Division	Order/Class	Identified Algal Genera	Total Strain
Chlorophyta	Volvocales	*Pandorina, Eudorina, Gonium*	06
	Chlorococcales	*Chlorella*	03
	Oedogoniales	*Oedogonium*	03
	Zygnematales	*Cosmerium, Sirogonium, Zygnema, Spirogyra*	07
Charophyta	Charales	*Chara, Chlorococcum*	06
Euglenophyta		*Phacus, Euglena*	04
Bascillariophyta	Pinnales	*Frustulia, Navicula, Gomphonia,* Diatom	08

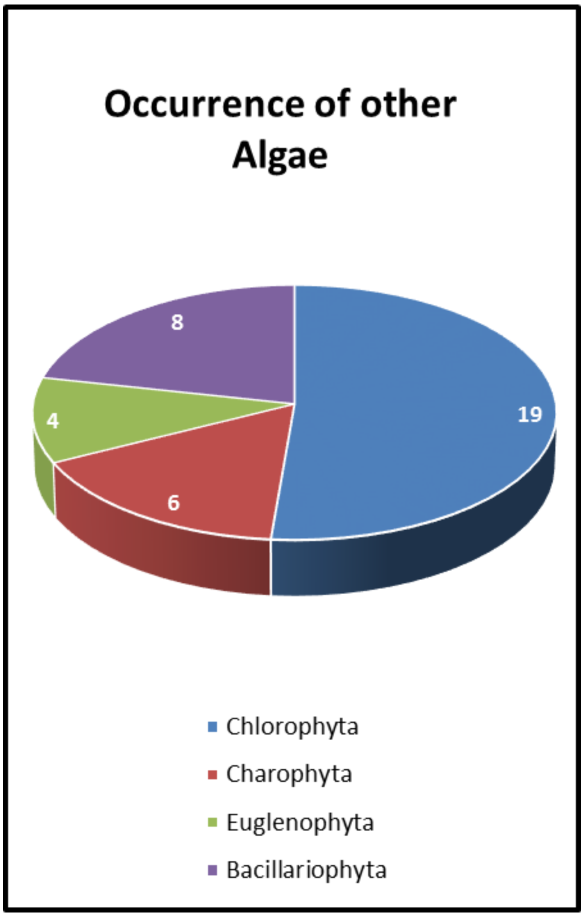

Figure 10. Occurrence of other Algae.

Table 8. Showing Recurrence of Cyanobacteria in rice field of West Bengal during cropping season from 2011 to 2014:

Rice Fields	Groups of Cyanobacteria	Cyanobacterial succession during rice cropping season from 2011 to 2014				
		Before plantation	After 10 -15 days of plantation	After 30 -40 days of plantation	After 50-60 days of plantation	After harvesting of rice crop
Upland Drainfed Rice Fields	Unicellular and colonial	*Aphanothece Aphanocapsa*	*Aphanothece*	*Aphanothece*	----	-----
	Non-Heterocystous filamentous	*Lyngbya, Oscillatoria Microcoleus*	*Oscillatoria Microcoleus*	*Microcoleus*	*Microcoleus*	-----
	Heterocystous	*Nostoc, Cylindrospermum*	*Nostoc, Cylindrospermum*	*Nostoc*	*Scytonema*	---
Up Land Irrigated Rice Fields	Unicellular and colonial	*Aphanothece Aphanocapsa*	*Aphanothece Aphanocapsa*	*Aphanothece*	*Aphanothece*	------
	Non-Heterocystous filamentous	*Lyngbya, Oscillatoria Microcoleus, Phormidium*	*Lyngbya, Microcoleus, Phormidium*	*Lyngbya, Microcoleus Phormidium Plectonema*	*Lyngbya, Microcoleus*	*Lyngbya*
	Heterocystous	*Nostoc Cylindrospermum Anabaena*	*Nostoc Anabaena Aulosira*	*Nostoc Aulosira*	*Nostoc, Aulosira Scytonema*	*Nostoc, Aulosira Scytonema*
Low Land Rice Fields	Unicellular and colonial	*Aphanothece Aphanocapsa Gloeocapsa*	*Aphanothece Aphanocapsa Gloeocapsa*	*Aphanothece Aphanocapsa, Chroococcus, Gloeothece Coelospaerium Merismopedia*	*Aphanothece Aphanocapsa Chroococcus Gloeothece Coelospaerium Merismopedia Gloeocapsa Microcystis Xenococcus Syechococcus*	*Aphanothece Aphanocapsa Gloeothece Merismopedia Gloeocapsa Xenococcus*
	Non-Heterocystous filamentous	*Phormidium, Lyngbya, Oscillatoria Microcoleus,*	*Phormidium, Lyngbya Oscillatoria Microcoleus*	*Plectonema, Limnothrix, Phormidium, Lyngbya, Oscillatoria Microcoleus*	*Plectonema Limnothrix Phormidium Lyngbya Oscillatoria Microcoleus*	*Plectonema, Lyngbya, Oscillatoria Microcoleus*
	Heterocystous	*Nostoc Cylindrospermum Anabaena Aulosira*	*Nostoc Anabaena Aulosira*	*Nostoc Chlorogloeopsis Gloeotrichia, Hapalosiphon Cylindrospermum Anabaena Aulosira Wollea Microchaete Rivularia*	*Nostoc Chlorogloeopsis Gloeotrichia Hapalosiphon Cylindrospermum Anabaena Aulosira Wollea Microchaete Rivularia Westiellopsis*	*Nostoc Aulosira Scytonema Tolypothrix Petalonema Gloeotrichia*

Meteorological and Effect of Physico-chemical parameters

Occurrence of Blue-green algae in rice growing fields of North 24 parganas district West Bengal has been under taken for the first time in this area. North 24 parganas is one of the best rice growing districts due to its soil classes, pattern of rain fall, and temperature and weather variability in India. It is situated between $20^0 11' 6''$ to $23^0 15' 2''$ Latitude and $88^0 20'$ to $89^0 05'$ E. Longitude and covers area about 4094 sq. kms in West Bengal.During three successive years (2011-14) surveyed Kharif crop season i.e., July to October and Rabi crop season i.e., November to June in rice cropping seasons studied the physio-chemical characters in different rice growing localities showed; The character of Soil color mostly Grayish to light blackish. Types of Soil was Sandy to loamy and clayey. Average temperature was in summer: 25^0C to 42 ± 2^0C but maximum were in May 42^0C ±2^0C. But minimum were in December to January. Its ranges were 9^0Cto 25^0C. ±2^0C. Average Humidity % range were July to September which were 69 - 92 ± 5 %. In Winter its were 41 to 60 % ± 5 (Table 1).

The range of pH was from 5.25 to 8.73 ± 0.50 in November to July i.e., in Rabi crop season. The range of pH was minimum in October to June i.e., 5.25±0.5 and maximum were in July to October i.e. 8.73±0.50. In Amdanga block, the pH range were maximum in July to September i.e. 6.01 to 8.73±1.and the minimum were in July i.e., 5.25. in Gaighata block, in kharif season and moderate in Habra block areas. Range of Available Phosphorus (ppm) were 2.89 to 6.72 ± 0.5. The range of Conductivity (E.C. in dS/m) were <1±0.30; Range of Organic carbon percentage were from 0.53 to 1.24±2. Range of Organic carbon percentage were minimum in Habra block and maximum in Amdanga areas and moderate in Gaighata block. The range of total Nitrogen percentage were0.18 to 0.86±0.2 which were minimum in Gaighata block i.e., 0.18±0.20 and maximum Range in Amdanga Block i.e., 0.86±0.2 and moderate in Habra block areas(Table 2 & 3).

During the early part of the cultivation cycle, the algal biomass increased and consist mainly of diatoms and unicellular green algae, from tillering to panicle initiation, the algal biomass reached its highest values, but after penicle initiation the total biomass decreased. If the plant cover was dense then heterocystous and filamentous BGA became dominant but if the cover was thin the green algae and some heterocystous BGA were found.

Diversity of Cyanobacteria

Cyanobacteria have been found not only to grow in highly alkali soils but also improve the physio-chemical properties of soils enriching them with carbon, nitrogen and available phosphorus Kaushik, (1994). Among the unicellular group, planktonic forms were fast growing and had less generation time. They were more sensitive due to environmental changes i.e. temperature, nutrients, pH level and water Level. Rice fields of surveyed localities particularly showed abundant occurrence of Nostocacean flora by Singh et al., 2015; Halder, 2015; Similar variations from rice fields had also been reported by other workers Deka and Bordoloi, 1992; Saika and Bordoloi, 1994; Tiwari et al., 2008; Singh et al., 2001. The incidence of total number of samples were recorded from different localities of 3 different blocks in North 24 parganas district with reference to genera as well as Eco-physiochemical studies which are presented in Table 1-8. Most of the paddy fields under investigations, they do not have submerged condition and a large number of BGA grow on soil surface. They form mixed and irregular patches of different colours and consistency. Among three areas Amdanga exhibited the maximum number of Cyanobacteria and the moderate at Gaighata block and minimum at the Habra block due to presents of different physio-chemical parameters. The paddy field ecosystem consists of diverse habitats for microorganism. The abundance of cyanobacteria in paddy fields was first observed by Fritsch (1907). During the study altogether 840 times representation of 47 species of Nitrogen fixing Cyanobacteria samples and it belonging to 26 genera and four orders i.e., Chroococcales, Oscillatoriales, Nostocales, and Stigonematale were isolated from various rice growing localities. Three major Cyanobacteial groups (Table 6): i. Unicellular and colonial were minimum i.e., 23.42 % and its 8 genera and 11 species. ii. Non-heterocystous filamentous Cyanobacteria were slight more i.e., 29.78% and its 6 genera and 14 species iii. Heterocystous filamentous Cyanobacteria were maximum i.e., 46.80%, its 12 genera and 122 species were occurrence in most of all low and upland rice growing areas. Habitat and encountered wise Distribution Pattern of Cyanobacteria genera (Table 4) were maximum in soil surface areas, there were 33.71% and the followed in upland i.e., 20.24% then followed wetland i.e., 19.15% then lowland i.e., 18.33% and minimum in Usar land i.e., 8.57% successively depending upon the pH, EC, Temperature, Humidity, Total Nitrogen, Organic Carbon, soil N, P, K and soil texture. Cyanobacteria benefits in rice plants by producing growth promoting substances followed by increasing the availability of phosphorus by excretion of organic acids was also exploited in the prevention of soil erosion process (Kumar and Rao, 2012). BGA inoculation popularly known as “Algalization” helps to provide an environmentally safe agro-ecosystem contributing to economic viability in paddy cultivation, reducing cost and energy inputs (Sunil, 2008). The favorable balance of soil nitrogen of rice fields wherein rice can be grown on the same land even without any addition of fertilizers and without any reduction in yield, confirms to the significance of cyanobacterial nitrogen fixation (Venkatraman, 1972; Nayak et al., 2001; Song et al., 2005). These habitats are micro environment physico-chemically different to each other and could exhibits biologically distinct properties. Such heterogeneity of the habitat should influence the structure and diversity of microbial communities in the paddy field ecosystem as a whole and may support various microbiological process occurring in paddy fields which are agronomic ally and bio geochemically important (Kimura, 2000; Kirk, 2004).

Discussion and Conclusion

Present investigations revealed that Depend on pH, presence or absence of nitrogen, phosphorus, level of CO_2 etc. have great role for the development of different other higher algal biomass which are vary from place to place. The pH range has a great role for the growth and succession of cyanobacteria. Low range of pH and more light intensity in Rabi crop season developed mostly the strains of unicellular groups of Cyanobacteria i.e., the order Chroococcales and minimum the order Oscillatoroales, and increasing the pH, and water label and decrease light intensity in Kharif crop season developed mostly filamentous group of Cyanobacteria i.e., Oscillatoroales, Nostocale, and Stigonematales. Among three Blocks, Amdanga exhibited the maximum number of cyanobacteria and the moderate at Gaighata blocks and minimum at the Habra blocks due to presence of different physio-chemical parameters for growth of BGA accordingly.

The common forms are *Nostoc, Anabaena, Lyngbya, Oscillatoria, Aphanocapsa, Scytonemaand Cylindrospermum, Microchaete Phormidium, Gloetorichia,* and *Lyngbya.* It is very clear that the growth of free floating separate and distinct colonies of BGA eg., *Aulosira, Anabaena, Aphanothece, Gloeotrichia,* and *Microchaete* largely dependent on water level and generally they do not grow attached on soil surface. When the pH was low, chlorophyceae became dominant i.e., under the division Chlorophyta and the orders are Volvocales, Chlorococcales, Oedogoniales, Zygnematales and the genera are 1.Pandorina, 2.Eudorina 3.Gonium,and 1.Chlorella; 1.Oedogonium; and 1. Cosmerium 2.Sirogonium 3.Zygnema 4.Spirogyrasuccessively. Absence of cover BGA grows slowly, but Chlorophyceac and Diatoms grows faster. High level of CO_2 also helps the green algae. Light intensity can play also a major role in the growth of BGA (Table 7).

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