

## Cyanobacterial Diversity in Agriculturally Fertile Soil of Patna and Their Population Density

Anand Mohan<sup>1</sup> and Baidyanath Kumar<sup>2</sup>

<sup>1</sup>Department of Biotechnology, College of Commerce, Arts and Science, Patliputra University, Patna

<sup>2</sup>Visiting Professor, Department of Biotechnology, Patna University (Patna)

Corresponding Author: Dr. Baidyanath Kumar

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**Abstract:** In the present investigation 187 species of cyanobacteria belonging to 45 genera were recorded from three groups of agriculturally fertile soils of Patna under investigation. But the number of species rounded from each soil group was quite variable depending upon the ecological condition at the soil. A comparatively large number of cyanobacterial species was recorded from soils of group A (138 species), followed by soils of Group:-B (135 species) and Group:-C (129 species). The total average density of cyanobacteria varied from  $560 \times 10^3$  to  $1650 \times 10^3/g$  of soil (dry weight). In all the three groups of soils the population density of cyanobacteria was minimum in June ( $565 \times 10^3/g$  in group A,  $560 \times 10^3/g$  in Group C and  $650 \times 10^3/g$  in group B) and maximum during rainy season (July to September).

**Key words:** Cyanobacteria, Biodiversity, Population density, Biofertilizer

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### I. Introduction

Cyanobacteria are oxygenic photosynthesizer commonly found in fresh water, marine water and soil. They are considered as an important group of microorganisms capable of fixing atmospheric nitrogen. They have a unique potential to contribute to productivity in a variety of agricultural and ecological situations. Many cyanobacteria fix nitrogen under aerobic conditions in specialized cells called heterocyst which comprise 5-10% of cells in a filament [1] (Ganter, 2000). Non- heterocystous cyanobacteria are also able to promote plant growth and can also be used as bio fertilizer.

Besides fixing atmospheric nitrogen, cyanobacteria play a major role in reducing soil erosion because of ability to secrete polysaccharides that bind soil [2] (Nayak and Prassana, 2007). They also control soil run off and increase soil organic matter content and in producing certain substances which enhance the growth of plants [3] (Ordog, 1999). Due to this important characteristic of nitrogen fixation, the utility of cyanobacteria in agriculture to enhance production is beyond doubt.

The algae constitute an important component of the soil micro flora. They act as a reserve for plant nutrients, influence soil structure, influence the activities of other organisms, and contribute to the organic carbon and fixed nitrogen status of the soil through their photosynthesis and nitrogen fixation.

Many soil algal species are ubiquitous in distribution and there are no characteristic algal associations formed in any particular geographical region or soil type. In general blue- green algae do not occur in soils of pH less than 4.

Solar radiation, water, and temperature are the more important abiotic factors regulating the distribution, metabolism, and life histories of soil algae, whereas ionic factors, e.g., pH, redox potential, and soil texture are somewhat less important factors [4] (Metting, 1981).

In the soil profile, most algal growth is confined to the upper few mm or cm, and the subterranean flora mainly includes resting stages or inactive cells carried along with the seepage water, agricultural activity, root growth, or soil animals. There is micro stratification of algal populations within the upper few mm or cm of soil profiles, rocks, or gravel deposits [5, 4] (Friedmann and Galum, 1974; Metting, 1981).

In semiarid environments, the upper surface tends to be colonized mainly by cyanobacteria (0-2cm), whereas the greatest cell numbers of Chlorophyceae and Bacillariophyceae occur at 4-6cm depths [6] (Nordin and Blinn, 1972). The blue- green algae dominate in deserts, forming surface crusts, The Oscillatoriaceae are common in cultivated and noncultivated desert in Arizona, whereas the other cyanophytes are common in virgin or fallow areas [7] (Cameron, 1963). In several tropical regions, *Schizothrix calcicola* is the most common blue-greens.

The algae are important in stabilizing the soil through aggregation of soil particles. They contribute to soil nutrients. Crusts of Cyanophyceae aid in the retention of silt and clay that produce a rearrangement of soil

particles. These crusts greatly increase organic carbon and nitrogen contents of soil, improve water infiltration, decrease erosion, and furnish a suitable habitat for seed germination.

The terrestrial blue-greens show good adaptations to live and grow in those climates and microenvironments in which available water is the main limiting factor, and some soil algae can survive very long periods without water. In soils, algal growth is largely influenced by the temperature range. Species of *Stichococcus*, *Microcoleus*, and *Schizothrix* have a marked capacity to resist extremely low temperatures (even -150°C) in crushed soil. Likewise, many soil algae when dry can tolerate abnormally high temperatures; thus, *Scytonema* is known to tolerate short exposure to 110°C, whereas *Spongiochloristypical* cells can survive a year in air-dried soil that has been dried in an oven at 100°C [8] (Trainor and McLean, 1964).

The upper millimeter of dry soil often appears crusty in nature due to growth of microorganisms comprising cyanobacteria, algae and/or lichens. Soil particles form an intimate association among these organisms, resulting in a biological crust that covers the surface of the soil as a coherent layer [9] (Belnep et al., 2001). Biological soil crusts often occur in hostile environmental regimes that include extremes in temperature and light, and scarcity of water. Many microorganisms withstand such adverse ecological conditions and respond to the onset of dry conditions by entering into a dormant resistant state, and thus have been distinguished as pioneers of succession on soil. As a component of the soil crust, the microflora acts as a reservoir of plant nutrients, as organisms influencing the soil structure and activity of other microorganisms, and as agents for the incorporation of organic carbon and nitrogen through photosynthesis and nitrogen fixation [10, 11, 12] (Smith et al., 1990; Adhikary et al., 2000; Johansen, 1993). It has been stated that biodiversity of biological crusts on the top soil surfaces is the most poorly researched habitats on earth [13, 14.] (Moore, 1998; Copley, 2000). In India, there are few reports of soil cyanobacteria and algae [15, 16] (Marathe and Kushaldas, 1997; Venkataraman et al., 1974).

Cyanobacteria evolved very early in the history of life, and share some of the characteristics of gliding bacteria on one hand and those of higher plants on the other. Cyanobacteria can both photosynthesize and fix nitrogen, and these abilities, together with great adaptability to various soil types, make them ubiquitous. Cyanobacteria also have a unique potential to contribute to productivity in a variety of agricultural and ecological situations. Cyanobacteria have been reported from a wide range of soils, thriving both on and below the surface. They are often also characteristic features of other types of sub-aerial environment and many intermittently wet ones such as rice fields. Most paddy soils have a natural population of cyanobacteria which provides a potential source of nitrogen fixation at no cost. Ammonia can be taken up by cyanobacteria through passive diffusion or as ammonium ( $\text{NH}_4^+$ ) by a specific uptake system. The amino acids arginine, asparagine and glutamine have also been reported to serve as nitrogen sources. Nitrate and nitrite are important sources, which later reduce into ammonia. Many cyanobacteria are also capable of using atmospheric dinitrogen ( $\text{N}_2$ ) as the source of nitrogen, and this is what most commonly termed nitrogen fixation. Like many other biological systems, nitrogen fixation in cyanobacteria is brought about by a high molecular weight, oxygen labile, metalloprotein enzyme known as nitrogenase. Nitrogenase reduces molecular nitrogen to ammonia in presence of hydrogen. Due to this important characteristic of nitrogen fixation, the utility of cyanobacteria in agriculture to enhance production is beyond doubt.

Many studies have been reported on the use of dried cyanobacteria to inoculate soils as a means of aiding fertility, and the effect of adding cyanobacteria to soil on rice yield was first studied in the 1950s in Japan. The term 'algalization' is now applied to the use of a defined mixture of cyanobacterial species to inoculate soil, and research on algalization is going on in all major rice producing countries. The average of the results from all these studies have shown an increase in grain yield of 15-20% in field experiments. It has been suggested that the cyanobacteria introduced as a result of algalization can establish themselves permanently if inoculation is done consecutively for 3-4 cropping seasons. The basic method of mass production involves a mixture of nitrogen fixing cyanobacteria in shallow trays or polythene lined pits filled with water kept in open air, using clean, sieved farm soil as a carrier material. To each pit 10 kg soil and 250 g single super phosphate is added and water is filled up to a height of 12-15 cm. Starter culture, a mixture of *Anabaena*, *Nostoc*, *Aulosira* and *Tolypothrix*, is inoculated in each multiplication unit. Malathion (5-10 ml per tank) or carbofuran (3% granules, 20 g per tank) is also added to prevent insect breeding. In hot summer months, the cyanobacteria form a thick mat over the surface after 10-12 days of growth in open sun. The contents are allowed to dry and the dried flakes are collected, packed and used to inoculate rice fields. The basic advantage of this technology is that farmers after getting the soil based starter culture can produce the biofertilizer on their own with minimum additional inputs. An inoculum of 10-12 kg is considered sufficient to inoculate one hectare of paddy field 3-4 days after transplantation [17] (Upasana Mishra and Sunil Pabhi, 2004).

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The Cyanobacteria and their products find a conspicuous usage in maritime countries where most people use some algae or algal products daily, either directly or indirectly. Species of *Nostoc*, *Aphanothece*, and *Spirulina* are examples of freshwater or terrestrial blue- green algae that are edible.

The algal mixture obtained as byproduct of sewage treatment process, and the protein- rich fresh water alga *Spirulina* grown on wastewater can be fed to fishes, poultry, and cattle to improve their health and productivity. For this purpose, *Spirulina* has been successfully cultivated in wastewater in Lucknow, Nagpur, and Varanasi.

In respect of the nitrogen status of natural habitats, the nitrogen- fixing blue- green algae deserve special mention. These algae grow luxuriantly in tropical habitats, e.g. rice fields. The experiments conducted with *Tolypothrix tenuis* in Japan and with *Aulosira fertilissima* in India have shown that the yield of paddy is substantially increased following the inoculation of fields with these algae. The algologists at the Central Rice Research Institute, Cuttak (India), inoculated rice fields with four species of nitrogen- fixing blue- green algae; the grain yield increased by nearly 30%.

Over 15 genera of free- living blue- green algae are known to fix nitrogen, e.g. *Anabaena*, *Cylindrospermum*, *Nostoc*, *Aulosira*, *Scytonema*, *Oscillatoria*, *Plectonema*, *Aphanothece* etc. Their contribution to nitrogen fertility is especially important in flooded soils (e.g., rice fields) where the prevailing microaerobic or anaerobic conditions are highly conducive to nitrogenase activity in the blue- green algae. In Japan and Philippines, in nonfertilized flooded plots, net gains of soil nitrogen range from 20- 70kgN/ha/year [18] (Watanabe and Roger, 1984). Fertilization with phosphorus and potassium increased these gains greatly. Some 25-30% of the nitrogen fixed by the blue- green algae is taken up by the rice plants [18] (Watanabe and Roger, 1984).

Cyanobacteria are unique in the sense that both photosynthesis and nitrogen fixation often occur in the same organism or even the same cell. It is this dual capacity to fix carbon as well as nitrogen that makes cyanobacteria attractive as sources of nitrogenous bio fertilizer.

The role of cyanobacteria as biofertilizer has largely been reviewed by [ 19; 2; 3; 20; 21; 22; 23; 24; 25; 26] DolaBhowmik et al., 2010; Nayak and Prassana, 2007; Ordog, 1999; Nanda, 1991; Haroun and Hussein, 2003; Lakshmi and Annamalai, 2008; Gallaband Salem, 2001; Venkataraman, 1972, Singh, 1961; Relwani and Surahmanyam, 1963. Radheyshyam Sharma et al., (2012) [27] have studied the role of algae and cyanobacteria in sustainable agriculture development. Marina et al., (2015) [28] have studied the algae and cyanobacteria in soils of Moscow.

The biodiversity of cyanobacteria, their population density and seasonal dominance in the agriculturally fertile soil of Patna has not been studied so far and hence the present work has been undertaken.

## II. Materials and Methods

**Isolation of Cyanobacterial flora:** Three groups of agriculturally fertile soil of Patna district were selected for isolation of soil cyanobacteria. These were:

1. Soil used to cultivate only cereal crops viz., wheat, rice and maize,
2. Soil used to cultivate cereals(rice) and then pulses crops(Moong, Massor), and
3. Soil used to cultivate only vegetable crops viz., potato, Brinjal, Tomato etc.

Crust samples were collected from the upper surface of dry soils from all the above categories of soils in replicates of five. Scraping of surface soil in open ground devoid of vegetation(in the immediate area) was collected, stored in pre sterilized screw cap Tarson bottles and transported to the laboratory for analysis. Soil crust samples were wetted with sterile water and examined under light microscope. Within 12 to 24h of wetting cyanobacterial filaments could be visualized; however, isolation and culturing under defined conditions was required for their identification. A pinch of each crust was transferred to agarized BG11 medium [29] ( Rippka et al., 1979) with or without combined nitrogen and incubated at  $25\pm 2^{\circ}\text{C}$  under continuous light from fluorescent tubes at an intensity of  $7.5\text{Wm}^2$ (1500 lux). Morphometric analysis of each species was made and identified following [30; 31; 32; 33] Desikachary (1959), Komerak and Anagnostidis (1989) and Anagnostidis and Komerak (1988; 1990). The dominant organism in each soil sample was purified following standard methods [34] (Venkataraman, 1969) and axenic culture of the cyanobacteria was used in the experiments. Bright field- illuminated photomicrographs of the cyanobacterial species were taken with a Meiji ML-TH-05 trinocular research microscope using a F-108 Nikon camera. Line drawing of cyanobacterial flora was made using prism type Camera Lucida (Erma-Japan).

### Preparation of BG11 Medium

**BG11 medium was prepared using following components**

Component	Amount	Stock solution concentration	Final concentration
1.NaNO <sub>3</sub> (FisherBP360-500)	10mL/L	30g/200mLdH <sub>2</sub> O	17.6mM
2.K <sub>2</sub> HPO <sub>4</sub> (SigmaP3786)	10mL/L	0.8g/200mLdH <sub>2</sub> O	0.23mM
3.MgS.7H <sub>2</sub> O(Sigma230391)	10mL/L	1.5g/200mLdH <sub>2</sub> O	0.3mM

4. CaCl <sub>2</sub> .2H <sub>2</sub> O(Sigma C-3881)	10mL/L	0.72g/200mLdH <sub>2</sub> O	0.24mM
5. Citric acid.H <sub>2</sub> O(Fisher-A104)	10mL/L	0.12g/200mLdH <sub>2</sub> O	0.031mM
6. Ferric ammonium citrate	10mL/L	0.12g/200mLdH <sub>2</sub> O	0.021mM
7. Na <sub>2</sub> EDTA.2H <sub>2</sub> O(Sigma ED255)	10mL/L	0.02g/200mLdH <sub>2</sub> O	0.0027mM
8. Na <sub>2</sub> CO <sub>3</sub> (Baker 3604)	10mL/L	0.4g/200mLdH <sub>2</sub> O	0.19mM
9. BG-11 Trace metals solution	1mL/L		
10. Sodium Thiosulphate Pentahydrate(for agar media) (Baker3946)	1mL/L	49.8g/200mLdH <sub>2</sub> O	1mM

**Liquid media:**

To approximately 900 mL of dH<sub>2</sub>O the first 9 components in the order specified were added while stirring continuously and the final volume was made to 1 L by adding more distilled water. The flask was covered by non absorbent cotton and autoclaved at 15lb/inch<sup>2</sup> pressure for 20 minutes. The medium was then allowed to cool and then stored at refrigerator temperature.

Similarly the BG agar medium was prepared by adding the first nine components in the order specified while stirring continuously to 400mL of distilled water and the final volume was then adjusted to500mL by adding more distilled water.

In a separate container 15 g of agar was added to 500 mL of dH<sub>2</sub>O. The flask was covered by cotton plug and autoclaved both solutions at 15lb/inch<sup>2</sup> pressure for 20 minutes. Both solutions were allowed to cool to 45-50°C in a water bath. Sterile Sodium Thiosulfate (Component 10) was then added to agar solution and mixed well. Both agar and liquid solutions were combined and mixed well and then stored at refrigerator temperature.

**Population density of Soil Cyanobacteria:**

The population density of Cyanobacteria was estimated using BG-11 media with or without combined nitrogen and these media were used in liquid or solidified form with 1.5% agar. Media were sterilized for 15min in an autoclave and then transferred into either sterile test tubes or sterile plastic Petri dishes.

Dilution on solid media was chosen for algal counts: 10g of soil were transferred to 90ml of sterile water and homogenized. Then serial 4-solid dilution of homogenate in liquid BG-11 was prepared. Each Petri dish containing solidified BG-11 was inoculated with 1 ml of dilute soil suspension; 4 replication of each dilution were used [35] (Lukesova, 1993). Incubation proceeded for 3 weeks in the environmental chamber equipped with florescent tubes (Philips “TL” D 840) provided light at 14h/10h light dark cycle. The chamber temperature was kept at 23-25°C. The number of algae was estimated by counting the algal colonies that developed on agar plates of the most suitable dilution (20-400 colonies/plate), taking 1 cell=1 colony. And the number of algae was estimated per 1g dry weight (d.w.) of soil.

**Statistical Analysis**

The results of each experiment have been expressed with standard error (SE) and Critical difference (CD) at 5% level. The standard error was calculated by formula:

$$SE = \sqrt{\frac{\text{Mean square of the error}}{\text{Number of replicates}}}$$

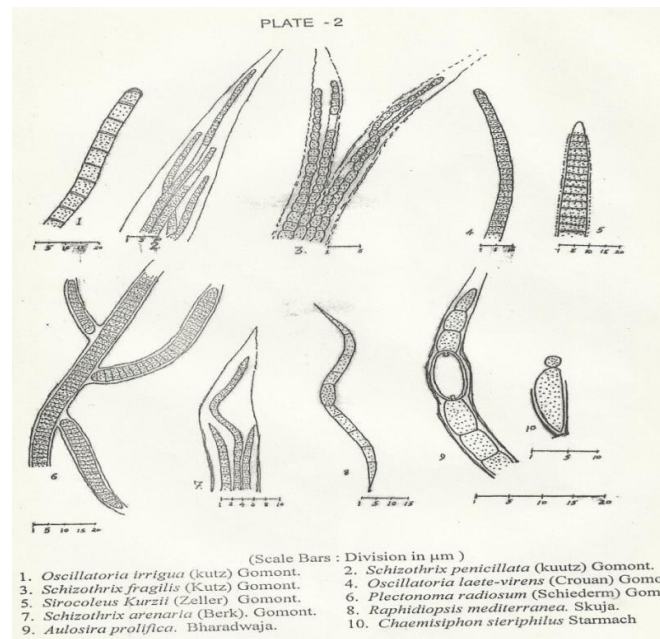
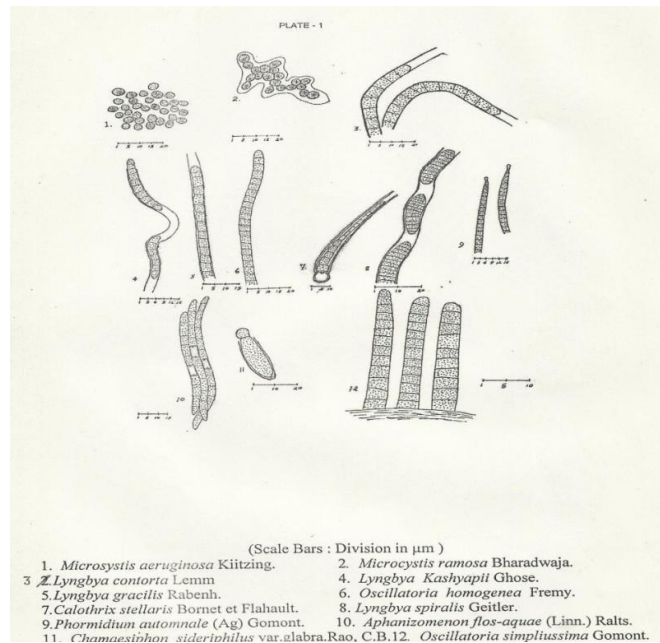
CD was calculated by the formula:

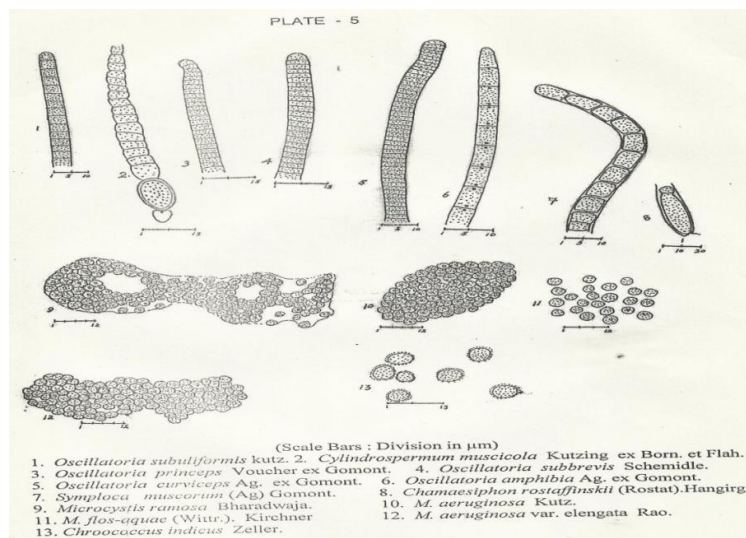
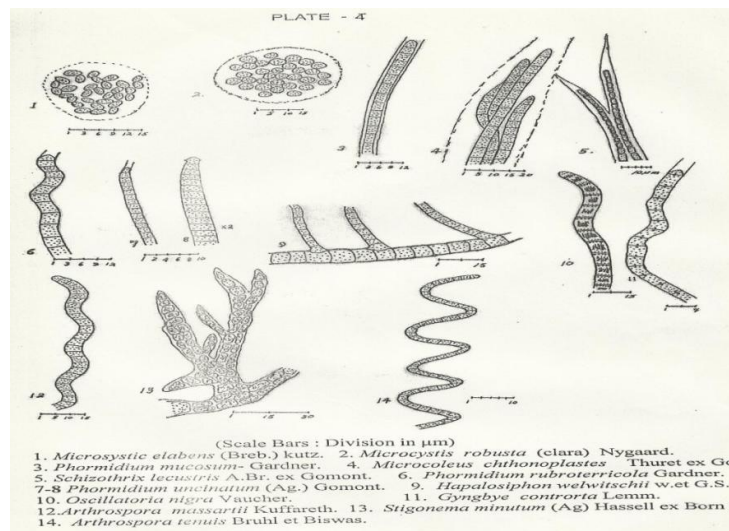
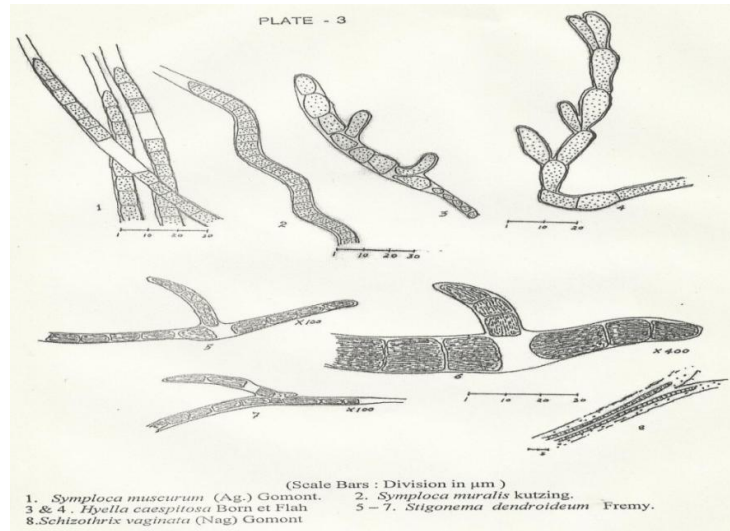
$$CD = SE \times P \times 2$$

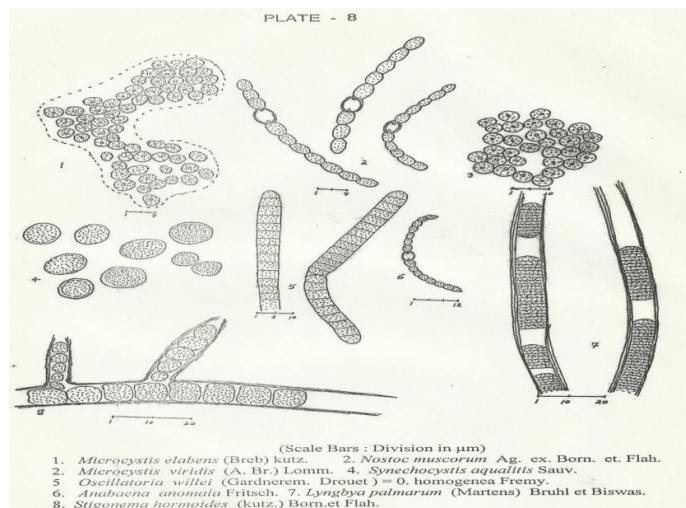
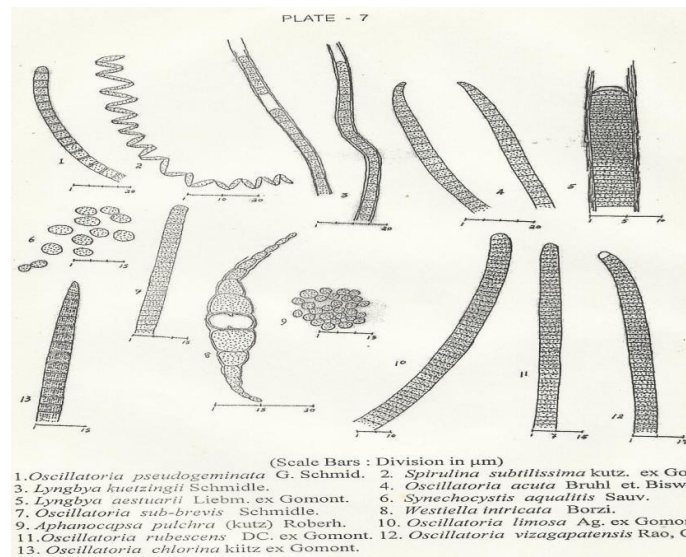
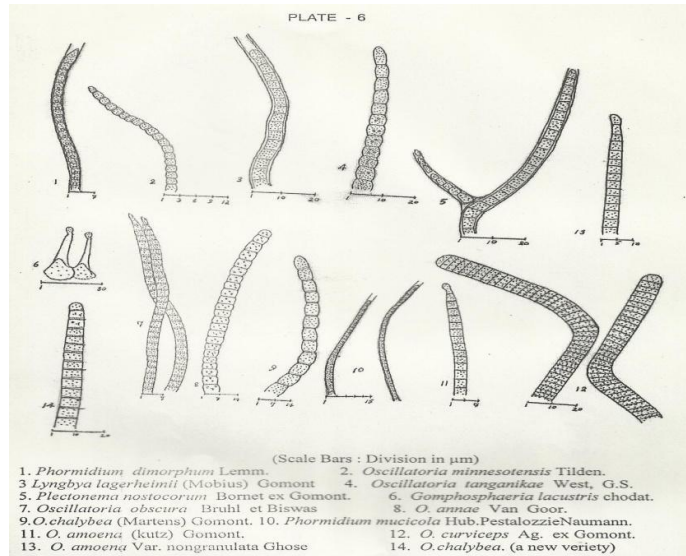
Where P is probability at 5% level

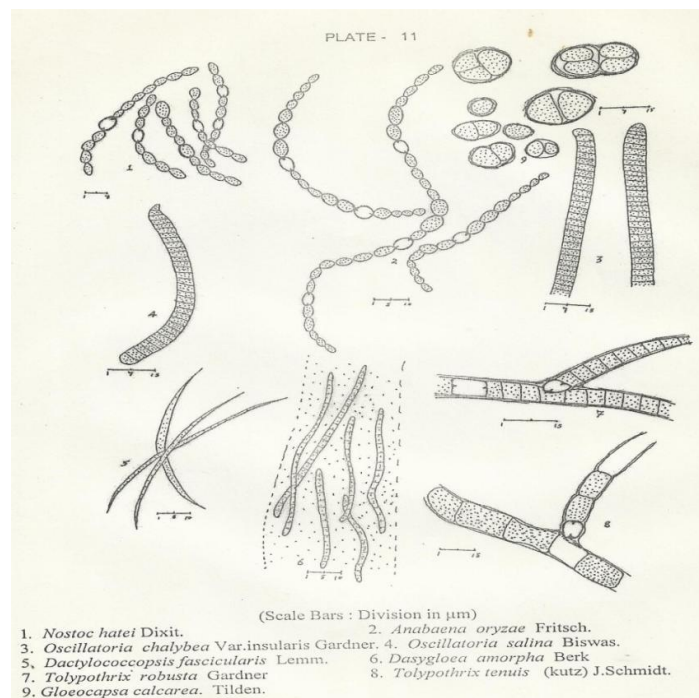
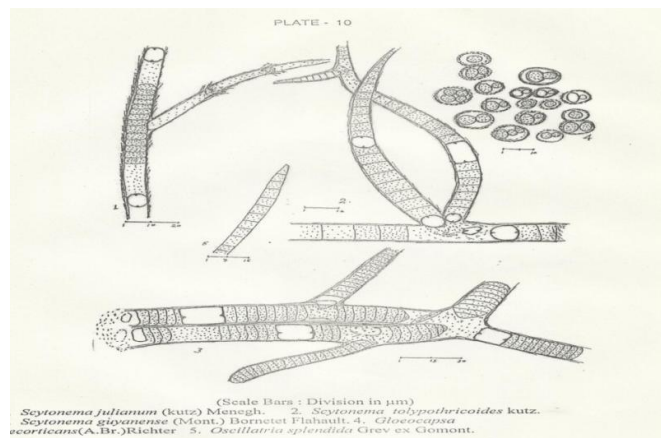
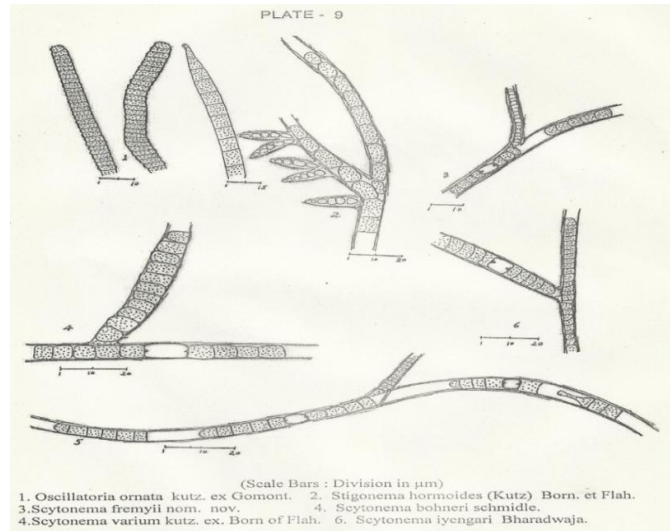
### III. Results

In course of a systematic study on soil cyanobacterial flora of three groups of agriculturally fertile soils 187 species of cyanobacteria belonging to 45 genera were recorded viz. *Microcystis* Kutzing, *Chroococcus* (Nag.), *Oscillatoria* (Vaucher), *Schizothrix* (Kutz.), *Lyngbya* (Ag.), *Phormidium* (Kutz.), *Ahanizomenon* (Morren.), *Calothrix* Ag., *Chamaesiphon* A. Br. et. Gurnow., *Sirocooleus* Kutzing., *Plectonema* Thuret., *Aulosira* Kirchnar., *Raphidiopsis* Fritsh et. Rich., *Symploca* Kutzing., *Hyella* Born. And Flah., *Stigonema* Ag., *Microcoleus* Desmazieres., *Arthrospora* Stizenberger., *Haplosiphon* Nag., *Cylindrospermum* Kutz., *Gomphosphaeria* Kutz., *Spirulina* Turpin em. Gardner., *Syneocystis* Sauvageau., *Westiella* Borzi., *Aphanocapsa* Nageli., *Nostoc* Vaucher., *Anabaena* Bory., *Scytonema* Ag., *Gleocapsa* kutzing., *Dactyloccopsis* Hansgirgi., *Dasyloea* Thwaites., *Tolypothrix* Kutzing., *Rivularia* (Roth) Ag., *Gloeotrichia* Ag., *Dermocarpa* Crouan., *Myxosarcina* Printz., *Synechococcus* Nag., *Coelosphaerium* Nag., *Hormothamnion* Solutum., *Rhabdoderma* Sp. Schmidle et Lautb., *Chlorogloea* Wille., *Aphanothece* Nag., *Gleoethece* Nag., *Merimopedia* Meyen. and *Johannesbaptistia* J.de Toni. (Photoplate-1-17)

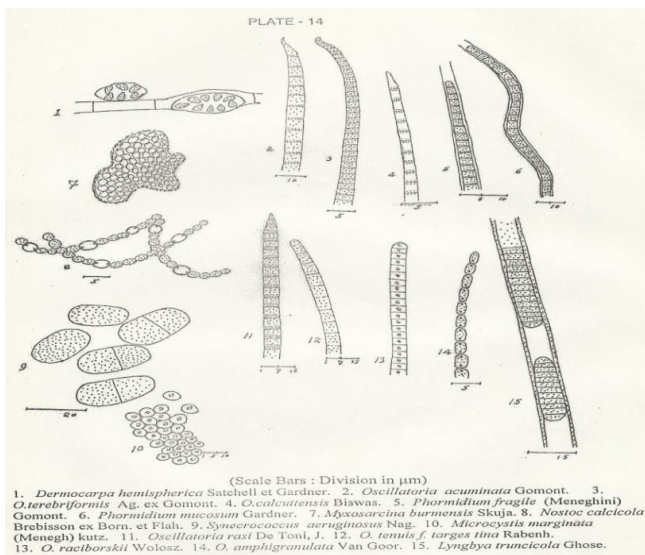
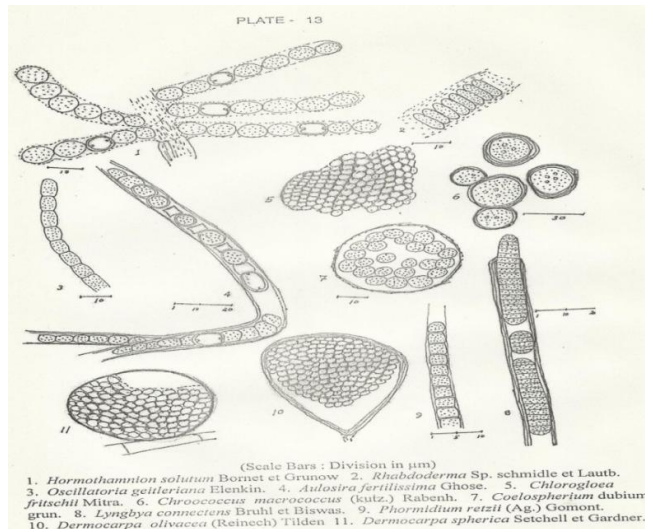
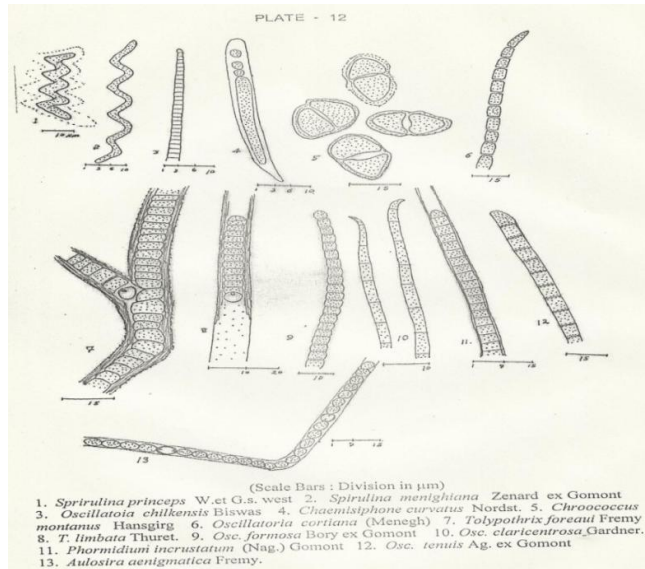


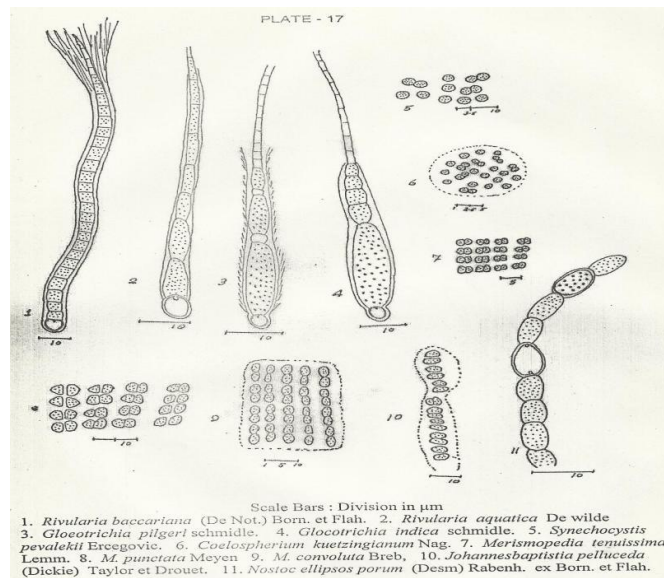
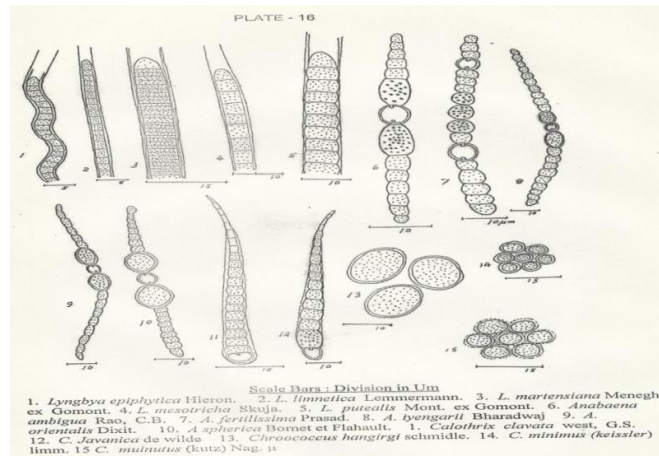
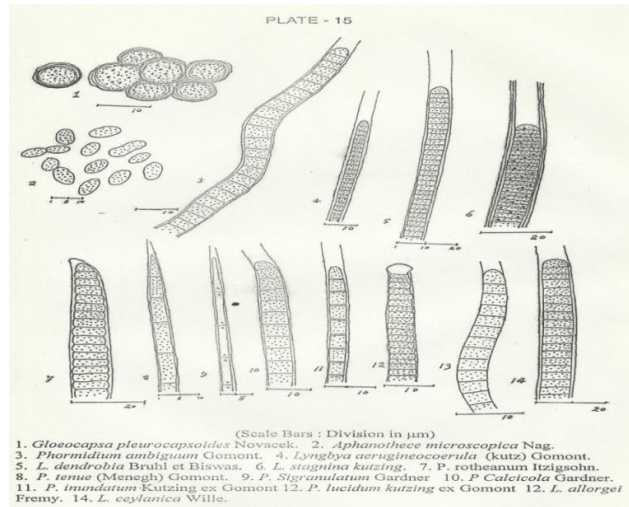












Altogether 187 species of cyanobacteria belonging to 45 genera were recorded from three groups of agriculturally fertile soils of Patna under investigation. But the number of species rounded from each soil group

was quit variable depending upon the ecological condition at the soil. Cyanobacteria flora founded in three groups of soil have been presented in Table-1.

**Table-1: Cyanobacteria in agriculturally fertile soil of Patna**

Cyanobacteria	Soil groupA	Soil groupB	Soil groupC
<i>M. aeruginosa</i>	+	-	-
<i>M. flos-aqual</i>	+	-	-
<i>M. ramosa</i>	+	-	-
<i>M.aerug. var elongate</i>	+	-	-
<i>M. robusta</i>			
<i>M.elabens</i>	-	+	-
<i>M. viridis</i>	-	+	-
<i>M.marginata</i>	+	+	+
	+	+	+
<i>Chroococcus. indicus</i>	+	-	+
<i>Chr. montanus</i>	+	-	+
<i>Chr. hansgirgi</i>	+	-	+
<i>Chr.minimus</i>	+	-	+
<i>Chr.minutus</i>	+	-	+
<i>Chr.macrococcus</i>	+	-	+
<i>Oscillatoria irrigua.</i>	+	-	-
<i>O. nigra</i>			+
<i>O.subuliformis</i>	+	+	+
<i>O.princeps</i>	+	+	+
<i>O.subbrevis</i>	+	-	+
<i>O.curviceps</i>	+	-	-
<i>O.amphibia</i>	+	+	-
<i>O.minnesotensis</i>	+	+	-
<i>O. tanganyikae</i>	+	+	+
<i>O. obscura</i>	+	-	+
<i>O. annae</i>	+	-	+
<i>O. chalybea</i>	+	-	-
<i>O. amoena</i>	+	+	+
<i>O. pseudogeminata</i>	+	-	+
<i>O. amonea var non granulated</i>	-	+	-
<i>O. chalybea</i>	-	+	-
<i>O. acuta</i>	-		-
<i>O. simplicissima</i>	-	+	-
<i>O. laete-virens</i>	+	-	+
<i>O. homogenea</i>	+	-	+
<i>O. limosa</i>	+	+	+
<i>O. rubescens</i>	+	+	+
<i>O. vizagapatensis</i>	+	+	+
<i>O. chlorinata</i>	+	-	+
<i>O. ornata</i>	+	+	+
<i>O. splendida</i>	+	+	+
<i>O. salina</i>	-	+	+
<i>O. chalybea</i>	-	+	+
<i>O. chickenis</i>	-	+	+
<i>O. cortiana</i>	-	+	+
<i>O. formosa</i>	+	+	-
<i>O. claricentrosa</i>	+	-	-
<i>O. tenuis</i>	+	-	-
<i>O. geitleriana</i>	+	-	+
<i>O. acuminata</i>	+	-	+
<i>O. terebriformis</i>	+	-	+
<i>O. culcuttensis</i>	+	+	-
<i>O. raoi</i>	+	+	-
<i>O. tenuis f.targestina</i>	-	+	-
<i>O. raciborskii</i>	-	+	-
<i>O. amphigranulate</i>			
	-	+	-
	+	-	+
	+	-	+
<i>Schizothrix vaginata</i>	-	+	+
<i>Sch. pencillata</i>			
<i>Sch. fragilis</i>	-	-	+
<i>Sch. arenaria</i>	-	-	+
<i>Sch. lLacustris</i>	-	-	+
	+	+	+
<i>Lyngbya gracilis</i>	+	+	+

<i>L. contorta</i>	+	+	+
<i>L. kashyapii</i>	+	+	+
<i>L. spiralis</i>	+	-	-
<i>L. lagerheimii</i>	+	+	+
<i>L. aestuarii</i>	+	+	+
<i>L. kuetzingii</i>	+	+	+
<i>L. palmarum</i>	+	+	+
<i>L. connectens</i>	+	+	+
<i>L. truncicola</i>	+	+	+
<i>L. dendrobia</i>	+	+	+
<i>L. aerugineo-coerulea</i>	+	+	+
<i>L. allorgei</i>			
<i>L. stagnina</i>	-	+	+
<i>L. ceylanica</i>	-	-	-
<i>L. epiphytica</i>	+	-	-
<i>L. limnetica</i>	+	-	-
<i>L. martensiana</i>	-	-	-
<i>L. mesotricha</i>	-	-	-
<i>L. putealis</i>	-	-	-
	-	+	+
<i>Phormidium</i>			
<i>automnale</i>	+	+	+
<i>P. muscosum</i>	+	+	+
<i>p. rubroterricola</i>	+	+	+
<i>p. uncinatum</i>	+	+	+
<i>p. dimorphum</i>	+	+	+
<i>p. mucicola</i>	+	+	+
<i>p. incrustatum</i>	+	+	+
<i>p. retzii</i>	+	+	+
<i>p. fragile</i>	+	+	+
<i>p. mucosum</i>	+	+	+
<i>p. ambigum</i>	+	-	+
<i>p. bigranulatum</i>	+	-	+
<i>p. calcicola</i>	+	-	+
<i>p. inundatum</i>	+	-	+
<i>p. lucidum</i>	+	-	+
<i>p. rotheanum</i>	+	+	+
<i>p. tenue</i>	-	+	+
<i>Aphanizomenon flos-</i>			
<i>aquae</i>	+	-	-
<i>Calothrix stellaris</i>	+	+	+
<i>C. clavata</i>			
<i>C. javanica</i>	+	+	+
	+	+	+
<i>Chamaesiphon</i>			
<i>C. sideriphilus</i>	+	+	-
<i>C. sideriphilus</i>	+	+	+
<i>C. rostafinskii</i>	+	+	+
<i>C. curvatus</i>	+	-	+
<i>Sirocoleus sp.</i>	-	+	-
<i>Plectonema</i>			
<i>P. radiosum</i>	-	+	+
<i>P. nostocarum</i>	-	+	+
<i>Aulosira</i>			
<i>A. prolifica</i>	+	+	+
<i>A. aenigmatica</i>	+	+	+
<i>A. fertilissima</i>	+	+	+
<i>Raphidiopsis</i>			
<i>mediterranea</i>	-	+	+
<i>Symploca muscurum</i>	-	+	+
<i>S. muralis</i>			
	+	+	-
<i>Hyella caespitosa</i>	-	+	-
<i>Stigonema</i>			
<i>dendroidum</i>	+	+	-
<i>S. minutum</i>	-	+	-
<i>S. hormoids</i>	-	+	-
<i>Microcoleus</i>			
<i>chthonoplastes</i>	+	+	-
<i>Arthrospora massartii</i>	+	+	+
<i>A. tenuis</i>	+	+	+
<i>Haplosiphon</i>	+	-	+

<i>welwitschii</i>			
<i>Cylindrospermum muscicola</i>	-	+	+
<i>Gomphosphaeria lacustris</i>	+	-	-
<i>Spirulina subtilissima</i>	-	+	+
<i>S. princeps</i>	-	+	+
<i>S. meneghiana</i>	-	+	+
<i>Synechocystis aquatilis</i>	+	+	+
<i>S. pevalekii</i>	-	+	+
<i>Westiella intricata</i>			
<i>Aphanocapsa pulchra</i>	+	+	+
<i>A. banarensis</i>			
<i>A. grevilli</i>	+	+	+
<i>A. Montana</i>	+	+	+
	+	+	-
<i>Nostoc muscorum</i>	-	+	+
<i>N. hatei</i>			
<i>N. calcicola</i>	+	+	+
<i>N. ellipsosporum</i>	+	+	+
<i>N. coeruleum</i>	+	+	+
<i>N. linckia</i>	+	+	-
<i>N. pruniformae</i>	+	+	-
<i>N. sphaericum</i>	+	+	-
	+	+	+
<i>Anabaena anomala</i>	+	+	+
<i>A. oryzae</i>			
<i>A. iyengarii</i>	+	+	+
<i>A. orientalis</i>	+	+	+
<i>A. spherica</i>	+	+	-
<i>A. ambigua</i>	-	+	+
<i>A. fertilissima</i>	-	+	+
	+	+	+
<i>Scytonemabohneri</i>	+	+	+
<i>S. varicum</i>	+	+	+
<i>S. freyiii</i>	+	+	+
<i>S. iyengari</i>	+	+	+
<i>S. julianum</i>	+	+	+
<i>S. tolypothrichoides</i>	+	+	+
	-	+	+
<i>S. quyanese</i>	-	+	+
<i>Gloeocapsa decorticans</i>	+	+	+
<i>G. calcarea</i>	+	+	+
<i>G. pleurocapsoides</i>	+	+	+
<i>G. punctata</i>			
<i>G. stegophilla</i>	+	+	+
	+	+	+
<i>Dictylococcopsis fascicularis</i>	+	-	-
<i>Dasygloea amorpha</i>	+	-	-
<i>Tolypothrix robusta</i>	+	+	-
<i>T. tenuis</i>			
<i>T. foreaui</i>	+	+	-
<i>T. limbata</i>	+	+	-
	+	+	-
<i>Rivularia beccariana</i>	+	+	+
<i>R. aquatic</i>			
	+	+	+
<i>Gloeotrichia pilgeri</i>	+	-	-
<i>G. indica</i>			
	-	-	+
<i>Dermocara hemispherica</i>	+	-	-
<i>D. olivacea</i>	-	+	-
<i>D. spherica</i>	-	-	+
<i>Myxosaricina burmensis</i>	-	+	-
<i>Synechococcus aeruginosa</i>	+	-	+

<i>Coelsphaerium dubium</i>	+	-	+
<i>C. kuetzingianum</i>	+	+	-
<i>Hormothamnion solutum</i>	-	+	+
<i>Rhabdoderma sp</i>	-	-	+
<i>Chlorogloea fritschii</i>	-	-	+
<i>Aphanothece microscopica</i>	+	+	+
<i>A. pallida</i>	-	+	+
<i>A castagnei</i>	+	+	+
<i>Gloeotheca rupestris</i>	+	+	+
<i>Merismopedia tenuissima</i>	+	+	+
<i>M. punctata</i>	+	+	+
<i>M. convolute</i>	+	+	+
<i>Johannesbaptistia pellucid</i>	-	+	+

Species of Cyanobacteria grow at any place and in any environment where moisture and sunlight are available. However, cyanobacterial species grow in specific environment and therefore, their distribution pattern, ecology, periodicity and occurrence differ widely. Ingress of industrial wastes, domestic wastes, sewage and plant debris etc, is main factors that determine the dominance of cyanobacteria in agriculturally fertile soils. With onset of favorable climatic conditions some cyanobacterial flora becomes dominant, increasing the fertility of soils.

From the results (Table-1) it is evident that a comparatively large number of cyanobacterial species was recorded from soils of group a. (138 species), followed by soils of Group:-B (135 species) and Group:-C (129 species). *M. aeruginosa*, *M. flos-aquae*, *M. ramosa*, *M. aeruginosa var elongata* *Oscillatoria irrigua*, *O.simplicissima* *O.formosa*, *O. clariontrosa*, *Aphanzomenon flos-aquae* *Gamphosphaeria lacustris*, *westiella intricate*, *Dictyococcopsis fascicularis*, *Dasgloea amarpha* *Gloeotrichia pilgeri* and *Dermocarpa hemispherica*, were recorded only from soils of group A, where cereal crops viz., Wheat, Rice and maize are being cultivated. *Microcystis robusta*, *M.elabens* *Oscillatoria raai*, *O.tenuis f.targestina*, *O.raciborskii*, *Lyngbya stagnina*, *L. limnetica*, *L. mariensiana*, *L.mesotricha*, *Sirocoleus sp.* *Raphidiopsis mediterranea*, *Hyella Caespiton* *Dermocara olivacea*, and *myxosarcina burmensis* were recorded from soils of group B where cereals and pulses crops both are being cultivated. *Rhadoderma sp.* and *chlorogloea fritshii* are isolated only from Group-C soils which are continuously used to cultivate only vegetable crops. *Oscillatoria homogenea*, *O.limosa*, *O.rubescus*, *P.chlorinata*, *O.ornata*. *Schizothrix lacustris*, *Lyngbya lagerheimii*, *L.aestuaryl*, *L.kutzingi*; *L. palmarum*, *L. connectens*, *L.runcicola*, *Phormidium autumnale*, *P. muscosum*, *p. rubroterricole*, *P. uncinatum*, *P. dimarphum*, *P. mucicola*, *P.incrustatum*, *P.retzil*, *P.fragile*, *P.mucosum*, *calothrix stellaris*. *Calolthrix clavata*, *c.javanica*. *Aulosira prolifc*, *A aenigmat*, *A. fertilissima*, *Arthrospora massartil*, *A, tenuis*, *Syecocystis aquatitit*, *Aphanocappa pulihera*, *A. banarensis*, *A grevillei*, *Nostoc hatei* *N. Callicola*, *N. ellipsoporom*, *N. sphaerium*, *Anabaena anomal*, *A.oryzae*, *A iyengarii*, *A.fertilissima*, *scytonema bohnerei*, *S. varicum*, *S. fremyil*, *S. iyengeri*, *S. julianum*, *Gloeopcsa decorticans*, *G. calcarea*, *G. pleurocapsoides*, *G. punctata*, *G. stegophila*. *Rivularia beccariana*, *R.aquatica*. *Aphanothece microscopie* *Gloeotheca rupestris*, *merismopedia tenuissima*, *M. punatata*, and *M.convolute* were recorded from all the three groups of soils. It reveals that maximum number of cyanobacteria species were recorded during spring (109 species) followed by rainy (68), winter (6) and summer (49).

The above findings suggest that the agriculturally fertile soils of Patna provide favourable conditions for the growth of wide range of cyanobacterial biodiversity. Soil habitats are the most important non-aqueous ecosystem for algae [36] (Zenova et al., 1995). The activities of algae contribute to soil formation, to the stability of mature soils [4] (Metting, 1981), and to the energy and matter fluxes in ecosystem [37] (Kuzyakhmetov, 1998a). Another important aspect of soil algae is nitrogen fixation. Algae contribute to the nitrogen content of the soil through the process of biological nitrogen fixation [38] (Goyel, 1997).

Green and blue-green algal populations and upper top soil are large and diverse, and they can perform valuable services for the soil ecosystems [4; 39] (Metting, 1981; Starks et al., 1981) and for agriculture too [40] (Ruble and Davis, 1988). One of the major benefits of algal functions in terrestrial habitats is the product of their photoautotrophic nutrition, the generation of organic matter from inorganic substances [41] (Alexander, 1977). They also serve as food source for bacteria and invertebrates, and biologically active compounds produced by algae can affect other components of soil communities, including plants [4; 36] (Metting, 1981; Zenova et al., 1995). Soil algal biomass in temperate lands are usually expressed as the number of cells per gram of dry soil, or as the number of cells per square meter of soil. In the former case, the algal biomass varies from 0 to 10<sup>8</sup> cells g<sup>-1</sup> soil (d.w.) [40; 41; 42; 43; 44] (Ruble and Davis, 1988; Sukala and Davis, 1994; Wohler et al., 1998;

Lukesova, 2001), while in the latter it ranges from 0 to 1011 cells m<sup>-2</sup> [ 45] (Lukesova and Hoffmann, 1996). Soil algae, especially cyanobacteria, are known to aggregate soil particles by producing extracellular polysaccharides [46] (Lynch and Bragg, 1985) and forming water-stable aggregates that reduce the impact of wind erosion [12] (Johansen, 1993).

In the present investigation month wise population density of cyanobacteria in three groups of soils was studied and the results obtained have been presented in Table-2

**Table-2: Showing population density of cyanobacteria in three groups of agriculturally fertile soil of Patna. Population density in (N×10<sup>3</sup> cells/g dry soil.)**

Month.	Group- A	Group- B	Group- C
January	960×10 <sup>3</sup> ±0.26	1050×10 <sup>3</sup> ±0.66	940×10 <sup>3</sup> ±0.38
February	910×10 <sup>3</sup> ±0.67	970×10 <sup>3</sup> ±0.47	906×10 <sup>3</sup> ±0.36
march	806×10 <sup>3</sup> ±0.6	905×10 <sup>3</sup> ±0.38	801×10 <sup>3</sup> ±0.49
April	715×10 <sup>3</sup>	811×10 <sup>3</sup> ±0.30	713×10 <sup>3</sup> ±0.53
May	610×10 <sup>3</sup> ±0.37	765×10 <sup>3</sup> ±0.41	640×10 <sup>3</sup> ±0.48
June	565×10 <sup>3</sup> ±0.45	650×10 <sup>3</sup> ±0.20	560×10 <sup>3</sup> ±0.27
July	1470×10 <sup>3</sup> ±0.63	1585×10 <sup>3</sup> ±0.26	1480×10 <sup>3</sup> ±0.38
August.	1476×10 <sup>3</sup> ±0.55	1650×10 <sup>3</sup> ±0.85	1485×10 <sup>3</sup> ±0.34
September.	1474×10 <sup>3</sup> ±0.73	1630×10 <sup>3</sup> ±0.80	1484×10 <sup>3</sup> ±0.26
October.	1263×10 <sup>3</sup> ±0.76	1445×10 <sup>3</sup> ±0.74	1365×10 <sup>3</sup> ±0.28
November.	1255×10 <sup>3</sup> ±0.37	1335×10 <sup>3</sup> ±0.67	1251×10 <sup>3</sup> ±0.69
December.	1130×10 <sup>3</sup> ±0.44	1275×10 <sup>3</sup> ±0.58	1150×10 <sup>3</sup> ±0.68

The total average density of cyanobacteria varied from 560×10<sup>3</sup> to 1650×10<sup>3</sup>/g of soil (dry weight). In all the three groups of soils the population density of cyanobacteria was minimum in June (565×10<sup>3</sup>/g in group A, 560×10<sup>3</sup>/g in Group C and 650×10<sup>3</sup>/g in group B) and maximum during rainy season (July to September). During rainy season i.e. the peak season of cyanobacterial growth the population density of cyanobacteria in three group of agriculturally fertile soil was in the following range.

$$\text{Group- B} \quad \text{Group- C} \quad \text{Group-A}$$

$$1585 \times 10^3 - 1650 \times 10^3 > 1480 \times 10^3 - 1485 \times 10^3 > 1470 \times 10^3 - 1476 \times 10^3.$$

The population density of cyanobacteria in group B and group C soils was more or less equal. Group-A soil is being used to cultivate only cereal crops and Group-C to vegetable crops. Soils of Group B which supported maximum cyanobacteria densities are being continuously used to cultivate both cereals and pulses crops.

#### IV. Discussion

At all the three sites, Cyanobacteria were represented with largest number of species, (138 species from group-A; 135 species from group-B and 129 species from group-C (Table-1).A comparatively large number of cyanobacterial species was recorded from soils of group a. (138 species), followed by soils of Group:-B (135 species) and Group:-C (129 species). *M. aeruginosa*, *M. flos-aquae*, *M. ramosa*, *M. aeruginosa var elongata* *Oscillatoria irrigua*, *O.simplicissima* *O.formosa*, *O. clariontrosa*, *Aphanzomenon flos-aquae* *Gamphosphaeria lacustris*, *westiella* intricate, *Dictylococcopsis fascicularis*, *Dasgloea amarpha* *Gloeotrichia pilgeri* and *Dermocarpa hemispherica*, were recorded only from soils of group A, where cereal crops viz., Wheat, Rice and maize are being cultivated. *Microcystis robusta*, *M.elabens* *Oscillatoria raoi*, *O.tenuis f.targestina*, *O.raciborskii*, *Lyngbya stagnina*, *L. limnetica*, *L. martensiana*, *L.mesotricha*, *Sirocoleus sp.* *Raphidiopsis mediterranea*, *Hyella Caespiton* *Dermocara olivacea*, and *myxosarcina burmensis* were recorded from soils of group B where cereals and pulses crops both are being cultivated. *Rhadoderma* sp. and *chlorogloea fritshii* are isolated only from Group-C soils which are continuously used to cultivate only vegetable crops. *Oscillatoria homogena*, *O.limosa*, *O.rubescus*, *P.chlorinata*, *O.ornata*. *Schizothrix lacustris*, *Lyngbya lagerheimii*, *L.aestuuaril*, *L.kutzingi*; *L. palmarum*, *L. connectens*, *L.runcicola*, *Phormidium autumnale*, *P. muscosum*, *p. rubroterricole*, *P. uncinatum*, *P. dimarphum*, *P. mucicola*, *P.incrustatum*, *P.retzil*, *P.fragile*, *P.mucosum*, *calothrix stellaris*. *Calothrix clavata*, *c.javanica*. *Aulosira prolifc*, *A aenigmat*, *A. fertilissima*, *A.grevillei*, *Nostoc hatei* *N. Callicola*, *N. ellipsoidorum*, *N. sphaerium*, *Anabaena anomal*, *A.oryzae*, *A iyengarii*, *A.fertilissima*, *scytonema bohneri*, *S. varicum*, *S. fremyil*, *S. iyengeri*, *S. julianum*, *Gloeocpsa decorticans*, *G. calcarea*, *G. pleurocapsoides*, *G. punctata*, *G. stegophila*. *Rivularia beccariana*, *R.aquatica*. *Aphanotheca* microscopic *Gloeotheca rupestris*, *merismopedia tenuissima*, *M. punatata*, and *M.convoluta* were recorded from all the three groups of soils. It reveals that maximum number of cyanobacteria species were recorded during spring (109 species) followed by rainy (68), winter (6) and summer (49). Species of *Microcystis*, *Oscillatoria*, *Schizothrix*,

*Lyngbya*, *phormidium*, *Aphanizomenon*, *Calothrox*, *Chamaesiphon*, *Chroococcus*, *Sytonema*, and *Gloeocapsa* and *Tolythrix* were recorded in all the four seasons. *Sirocoleus kurzii*, *Aulosira prolifica*, *Raphidiopsis mediterranea*, *Symploca muscorum*, *S. muralis*, *Hapalosiphon welwitschii*, *Spirulina Subtilissima* and *plectonema radiosum* were observed only during the monsoon months.

Similarly the species of *Merismopedia*, *Gloeothece*, *Aphanothece*, *Harmothamnion*, *Rhabdoderma*, *Chlorogloea Coelosphaerium*, *Myxosarcina*, *Synechococcus*, *Dermocarpa*, *Anabaena fertilissima*, *A. ambigua*, *A. sphaerica*, *A. orientalis*, *A. iyengarii*, *Tolypothrix limbata*, *T. foreaui*, *Synechocystis pevalekii* *Nostoc calcicola*, *N. elliposporum*, *N. Coeruleum*, *N. linckia*, *N. pruniformae*, *N. spraricum*, *Chroococcus micrococcus*, *C. montanus*, *C. hansgirgi*, *C. minimus*, *C. mintus*, *Spirulina princeps*, *S. meneghiana*, *Aulosira aenigmatica*, *Chamaesiphon curvatus*, *Calothrix javanica*, *C. clavata*, *Microcystis marginata*, *Johannesbaptistia pellucid*, *Rivularia beccariana*, *R. aquatic*, *Gloeotrichia pilgeri* and *G. indica* were recorded only during spring season. The species that were observed only in winter season are *plectonema nostocorum*, *Stigonema hormoides*, *Scytonema igengari*, *S. julianum*, *Glococapsa decorticans*, *Nostoc hatei*, *Westiella intricate*, *Aphanocapsa pulchra*, *Anabaena anomala*, *Dactylococcopsis fascicularis* and *Tolypothrix robusta*.

The above findings suggest that the agriculturally fertile soils of Patna provide favourable conditions for the growth of wide range of cyanobacterial biodiversity. The present findings is also in accordance with [49; 50; 47; 48] Santina Zancan et al., 2006; Goyal, S.K., 1997; Hoffmann., 1989; Hunt et al., 1979 who have recorded a wide varieties of cyanobacteria in terrestrial habitat.

As in other studies on soil algae where Cyanobacterial species were identified [45; 44] (Lukesova and Hoffmann, 1996; Lukesova, 2001), a great variety of species was recorded at each site. Though critical comparisons with published data are often hindered by different experimental conditions and quantization methods, often linked to the difficult and tedious nature of species identification, my finding confirm that *Oscillatoria homogenea*, *O.limosa*, *O.rubescens*, *O.chlorinata*, *O. crnata*, *Schizothrix lacustris*, *Lyngbya lagerheimii*, *L.aestuarii*, *L.kuitzingi*, *L.palmarum*, *L.connectons*, *L.truncicola*. *Phormidium automnale*, *P.muscorum*, *P.rubroterricola*, *P.unicinatum*, *P.dimorphum*, *P. mucicola*, *P.incrustatum*, *P.retzi*, *P.fragile*, *P.mucosum*. *calothrix stellaris*, *C. clavata*, *C. javanica*. *Aulosira prolifc*, *A.aenigmata*, *A.fertilissima*. *Arthrospora massartii*, *A.tenuis*. *Synechocystis aquatilis*, *Aphanocapsa pulechra*. *A. panarensis*, *A.grevillei*, *Nostoc hatei*, *N.calcicola*, *N.elliposporum*, *N.sphaericum*, *Anabaena anomala*, *A.oryzae*, *A.iyengarii*, *A.fertilissima*, *Scytonema bohnerei*, *S.varium*, *S.fremyii*, *S.iyengeri*, *S.julianum*, *Gloeocapsa decorticam*, *G. calcarea*, *G. pleuracapsaides*, *G.punitata*, *G. stegophila*, *Riuularia beccariana*, *R.aquatica*, *Aphanothece microscopic*, *Gloeothece repestris*, *Merismopedia tenuissima*, *M.puctata* and *M.convoluta*, can be considered cosmopolitan and widespread in different agriculturally fertile soils. In the present findings, these species were found in all the three groups of agriculturally fertile soil. The present findings are in accordance with and Starks and Shubert (1981) [50] who also identified a large number of Cyanobacteria in agriculturally soils. In the present findings it was found that all the three groups of soil support numerous heterocystous and non-heterocystous cyanobacteria.

Cyanobacteria were found abundant in all the three groups of soils, always more than  $100 \times 10^3$  cells/g of soil (d.w) with peaks of about  $1650 \times 10^3$  cells/g of soil (dw). Favorable temperature, pH and moisture content, as well as adequate light and abundant essential mineral nutrients would seem to be important in favoring such high cell concentration [40] (Ruble and Davis, 1988).

The group-B soil, in which both cereal and pulses crops are being cultivated, supported highest population density of cyanobacterial ( $1585 \times 10^3$ -  $1650 \times 10^3$  cells/g of soil (dw) followed by group-C ( $148$  to  $1485 \times$ cells/g of soil) and Group-A ( $1470$  to  $1476 \times 10^3$  cells/g soil d.w. (Table-3). The finding is also in accordance of Ruble and Davis, 1988 and Kuzyathmetov, 1998a) [40; 37].

## V. Conclusion

Cyanobacteria evolved very early in the history of life, and share some of the characteristics of gliding bacteria on one hand and those of higher plants on the other. They can both photosynthesize and fix nitrogen, and these abilities, together with great adaptability to various soil types, make them ubiquitous. Cyanobacteria also have a unique potential to contribute to productivity in a variety of agricultural and ecological situations. Cyanobacteria have been reported from a wide range of soils, thriving both on and below the surface. They are often also characteristic features of other types of sub-aerial environment and many intermittently wet ones such as rice fields. Most paddy soils have a natural population of cyanobacteria which provides a potential source of nitrogenfixation at no cost. Ammonia can be taken up by cyanobacteria through passive diffusion or as ammonium ( $\text{NH}_4^+$ ) by a specific uptake system. The amino acids arginine, asparagine and glutamine have also been reported to serve as nitrogen sources. Nitrate and nitrite are important sources, which later reduce into ammonia. Many cyanobacteria are also capable of using atmospheric dinitrogen ( $\text{N}_2$ ) as the source of nitrogen, and this is what most commonly termed nitrogen fixation. Like many other biological systems, nitrogen fixation in cyanobacteria is brought about by a high molecular weight, oxygen labile, metalloprotein enzyme known as



nitrogenase. Nitrogenase reduces molecular nitrogen to ammonia in presence of hydrogen. Due to this important characteristic of nitrogen fixation, the utility of cyanobacteria in agriculture to enhance production is beyond doubt.

Species of Cyanobacteria grow at any place and in any environment where moisture and sunlight are available. However, cyanobacterial species grow in specific environment and therefore, their distribution pattern, ecology, periodicity and occurrence differ widely. Ingress of industrial wastes, domestic wastes, sewage and plant debris etc, is main factors that determine the dominance of cyanobacteria in agriculturally fertile soils. With onset of favorable climatic conditions some cyanobacterial flora becomes dominant, increasing the fertility of soils.

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