CHAPTER I

INTRODUCTION

Cyanobacteria, which was once known as ‘Blue Green Algae’ (BGA) is one of the ancient groups of lower cryptogams. The origin of cyanobacteria is estimated to be around 2.7 billion years ago (Buick, 1992; Brasier et al., 2002; Dalton, 2002). They are the members of a fascinating group which represent a link between bacteria and algae whose cellular organization is prokaryotic and are characterized by the lack of membrane-bound organelles including nucleus, chloroplast and mitochondria. On the other hand, they possess an O\textsubscript{2}-evolving photosynthetic system similar to that of eukaryotic algae and higher plants (Vaishampayan et al., 2001). Therefore, due their superficial resemblance to eukaryotic green algae, they were once classified as “Blue Green Algae”. Later, BGA have been proposed to be named as ‘cyanobacteria’ by some bacteriologists under the rules of ‘International Code of Nomenclature of Bacteria’ in the year 1978 (Oren, 2004). Cyanobacteria were the first organisms to have two photosystems and produce organic materials as well as release off O\textsubscript{2} as a byproduct. Hence, being the first batch of cryptogams, they played an important role to the evolution of the earth’s oxidizing atmosphere and are also known to be one amongst the earliest colonizers on land (Booth, 1941).

Cyanobacteria are also the most genetically diverse group of organisms that occupy 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, (Hoffmann, 1989) or hot springs (Bhardwaj et al., 2010; Roy et al., 2014). They are generally free living and are also found in symbiotic association with all higher groups of plants. Their ability to form symbiotic association with algae (endophytically), fungi, bryophytes, pteridophytes, gymnosperm and angiosperm marked them as a unique group among micro-organisms (Adam and Duggan, 2008). Cyanobacterial study thus, had become a great topic of interest for the researcher from every corner of the globe.

The main photosynthetic pigments of cyanobacteria are chlorophyll a, carotenes and xanthophylls together with phycobilproteins, c-phycocyanin (blue) and c-phycoerythene (red). These pigments along with mucilage develop different colouration in nature, which ranges from dirty yellow, through various shades of blue-green to brown or black (Roger and Kulasooria, 1980). Their ranges in vegetative form extend from simple unicellular to colonial, simple filamentous to branch filamentous (uniseriate or multiseriate). Cyanobacteria form gelatinous masses, furry cushions, skin sheets or powdery covering on the substratum and constitute up to 75% of the total algal flora in India (Pandey, 1965).

The majority of the cyanobacteria are capable of fixing the atmospheric nitrogen and their presence in various ecosystems is thought to maintain the nitrogen-level in the soil. 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). Nitrogen fixing capacity of cyanobacteria were indicated early in 1901 by Beijerinck (Wagner, 2012) and their importance in maintaining the fertility of Indian rice fields was first discussed by De in
1936 (Pabbi, 2008). Fogg (1949) initiated the study of this organism in axenic culture and demonstrated that heterocystous forms fix the atmospheric nitrogen. The protein responsible for the nitrogenous activity was discovered to be located in the heterocysts (Fleming and Haselkorn, 1973). This heterocyst, according to Fogg (1949) is a specialized thick-walled, hollow looking larger cell that contains the nitrogen fixing capacity. The involvement of this protein in nitrogen fixation was proved by immune-labeling studies by different researcher after Fogg’s discovery (Stewart, 1980). The differentiation of ‘heterocyst’ takes place mainly due to the nitrogen limitation in the particular medium (Desikachary, 1959; Stanier and Cohen-Bazire, 1977). At the onset of adverse environmental condition, some cyanobacteria develop climate-resistant spores, called an ‘akinetes’. Akinetes contain large reserves of carbohydrates and can tolerate harsh condition such as the complete drying of water bodies, extremely hot soil or severe low temperature. When favorable condition returns akinetes serve as “seeds” for the growth of the juvenile filaments. Therefore, heterocysts and akinetes are considered as unique morphological structure of cyanobacteria. Heterocyst formation occurs mainly in filamentous cyanobacteria, both in non-branching and branching forms. It was estimated that over 50% of cyanobacterial genera occurring in rice fields are heterocystous filamentous forms, belonging to orders Nostocales and Stigonematales (Venkataraman, 1993). Examples of few free-living filamentous heterocystous genera are Anabaena, Nostoc, Calothrix, Rivularia, Tolypothrix, Scytonema etc. Few are filamentous, but non-heterocystous (Lyngbya, Pseudoanabaena, plectonema etc.). Among non-heterocystous genera, the unicellular Cyanothecce (Reddy et al., 1993), Aphanotheca, Gloeocapsa,
Synechococcus (Vaishampayan et al., 2001), Gloeethece (Wyatt and Silvey, 1969), Synechococcus (Mitsui et al., 1986) have also been reported to fix nitrogen aerobically.

Besides free living cyanobacteria, Azolla-Anabaena, a symbiotic association where the cyanobacterium Anabaena azollae lives in association with the free floating water fern Azolla. This existence of symbiotic association of Azolla-Anabaena complex has been reported by Peters (1975). This symbiotic complex has emerged as an important input in rice cultivation as green manure crop, dual crop, water purifier, hydrogen producer, cattle feed, fish and poultry feed. It has been widely using as bio-fertilizer in different countries like Vietnam, China, Thailand (Liu, 1979; Tuam and Thuyet, 1979; Wagner, 1997). In Vietnam dual cropping of rice and Azolla-Anabaena complex has been reported to increase rice yield by 14.40% (Tuam and Thuyet, 1979) and an increase of about 18% in China as reported by Liu (1979).

Among the diverse habitats, rice fields provide a very congenial condition for abundant growth of N\textsubscript{2}-fixing cyanobacteria (Whitton, 2000; Nayak et al., 2001). There occurrence is controlled by environmental factors like temperature, light intensities, nutrients availability and water stability (Roger and Kulasooria, 1980). Therefore, higher abundant of cyanobacteria, comprising more than half the population of heterocystous are reported to be grown at or floating above the surface of water logged rice fields and have helped in enhancing nitrogen budget (Ladha and Reddy, 1995). N\textsubscript{2}-fixing cyanobacteria thus play an important role in the fertility of rice field ecosystems. Considerable amount of research have been carried out on diversity and distribution of the cyanobacterial flora of rice fields in India by various author (Singh, 1961; Aiyer,
1965; Amma et al., 1966; Singh, 1978; Singh, 1985; Khan et al., 1994; Singh et al., 1996; Singh et al., 1997c; Mandal et al., 1998; Tiwari et al., 2000; Mishra and Pabbi, 2004; Prasanna and Nayak, 2007; Begum et al., 2008; Digambar Rao et al., 2008; Choudhary and Bimal, 2010; Dey et al., 2010; D’souza E Gomes et al., 2011).

Rice-fields are considered as one of the highly dynamic ecosystems. Changes in the physico-chemical properties of the rice fields’ soil could be well monitored due to changes in seasons and cultivation cycle (Roger and Kulasooria, 1980; Song et al., 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 dynamics of cyanobacterial populations, the ways in which their numbers grow and shrink in times are also related to the variation of environmental factors prevailing in the rice fields. Therefore, along with the study of the diversity, it is necessary to evaluate the environment that promotes the N$_2$-fixing cyanobacterial persistence and the effect of soil physico-chemical factors on its population dynamics. A little survey has so far been done on quantitative estimations of cyanobacteria as affected by environmental factors and soil qualities in relation to rice field agro ecosystem (Okuda and Yamaguchi, 1955; Chunleuchanon et al., 2003; Song et al., 2005; Thamizh Selvi and Sivakumar, 2011; Das et al., 2011). Regarding the rice fields of Assam, a few attempts though have been made in finding the diversity of cyanobacteria (Saikia and Bordoloi, 1994; Ahmed, 1999; Hazarika et al., 2001; Das et al., 2003; Dihingia and Baruah, 2012; Dihingia et al., 2013; Bharadwaj and Baruah, 2013; Deb et al., 2013; Dasgupta and Ahmed, 2013; Kemprai,
studies on cyanobacterial population dynamics in relation to the environmental factors have not yet been reported.

Earlier studies depict that the population dynamics of cyanobacteria are closely related to some soil physico-chemical parameters. They are generally sensitive to high light intensities and so are regarded as low light species (Roger and Reynaud, 1979). Furthermore, a very high temperature has a deleterious effect on cyanobacteria and their nitrogenous activity (Roger and Reynaud, 1979). In addition to light and temperature, the pertinent factors that affect cyanobacterial presence in soil are moisture, pH, mineral nutrients (Granhall, 1975) and other added chemicals (Singh, 1975; Huang, 1978; Roger and Reynaud, 1979).

Cyanobacteria, in turn improve water holding capacity of soil and increases soil aggregation (Ibraheem, 2007) other than nitrogen fixation. They also improve soil structure and increase humus content by dissolving certain soil minerals. In addition, they also add substantial amount of organic matter to the soil (Goyal, 2002). This organic matter acts as a storehouse of nutrients like nitrogen, phosphorus and micronutrients. Cyanobacteria excrete organic acids that render phosphorus solubilisation, making phosphorus available to plants (Fuller and Rogers 1952; Singh et al., 1981). Therefore, due to these innate properties, N₂-fixing cyanobacteria shot into fame and popularity as an alternate source to chemical fertilizers which make them an ideal organism for maintaining a sustainable agricultural system.
Agriculture, the mainstay of Indian economy, involves more than two thirds of the population. Our country has already attained 1-billion population marks whereas we are still trailed behind in food production roughly by 206 million tones (Tripathi et al., 2001). It has been estimated that by 2025, the population will reach 1.3 billion with the potential demand of 315 million tones food grains (Tripathi et al., 2001). The average agricultural productivity of Northeastern region is below the National average. The total rice production in NE region is estimated to be around 5.50 million tones only, with an average productivity of 1.57 t/ha, which is far behind the national average of 2.08 t/ha (Pattanayak et al., 2006). Kamrup is one of the oldest landmasses of lower Brahmaputra flood plain of Assam. Guwahati, the only major city in Kamrup district and entire Assam, is considered as one of the fastest developing cities in India.

So, to overcome the ever increasing demand of the food grain, extensive chemical fertilizers are in continuous use. Chemical fertilizers and pesticides boost the productivity of crops in the past and also are going to play an important role in the near future. But continuous application of chemicals in an unplanned way led to adverse environmental, agricultural and health consequences. Soil fertility has been decreasing gradually due to soil erosions, loss of nutrient, accumulation of salts and other toxic elements, water logging and un-balanced nutrient compensation. In that case, N$_2$-fixing cyanobacteria can be used as biofertilizer which, in turn, will reduce the total input of chemical fertilizers and will stop further degradation of arable soil.

If the present scenario of adoption of agricultural technology continue, it will be difficult to catch up with required rate of productivity to meet the demand of food
arising from this growing population. So, in this context it becomes utmost important to understand the rice field soil microflora with special reference to $N_2$-fixing cyanobacteria so that an alternate low cost and renewable source of nitrogen fertilizers could be suggested for upliftment of rice production and also to improve the carbon content of the soil of the district.

Therefore, in this endeavor, effect of environmental factors influencing the $N_2$-fixing cyanobacterial persistence and population dynamics was evaluated in different rice fields of Kamrup district, Assam with the following objectives:

1. Estimation of $N_2$-fixing cyanobacterial abundance and species diversity in different rice fields of Kamrup district, Assam.

2. To study the periodicity and population dynamics of $N_2$-fixing cyanobacteria therein.

3. To assess the physico-chemical properties of the soil of the selected sites.

4. To formulate management action plan for different rice fields of the district.