IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

A STUDY ON N2-FIXING CYANOBACTERIAL BIO-DIVERSITY OF RICE FIELDS SOILS OF BHUVANAGIRI AND SRIMUSHNAM TALUK CUDDALORE DISTRICT, TAMILNADU

Mrs. P.PREMALATHA, [1] Dr. R.ARUNADEVI, [2]

Ph.D., Research Scholar, [1] Assistant Professor [2]

P.G & Research Department of Botany,

Government Arts College, C.Mutlur, Chidambaram,

Cuddalore-Dist, Tamilnadu, India – 608 102.

Abstract: Freshwater and the surface of soil provide the sites for aerobic phototrophic nitrogen (N2) fixation by free-living cyanobacteria and the Azolla-Anabaena symbiotic N2-fixing complex. Free-living cyanobacteria, the majority of which are heterocystous and nitrogen fixing, contribute an average of 20-30 kg N2, ha-1, whereas the value is up to 600 kg ha-1 for the Azolla-Anabaena system (the most beneficial cyanobacterial symbiosis from an agronomic point of view). Synthesis and excretion of organic/growth-promoting substances by the cyanobacteria are also on record. Recent results indicate a strong potential for cyanobacterial biofertilizer technology in rice-growing countries, which opens up a vast area of more concerted basic, applied, and extension work in the future to make these self-renewable natural nitrogen resources even more promising at the field level in order to help reduce the requirement for inorganic N2 to the bare minimum, if not to zero.

Keywords: Bio-Diversity, Cyanobacteria, N2-fixing, Rice field soils, fertilizers, Seasonality

I. INTRODUCTION

N2 is an essential and most limiting nutrient for plant growth in most of the world's agricultural soils, and hence, crop production worldwide relies heavily on its inputs. Plant mainly depends upon combined or fixed forms of nitrogen such as ammonia and nitrate. With exponential growth of human population, as well as subsequently increasing demands of rice lead to put pressure on farmers and the other stakeholders to produce and procure huge amount of rice. Some farmers hence, has started using chemical nitrogenous fertilizer to acquire more production without knowing the negative effects of those chemicals. Biological nitrogen fixation, on the other hand, offers a natural means of providing nitrogen for plants. N_2-fixing cyanobacteria are one of the main components of the micro-biota in rice field soils (Ladha and Reddy, 2003) that make a valuable contribution to soil fertility by fixing atmospheric nitrogen (Tiwari et al., 2001). Nitrogen fixed by this biological process was estimated to contribute about 60% of the nitrogen requirement of the living organism (Venkataraman, 1993). They are hence, considered as natural biofertilizer (Baftehchi et al., 2007). Different authors attributed cyanobacteria to be an important organism in controlling organic pollutants through biodegradation (Cerniglia et al., 1980 and Chaillan, 2006). They further enhance plant growth by synthesizing and liberating growth promoting substances (Pandey et al., 2005; Karthikeyan et al., 2007; Zulpa et al., 2008).

Cyanobacteria excrete organic acids that render phosphorus solubilisation, making phosphorus available to plants (Fuller and Rogers 1952; Singh et al., 1981). They also increase the humus content, improve soil structure and dissolve certain soil minerals. In addition, they also add substantial amount of organic matter to the soil. These organic matters thus gathers, acts as a storehouse of nutrients like nitrogen, phosphorus and micronutrients and take part in soil fertility and increase the water holding capacity (Goyal, 2002). Though cyanobacteria are ubiquitous in nature, higher amount of them, comprising more than half the population of heterocystous are reported to be grown at or floating above the surface of water logged rice fields (Ladha and Reddy, 1995), as rice fields provide a very congenial condition for abundant growth of N_2-fixing cyanobacteria (Whitton, 2000; Nayak et al., 2001).

Rice-fields are considered as one of the highly dynamic ecosystems. Fernández-Valiente and Quesada, 2005) and could be attributed to the variation in cyanobacterial diversity, distribution, density (Watanabe et al., 1978), biomass (Gupta, 1966) and contribution to the total nitrogen fixed in rice field soils (Watanabe and Cholitkal, 1979). The present endeavor, therefore, was aimed to study the N_2-fixing cyanobacterial diversity along with their seasonal variation in rice fields' soils of Bhuvanagiri and Srimushnam Taluk Cuddalore district, Tamilnadu.

II. Nitrogen Fixation in Cyanobacteria

Biological nitrogen fixation (BNF) is the conversion of inert dinitrogen (N_2) into a combined form by living organisms through catalytic process of the enzyme nitrogenase. Among the vast myriad of diverse organisms present on the Earth, this process is confined to certain prokaryotic microorganisms. Nitrogen fixing prokaryotes could be autotrophic or heterotrophic, aerobic, micro-aerobic or anaerobic, and among them the cyanobacteria are the only prokaryotes that fix nitrogen and simultaneously carryout oxygen evolving photosynthesis. How the prokaryotic microorganisms which do not possess a compartmentalised cellular organisation with intracellular organelles carry out these two incompatible processes of oxygen sensitive nitrogen fixation and oxygen evolving photosynthesis has drawn a variety of explanations but this enigma is not yet completely resolved.

From phylogenetic studies it has been postulated that all the nitrogenases have been derived from a common prokaryotic ancestral group that existed prior to the oxygenation of the Earth's atmosphere (Berman Frank et al., 2003). This anoxic atmosphere was quite different from the present day atmosphere and had a predominance of CH_4, CO_2, N_2 and NH_3. Ultra-violet radiation would have dissociated NH_3 releasing N_2 and H_2 to the atmosphere. Primitive nitrogenases could have initially arisen as respiratory enzymes where N_2 acted as a sink for anaerobic respiration of certain heterotrophy.

The slow process of oxygenation over several millions of years, primarily due to the oxygenic photosynthesis of cyanobacteria, resulted in the partial pressure of atmospheric oxygen to increase from 0.0004 % to> 0.03 %. This oxygenation was inimical to N_2 fixation and all the organisms had to adopt various methods to protect their nitrogenase enzyme from damage by O_2 (Gallon, 1981). Such evolutionary processes of adaptation would have been slow and gradual over a long period of time because the anaerobic atmosphere went through a long journey of micro-aerobic conditions to reach oxygen concentrations inimical to the nitrogenase enzyme. Gallon et al. (1991) and Gallon (1992) have described these mechanisms as behavioural adaptations, physical barriers, physiological and biochemical strategies and structural changes, and treated the cyanobacteria as a special group and these have been discussed by Kulasooriya (2008).

III. Diversity of Nitrogen Fixing Cyanobacteria

According to early reports, although N_2 fixation was widespread among cyanobacteria, all species did not fix nitrogen. Many did so both under aerobic and micro-aerobic conditions while a lesser number fixed N_2 only under aerobic conditions (Fogg et al., 1973). The information in this report has undergone major revisions during the past four decades particularly with respect to the number of unicellular species, which play a major role in nitrogen fixation in the deep open seas (Montoya et al., 2004; Moisander et al., 2010; Thompson & Zehr, 2013). During the 19th century cyanobacteria (considered as blue-green algae at that time) were first suspected to fix atmospheric nitrogen due to their ability to grow well under nitrogen deficient conditions (Frank, 1889; Schloesing & Laurent, 1892), but the observations were not based upon axenic cultures.

Conclusive proof was provided by Drewes (1928) and this work was supported by De (1939), who showed that nitrogen fixing cyanobacteria were abundant in paddy fields of India. This showed the economic importance of these organisms as contributors for sustaining the natural fertility of rice soils (Singh, 1961). Until the late 1950s and mid 1960s the difficulty of demonstrating N_2 fixation in cyanobacteria was due to the limitation of reliable methods. The common methodology was based upon the demonstration of significant growth of the test organism under nitrogen free conditions.

First it was necessary to ensure that the experimental cultures were axenic because it was tedious to remove the associated bacteria, which sometimes dwell within mucilage sheaths of the test organisms. Secondly it was necessary to establish unequivocally that the culture set up was completely free of combined nitrogen including those present as traces in the air bubbled through the cultures.

The meticulous set up to remove such traces by Fogg (1942) was followed by many others until the acetylene reduction assay (Dilworth, 1966; Schollhorn & Burris, 1966) became available. This simple technique enabled the rapid demonstration of nitrogenase activity by any organism, symbiotic association, and cell-free extracts and purified nitrogenase enzyme under a variety of incubation conditions. However unequivocal confirmation of N_2 fixation by any organism or system had to come from evidence supported by techniques using isotopes of nitrogen.

Filamentous, heterocystous cyanobacteria During the early 1960s there was no clear idea as to whether any specialised cell/s in filamentous cyanobacteria perform N_2 fixation, and the role of heterocysts was not fully understood (Cox, 1966). The classical research article by Fay et al. (1968) brought a new dimension proposing that a specialised cell, the heterocyst is the site of N_2 fixation in cyanobacteria. Accordingly the major adaptation for N_2 fixation under aerobic conditions among the filamentous cyanobacteria appeared to be due to structural changes of their cells. A majority of filamentous cyanobacteria that exhibit aerobic nitrogen fixation possess heterocysts.

IV. Ecosystem diversity

Nitrogen fixing cyanobacteria are ubiquitous in their global distribution and occupy a broad range of habitats across all latitudes and longitudes perhaps indicating their pioneering ancestry in the primitive Earth. They are widespread in freshwater and terrestrial habitats, but not so common in marine ecosystems except in certain oligotrophic deep ocean areas. They are frequent inhabitants of extreme habitats such as freezing conditions of the glaziers in the Arctic and Antarctic regions, hot spring micro-flora and epilithic and endolithic species found as the only inhabitants of the arid Atacama Desert. Many lichens with cynobacterial endosymbionts demonstrate their ability to colonise habitats that are inhospitable to other organisms.

4.1 Freshwater and wetland ecosystems

Cyanobacteria are common inhabitants among the phytoplankton of lentic water bodies such as ponds, irrigation tanks, reservoirs, lakes and wetlands like rice paddies. Very often certain cyanobacteria become dominant when freshwater ecosystems get polluted and sometimes they are even used as bio-indicators of pollution. Kulasooriya (2005) reported on the changes of cyanobacterial diversity in inland freshwater bodies of Sri Lanka during the 20th century primarily due to pollution by anthropogenic activities. With the increase of pollution the overall diversity of phytoplankton diminishes and a few cyanobactrial species particularly toxigenic ones become predominant suppressing the more sensitive species. In eutrophic waters such cyanobacteria form 'algal blooms' and a number of them are toxigenic. Limnologists often attribute algal bloom formation to pollutants particularly those containing phosphorus and nitrogen.

A number of toxigenic, bloom forming members such as Anabaena, Cylindrospermopsis, Nodularia and Aphanizomenon are N_2 fixing genera and they have the advantage of rapid growth in phosphorus rich waters even under N-limiting conditions. However, a common bloom forming toxigenic genus Microcystis is a non-fixing, unicellular colonial form. In wetland rice fields N_2 fixing cyanobacteria have been observed as epiphytes on rice field weeds (Kulasooriya et al., 1981a) and on rice plants (Roger et al., 1981).

Occasionally they have been reported even as endophytic organisms in deepwater rice (Kulasooriya et al., 1981b) providing fixed nitrogen to the host plants (Watanabe et al., 1981). The common occurrence of cyanobacteria in wetland rice field ecosystems has been utilised in rice production in certain countries by the application of selected nitrogen fixing genera as biofertiliser inoculants (Venkataraman, 1972; Roger & Kulasooriya, 1980). In a study on the relationship between the abundance of N_2 fixing cyanobacteria and environmental features in Spanish rice fields, Quesada and Fernandez-Valiente (1996) investigated 25 sampling sites in rice fields in Valencia, three times a year for two years.

Correlation analysis with environmental features showed that cyanobacterial abundance was influenced more by water than soil properties. Salinity, mineralisation variables and soluble reactive phosphate (SRP) correlated positively with the presence of heterocystous cyanobacteria. Dissolved inorganic nitrogen (DIN) and the ratio DIN: SRP correlated negatively with cyanobacterial abundance.

Cyanobacteria are seldom found in the phytoplankton of fast flowing rivers and streams. In slow moving waters and streams they are more frequent as the benthic flora as well as attached to stream substrates as the periphyton (Maracarelli et al., 2008; Stancheva et al., 2013). Marcarelli et al. (2008) in their review report states that in streams the dominant autotrophic N_2 fixers are heterocystous cyanobacteria such as Nostoc, Anabaena and Calothrix and that no researcher has confirmed the presence of unicellular free-living N_2 fixing members except those in endosymbiosis with diatoms such as Rhophalodia and Epithemia.

They also record that N2 fixing cyanobacteria rarely contributes more than 5 % to the N_2 budget of in-stream ecosystems and this was significantly lower than denitrification and dissolved inorganic nitrogen uptake rates. However they concludethat studies on in-stream N_2 fixation are limited and have the drawback of not recording all the inputs and outputs of this ecosystem simultaneously. Stancheva et al. (2013) have confirmed the dominance of heterocystous free living cyanobacteria among the phototrophic N_2 fixers in the stream micro-flora,together with the diatoms Epithemia and Rhopalodia with their endosymbioti ccynobacteria.

Recording data from a total of 104 stream sites across 29 watersheds in southern California, they report threshold concentrations of low inorganic N and low N: P ratios and high N_2 fixation by cyanobacteria. They suggest that changes of these organisms can be used for the bio-assessment of nutrient content of stream ecosystems.

4.2 Biochemistry and genetic Regulation of N_2 fixing Cyanobacteria

The basic enzyme complex nitrogenase that catalyses N_2 fixation is common to all N_2 fixing organisms. Information available up to 2006 on the biochemistry including the structure of the nitrogenase enzyme complex, the proposed mechanism of nitrogen reduction and its genetic regulation has been reviewed by Kulasooriya (2008). The nitrogenase complex is composed of two components: a Fe-protein or component II, which is a dimer having a molecular weight ranging from 62,000 to 64,000 daltons and a Mo-Fe-protein or component I, a tetramer having a molecular weight ranging from 220,000 to 230,000 daltons. The latter also possess the Fe-Mo-co-factor, which has no peptide residues and has inorganic constituents with an approximate stoichiometry of $Fe_6Mo_4S_4$.

The N_2 molecule gets attached to the site of the co-factor and undergoes stepwise reduction through the enzyme bound intermediates N_2H_2 and N_2H_4 to finally produce the enzyme free key intermediate NH_3. This enzyme free end product NH_3 is released in cell free extracts and in vitro demonstrations using purified nitrogenase, but immediately incorporated in the intact organisms into the amino acid glutamine by the glutamine synthetase and L-glutamine 2-oxo glutarate 2-amino transferase (glutamate synthase) (GS/GOGAT) pathway. These basic biochemical mechanisms of nitrogen fixation were extensively studied and initially demonstrated in the anaerobic bacterium Clostridium pasteurianum and the facultative anaerobe Klebsiella pneumonia, but have been found to be similar in all N_2 fixing organisms including the cyanobacteria.

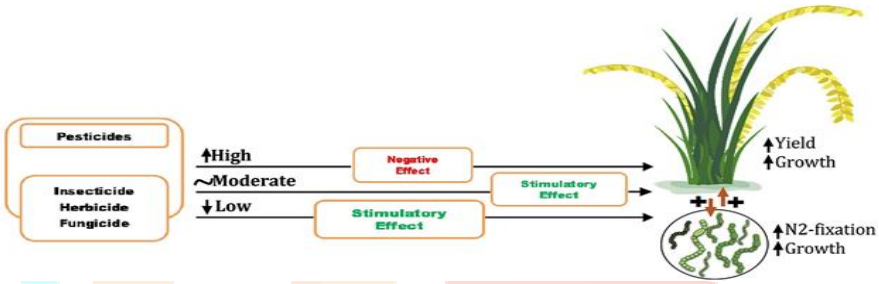
Among cyanobacteria the common experimental species studied have been Anabaena variabilis and Anabaena (Nostoc) PC 7220. A generalised scheme has been accepted for all N_2 fixing microorganisms with minute variations between the major groups such as the proteobacteria and the cyanobacteria. Besides the 2-component nitrogenase complex, other anciliary reactions are needed for the complete N_2 fixation process. It requires Mg^{2+} ions to activate the ATP and accept electrons (reducing power) transported by electron carriers ferredoxins and flavodoxins. This MgATP complex binds to the Fe-protein, which makes it highly reductive and energised.

This complex then binds to the Fe-Mo-co centre of the Mo-Fe-protein and makes this site highly energised and reductive. Energy and reducing power (electrons) are accepted by the Mo-Fe-protein only from the reduced and energised Fe-protein and no other source. The energised and highly reduced Fe-Mo-co centre is now capable of reducing triple bonded substrates such as N_2, C_2H_2, CN, iso-CN, azides etc.Accordingly the Feprotein component is termed dinitrogenase reductase and the Mo-Fe-protein component as dinitrogenase to reflect their major functions in the overall enzymatic process.

Besides the Mo-Fe protein of the nitrogenase complex, it has been revealed that there are two other complexes: Vanadium-Iron (V-Fe protein) and Iron-Iron (Fe-Fe protein) formed in most N2 fixing organisms including the cyanobacteria, specially under Mo deficient conditions. In an extensive review article Bothe et al. (2010b) have reported more recent information on the mechanisms of N_2-fixation and H_2 metabolism in cyanobacteria including their genetic regulation.

According to this article the pattern of pyruvate degradation, ammonium and H_2 formation by nitrogenase, and H_2 uptake by hydrogenases is not only typical of strict and facultative anaerobic bacteria, but also proceeds in cyanobacteria. It has been reported that a transitional 2:1 state is formed between the two nitrogenase components with the larger Mo-Fe component in the Centre and one molecule of the smaller component (Fe-protein) attached at each end. It is the prosthetic group (4Fe-4S) cluster that bridges the sub-unit interface and is ligated by 2 cysteinyl residues from each sub-unit. This cluster accepts reducing equivalents from the electron carriers and also binds MgATP/MgADP. The overall reaction of the reduction of one molecule of nitrogen to produce two molecules of ammonia can be depicted by the following equation from Bothe et al. (2010b). $8H^+ + 8e^- + N_2 + 16\text{Mg-ATP} \rightarrow 2NH_3 + 2H^+ 16\text{Mg-ADP} + 16\text{Pi}$

Picture -1- Regulation of N2 fixing Cyanobacteria



v. MATERIALS AND METHODS

Soil Sampling site was located at Azhichikudi Panchayath Nalanthethu 11.47°N 79.63°E) which is selected for the present investigation were located in the Bhuvanagiri Taluk and Srinedunchery: 11° 45′ 0″ Latitude N and 79° 45′ 0″ Longitude) which is located in Srimushnam Taluk Cuddalore District of Tamilnadu, India.

The research was carried out during two crop cycles (from April 2019 to March 2020). Each cycle consisted of four stages rice growth: land preparation (1 d), transplanting (30 d), panicle initiation (90 d), and harvest (120 d). Composite soils were collected from four plots of 20 ha rice field. At each plot (size about 120 m2), three random sampling site were selected.

From each sampling-site, three sample soils from 1 cm to 5 cm depth were collected with the 3 cm diameter plastic cylinder, which later mixed at the laboratory as composite soil samples. So that, a total of 90 composite 55 samples (2 cycles × 4 stages × 4 plots × 3 sampling sites) were analyzed at the laboratory.

VI. Culture Maintenance

Cyanobacteria are photosynthetic bacteria and live on the soil surface. The representative sample used for analyses was soil from 1 cm depth-soil layer. Dilution of 10-1 was made with a certain volume of sterilized distilled water and was adjusted to the volume of sample which was calculated using formula $\pi r^2 \times t$ (tube radial (r) = 1.5 cm; a depth-soil layer (t) = 1 cm). Serial dilutions of 10-1 until 10-6 were carried out and each dilution was plated on the Nitrogen-free BG11 agar medium. The colonies that grow on the agar medium were isolated to make colony library.

The isolates were maintained at 23 ± 2 °C under the continuous light intensity of 1200 lux to 1400 lux. Identification the morphological characters used for identification was grouped into macroscopic and microscopic characters. Macroscopic characters observed were the nature of filaments, growth pattern color, and surface texture of the colony. Microscopic characters were as followed: the shape and size of vegetative cells, heterocysts, akinets (if found); location and color of heterocyst, the nature of mucilaginous sheath. Stereo Olympus SZX16 and light microscope Olympus 1 X73 were used for morphological examination. Identification of isolates was done using monographs.

Picture -2 Culture Maintenance of N2 fixing Cyanobacteria

VII. DATA ANALYSIS

7.1. Relative abundance: The relative abundance of a particular cyanobacteria type was calculated by employing the following formula:

$$\text{Relative abundance} = \frac{A}{B} x100$$

Where,

A = total number of samples collected

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

7.2. Diversity Index: The Diversity Index (Sambha- Kuruvai) was been studied following the formula:

$$H_S = - \sum_{i=1}^{S} (Pi)(1n\ Pi)$$

Where,

H_s - 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, = n_i/N

N - Total number of individuals of all kinds

n_i - number of individuals of i^{th} species

ln - log to base 2

7.3. Similarity coefficient:

Similarity coefficient of cyanobacteria in different sites and seasons were studied following the formula:

$$\frac{X\ Y\ Z}{Z}$$

Where,

X and Y represent number of species present at any two different study sites

Z = Common species between any two study sites.

VIII. RESULTS AND DISCUSSION

8.1 Species diversity

A total of 55 species of N_2-fixing cyanobacteria was identified belonging to 20 genera under 9 families. Out of them, 45 species were filamentous heterocystous under 14 genera and 7 families. 10 species were unicellular under 5 genera and 1 family and 4 species belonging to single genus were filamentous non-heterocystous. Among filamentous heterocystous, Nostocaceae topped with 4 genera (Anabaena, Anabaenopsis, Aulosira and Nostoc), followed by rice field with 3 genera (Calothrix, Gloeotrichia, Rivularia) and Scytonemataceae (Scytonema, Tolypothrix) and Stignemataceae (Hapalosiphon, Westiellopsis) with 2 genera. The rest of the filamentous heterocystous families had only 1 genus each and were belonged to Mastigocladaceae (Mastigocladus), Mastigocladopsidaceae (Mastigocladopsis) and Microchaetaceae (Microchaete). Oscillatoriaceae was the lone filamentous non-heterocystous family with the genus Lyngbya. Whereas, Chroococcaceae, the only recorded unicellular family had 5 genera (Aphanocapsa, Aphanothece, Chroococcus, Gloeocapsa and Synechococcus)

Table: 1 Thus, the filamentous heterocystous (80%) cyanobacteria showed clear dominance over the unicellular/colonial (14%) and filamentous non-heterocystous (6%) forms. The dominance of filamentous heterocystous forms over other forms (unicellular and filamentous non-heterocystous) was also recorded in other rice field soils of India (Nayak and Prasanna, 2007). Hazarika (2007) reported 20.83% of unicellular/colonial and 30.56% of filamentous non-heterocystous form which was outnumbered by filamentous heterocystous cyanobacteria (48.61%) in soils of greater Vellaaru River.

Nostocaceae was the dominant family with 54% of species, followed by Chroococcaceae (14%), Rivulariaceae (13%), Scytonemataceae (7%), Oscillatoriaceae (6%), Stignemataceae (3%).Whereas, Mastigocladaceae, Mastigocladopsidaceae and Microchaetaceae contributed with only 1% of species each (Fig.1). Species of Nostocaceae, Chroococcaceae, Rivulariaceae, Oscillatoriaceae, Scytonemataceae and Mastigocladopsidaceae were reported from all the three sites of the district. Species of Stigonemataceae and Mastigocladaceae were reported in the rice fields of Bhuvanagiri and Srimushnam Taluk Cuddalore district, Tamilnadu.

Anabaena was reported to be the dominant genera with a total of 31% species. Nostoc (17%) and Calothrix (8%) were the second and third biggest genera followed by unicellular cyanobacteria Aphanocapsa (6%) (Fig.2). The predominance of Anabaena and Nostoc irrespective of chemical/biofertilizers supplementation and stage of crop growth was reported in different rice growing areas of India (Singh et al., 1996; Singh et al., 1997; Nayak et al., 2001, 2004). Thamizhselvi and Sivakumar (2011) also reported Anabaena and Nostoc as the dominant genera among the heterocystous form of cyanobacteria in rice fields of Bhuvanagiri and Srimushnam Taluk Cuddalore district, Tamilnadu,

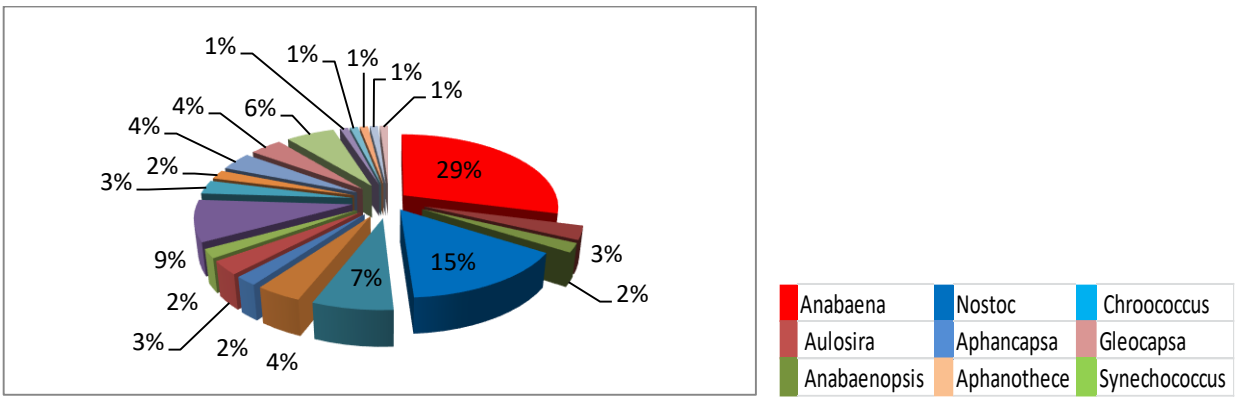
Table.1. Diversity of N_2-fixing cyanobacteria enumerated in all the rice fields of Bhuvanagiri and Srimushnam Taluk Cuddalore district, Tamilnadu

Sp. No.	Species Name	Family	Bhuvanagiri Taluk			Srimushnam Taluk			Chidamabaram Taluk		
			1	2	3	4	5	6	7	8	9
1	Aphanocapsa littoralis Hansgirg	Chroococcaceae	-	+	+	-	-	-	-	-	-
2	Aphanocapsa roeseana De Bary	Chroococcaceae	-	+	-	-	-	+	-	-	-
3	Aphanothece microscopica Nägeli	Chroococcaceae	-	-	-	-	-	-	+	+	-
4	Aphanothece naegelii Wartmann	Chroococcaceae	-	-	-	-	-	-	+	+	+
5	Chroococcus montanus Hansgirg	Chroococcaceae	+	-	-	-	-	-	+	+	-
6	Gloeocapsa quaternata Kützing	Chroococcaceae	-	-	+	-	-	-	-	-	-
7	Synechococcus aeruginosus Nägeli	Chroococcaceae	+		+	-	-	-	-	-	-
8	Mastigocladus laminosus Cohn ex Kirchner	Mastigocladaceae	+	+	-	-	-	-	-	-	-
9	Mastigocladopsis jogensis Iyengar & Desikachary	Mastigocladopsidaceae	-	+	+	+	-	-	-	-	-
10	Microchaete aequalis (Frémy) Desikachary	Microchaetaceae	-	-	-	+	-	-	-	-	-
11	Anabaena anomala F.E.Fritsch	Nostocaceae	-	+	+	+	+	-	+	+	-
12	Anabaena constricta (Szafer) Geitler	Nostocaceae	-	+	-	-	-	-	+	+	+
13	Anabaena doliolum Bharadwaja	Nostocaceae	-	-	+	+	+	-	-	-	-
14	Anabaena fertilissima C.B.Rao	Nostocaceae		+	+	+	+	+	-	-	-
15	Anabaena fuellebornii Schmidle	Nostocaceae	+	-	-	-	-	-	-	-	-
16	Anabaena gelatinicola Ghose	Nostocaceae	-	-	-	-	+	-	-	-	-
17	Anabaena iyengari Bharadwaja	Nostocaceae	-	-	-	-	-	-	-	-	+
18	Anabaena orientalis S.C.Dixit	Nostocaceae	-	-	+	-	-	-	-	-	-
19	Anabaena oryzae F.E.Fritsch	Nostocaceae	+	+	+	-	+	+	-	-	-
20	Anabaena sphaerica Bornet & Flahault	Nostocaceae	-	+	-	-	-	-	-	-	+
21	Anabaena spiroides Klebahn	Nostocaceae	+	+	+	+	-	+	-	-	-
22	Anabaena unispora N.L.Gardner	Nostocaceae	+	-	-	-	-	-	-	-	-
23	Anabaena vaginicola F.E.Fritsch & Rich	Nostocaceae	-	-	+	-	+	+	-	-	-
24	Anabaena variabilis Kützing ex Bornet & Flahault	Nostocaceae	+	+	+	+	-	-	+	+	+
25	Aulosira aenigmatica Frémy	Nostocaceae	-	-	-	-	-	-	-	-	+
26	Aulosira bombayensis Gonzalves	Nostocaceae	-	-	-	-	-	-	+	-	-

27	Aulosira prolifica Bharadwaja	Nostocaceae	-	+	+	-	-	-	-	-	-
28	Nostoc calcicola Brébisson ex Bornet & Flahault	Nostocaceae	-	-	+	+	-	-	-	-	+
29	Nostoc carneum C.Agardh ex Bornet & Flahault	Nostocaceae	-	+	+	+	-	-	-	-	-
30	Nostoc commune Vaucher ex Bornet & Flahault	Nostocaceae	+	-	-	-	-	-	-	-	-
31	Nostoc linckia Bornet ex Bornet &Flahault	Nostocaceae	+	+	+	+	+	+	-	-	-
32	Nostoc muscorum C.Agardh ex Bornet & Flahault	Nostocaceae	-	+	+	-	-	-	+	+	-
33	Nostoc passerinianum Bornet & Thuret ex Bornet & Flahault	Nostocaceae	-	+	+	-	-	-	-	-	+
34	Nostoc paludosum Kützing ex Bornet & Flahault	Nostocaceae	-	-	+	+	+	-	+	+	+
35	Nostoc piscinale Kützing ex Bornet & Flahault	Nostocaceae	+	+	+	+	+	+	-	-	-
36	Nostoc punctiforme Hariot	Nostocaceae	+	+	+	-	-	-	+	+	-
37	Nostoc spongiaeforme C.Agardh ex Bornet & Flahault	Nostocaceae	-	-	-	+	+	+	-	+	+
38	Lyngbya allorgei Frémy	Oscillatoriaceae	-	-	-	-	-	-	+	+	+
39	Lyngbya palmarum Martens ex Brühl & Biswas	Oscillatoriaceae	-	-	-	+	+	+	+	-	-
40	Lyngbya rubida Frémy	Oscillatoriaceae	+	+	-	+	-	-	-	+	-
41	Lyngbya perelegans Lemmermann	Oscillatoriaceae	+	-	+	-	-	-	-	-	-
42	Calothrix clavatoides Ghose	Rivulariaceae	+	+	-	-	-	-	-	-	-
43	Calothrix marchica Lemmermann	Rivulariaceae	+	+	-	+	+	+	+	+	-
44	Calothrix membranacea Schmidle	Rivulariaceae	-	-	+	-	+	+	-	-	-
45	Calothrix weberi Schmidle	Rivulariaceae	-	+	+	-	-	-	+	-	+
46	Gloeotrichia longicauda Schmidle	Rivulariaceae	+	+	-	-	-	-	-	+	-
47	Gloeotrichia pilgeri Schmidle	Rivulariaceae	+	-	-	-	-	-	-	-	-
48	Rivularia hansgirgi Schmidle	Rivulariaceae	-	-	+	+	+	-	-	-	+
49	Scytonema fritschii S.L.Ghose	Scytonemataceae	-	+	+	-	-	-	-	-	+
50	Scytonema hofmannii C.Agardh ex Bornet	Scytonemataceae	-	-	-	+	-	-	-	-	-
51	Scytonema simplex Bharadwaja	Scytonemataceae	-	+	-	+	+	-	-	-	-
52	Tolypothrix nodosa Bharadwaja	Scytonemataceae	-	+	+	-	-	-	-	-	-
53	Tolypothrix tenuis Kützing ex Bornet & Flah	Scytonemataceae	-	-	-	-	-	-	+	+	-
54	Hapalosiphon welwitschii West & G.S.West	Stigonemataceae	+	+	+	-	-	-	-	-	-
55	Westiellopsis prolifica Janet	Stigonemataceae	-	+	+	-	-	-	-	-	-

Figure.3. Percentage composition of N_2-fixing cyanobacterial families in rice fields' soil of Bhuvanagiri and Srimushnam Taluk Cuddalore district, Tamilnadu

Figure.4. Percentage composition of N_2-fixing cyanobacterial genera in rice fields' soil of Bhuvanagiri and Srimushnam TalukCuddalore district, Tamilnadu



Anabaena, Calothrix and Nostoc were recorded with maximum number of species in rice grown areas of Tripura (Singh et al., 1996). Singh et al. (1997) Anabaena, Aulosira, Anabaenopsis, Nostoc, Aphancapsa, Aphanothece, Chroococcus

Gleocapsa, Synechococcus also recorded highest number of species belonging to genera Anabaena and Nostoc in rice fields of Bhuvanagiri and Srimushnam Taluk, Cuddalore district, Tamilnadu. Nostoc and Anabaena can be considered as one of the most versatile and highly competitive genera observed in all types of environments that have the capacity to colonize as floating assemblages or as edaphic forms in rice field's soil (Singh et al., 1996; Singh et al., 1997a; Nayak et al., 2001, 2004; Prasanna and Nayak, 2007; Thamizhselvi and Sivakumar, 2011).

N_2-fixing cyanobacteria found in an organic rice field in Bhuvanagiri and Srimushnam Taluk, Cuddalore district, Tamilnadu. Land Preparation (1 d) Transplanting (30 d) Panicle Initiation (90 d) Harvest (120 d) species found in the organic rice field during the cycle of rice crops. Genus Nostoc had the highest number of species (six species) followed by Anabaena (five species). Other species were identified as C.fusca, Cy. muscicola, Nodularia sp., Scytonema sp., Trichormus sp., and Stigonema sp. Examination of soil samples from panicle initiation stage showed that non-heterocyst N_2-fixing Oscillatoria became more abundant, both in number and in species diversity. Mostly, N_2-fixing cyanobacteria fix the atmospheric nitrogen inside the specialized cell called heterocyst. Because nitrogenase enzyme that responsible for the N_2 fixation is oxygen-sensitive, heterocysts function as a compartment, keeping the separation of nitrogenase and photosynthetic-oxygen. Although having the ability to fix N_2, Oscillatoria lack heterocyst. Oscillatoria uses a temporal strategy which is the cells reduce N_2 into ammonium during the night when photosynthesis is halted.

One cycle of rice crop took three to four months. At the beginning of rice growth on the first cycle (Apr 2019), 20 representative isolates have been cultured, while on the second cycle (June 2020) only 11 isolates were collected. Five of the 11 isolates were also found from December sampling and were identified as C.fusca, N.muscorum, N.paludosum, N. punctiforme, and Stigonema sp. Morphological identification of 31 isolates has been recognized as 10 species. At the end of rice growth (harvest stage), the number of species that has been identified was almost the same from the transplanting stage (11 species).

The highest number of species was reached at panicle initiation stage (55 species). A research reported the occurrence of 6, 12, and 26 species on the 20th, 40th, and 60th day of the rice plantation, respectively. The up-down pattern of the number of species found at each stage of rice growth indicates that rice plants influence the cyanobacterial population on the surface of the soil. During rice growth, the root continues to produce many organic Substances, like polysaccharide, amino acid, and vitamin.

The root exudates act as a chemical signal to attract microorganism approaching the roots. Microorganisms, in turn, give benefits to roots by supplying minerals like NH_4, PO_4^{2-}, and Fe^{2+}. In this study, soil sample analysis showed an increase of phosphate (P_2O_5) content from 3.64 % at rice transplanting to 4.83 % at panicle initiation stage. Phosphate is one of macro elements, responsible for the ATP building as well as nucleic acid synthesize. The availability of phosphate in the soil during panicle initiation of rice plants advantages the significant growth of cyanobacterial population. Another explanation for the increase of the cyanobacterial population at panicle initiation stage is rice canopy.

During rice growth, plant canopy is broadened. The canopy protects cyanobacteria below from UV light so that more colonies can germinate. Three species were found at all stages of rice crop: N. punctiforme, N. muscorum, and A. fertilissima. Many studies reported the presence of N. punctiforme and N. muscorum on rice fields Those studies showed that the two species were well adapted to many habitats from poorly drained soils to flooded soil such as wetlands. The condition of the soil surface in every stage of rice growth impersonated the large scale of the global environment.

During land preparation until transplanting, the soil surface was open to any environmental influence such as light and dryness. The condition was changed as rice growth and the plant canopy cover the soil surface. N.punctiforme and Nostoc commune are two species that can adapt well to such changing. Another study showed that N.punctiforme grew rapidly under heterotrophic condition. This species is also competence as model species under laboratory conditions N.Commune is an adaptive species which characteristic of their large colony. The production of large amounts of sheath material covered the colony as a packed, which is difficult to consume by a predator. This benefits the population of N_2. commune to survive in the environment.

Table: 2 Identified N_2-fixing cyanobacteria found in an organic rice field in Bhuvanagiri and Srimushnam Taluk, Cuddalore district, Tamilnadu.

Land Peparation (1 d)	Transplanting (30 d)	Panicle Initiation (90 d)	Harvest (120 d)
Calothrix fusca, Anabaena iyengani, Anabaena torulosa, Nostoc muscorum, Nostoc padulosum, Nostoc punctiforme (two morphotype: strain SO-101 and strain SO-104), Nostoc spp. (SO-132, SO-134), Stigonema sp.	*Calothrix fusca, Anabaena spiroides, Anabaena fertilissima, Nostoc carneum, Nostoc ellipsosporum, Nostoc muscorum, Nostoc linckia, Nostoc paludosum, Nostoc punctiforme (strain SO-104), Stigonema sp.*	*Calothrix fusca, Anabaena fertilissima, Anabaena oscillarioides, Anabaena torulosa, Nostoc muscorum, Nostoc linckia, Nostoc punctiforme (strain SO-104), Nostoc spp. (strain SO-141, SO-142, SO-143, SO-144), Oscillatoria spp., Scytonema sp., Stigonema sp.*	*Cylindrospermum muscicola, Anabaena fertilissima, Anabaena oscillarioides, Nostoc muscorum, Nostoc padulosum, Nostoc punctiforme (strain SO-101 and SO-104), Nodularia sp., Trichormus sp., Nostoc spp. (SO-118, SO-122) 020011-*

IX. CONCLUSION

The diversity of N_2-fixing cyanobacteria in Sarinah Organic rice fields is dominated by genus Nostoc. Other genera that were found including Anabaena, Calothrix, Cylindrospermum, Trichormus, and Stigonema. The highest number of species was found at panicle initiation's stage. Nostoc muscorum, Nostoc punctiforme, and Anabaena sp. were found in all stages of rice crop cycle.

Originating as one of the earliest photo-autotrophic organisms of the primitive Earth, nitrogen fixing cyanobacteria have eventually established themselves as the only oxygenic photosynthetic prokaryotic group that could sustain the oxygen sensitive nitrogenase activity in them under aerobic conditions. A number of evolutionary trends appear to have been followed for this purpose as evident from the different adaptations shown by the present day species. Among these adaptations the development of the thick walled specialised cell, the heterocyst, appears to be widespread among filamentous species, while unicellular species exhibit a variety of adaptations, the exact mechanisms of some of which have yet to be elucidated. Similarly the enigmatic situation with the non-heterocystous, filamentous cyanobacterium Trichodesmium, which contributes significantly to the N budget of the oligotrophic deep oceans through its biological N2 fixation remains a challenge for future research.

Nitrogen fixation by free living cyanobacteria and their N2 fixing symbiotic associations make a significant contribution to the global natural nitrogen budget both in the marine and terrestrial ecosystems. Their ubiquitous global distribution including extreme habitats, which depicts their archaic ancestry, have enabled them to become frequent pioneers of natural colonisation. Their nitrogen fixation is perhaps crucial for the sustenance of certain vegetations in the Arctic, Antarctic, Tundra and Boreal forests. The only negative characteristic shown by certain species is the formation of toxigenic blooms in water bodies, but this should also be viewed as a natural defense mechanism, which provides them a survival advantage. Utilisation of these organisms and their symbiotic associations has the highest potential in rice cultivation, and with the other potential diverse uses cyanobacteria should be considered as an invaluable group of prokaryotic microorganisms that warrants care and conservation.

X. References

[1] Ahmed, S.U. (2001). Distribution pattern of blue green algae in rice field soils of Hojai sub-division of Assam. Phykos 40 (1 and 2): 33–38.

[2] Allen, M. & R.Stanier (1968). Selective isolation of blue green algae from water and soil. Journal of General Microbiology 51: 203–209.

[3] Allen, M.(1968).Simple conditions for growth of unicellular blue green algae on plates. Journal of Phycology 4: 1-4.

[4] Anand, N. & G.Revathi (1987). Blue green algae from rice fields of Tamil Nadu. Phykos 26: 17–21.

[5] Anand, N. (1990). A hand book of Blue green algae. Bishen singh, Mahendra pal singh, Dehra Dun. pp.1–79.

[6] Anand, N., R.S.Hooper & S.Kumar (1995). Distribution of blue -green algae in rice fields of Kerla state, India. 35:55-64.

[7] Anand, N.,S.Kumar & R.S. Hooper (1987).Blue-green algae from rice fields in Kerala state, India.Hydrobiologia 144:223-32

[8] Auti, B.K. & S.D. Pingle (2006). Nostocales from Northern circle of Ahmednagar district (M. S.) Indian Hydrobiology 9(2): 147–150.

[9] Banarjee, J.C.(1935).On algal found on soil samples from alluvial paddy field of Faridpur, Bengal, Science Culture. Journal: 298

[10] Castenholz, R.W. & J.B. Waterbury (1989). In: Staley, J.T., M. P. Bryant, N.P. fenningand & J.G. Holt (eds.). Bergey's Mannual of Systematic Bacteriology - Vol. 3. Williams and Wilkins, Baltimore, 1710–1727.

[11] Cerniglia, C.E., Van Baalen, C. and Gibson, D.T. 1980 Oxidation of biphenyl by the cyanobacterium Oscillatoriasp., strain JCM. Arch Microbiol. 125: 203-207.

[12] Chaillan, F., Gugger, M., Saliot, A., Couté, A. and Oudot, J. 2006 Role of cyanobacteria in the biodegradation of crude oil by a tropical cyanobacterial mat. Chemosphere. 62:1574-82.

[13] Dasgupta, M. and Ahmed, S.U. 2013 Some potential rice field BGA isolates from Sonitpur district Assam, North east India. J. Nat .Prod. Plant Resour. 3(4): 17-23.

[14] Desikachary, T.V. (1959). Cyanophyta. Indian council of Agricultural Research, New Delhi, 685pp.
Desikachary, T.V. 1959 Cyanophyta. Indian Council of Agricultural Research, New Delhi. pp. 99-616.

[15] Feldgarden, M.N. Byrd M. S. & F.M. Cohan (2003). Gradual evolution in bacteria: evidence from Bacillus systematics. Microbiology 149: 3565–3573.

[16] Fuller, W.H. & R.N. Roger (1952). Utilization of the Phosphorous of the algal cells as measured by the Neubauer technique. Soil Science 74: 417

[17] Fuller, W.H. and Rogers, R.N. 1952 Utilization of the phosphorus of the algal cells as measured by the Neubauer technique. Soil Sci. 74: 417-430.

[18] Gonzalves, E.A. & K.S.Gangla Observations on the algae of paddy field soils. Journal of University Bombay 18 : 51–59.

[19] Goyal, S.K., B.M. Shrama & R.S. Gupta (1984). Algal flora of rice field soils of Jammu and Kashmir states. Phykos 23: 59–64.

[20] Gupta, A.B. (1964). Algal flora and its importance in the economy of rice fields. Hydrobiologia 38: 213–222.

[21] Jawale, A.K. & D.A. Kumawat (2004). Some blue green algae from Maharashtra in National Symposium on Biology and Biodiversity of fresh water algae. Chennai, 37pp.

[22] Kamat, N.D. (1962). The Cyanophyceae and Chlorophyceae of Panhala. Journal of University Bombay 30: 22–31.

[23] Kamat, N.D. (1963). The Algae of Kolhapur, India. Hydrobiologia 22 (3 & 4): 209–305.

[24] Kamat, N.D. (1964). The Algae of Mahabaleshwar. Journal of University Bombay 31: 28–41.

[25] Kamat, N.D. (1974). Algae of Marathwada, Maharashtra. Phykos 13(1 & 2) : 22–32.

[26] Kamble, P.B. (2010). Isolation purification and biochemical characterization of blue green algae from paddy field soils of Satara district - MPhil Dessertation submitted to Shivaji university, Kolhapur, 125pp.

[27] Kamble, P.B. (2017). Studies on Filamentous blue green algae from Satara district Maharastra. PhD Thesis submitted to Shivaji University, Kolhapur, 278pp.

[28] Karande, C.T. (2009). Cyanobacterial biodiversity in paddy fields from Satara district - A project submitted to UGC.

[29] Kolte, S.O. & S.K. Goyal (1985). Distribution pattern of blue green algae in rice field soils of Vidarbha region of Maharashtra state. Phykos 24: 156–162.

[30] Kondo, M. & [1] M. Yasuda (2003). Seasonal changes in N2 fixation activity and environment in paddy soils of Japan. Japan Agricultural Research Quarterly 37: 105–111.

[31] Madane, N.P. & P.A. Shinde (1993). Blue-green algae in salt affected soils of Kolhapur district (M. S.) Journal of Maharashtra Agricultural Universities 18: 289–290.

[32] Nayak, S. & R. Prasanna (2007). Soil pH and its role in cyanobacterial abundance and diversity in rice field soils. Applied ecology and environmental Research 5(2): 103–113.

[33] Nayak, S., R. Prasanna, T.K. Dominic & P.K. Singh (2004). Effect of BGA – Azolla biofertilizers on nitrogen fixation and chlorophyll accumulation at different depths in soil cores. Biology and Fertility of Soils 40: 67–72.

[34] Nayak, S., R. Prassana, T.K. Dominic & P.K. Singh (2001). Floristic abundance and relative distribution of different cyanobacterial genera in rice field soil at different crop growth stages. Phykos 40: 14–21.

[35] Nikam, T.D., J.N. Nehul, Y.R. Gahile, B.K. Auti, M.L. Ahire, K.M. Nitnaware, B.N. Joshi & N. Jawali (2013). Cyanobacterial diversity in Western Ghats region of Maharashtra, India. Bioremediation, Biodiversity and Bioavailability 7[1]: 70–80.

[36] Patil, P.L. & S.D. Satav (1986). A study of nitrogen fixing blue green algae from rice fields of Western Maharashtra Phykos 25(1 & 2): 113–116.

[37] Patil, S.R. & B.B. Chaugule (2009). Diversity of blue green algae in paddy fields of Western Maharashtra Indian Hydrobiology 12: 89–94.

[38] Prasad, B.N. & R.K. Mehrotra (1980). Blue green algae of paddy fields of U.P. Phykos 19: 121–128.

[39] Rippka, R., J. Deruelles, J.H. Waterbury, M. Berdman & R.Y. Stanier (1979). Generic assignments and strain histories and properties of pure cultures of Cyanobacteria. Journal of General Microbiology III: 1-161

[40] Roger, P.A. & S.A. Kulasooriya (1980). Book of blue green algae and Rice. The International Rice Research Institute, Los Banos, Laguna, Philippines, 117pp.

[41] ThamizhSelvi K. & Sivakumar K. (2012). Distribution of heterocystous cyanobacteria in rice fields of Cuddalore Nitrogen fixing cyanobacteria 127 Journal of the National Science Foundation of Sri Lanka 44(2) June 2016. International Journal of Life Sciences and Pharmaceutical Research 2(4): 30 – 39.

[42] Sahu, J. K., H. Naik & S.P. Adhikary (1997). Blue green Algae of rice field soils of Orissa state - I Distributional pattern in different agroclimatic zones. Phykos 35: 93–110.

[43] Santra, S.C. (1991). Rice field blue green algae (Cyanobacteria) and its utilization prospects as biofertilizer in West Bengal, India, Proceeding of National Symposium On Cyanobacterial Nitrogen fixation New Delhi, 385–389pp.

[44] Santra, S.C. (1993). Biology of Rice Fields Blue Green Algae. Daya Publishing House, New Delhi, 184pp.

[45] Sardeshpande, J.S. & S.K. Goyal (1981a). Distributional pattern of blue green algae in rice field soils of Konkan region of Maharashtra State. Phykos 20: 102–106

[46] Sardeshpande, J.S. & S.K. Goyal (1981b). Distribution pattern of blue green algae in rice field soils of Konkan region of Maharashtrra State. Phykos 20: 102–106.

[47] Singh, P.K. (1978). Nitrogen economy of rice soils in relation to nitrogen fixation by blue green algae and Azolla, pp. 12–23. In: Proceeding of National, Symposium On increasing rice yield in Kharif (CFRI, India).

[48] Singh, R.N. (1961). Role of blue green algae in the nitrogen economy of Indian agriculture. Indian council of Agricultural Research, New Delhi, 175pp.

[49] Sinha, J.P. & D. Mukherjee (1975a). On Blue green algae from the paddy fields of Bankura district of West Bengal - I. Phykos 14(1 & 2): 117–118.

[50] Sinha, J.P. & D. Mukherjee (1975b). On Blue green algae from the paddy fields of Bankura district of West Bengal - II. Phykos 14 (1 & 2): 119–120.

[51] Sinha, J.P. & D. Mukherjee (1984). Blue green algae from the paddy fields of Bankura District of West Bengal - III. Phykos 23(1 & 2): 142–143.

[52] Somawanshi, R.B., A.D. Kadlag, A.N. Deshpande, B.D. Tamboli, P.P. Kadu & B.D. Bhakare (1999). A Book of Laboratory methods for analysis of soils, irrigation water and plants. 230pp.

[53] Tiwari, G.L. (1972). Study of blue green algae from paddy field soils of India. Hydrobiologia 29: 335–350.