ISOLATION AND CHARACTERISATION OF NOSTOCALES (CYANOBACTERIA) OF THE PADDY FIELDS OF KERALA

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IN

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By

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Under the guidance of

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Dedicated

to my

family members

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CERTIFICATE

Certified that the thesis entitled "**Isolation and Characterisation of Nostocales (Cyanobacteria) of the Paddy fields of Kerala**" submitted by Mrs. Umamaheswari, N.A. for the degree of **Doctor of Philosophy** in Botany of the University of Calicut is a bonafide record of research work done by her in this Department under my supervision. This has not previously been formed the basis for the award of any degree/ diploma.

Calicut University
30 May 2005

Prof. (Dr.) P. V. Madhusoodanan

DECLARATION

This thesis entitled **"Isolation and Characterisation of Nostocales (Cyanobacteria) of the Paddy fields of Kerala"** submitted by me for the degree of **Doctor of Philosophy** in Botany of the University of Calicut has not been formed the basis for the award of any other degree/diploma to the best of my knowledge.

Calicut University
30-05-2005

UMAMAHESWARI, N.A.

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Introduction

INTRODUCTION

Rice is the major crop of cultivation in India since centuries. Yet our National Average Production (NAP) is only 1.7 tonnes/hectare which is too less when compared to other rice producing countries. This is mainly because, more than 80% of the farming community, for obvious reasons, is unable to make use of the advanced agricultural technology especially, the use of nitrogenous fertilizers. It is known that rice crop is capable only to utilize about 35% of the added nitrogenous fertilizers because of the waterlogged conditions. Therefore, it is essential to develop a system that not only supplements nitrogen, but also enables the crop to utilize much of the applied nitrogen and thereby ensure sustainability in production. In this context, the nitrogen fixing cyanobacteria form the most potent biological system that promises nitrogen supply and its utilization as well (Upasana *et al.*, 2001). These are self-supporting, photoautotrophic microorganisms and can be produced in bulk with ease where water and CO_2 is available and temperature is suitable (Venkataraman, 1981 b, d). It has been reported that the genus *Nostoc* can improve the quality of nutrient poor soil and increase the productivity of unfertilized rice crops (Potts, 2000; Whitton, 2000).

Cyanobacteria, known as Blue-green algae until recently are ubiquitous prokaryotes. They are unique in their capacity for simultaneous aerobic phosphorylation and nitrogen fixation. Some time ago, they were considered to be the most primitive algae *i.e.*, Cyanophyceae/ Myxophyceae/ Blue-green algae. Being more akin to eubacteria in their structure and chemical composition they are currently being considered as oxygenic photosynthetic bacteria despite their structural specialization and non-cyclic photophosphorylation. They are related to eubacteria in their prokaryotic cellular organization, possession of a gram-negative cell wall made up of peptidoglycan and a gelatinous sheath liable to lysozyme treatment, absence of sexual reproduction, tolerance to high temperature and susceptibility to antibiotics, high degree of adaptability, non-sexual genetic recombination and ability to fix nitrogen. However, they show advancement in their structural diversity, inter-cellular transport, division of labour of cells into photosynthetic (vegetative)

cells, heterocysts and akinetes, absence of any type of motile organs (flagella/cilia) and oxygenic photosynthesis.

Cyanobacteria have been known to occur in the sediments from the early Precambrian period as components of stromatolitic microbial mats, which appear to have been formed as they are today (Schopf, 1983, 1992). This fact serves as a clue that these microorganisms played a major role in the evolution of an oxygenic environment (Fay, 1983). Ever since they originated, they evolved several acclimation and adaptive mechanisms, both structural and functional, as strategies for their survival and growth (Mann, 2000; Bhaya *et al.*, 2000; Hagemann, 2002; Komarek and Kastovsky, 2003). Thus these oxy-photosynthetic bacteria can be considered as the ancestors of the present day plants with chloroplasts (Chlorophyceae and other green plants) and red algal pigments (Rhodophyceae). They also share the common features of genomic structures and functions with bacteria (Ris and Singh, 1961; Lang, 1968; Stanier, 1977) and differ in possessing the faculty of aerobic phosphorylation (Haselkorn, 1978).

Cyanobacteria are either free living or symbiotic and can be broadly divided into three major groups-unicellular, filamentous non-heterocystous and filamentous heterocystous. The unicellular members consist of simple, single cells that may be ovoid, spherical, or cylindrical occurring as free cells or aggregated into a mucilaginous matrix. The cells in filamentous forms are arranged one above the other to form a 'trichome'. The trichome with a mucilaginous sheath around would constitute a 'filament'. The filamentous forms are being categorized based on the presence or absence of specially differentiated cells, the heterocysts into non-heterocystous and heterocystous filamentous forms. Uniform cell morphology in a filament is the characteristic feature of non-heterocystous filamentous forms like *Lyngbya, Phormidium* and *Oscillatoria.* Nevertheless, heterocystous filamentous forms may be of uniform width or showing base-apex difference and there are forms, which exhibit various types of false and true branching. Those exhibiting true branching constitute heterotrichous forms and they form the highest level of differentiation in cyanobacteria.

Based on the above characters the cyanobacteria are classified in both classical and modern systems. Recently, the blue-green algae are included under bacteria and termed cyanobacteria (Rippka *et al.*, 1979) mainly based on their similarity to bacteria in the prokaryotic organization of cell structure as revealed in electron microscopic investigation. In the classification proposed by Desikachary (1959) and Rippka *et al.* (1979), the cyanobacteria have been distinguished into five sub groups that more or less correspond to the earlier orders designated as Chroococcales, Chamaesiphonales, Pleurocapsales, Nostocales (including the families Oscillatoriaceae, Nostocaceae, Rivulariaceae, Microchaetaceae and Scytonemataceae) and Stigonematales.

In nitrogen fixing filamentous cyanobacteria, thick walled hyaline cells called heterocysts interrupt the trichome or filaments. Under conditions when combined nitrogen is depleted in the culture medium the gradual transformation of certain vegetative cells in the cyanobacterial filaments lead to the development of morphologically, physiologically, biochemically and functionally specialized cells called heterocysts (Stewart, 1980; Wolk, 1982, 2000). It has been reported that heterocysts are the sole site of aerobic nitrogen fixation among heterocystous cyanobacteria (Fay *et al.*, 1968). The heterocysts are specialized vegetative cells attributed with a number of functions. They vary in size (bigger, smaller or equal to the vegetative cells), shape (spherical, oval or cylindrical), position (terminal and / or intercalary) and number and are considered to be of physiological significance. Heterocystous cyanobacteria include *Anabaena, Nostoc, Cylindrospermum, Aulosira, Calothrix, Rivularia, Scytonema, Tolypothrix, Hapalosiphon* and *Westiellopsis.* It has been established that they are the seats of the enzyme nitrogenase-an exclusive and elusive enzyme of prokaryotes-that is responsible for nitrogen fixation (Fleming and Haselkorn, 1973; Bothe, 1982; Kannan, 2004). They play a role in reproduction (Anand, 1980) and in some cases they regulate sporulation also (Wolk, 1965, 1982; Singh *et al.*, 1972; Anand, 1978).

Differentiation of heterocyst creates an intracellular anaerobic atmosphere wherein the enzyme nitrogenase can perform optimally. The vegetative cells and the heterocyst need to function in harmony and they represent one of the best examples of biochemical symbiosis - the vegetative

cell supplying the photosynthate to the heterocyst and the latter fixing nitrogen to the vegetative cell in return (Haselkorn, 1978; Stewart, 1980; Apte, 1992). The co-existence of oxygenic photosynthesis and nitrogen fixation in a single organism is an obvious case of paradox, since oxygen is completely inimical to the intrinsically anaerobic process of nitrogen fixation. The same photosynthesis that provides the requirements of nitrogen fixation becomes the cause of its inhibition. This is because; the oxygen released during photosynthesis brings about the following changes-

1. Degrades the oxygen liable nitrogenase proteins.
2. Destroys the anoxic environment essential for the enzyme activity.
3. Represses the transcription of *nif* genes.

The biochemical modification of heterocysts to create an anoxic environment/niche is by preventing oxygen evolution of PS II through the degradation of phycobilin proteins (Fay, 1969; Thomas, 1970).

The nitrogenase enzyme complex comprises of two component proteins:

1. Mo-Fe protein which is also known as dinitrogenase and
2. The Fe protein also known as dinitrogenase reductase.

Both enzymes are essential for substrate reduction (Hallenbeck, 1987). The overall reaction of nitrogenase catalysis can be represented by a simple equation.

$$N_2 + 8e^- + 8H^+ + 16ATP \xrightarrow[\text{Absence of oxygen}]{\text{Nitrogenase enzyme complex } Mg_2^+} NH_3 + H_2 + 16ADP + 16iP$$

The source of ATP for cyanobacterial nitrogenase activity is obtained through photophosphorylation (Bottomley and Stewart, 1976). Oxidative photo-phosphorylation supports ATP pool during short periods of darkness. Local ATP pool governs the N_2 fixation in heterocyst rather than the pool adjoining the vegetative cells.

In addition to heterocysts, certain genera of filamentous cyanobacteria possess specialized resting cells called akinetes. There is an apparent relationship between akinetes and heterocysts (Stulp and Stam, 1982). In *Anabaena, Cylindrospermum, Gloeotrichia* and *Calothrix* akinetes have been

seen to arise adjacent to the heterocyst (Geitler, 1925) and in *Nostoc* sp. a chain of akinetes arise midway between two heterocysts. Formation of akinetes in cyanobacteria enables them to survive during long periods of desiccation and then germinates to produce new filaments (Fay, 1983).

An akinete develops from a vegetative cell that becomes enlarged and filled with food reserves and augments its wall extremely by additional nutrients. After a period of dormancy, the akinete germinates and gives rise to trichome (Sutherland *et al.*, 1979; Nichols and Adams, 1982). The protoplasm of akinete has reduced amounts of photosynthetic pigments, but accumulates large amounts of cyanophycin granules (protein reserves). The distribution of akinetes in cyanobacterial filaments varies according to the species. In strains of *Cylindrospermum,* akinetes always develop adjacent to the terminal heterocyst, whereas in other members of Nostocaceae they may occur singly or in chains either next to or away from the heterocyst.

The normal growth pattern of cyanobacteria is affected by various factors such as physico-chemical, biotic, *etc.*, which affect their vegetative growth, heterocyst induction and the pattern of akinete development. Since these members have greater adaptability to any type of extreme environments they escape from the adverse situations and survive in that condition for many years. This is achieved by changing their lifestyle aptly to the surroundings. The release of various extra-cellular compounds by cyanobacteria to withstand the various stresses during their life period makes the surroundings or habitat congenial for their life activities.

Though cyanobacteria are known for their remarkable adaptability to any type of surroundings their growth and establishment in paddy fields are affected by a number of factors-both abiotic and biotic. An analysis of 2213 soil samples from 15 states of rice growing areas of India revealed the presence of cyanobacteria only in 33% samples dominated by *Aulosira fertilissima* and *Nostoc* sp. (Venkataraman, 1975) - even though hundreds of species have been found enhancing crop production. The lack of knowledge about the various factors that affect the growth of this biofertilizer has very often resulted in their marked decrease and death. In several parts of the country,

cyanobacterial production has been commercialized (Venkataraman, 1981c,d; Goyal, 1993; Kannaiyan, 1993). In Orissa, several training programmes are organized to appraise farmers, members of youth clubs, NGOs and officials of agriculture regarding production, quality testing and economics of the technology for capitalist development (Adhikary *et al.*, 2001). Most of the studies in algalization (in relation to cyanobacteria) have been uncritical (Roger, 1991). Though cyanobacterial inoculation trials (Subramanyan, 1972; Watanabe, 1973; Singh & Singh, 1987; Naik, 2000) have been carried out using inoculum developed from a mixture of laboratory cultures, almost none of the published inoculation experiments have paid attention to the establishment of inoculation strains. These studies have not given apt importance to biological and environmental factors that may lead to the subsequent establishment and multiplication of cyanobacteria in the inoculated environment. Earlier studies have shown a remarkable strain variation amongst the cyanobacteria with respect to their growth and nitrogen fixing capacity (Kolte and Goyal, 1986; Roychoudhary *et al.*, 1986). Existence of remarkable variations even at intra-specific level that alter the nitrogen fixing ability and biomass production has thrown open the possibility of selecting the desired and suitable strains through screening the autochthonous cyanobacteria (Goyal, 1989).

Importance of the study

Paddy is one of the important food crops of the world. It is the staple food for over half of the world's population and it provides 27% of dietary energy supply and 20% of dietary protein intake in the developing world (FAO, 2004) and in India, it is the most important and extensively grown cereal occupying an area of 40 million hectares with an average production of 17 quintals / hectare. It provides 20% of the global human per capita energy and 15% of the per capita protein (Roger, 1995). Of the entire nutrient that the rice plants need, nitrogen is perhaps the most vital, as it governs planned crop growth, yield and ultimately, the productivity. The nitrogen requirement of rice accounts for about ⅓ of the total nitrogen consumption in the country (Swaminathan, 1983).

Rice plants are incapable of utilizing atmospheric nitrogen (which occupy 78% of the atmospheric gases) directly, as they require N_2 in the form of nitrates

or ammonium compounds. Today, for a developing country like India, one of the most important limiting factors in crop production is the synthetic nitrogen fertilizer which is on great demand and quite expensive. As the over use of chemical fertilizers lead to coupled crop destruction and soil deterioration, a wide interest in this aspect is now on *Biological Nitrogen Fixation* as a supplement. Here, the cyanobacteria and *Azolla* (an aquatic fern) play an important role in increasing soil fertility as well as productivity of rice (Upasana *et al.,* 2001). The use of these biofertilizers exhibit several advantages over chemical fertilizers, including their one time application, efficient uptake, no leaching, balanced nutrition of crops, no destruction of soil qualities, enhancing soil binding, allowing effective check of pest attacks, weed suppression and high cost benefits. They are very useful for marginal farmers, in land reclamations and in rainfed areas. Moreover, they are non-polluting, used in low dosages, and therefore, have low transportation and storage costs (Vivek, 2005). It has been suggested that carrier based basal and foliar cyanobacterial biofertilizers provide a complete range of the necessary nutrients for crop growth and productivity (Malliga, 2004).

One of the major functions of nitrogen cycle in the soil is the cyanobacterial nitrogen fixation. These unique prokaryotic microbes augment the nitrogen levels and hence this area of study has evoked considerable interest. These, being congenial to the low land ecosystem, are first-rate biofertilizers in paddy fields. The information regarding the native cyanobacteria of Kerala is essential so that they can be successfully employed in paddy fields as a cost-effective and safe biofertilizer. Once successful, cyanobacterial production can be commercialized which will be a very promising field assuring high yield to farmers at low cost. Hence the study is highly essential.

Objectives of the study

The success of cyanobacterial application in rice fields depends on the local circumstances and local awareness. The information regarding the native cyanobacteria (of Kerala) being inadequate, a vivid knowledge in this aspect is essential for its application as a biofertilizer.

Of the various orders of cyanobacteria, the Nostocales include the dominant cyanobacterial biofertilizer actively participating in BNF. Hence it has been chosen for the present study. The present study is aimed at the assessment, both quantitatively and qualitatively of the cyanobacterial wealth in the rice fields and adjoining areas of different regions of Kerala (belonging to the order Nostocales) in a random way. This includes isolation, identification, and characterization of some of the nitrogen fixing cyanobacteria in field and laboratory conditions. The characterization study includes:

1. The systematic assessment of cyanobacteria at species level based on field collection.
2. Selection of suitable growth media and elucidation of growth and thallus characteristics of some selected cyanobacterial isolates in solid and liquid media based on morphological parameters.
3. Study of responses of selected cyanobacterial strains to various parameters like, pH, salinity, nutritional amendments, agroprotectors like, pesticide, insecticide and fertilizers under laboratory conditions.

Major characteristics of the rice field soils of Kerala

Based on fertility status and, eco-geographical features and physico-chemical properties, the rice fields of Kerala have been classified into eight types (Karmachandran, 1987; Anilakumar, 1989). They are, (Fig. 1)

1. **Karappadom soil:** Seen in the upper part of the Kuttanad range along the inland waterways. The soils are generally deep with poor drainage, acidic-saline (pH 4.35) and dark grey in colour.
2. **Kari soil:** Seen in the Alappuzha-Kollam areas (Kuttanad range). Dark coloured soil, rich in acid PO_4 (pH 3.61) and organic matter.
3. **Kayal soil:** Found in the reclaimed lakebeds of Kottayam and Alappuzha areas, in Kuttanad range. The soils are deep, poorly drained, acidic (pH 4.18) and dark brown in colour.
4. **Kole soil:** Occurs in Thrissur (Thrissur and Mukundapuram taluks) and Malappuram (Ponnani taluk) districts; rich in organic matter, acidic (pH 4.56) and resembles Kuttanad soil.

5. **Laterite soil:** Spreads along the mid land physiographic divisions and also the terrace soils of Wayanad and Idukki areas of Kerala - acidic (pH 5.17), deep and fine textured.
6. **Pokkali soil:** Distributed in Alappuzha, Ernakulam and Thrissur areas; acidic (pH 3.51) but salinity is observed due to inundation of seawater.
7. **Poonthalpadom soil:** Seen in different taluks of Palakkad district. Clayey, sticky, plastic, deep and alkaline (pH 7.74).
8. **Sandy Alluvium:** Found in the Karunagappally and Mavelikkara taluks of Alappuzha-Kollam areas and coastal belts of other districts; coarse textured, coloured grey-brown, acidic (pH 5.04) and contains poor organic matter.

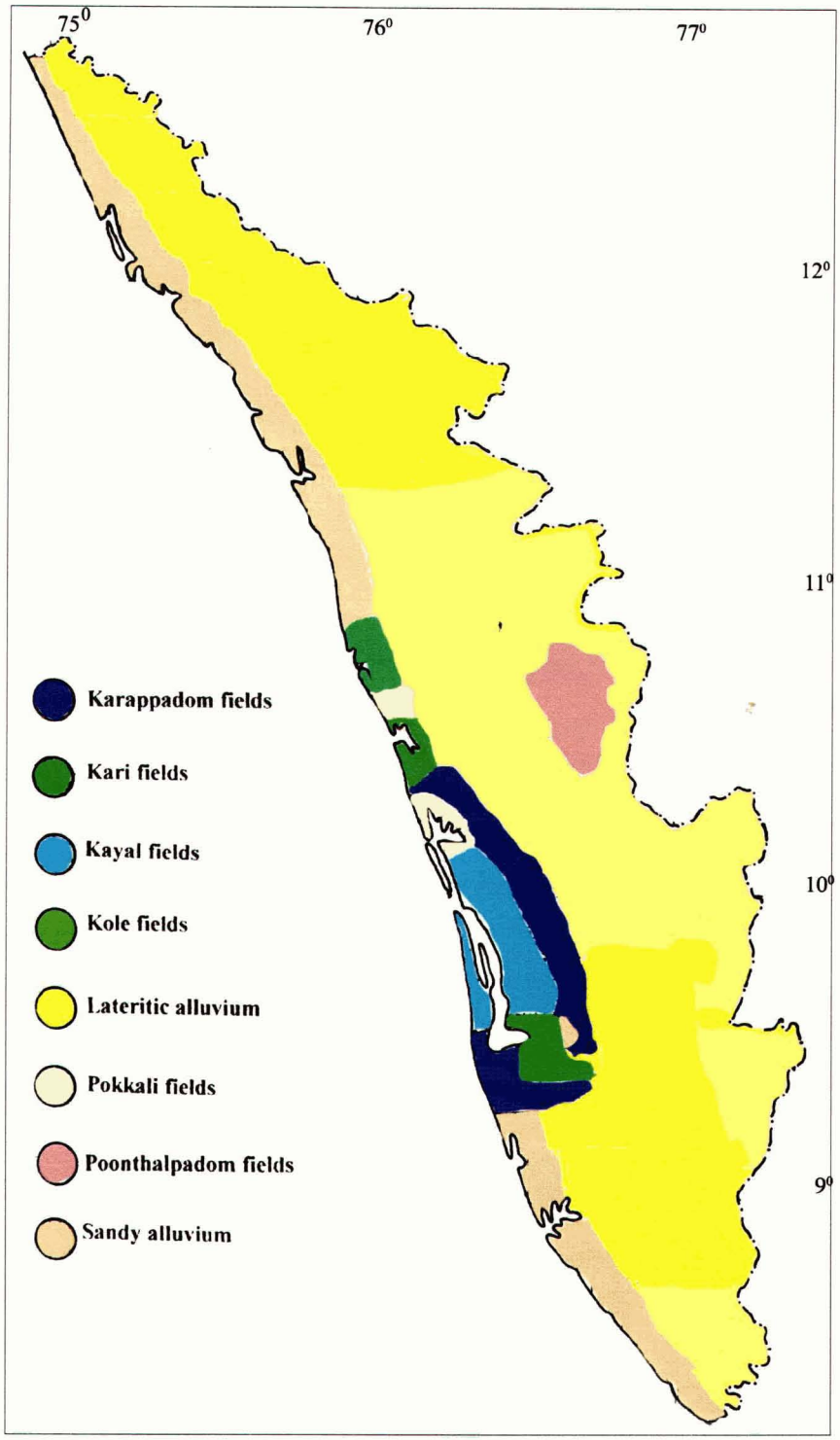


Fig. 1. Map of Kerala showing different soil types with special reference to rice fields

REVIEW OF LITERATURE

Umamaheswari N. A. "Isolation and Characterisation of Nostocales (Cyanobacteria) of the Paddy fields of Kerala " Thesis. Department of Botany , University of Calicut, 2005

Review of the Literature

REVIEW OF LITERATURE

The significance of cyanobacteria (BGA) was first recognized by De (1936,1939) and Singh (1942), who held them responsible for the natural fertility of the rice field soils. Since then, a series of reports have appeared emphasizing their role in nitrogen cycle in general and rice field soils in particular (De and Mandal, 1956; Cameron and Fuller, 1960; Singh, 1961a; Stewart *et al.*, 1979; Roger and Kulasooriya, 1980; Venkataraman, 1981a,b and Goyal, 1982). It has been reported that cyanobacteria may contribute 20-30 Kg biologically fixed nitrogen per hectare in rice cultivation with an economic efficiency of 1: 25 (Venkataraman, 1972, 1981a,b,c; Goyal, 1982; Sharma and Gupta, 1983) and also improve the physico-chemical properties of the soil significantly (Aiyer *et al.*, 1972; Marathe, 1972 and Roychoudhury *et al.*, 1979), thereby maintain soil fertility and sustainability (Goyal, 1992; Kannaiyan, 2001). Roger (1991) after 260 field experiments concluded that the cyanobacterial inoculation might enhance the average grain yield by 3 q/ha. Singh *et al.* (1992) noted a maximum increase in grain yield up to 6.25 q/ha in different varieties of paddy by cyanobacterial inoculation in 10 districts of U.P. Further use of cyanobacteria in the paddy fields can add something more than BNF, for example, vitamins, amino acids and other growth regulating substances which may be beneficial to the paddy plants (Singh *et al.*, 1992). In addition, they excrete polysaccharides that bind the soil particles and improve soil aggregation (Kaushik, 2001). Considering the economy of cyanobacterial application, it was noticed that by using cyanobacteria as a source of 30 Kg nitrogen/ha, an average increase of 2.56 q/ha in paddy yield could be achieved which results in an additional income of Rs 766.90/ha (Singh *et al.*, 1998).

Reports are available on the cyanobacterial flora of the rice fields of various states of India. Some of them are:

1. Andhra Pradesh: Laloraya and Mitra, 1973; Dikshit and Pandey, 1986.
2. Arunachal Pradesh: Singh *et al.*, 1997c.
3. Assam: Deka and Bordoloi, 1991; Saikia and Bordoloi, 1994.
4. Bihar: Laloraya & Mitra, 1973; Jha *et al.*, 1986; Verma *et al.*, 1990.
5. Goa: Bongale, 1981.

6. Jammu and Kashmir: Goyal *et al.*, 1984; Sharma and Kemi, 1992.
7. Karnataka: Bongale and Bharati, 1980; Shivaram and Shetty, 1983,1988; Bongale, 1985; Singh and Bongale, 1990.
8. Kerala: Parukutty, 1940; Aiyer, 1965; Amma *et al.*, 1966; Tiwari, 1972,1975; Laloraya and Mitra, 1973; Anand and Hopper, 1987,1995; Shaji and Panikkar, 1994; Madhusoodanan and Dominic, 1995.
9. Madhya Pradesh: Tiwari, 1972,1975; Mishra and Purohit, 1979; Sangita and Naik, 1996.
10. Maharashtra: Marathe, 1960; Tiwari, 1972, 1975; Kamat & Patel, 1973; Goyal, 1964; Sardeshpande and Goyal, 1981a; Kolte and Goyal, 1985; Patil and Satav, 1986.
11. Mizoram: Singh *et al.*, 1996.
12. Nagaland: Singh *et al.*, 1997b.
13. North Eastern region: Reddy *et al.*, 1986; Deka and Bordoloi, 1991; Saikia and Bordoloi, 1994.
14. Orissa: Parija *et al.*, 1969; Laloraya and Mitra, 1973; Singh, 1978; Padhy *et al.*, 1992; Sahu *et al.*, 1996.
15. Punjab: Grover and Pandhol, 1975.
16. Tamil Nadu: Tiwari, 1972,1975; Laloraya and Mitra, 1973; Anand and Revathi, 1987; Ramakrishnan and Kannan, 1992.
17. Tripura: Singh *et al.*, 1997d.
18. Uttar Pradesh: Singh, 1942; Pandey, 1965; Shukla, 1971.
19. West Bengal: Laloraya & Mitra, 1973; Saha and Mandal, 1979; Chatterjee & Chatterjee, 1983.

Thus, the potential of cyanobacteria in rice cultivation has been well documented.

During the last two decades, apart from the biofertilizer efficiency, cyanobacteria have grabbed the attention from other biotechnological fields such as biological fuel production, amino acid production, antibiotic production, single cell protein production and the production of other secondary metabolites and pigments used in food and other industries. Ge-Xian Mi *Nostoc* is an edible cyanobacterium and studies reveal that small sized colonies grew faster and produced higher biomass than large sized ones (Gao and Ai, 2004).

Cyanobacteria dominated biofilms of rocky shores often form high quality food resource for intertidal coastal ecosystems (Nagarkar *et al.*, 2004). Cyanobacteria play a vital role, in environment management (Subramanian and Uma, 1999). They are considered to be alleviating the pollutants to some extent. For example, *Oscllatoria–Lyngbya* mixed culture is reported to have maximum efficiency in removing arsenic compounds from the surroundings (Samal *et al.,* 2004). Using environmentally friendly biofertilizer like cyanobacteria mixed with lesser amounts of nitrogenous fertilizers, a reduction in mosquito larval breeding that promote Japanese encephalitis in rice fields and at the same time a better crop yield has also been assured by the scientists (Anil, 2000). Recently the hyper saline (salinities exceeding those of normal sea water) cyanobacerial mats are found to be acting as sensitive short and long-term indicators of elevated tropical hurricane activity and associated climatic and ecological changes impacting these and other water stressed environments (Paerl *et al.*, 2003).

Distribution, occurrence and structure:

Cyanobacteria are everywhere, where their life is possible. In their habitat and distribution, they are equaled to bacteria (Fogg *et al.*, 1973). The diazotrophic nature coupled with the great adaptability to thrive in the climatic extremes as well as their tolerance to other factors in the environment make these organisms ubiquitous (Roger and Kulasooriya, 1980; Kaushik, 1987). They occur in aquatic and terrestrial environments, in high extreme habitats and also in N_2- fixing association with almost all groups of plants (Sprent and Sprent, 1990). They occur as biological crusts on soil and rock in semiarid, arid and under arid microclimatic conditions in all ecoregions in earth (Budel, 1999, 2002; Tirkey and Adhikary, 2004). Budel *et al.* (2002) reported the existence of cyanobacteria on exposed rock surfaces of Inselbergs of the Atlantic rain forest zone in South-eastern Brazil, uninfluenced by higher vegetation. Fritsch (1945) aptly stated that the cyanobacteria are highly successful group, which establish themselves on new habitat. Since they are photoautotrophic, they are used as model organisms for the study of the mechanism and regulation of oxygen producing photosynthesis (Takakazu and Tabata, 1997).

Cyanobacteria are prokaryotic organisms with unique quality of nitrogen fixation and oxygenic photosynthesis. The name `Cyanobacteria' emphasizes two aspects: Its prokaryotic nature and fairly close resemblance to eubacteria. In cellular organization, they resemble bacteria and their photosynthesis is akin to eukaryotic algae and higher plants. They contain the pigments chlorophyll *a* and the phycobilin, the water-soluble biloproteins (phycocyanin, phycoerythrin and allophycocyanin). They do not possess flagella. Food reserves are glycogen and cyanophycin protein. Sexual reproduction is absent and asexual reproduction is by fragmentation, hormogones and by akinetes.

Cyanobacteria are included under two broad categories of morphological organizations namely, unicellular or coccoid forms and filamentous forms. The filamentous forms are separated on the basis of the presence or absence of specially differentiated cells called heterocysts, into heterocystous or non-heterocystous filamentous forms. The heterocystous forms may be unbranched or branched. If branched, they exhibit various types of true or false branching.

In majority of the N_2-fixing filamentous cyanobacteria, there are three distinct type of cells arranged in an intercalated pattern at certain stage of growth period. Of these, two cells namely, the heterocyst and the akinete arise from the differentiation of the third type of cells, the vegetative cells. Heterocysts are specialized structurally and physiologically, so as to perform the act of nitrogen fixation by a complex series of ultra structural and chemical changes (Haselkorn, 1978; Wolk, 1989). It has been established that bulk of nitrogen fixation occurs in the thick walled heterocysts (Wolk, 1982, 2000).

The relationship between the heterocyst and the vegetative cells is one of the mutual interchange of materials. The vegetative cell lacking the ability of nitrogen fixation is dependent on the heterocyst and the heterocyst is dependent on vegetative cells for the supply of carbohydrate (Plazinski, 1997). Heterocysts can be distinguished from vegetative cells by the hyaline protoplasts, which are often yellowish and are characterized by the absence of granular reserve materials and gas vacuoles. Polar nodules are present. The heterocyst cell wall consists of three layers – an outer polysaccharide fibrous layer, a homogeneous central layer and an inner glycolipid layer (Wolk, 1982). The polar nodule is

pierced by several fine canals, which connect the heterocyst with adjacent vegetative cells. The thick wall of heterocyst is found to reduce the diffusion of atmospheric gases in the cell to such an extent that all the oxygen is used up for respiration. A further adaptation of the heterocyst is the loss of the oxygen-evolving component of the photosynthetic apparatus, photo system II (including the phycobilin proteins). Photo system I is retained and in the light, generates the energy (reducing power) necessary for the reduction of nitrogen (in the form of ATP). Additional energy and reducing power are provided by dark respiration, using the oxygen that seeps into the heterocyst. In the heterocyst fixed nitrogen is at first stored in the form of cyanophycin and later exported to the neighbouring vegetative cells as glutamine. Carbon is probably imported from adjacent undifferentiated vegetative cells (Wolk, 1968) in the form of low molecular disaccharides (maltose). Carbon and nitrogen transports are found to take place via the fine channels in the polar nodule.

Heterocysts occur singly and at regular intervals along the cyanobacterial filament. Wilcox *et al.* (1973) observed that the normal pattern of heterocyst spacing is genetically determined. This has put forth the suggestion that vegetative cells produce an inhibitor of differentiation of heterocysts that requires activation by a co-inhibitor produced by heterocysts (Adams, 1992). Vegetative cells known to possess a factor (VF_1) that binds to *nif* H and also to *sis* A, a gene involved in heterocyst differentiation. A special type of genes in groups the *nif* genes controls the art of nitrogen fixation (Haselkorn, 1986). In *Anabaena* 12, these genes are localized in the chromonema. By conducting mutation studies, the nature of various products of *nif* genes has been determined. Now it is confirmed that *nif* HDK operon encodes nitrogenase, whereas *nif* LA has regulatory function. In the *nif* HDK genes, the genes H, D, and K encode for subunit of Fe-protein, Mo protein and Mo-Fe protein respectively. Although there is a fairly sophisticated level of understanding of nitrogen fixation in cyanobacteria, there are still a number of uncertain aspects: for example, an oxygen sensor responsible for the regulated expression of *nif* genes have to be described (Bohme, 1998).

Certain filamentous cyanobacteria possess thick walled resting spores called akinetes, which carry out the multiplication of the algae, generally after

tiding over unfavourable conditions. There is a relationship between the heterocysts and akinete related to the position and number of akinetes in a filament, which is controlled by a sporulation chemical substance synthesized solely by heterocysts (Hirosawa and Wolk, 1979).

Biological Nitrogen Fixation:

N_2-fixing agents in soil and water are 'Natural fertilizer factories', promoting their growth and N_2-fixing activity is an important task for sustaining crop yield. These agents ensure long-term maintenance of soil fertility by fixing nitrogen and scientists use the term Biological Nitrogen fixation (BNF) for the process and BNF technologies are encouraged now to promote long-term soil fertility. If benefits such as environment safety, fertilizer savings, improved soil properties, reduced pests and diseases and high crop yield are considered, BNF technologies are often economically feasible (Watanabe *et al.*, 1992; Anand, 2001; Malliga, 2004; Vivekanandan *et al.,* 2004).

The capacity of several cyanobacterial members especially species of *Nostoc* and *Anabaena* to fix atmospheric nitrogen is a significant biological process of economic importance. The process of Biological Nitrogen Fixation (BNF) that helps to maintain the nitrogen status of the irrigated rice field soils in the tropics has mainly been due to the cyanobacteria. Among these only heterocystous filamentous forms were once considered to be accomplishing aerobic nitrogen fixation, but recently, many coccoid and non-heterocystous filamentous forms also have known to fix nitrogen under conditions of low oxygen tension (micro-aerophilic condition), under laboratory conditions (Stewart, 1980; Gunaseeli *et al.,* 2004). It has been suggested that respiration plays an important role in protecting the nitrogenase by scavenging intracellular oxygen. In nature, the rice field ecosystem could possibly provide micro-aerobic condition, through root respiration where considerable nitrogen fixation by non-heterocystous forms or even coccoid forms takes place actively. Gallon and Chaplin (1988) and Gallon *et al.* (1988) demonstrated that axenic cultures of *Gloeothece* with nitrogenase activity is temporarily separated from oxygenic photosynthesis, while N_2 fixing cultures of *Synechococcus* (Mitsui *et al.*, 1987) and *Plectonema boryanum* (Bergman *et al.*, 1997) growing continuously in light are capable of temporarily alternating nitrogenase activity and photosynthetic

oxygen evolution. Biological nitrogen fixation has an absolute requirement of ATP (Burris, 1974) generally obtained through photosynthetic phosphorylation (Cox and Fay, 1969; Stewart, 1980; Fay, 1981) in photosynthetic organisms. Thus cyanobacterium, a unique organism in which diazotrophism is met with, would have developed some million years ago, from an ancient phototrophic prokaryotes having these qualities (Olson, 1970). The above quality may have achieved due to the absence of free oxygen in the atmosphere.

The term biofertilizer denotes all those " nutrient inputs of biological origin for plant growth" (Rao, 1982). Venkataraman coined the term 'algalization' to denote the process of application of cyanobacterial culture to field as biofertilizer. It has been reported that cyanobacterial application induced early flowering in rice plants (Jayaraman and Shanmugasundaram, 1993).

Biofertilizer programmes using cyanobacteria have repeatedly emphasized on the understanding and utilization of the native inhabitants or the local strains. The first step in this effort is a comprehensive taxonomic survey and thereby screening the distribution of various native strains located in various geographical conditions and affected by climatic conditions.

Several approaches toward developing rice capable of fixing N_2 are also considered, such as the establishment of effective endophytic symbiosis, the development of legume-like nodulation, and the introduction and expression of nitrogen fixing genes in rice plants (Reddy *et al.*, 2002). Developing rice with biological nitrogen fixation capacity could help the farmers to overcome the nitrogen nutrition limitations and increase the productivity (Shenoy *et al.*, 2001).

Taxonomy:

Rippka *et al.* (1979) separated the cyanobacteria from algae and included them under a special group of bacteria called cyanobacteria. The taxonomy of cyanobacteria has long been in a highly confused state (Gibbons and Murray, 1978). Moreover, different phycologists have attempted and erected a large number of new species and genera on trivial grounds that made the taxonomy of cyanobacteria extremely cumbersome to define especially at generic and species level (Castenholz, 1992). The numerous cyanobacterial taxa scattered in the literature were critically compiled by Geitler (1932), Elenkin

(1936), Starmach (1966), and Bourrelly (1970) on the basis of their morphological diversity (Geitlerian system).

Morphological characteristics of cyanobacteria formed the basis of the classification in both classical and modern systems. The grouping of these organisms under various orders according to Geitler (1932), Fritsch (1945) and Desikachary (1959) or under separate families without orders as done by Drouet (1981) were based on mere basic morphology. The various revisions made in various groups of the algae, *viz.*, coccoid forms (Drouet and Daily, 1956) Oscillatoriaceae (Drouet, 1968) Nostocaceae with cylindrical trichome (Drouet, 1973) and Nostocaceae with constricted trichome (Drouet, 1978) were based on nomenclatural synonymy reducing the number of taxa to very few. The ranking of cyanobacteria to a special group, namely, cyanobacteria, (Rippka *et al.*, 1979) was mainly based on their prokaryotic organization of the cell structure as revealed by electron microscopic investigation. Even for this classification of cyanobacteria into various typographical groups, the major criteria used have been the morphological diversity of the organisms.

However, studies in the structure and development of cyanobacteria in culture indicate that their diversity although considerable is not as great as the present taxonomic treatment of the group suggests. For example, phycologists (Geitler, 1932; Desikachary, 1959; Starmach, 1966) have given considerable taxonomic significance on the presence or absence of false branching. However, the property is highly variable in culture. In many cases, its expression is determined by culture conditions (Stanier and Cohen-Bazire, 1977).

An entirely new design for classification was suggested by Drouet and Daily (1956) followed by a series of monographs by Drouet (1968,1978,1981) thus, completely rearranging the taxonomy of cyanobacteria (Ecophenes approach). By treating many of the genera and species as ecophenes, Drouet reduced the number of taxa in cyanobacteria from 140 genera and 2000 species to 24 genera and 62 species. Because of the broader concept and nomenclature simplicity, many experimental workers welcomed Drouet's system of classification, but the phycologists have not accepted it. The existence of

genetically stable types with very narrow genetic variations has been proven experimentally (Stanier *et al.*, 1978; Stulp, 1983) and it was found that Drouet's interpretation does not suit the diversity of cyanobacteria in nature or in cultures. But, Tiwari *et al.* (2004) emphasized that the concepts of Drouet (1981) need to be understood, reinvestigated and appreciated.

Stanier and his associates (1971) tried another new and entirely different method to cyanobacterial taxonomy. They studied these prokaryotic organisms using bacteriological methods. Accordingly, a compact and economic system was proposed by Stanier *et al.* (1971), Waterbury and Stanier (1978) and Rippka *et al.* (1979). It was on the basis of characters evident in pure cultures. Gibbons and Murray (1978) suggested placing the cyanobacteria on the ordinal level into the subclass Oxy-photobacteriae, class Photobacteriae, division Gracilicutes of kingdom Prokaryotae. Stanier *et al.* (1978) proposed that "the nomenclature of cyanobacteria be placed under the rules of the international code for nomenclature of bacteria", which raised a lot of objections from phycologists (Bourrelly, 1979; Golubic, 1979 and Lee, 1989). Lee argued that cyanobacteria are phototrophic organisms, which were traditionally studied together with eukaryotic algae.

Anagnostidis and Komarek (1985,1990) pointed out that cultures of cyanophytes are very useful in taxonomy since they furnish additional information about the taxa. Anand (1988) emphasized on the precise study on the variability of culture material combined with perfect knowledge of the natural populations since these are important for the correct taxonomic classification of the cyanophytes.

Rippka *et al.* (1979) tried to modify Geitlerian taxonomy of cyanobacteria. This led to the redefinition of some genera based on morphological, physiological, developmental and DNA base composition studies. The organisms were divided into five sections by simple morphological and reproductive characters that are stable in pure cultures. Herdman *et al.* (1979), however, found out that there is no correlation between DNA composition and structural and developmental diversity of the organisms that distinguish the five sections of Rippka *et al.* (1979). Nevertheless, it is undoubtedly sure that the

taxonomic information resides in the DNA molecule (Krogmann, 1981). Evidence from RNA homology (Giovannoni *et al.*, 1988) suggests that two of the five sections proposed by Rippka *et al.* (1979) are heterogeneous. In short, a satisfactory taxonomy of cyanobacteria is lacking even now, which in turn poses problems to identify strains (Roger *et al.*, 1991). Use of RAPD markers (Prabina *et al.*, 2003) and 16S rRNA (Thajuddin and Nierzwicki-Bauer, 2004) as rapid methods to detect genetic variation or relatedness of the cyanobacterial strains has been suggested recently but this will also help accurate identification up to generic level only (Prabina *et al.*, 2003).

Eco-physiology of cyanobacteria in rice fields:

The eco-physiological and agricultural importance of cyanobacteria depends on the diazotrophic nature of most of the species *i.e.*, they can perform both photosynthesis and nitrogen fixation. Since these algae have to function from outside the crop plant, that is, paddy, they are always subjected to various stresses of the environment, which may be, natural or induced (Goyal, 1993). In India, out of 2213 samples collected from the rice fields of various states, about 33% were recorded to contain nitrogen-fixing cyanobacteria (Venkataraman, 1981 a). A rice field can be compared to an artificial ecosystem, and the physico-chemical and climatic factors and cultivation practices disturb this ecosystem, which ultimately affect the cyanobacterial population. Rice is the only crop, which shows better performance under flooded soil conditions. The high moisture content and flooded conditions of many paddy fields are not natural always (Brady, 1981). The soil types, field / land preparation for paddy cultivation, crop rotation, agro-chemicals and fertilizer treatments have high impact on cyanobacerial diversity. It is reported that the waterlogged conditions of tropical rice field soil is quite congenial for cyanobacterial growth (Kannaiyan, 1979). The cyanobacteria grow together with the rice plants and form patches of slimy bluish, blue-green, yellowish or brownish mass on the surface of water or attached to the paddy plants as mucilage balls (Whitton *et al.*, 1988 b). Species distribution is found to be heterogeneous between distantly located rice fields most probably because of variations in the physico-chemical factors. This heterogeneous and sometimes limited distribution of nitrogen fixing cyanobacteria is still, not understood, because no survey has correlated the

presence or absence of cyanobacteria with the rice field environmental factors (Lowendorf, 1980). The above reports show that the growth of cyanobacteria in the rice fields is possibly and undoubtedly influenced by many physical factors like light, temperature, rainfall, desiccation, *etc.* and chemical factors like organic carbon content, pH of the soil and water, phosphate, combined nitrogen, various minerals, salinity/acidity of the soil, agro-chemical and fertilizer treatments/amendments, *etc.* Some of the factors are mentioned below:

Light:

Being photoautotrophic cyanobacteria show definite responses to light both quantitatively and qualitatively (Fogg *et al.*, 1973). Light reaching the water layer of rice fields is the control factor over many physico-chemical characteristics, *viz.,* temperature, pH, salinity, CO_2 concentration and O_2 concentration, since the quality and quantity of light modify the photosynthetic rate (Mallin and Paerl, 1992). However, most of the cyanobacteria are sciophilous (Reynaud and Roger, 1979). Some forms like *Cylindrospermum, Aulosira, etc.* grow well under high light intensities. Light plays an important role in the nitrogen fixation of non-heterocystous cyanobacteria (Traore *et al.*, 1978) in which there is temporal separation of photosynthetic O_2 evolution (in the light) from N_2 fixation (in the dark). The requirements for efficient utilization of high light fluxes in cultures of *Spirulina platensis* were elucidated by Hu *et al.* (1998); the most important of these was a narrow light path coupled with a highly turbulent flow. These conditions facilitated ultra high optimal high densities *i.e.* above 100 mg chl/l, which resulted in very "high" output rates outdoor. Subramanian and Shanmugasundaram (1987) noticed that growth is proportional to the duration of light and the maximum growth could be obtained with continuous illumination.

In a submerged rice field, the light availability depends on the seasons and latitude, the cloud cover, the plant cover and the turbidity of water (Roger and Reynaud, 1979). The canopy of transplanted rice decreased light by 50 % when plants were 15 days old, 85 % after one-month and 95% after 2 months of growth (Kurosawa, 1956). This decreases the light reaching the cyanobacteria, which in turn affect their growth and activity.

Being photoautotrophic, cyanobacteria show definite response to the quality and quantity of light. The photosynthetic system of cyanobacteria becomes limited in its activity at low irradiance, saturated at high irradiance and inhibited at very high irradiance. Based on the response of cyanobacteria to light quality, they have been divided into three groups (Marsac, 1977). Group I Cyanobacteria can alter phycobilisomes (PBS) size and number along with photo system stoichiometry when grown in different light qualities, but do not change the absorbance characteristics of their PBS. Group II cyanobacteria can alter the levels of PE in the PBS, and group III members can modulate both PC and PE levels. The group II and group III members exhibit complementary chromatic adaptation and the phenomenon has been observed in a number of cyanobacteria (Marsac, 1977; Bryant and Cohen-Bazire, 1981; Bryant, 1981, 1982). In chromatic adapting cyanobacteria, red light promotes PC synthesis, while green light promotes PE synthesis. The differential expression of PE and PC under different light qualities is suggested to be due to differential transcription of the genes (Mazel *et al.*, 1986; Grossman *et al.*, 1988).

In addition to light quality, light intensity also modulates the stoichiometry of PSI / PSII. The PSI / PSII ratio was higher under low light intensities and vice versa (Kawamura *et al.*, 1979). The light regulations of PSI/PSII ratio have been demonstrated to be due to changes in PSI abundance (Murakami and Fujitha, 1991) (and not of the PSII and/or cytochrome b, f). Branching of *Westiellopsis prolifica* Janet has found to be influenced by light intensity (Adhikary, 1987). It has been reported that enhanced uptake of nitrate was observed in high light intensity in *Chroococcus sp.* (Dhandayuthapani *et al.*, 2004).

Cyanobacteria are restricted to the photic zone and usually located in the upper 0.5 cm horizon. They also exist in deeper horizons in dormant conditions as spores or filament fragments (Chapman and Chapman, 1973; Roger and Reynaud, 1976). In the moist shaded soils, cyanobacteria are abundant as encountered in enrichment cultures (Reynaud and Roger, 1978). A beneficial effect of the plant canopy shading on the abundance of cyanobacteria was also reported in sugarcane fields, maize fields and grass lands in India (Singh, 1961a).

Though cyanobacteria are autotrophic in nature they can survive in heterotrophic conditions also. Heterotrophic growth seems to be widespread in cyanobacteria and various reports reveal this fact indisputably (Khoja and Whitton, 1971; Adhikary and Pattnaik, 1979; Rippka *et al.,* 1979; Smith, 1982 and Padhi *et al.,* 1987). Stanier (1973) suggested that DCMU procedure is a foolproof method to find out the heterotrophic capability of cyanobacteria. Carbohydrates were preferred (chiefly glucose and sucrose) to other carbon compounds by cyanobacteria for supporting heterotrophic growth (Padhi *et al.*, 1987 and Dash and Padhi, 1988).

Temperature:

Rarely temperature plays a role as a limiting factor for cyanobacteria in the rice fields. Daily variations in the temperature are noticed in submerged rice fields (up to 10°C). The temperature was maximum during mid afternoon in flooded paddy fields, which often recorded 36-40°C (Grant *et al.*, 1986). The paddy field water itself is a good temperature conditioner. In addition, the range of temperature favourable for cyanobacerial growth is wider than that required by rice. Hence, the cyanobacteria are rarely affected by any violent temperature extremes in low land tropical field (Roger and Reynaud, 1979). However, it influenced cyanobacterial growth and biomass composition as well as productivity (Roger and Reynaud, 1979,1982). The optimum temperature required for luxuriant growth of cyanobacteria is about 30-35°C (Roger and Kulasooriya, 1980). Lower temperature results in lower activity and thereby lower productivity of cyanobacteria and better growth of eukaryotic algae, which poses problems in cyanobacterial growth. Green algae when present inhibit the growth of cyanobacteria. High temperature has been found to encourage cyanobacterial growth (Singh and Singh, 1989; Adhikary and Naik, 2001).

Singh (1976) reported that a temperature range of 34-39°C was favourable for growth of *Aulosira fertilissima* in paddy fields. Venkataraman (1964) reported a harmful, sometimes fatal effect of high temperature on growth of cyanobacteria. There are also reports on the relevance of growth due to low temperature (Roger and Reynaud, 1979; Subramanyan *et al.*, 1965a,b).

pH :

Amongst the various factors affecting the augmentation of cyanobacteria, hydrogen-ion concentration (pH) has an important role in determining their distribution, growth and establishment in the soil (Prasad *et al.,* 1978; Roger and Kulasooriya, 1980; Sardeshpande and Goyal, 1981b). Since in India, rice is cultivated in a variety of habitats with pH ranging from 3.5-8.5, it is essential to examine the response of cyanobacteria to different pH levels in terms of their growth and nitrogen fixation. This is again necessary because soil pH has a selective power in determining their density and diversity. The diurnal pH fluctuation in rice fields occurs due to photosynthetic removal of HCO_3 (Whitton *et al.*, 1988b). The pH values varied during the day, reaching the maximum of 8.4 at 2.00-3.00 P.M., according to the changes in the irradiance in the layer of water. Photon flux density at water surface varied during various phases of crop growth also, due to plant height, which affect the pH. Hence, pH depends on the time/hrs of the day and also of the crop phase. Quesada *et al.* (1995) observed that almost every sampling point in a study of rice field of Spain was above neutral and mean values were closer to pH 8. Cyanobacteria are generally reported to prefer neutral to slightly alkaline medium for their optimal growth (Gerloff *et al.*, 1950; Fogg, 1956; Venkataraman, 1961; Whitton and Sinclair, 1975; Singh, 1978). In culture media the optimal pH for cyanobacterial growth ranged from 7.5-10.0 in the upper limit and the lower limit was about 6.5-7.0 (Chapman *et al.*, 1972; Prasad *et al.,* 1978; Roger and Reynaud, 1979). The beneficial influence of pH (8.0-10.0) on cyanobacterial growth was demonstrated by the fact that addition of lime increased the cyanobacterial biomass and N_2 fixation (Roger and Kulasooriya, 1980; Venkataraman, 1981a). Liming the soil increases the pH from 6.5 to 7.5 and this increase in pH increases the availability of several nutrients in flooded soil, which in turn promotes growth and N_2 fixation (Amma *et al.*, 1966; Saha and Mandal, 1980) and establishment of cyanobacteria (Venkataraman, 1972; Singh, 1975). Better growth of many species of cyanobacteria has been reported at the neutral range (Gopal *et al.*, 1975; Subramanian and Shanmugasundaram, 1987; Fernadez-Valiente and Leganes, 1989; Singh *et al.*, 1995; Rath and Adhikary, 1996). High growth and nitrogen fixation occurred in the pH range of 7.3-9.8 in *Aphanothece* species (Singh,

1974). Thus, growth and N_2 fixation in cyanobacteria was affected by change in pH of the soil and the medium (Stewart, 1973; Singh, 1974).

There are, however, reports on the existence of cyanobacterial strains in soils with low pH values between 5.0-6.0 (Prasad *et al.*, 1978). Out of a total of 157 million ha of cultivable area 49 million ha of land are acidic in nature (Gopalaswamy, 2001). Dense cyanobacterial bloom has been reported in a soil of pH 5.5 in Japan (Matsuguchi and Yoo, 1979) and in acidic-bog lands in Swedish soils (Granhall, 1970). Aiyer (1965) reported the occurrence of 19 species of cyanobacteria in the acid and acid-sulphate soils of Kerala, with rich distribution of *Aulosira fertilissima* and *Calothrix brevissima* even from soils with pH as low as 3.5. Similar observations of existence of cyanobacteria in acidic environments were made by Dominic and Madhusoodanan (1996,1999), Tamilselvam *et al.* (2001) and Kavitha *et al.* (2004). Although many cyanobacteria, like, *Nostoc muscorum*, *Anabaena torulosa*, *Aulosira* and *Calothrix* spp. have been reported from acidic habitats with pH as low as 3.5 (Durrell, 1964; Aiyer, 1965), algal inoculation in such habitats requires proper soil amendment for pH, to have desired effect on the crop yield (Aiyer *et al.*, 1972). It is therefore, necessary either to apply appropriate quantity of lime to neutralize the acid or search for such strains, which are able to proliferate under such low pH conditions. Isolation and performance of such cyanobacteria from acid soils have been attempted in laboratory conditions and the results were reported to be encouraging (Gopalaswamy *et al.*, 2002). To improve the performance of promising acid tolerant strains, mutations have been induced and the performance of the mutants with the wild varieties have been compared and analyzed (Malathy *et al.*, 2001).

The major physiological change in response to variations in external pH was observed in the nitrogenase activity and almost no change was observed in photosynthetic rate (Fernadez-Valiente and Leganes, 1989). Some alkalophilic cyanobacteria circumvent pH increase by synthesizing more polysaccharides (Singh *et al.*, 1995). Both growth rate and chlorophyll-*a* content of the branched cyanobacterium *Westiellopsis prolifica* Janet are observed maximum in pH 8.0 but, the frequency of branching reaches its optimum at a still higher pH level *i.e.*, 8.5 (Biswas *et al.*, 1975). Another branched cyanobacterium *Mastigocladus*

laminosus exhibited maximum branching at pH 9.5 and growth at 8.5 (Sahu and Adhikary, 1987).

Salinity:

Sodium content in the soil is 0.1-1% of the sodium (Na) that is available in the earth crust (sodium content of the earth crust is 2.8 %). Sodium exists in the soil as solutions, exchangeable Na, and silicate minerals (NaCl, Na_2SO_4, and Na_2CO_3). Plants and photosynthetic microbes consume it as Na^+ ion from soil solutions. The amount of Na^+ varies greatly among soils. It may sometimes lead to salinity to sodicity especially in poorly drained soils of arid and semiarid regions.

Salinity is one of the most important factors in nature causing severe crop loss every year and it is an ever-increasing problem in agriculture (Apte, 1990). The occurrences of cyanobacteria in varying saline situations have drawn much interest in recent years especially on their levels of halo tolerance mechanism of adaptation and role in amelioration of salt affected soils. Occurrences of cyanobacteria in saline terrestrial habitat and their possible role in the reclamation have been reported by many workers (Singh, 1961b; Kaushik *et al.*, 1981; Subhashini and Kaushik, 1981; Kaushik and Krishnamurti, 1981; Kaushik and Venkataraman, 1982; Thomas and Apte, 1984; Kaushik and Subhashini, 1985). Bio amelioration of barren saline soils of Goa has been carried out recently using cyanobacterial consortium (Shaik and Furtado, 1995, 1998).

Cyanobacteria are known to require Na^+ for growth (Allen and Arnon, 1955), nitrogen fixation (Apte and Thomas, 1980), nitrate reduction, intracellular pH regulation (Kaplan *et al.*, 1984) and effect of photosynthesis (Garcia-Gonzalez *et al.*, 1987). Many cyanobacteria exhibit considerable tolerance to salt (NaCl) (Padhi *et al.*, 1998).

Cyanobacteria reveal varying degrees of behavioural diversity in response to salt stress. Some are halotolerant, while some are halosensitive. Some other types exhibit an initial reduced metabolic activity, which are transient for a short period and soon adapt to salinity stress (Moore *et al.*, 1985; Anand *et al.*, 1987). The halo sensitivity is expressed by filament breakage, cell disruption, leaching of pigments and nitrogenase inhibition (Smith *et al.*, 1983; Anand *et al.*, 1987). High

concentration of NaCl inhibited growth by ionic stress than by osmotic stress (Batterton and Van Baalen, 1971).

The physiological basis of salt tolerance may be due to extrusion of Na^+ and maintenance of low intracellular Na^+ (Dewar and Barber, 1973; Paschinger, 1977; Apte and Thomas, 1986), or, ionic balance between K^+ and Na^+ (Jha and Kaushik, 1988), or, synthesis of internal osmotica in the form of carbohydrates (Tel-Or, 1980; Blumwald and Tel-Or, 1982b; Blumwald *et al.*, 1983; Reed *et al.*, 1986; Anand and Parameswaran, 1990; Kumar and Kaushik, 1994; Padhi *et al.*, 1998), Proteins (Shobhana and Kaushik, 2002) and amino acids (Reed *et al.*, 1986; Saxena and Kaushik, 1992; Padhi *et al.*, 1998). Many cyanobacteria exhibit salt tolerance due to the presence of extra cellular polysaccharides (Jha *et al.*, 1987; Apte and Thomas, 1997).

The soil pH is considerably declined when cyanobacteria was inoculated before and after transplanting (Pandiarajan and Nagarajan, 2001) of rice in sodic soils. Application of *Azolla microphylla, Sesbania aculeata* and inoculation of salt tolerant cyanobacterial cultures have improved the nitrogen, phosphorus and organic content of saline and sodic soils (Amsaveni and Kannaiyan, 2001b). Isolation and characterization of salt tolerant cyanobacteria from saline and sodic soils and their mass multiplication and application in the salt prone areas of rice soil enhance the nitrogen status of the soil (Amsaveni and Kannaiyan, 2001a). For this proper screening is essential.

Desiccation and rewetting:

The effectiveness of soil inoculation of suitable diazotrophic cyanobacteria so as to effect and maintain soil fertility depends primarily on the capability of the organism to grow, establish and survive in the soil especially under desiccated conditions during the summer months. Cyanobacteria are capable of resisting water stress and desiccation under terrestrial and aquatic conditions. This ability of cyanobacteria plays an important role in their nitrogenase activity (Stewart, 1980). Resistance to desiccation increases with the dryness of the biotype and can be related to the floristic composition of desiccated soil (Roger and Reynaud, 1979).

The mechanism of desiccation tolerance is not well understood despite the fact that numerous prokaryotic and eukaryotic organisms are capable of surviving more or less complete dehydration and the current intense interest is in long-term storage and survival of cells (Kennedy *et al.*, 1994; Oliver *et al.*, 1998; Bill and Potts, 1999). Available evidence suggests that desiccation tolerance reflects the sum of numerous simple and complex interactions at the structural, physiological and molecular levels. For example, the effects of reactive oxygen species in desiccation damage are exacerbated by high light and UV radiation (Garcia-Pichel and Castenholz, 1999). One cyanobacteria *Nostoc commune* Vaucher has been studied to understand desiccation tolerance at the molecular level (Potts, 2000). The thick pigmental layers, containing the pigment scytonemin of *Aulosira* sp. help the strain to tide over the environmental condition under extreme desiccation (Adhikary and Sahu, 2000).

Cyanobacteria dominate the bacterial population of many extreme environments (Whitton and Potts, 2000) such as thermal springs (Ward *et al.*, 1989), frigid lakes (Orcutt *et al.*, 1986), *etc.* Cyanobacteria that experience extreme desiccation include intertidal marine members often dominated by a form species of *Microcoleus* (Pentecost, 1985; Potts and Whitton, 1977,1980), growths of *Gloeocapsa* in Tintenstrich communities and terrestrial crusts of *Tolypothrix, Calothrix* and *Nostoc* (Whitton *et al.*, 1979; Potts and Whitton, 1980; Amarpalli *et al.*, 1997 and Tripathy *et al.*, 1997). Some cyanobacteria and eukaryotic algae are sufficiently desiccation tolerant to survive long time transport in aerosols in Antarctica (Marshall and Chalmers, 1997). Many of these communities include forms that resist the effects of high doses of ionizing radiation.

One aspect of the eco-physiology, biochemistry and molecular biology of cyanobacteria that deserves attention with regards to desiccation tolerance is the synthesis, identity and function of extra cellular polysaccharides (Phillipis and Vincenzini, 1998; Adhikary, 1998). These biopolymers regulate the loss and uptake of water from cells, serves as a matrix for the immobilization of other components of the cell which may offer protection (e.g. urea absorbing components) and may protect cell walls from damage during swelling and

shrinkage (Caiola *et al.*, 1993,1996). There are reports on the isolation of *Nostoc muscorum* and *Nodularia heyniana* in culture from a soil that had been dry for 70 years (Chapman and Chapman, 1973). *Nostoc commune* has been recovered from 87 years old herbarium specimens (Trainor, 1985).

The ability of cyanobacteria to withstand desiccation has been attributed to various physiclogical and morphological characteristics like fatal plasmolysis, lack of cell vacuoles, formation of cysts in some genera and presence of mucilaginous sheath that absorbs water quickly and retains for long periods (Roger and Reynaud, 1982). This explains the dominance of mucilaginous species of *Nostoc* and *Cylindrospermum* in paddy fields with dry or moist but shaded soil.

Remoistening or rewetting after desiccation generally favours growth and regeneration of cyanobacterial species. Alternation of desiccation and remoistening periods has a great influence on the quality, yield and quantity of cyanobacteria.

Effect of seasonal changes:

The algal biomass and N_2 fixation vary greatly with variation in physico-chemical factors and climatic factors (Singh, 1976,1981; Reynaud and Roger, 1978; Watanabe *et al.*, 1978; Roger and Kulasooriya, 1980). Although seasonal variations (water, temperature (maximum & minimum), solar radiation, sunshine hours, rainfall, *etc.*) are recognized as primary determinants of cyanobacteria growth and N_2 fixation, the relationship between cyanobacterial growth and N_2 yield and seasonal variations has not been specifically measured till 1988. Bisoyi and Singh (1988b) made attempts to establish relationship between seasonal changes as independent variables and cyanobacterial growth and nitrogen fixation as dependent variables. The analysis showed that solar radiation was the prime factor (Watanabe *et al.*, 1978; Bisoyi and Singh, 1988b). The variations explained by seasonal changes were 52.3% and 50.3% for biomass production and nitrogen yield of cyanobacteria respectively. Higher water temperature showed a significant effect on biomass production and nitrogen yield of cyanobacteria (Stewart *et al.*, 1978; Roger and Reynaud, 1979; Bisoyi and Singh, 1988b). The number of hours of sunshine showed a positive

effect on biomass production and N_2 yield of BGA, but the effect was less significant. Clear sky conditions encouraged higher growth and nitrogenase activity of cyanobacteria (Roger and Kulasooriya, 1980).

Other factors like rainfall (Singh, 1976; Bisoyi and Singh, 1988b) and typhoons (Roger and Kulasooriya, 1980) affect cyanobacterial growth by reducing light availability and washing away of cyanobacterial biomass. The splashing raindrops break up the cyanobacterial colonies and there by increase the water turbidity, which in turn reduce the level of light reaching the water surface (Bisoyi and Singh, 1988b).

Minerals and other nutrients:

The process of biological nitrogen fixation is affected by a variety of physical and physiological processes (Stewart, 1980). While the physical and chemical status of the soil have an influence on the fertility of the soil, the productivity of the soil largely depends upon the availability of nutrients. In cyanobacteria, regulation of synthesis and activity of nitrogenase by combined nitrogen is well known (Wolk, 1982; Misra and Kumar, 1984a). Mineral nutrients may limit nitrogen fixation by affecting growth and synthesis of nitrogenase proteins (Robson *et al.*, 1981; Misra and Kumar, 1984 a,b; Thomas and Apte, 1984; Burns *et al.*, 1985 and Dimroth, 1987). The role of some of the mineral elements in cyanobacterial growth is as follows.

Phosphorus:

It is one of the most important macro elements responsible for biodiversity and bioaccumulation of photosynthetic microorganisms including cyanobacteria in nature. It is one of the key substrate involved in energy metabolism for the synthesis of nucleic acids and membranes and plays an important role in photosynthesis, respiration and regulation of a number of enzymes. Phosphate is available in nature as organic phosphate esters by the break down of animal or plant residues or by living cell excretes. These phosphate esters have to be enzymatically hydrolyzed to liberate the orthophosphate, the only form of nutritionally utilizable phosphorus directly taken up by the plants and microbes.

All nitrogen fixing cyanobacteria irrespective of their morphological organization, ecological distribution and nature of carbon and nitrogen assimilation, require phosphorus, sodium and molybdenum for their growth on molecular nitrogen (Apte and Thomas, 1980; Lang and Brown, 1981;Thomas and Apte, 1984). Robson *et al.* (1981), Thomas and Apte (1984) have demonstrated the role of phosphate and sodium in nitrogen fixation. While molybdenum deficiency enhanced heterocyst differentiation, phosphate deficiency inhibited it in *Nostoc linckia* (Kumar and Kumar, 1989). It has been reported that *Nostoc calcicola* grown under phosphate deficient condition showed a decrease in specific growth rate, phycocyanin content (50%), oxygen evolution (50%) and reduced ATP pool (Prasad and Kashyap, 1991). Kannaiyan *et al.* (1992) have demonstrated better biomass production of salt tolerant cyanobacterial cultures grown in the presence of single super phosphate than rock phosphate grown cultures. Singh *et al.* (1994) reported that the protein content of *Nostoc calcicola* cells were decreased by 7% in phosphorus starved cells. It has been found out that even a low concentration of 10 ppm single super phosphate itself supported maximum growth and ammonium excretion in certain salt tolerant cyanobacterium (Amsaveni and Kannaiyan, 1998). Algalization increases the available phosphorus in the soil because of excretion of organic acids and also solubilizes the unavailable phosphate (Arora, 1969; Kaushik, 1985). Limitation of phosphorus for cyanobacterial growth in the environment was evident by a positive correlation between available phosphorus and the abundance of cyanobacteria in rice fields (Roger *et al.*, 1987).

In general, cyanobacterium adopts two strategies to cope with the phosphate deficiency in the environment. (1) When phosphate is present in the environment, the cell accumulate the excess phosphate in poly phosphate bodies (Batterton and Van Baalen, 1968) which are broken down to release phosphate under conditions of phosphate deficiency. (2) When phosphate is limiting, the cell induces systems that help to scavenge the available phosphate.

Results of the several experiments have demonstrated that phosphorus application stimulates cyanobacterial growth and BNF in rice fields (De and Mandal, 1956; Roger and Kulasooriya, 1980; Roger *et al.*, 1987) The results of

Stewart *et al.* (1971) suggests that blooms of N_2- fixing cyanobacteria develop in aquatic system when phosphorus is available and combined nitrogen level is low. However, an IRRI report says that greater is the phototrophic nitrogenase activity in unfertilized plots than in those receiving NPK fertilizers (Watanabe, 1978). Phosphorus levels in aquatic system are related to cyanobacterial growth and nitrogen fixation (Vanderhoef *et al.*, 1974; Baral and Kumar, 1994). The phosphate requirement of cyanobacteria is 40-80 kg P_2O_5 ha^{-1} in Indian paddy fields. Application of 40 kg of P_2O_5 ha^{-1} increased yield of *Aulosira, Aphanothece* and *Gloeotrichia* by 2.5, 3.5 and 5 times respectively (Bisoyi and Singh, 1988a). Split application of phosphorus was found to be better than one basal application. Phosphorus use efficiency is generally very low in acidic soil. The availability of phosphorus in the soil is greatly limited by soil pH and it tends to become bound as calcium phosphate especially in high pH (Bisoyi and Singh, 1988a). It has been reported that a pH of 10 -10.2 and a temperature of 35-37.5°C is quite favourable for PMEase activity in cyanobacteria (Meenakshee, 2001)

Induction of periplasmic or extra cellular phosphatases (a periplasmic protein) is a common response to phosphorus deficiency among cyanobacteria (Doonan and Jensen, 1977; Block and Grossman, 1988). The process of phosphorus assimilation by *Synechococcus* and other species is energy dependent (Avendano and Valiente, 1994; Ritchie *et al.,* 1997) but does not hold true for all species (Istvanovics *et al.*, 1993). The production of multiple periplasmic phosphatases under phosphorus deficient condition allows the cells to scavenge phosphate by hydrolyzing it from various sources (Doonan and Jensen, 1980; Whitton *et al.*, 1990; Islam and Whitton, 1992). Some cyanobacteria are able to use hydroxyapatite and tri-calcium phosphate as source of both calcium and phosphorus (Bose *et al.*, 1971; Cameron and Julian, 1988; Roychoudhury and Kaushik, 1989; Natesan and Shanmugasundaram, 1992).

The rate of phosphate uptake depends upon many factors. It is temperature dependent in *Anabaena variabilis* (35°C) but declined with time (Healey, 1973), light dependent in presence of Na^+ in *Synechococcus* R-2 PCC 7942 (Ritchie *et al.*, 1997) and pH dependent (pH 7.5-9.0). The phosphorus

uptake declined sharply in the acidic range (Healey, 1973; Lawry and Jensen, 1979) and also at high alkaline conditions (Ritchie *et al.*, 1997). Alkaline phosphatase exhibited optimal activity between pH 8 and pH 10 (Healey, 1973; Healey and Hendzel, 1979).

Nitrogen:

Nitrogen plays an important role in sustaining crop productivity because of high crop requirement equated to population and it is universally deficient in tropical soils. In India, as far as nitrogen content, 77% of the soils are recognized as low and 23% medium. In semi-tropical arid soils the total nitrogen content comes to about 0.1% (Tandon and Kanwar, 1984).

Cyanobacteria mainly use inorganic nitrogen (nitrate, ammonia and di-nitrogen) to fulfill their nitrogen requirements. But urea and other organic sources of N_2, such as amino acids can also be assimilated by certain cyanobacteria (Kratz and Myers, 1955; Neilson and Larsson, 1980; Rawson, 1985; Kaushik, 1986). Nitrate has been considered to be a preferred non toxic source of combined nitrogen for most cyanobacteria and is usually made available to the organism as a result of high degree of nitrification under water logged conditions. The assimilation of nitrate by cyanobacteria involves nitrate uptake and reduction of intracellular nitrate to ammonium. The nitrate uptake takes place through a transport system having high affinity for nitrate (Flores *et al.*, 1983) and the intracellular accumulation of nitrate induce the nitrate reductase to function. Nitrate also has been shown to repress nitrogenase synthesis in *Anabaena* sp. (Meeks *et al.*, 1983; Martin-Nieto *et al.*, 1991) and *Plectonema baryanum* (Nagatani and Haselkorn, 1978). This operates at the level of *nif* HDK (Fujitha *et al.*, 1991; Huang and Chou, 1991; Martin Nieto *et al.*, 1991). They express the system for nitrogen fixation only in the absence of a suitable source of combined nitrogen (Stewart and Rowell, 1975). Comparatively higher levels of nitrate nitrogen have been reported to increase the growth of cyanobacteria without affecting the process of nitrogen fixation.

The subterranean cyanobacterial floras are influenced by type and mode of application of inorganic fertilizer like nitrogen, phosphorus and potassium (Roger *et al.*, 1980). In general, nitrogen fertilizers show a selective action on

the nitrogen fixing and non-nitrogen fixing cyanobacteria (Subramanyan *et al.*, 1965b; Yoshida *et al.*, 1973; Rinaudo, 1974). Studies at IRRI (1987,1988) have showed strong inhibition of photo-dependent ARA by urea broadcasting in about 75% while in others a significant ARA was observed. The ARA estimates under laboratory conditions by *Aphanothece pallida*, *Gloeocapsa decorticans* and *Nostoc piscinale* grown with nitrate, ammonia and urea nitrogen showed wide variations (Singh and Bisoyi, 1989). The inhibitory effect of ammonia was higher than that of nitrate and urea. Filamentous forms of cyanobacteria were less inhibited as compared to unicellular forms. It has also been reported that nitrogenase activity was significantly reduced/inhibited by NH_4Cl, KNO_3 and glutamine in *Anabaena variabilis* (Dolly and Jolly, 1997). Stewart (1964) and Venkataraman (1979) did not observe any reduction in presence of ammonium nitrogen up to 50 ppm. Anand (1992) reported that several cyanobacteria grow luxuriantly in ammonia based commercial fertilizers. Some mutants of *Anabaena* have been reported to grow only with molecular nitrogen (Gotto *et al.*, 1980). A clear inhibition of N_2-fixing cyanobacteria by urea late in the rice cycle has been reported by Roger *et al.* (1984).

Under field conditions in presence of higher levels of nitrogen fertilizers, the cyanobacteria grow faster and may fix less amount of nitrogen. However, they revert back to the N_2-fixing state, when the combined nitrogen source is exhausted in the soil due to progressive utilization and/or natural loss. The increased cyanobacterial biomass is now expected to contribute much more nitrogen than what it could have contributed in the absence of chemical nitrogen fertilizer. The assimilated combined nitrogen and the biologically fixed nitrogen subsequently become available in a gradual manner through the process like exudation and decomposition enrich rice fields.

Cyanobacteria are exposed to ammonium also under field conditions, as it is the common form of fertilizer nitrogen used in rice cultivation. Goyal and Marwaha (1985) reported a concentration dependent increase in growth and ammonium absorption by *Anabaena fertilissima* up to 50 ppm, and concentration higher than 50 ppm resulted in the decrease in nitrogen fixation and it was more drastic than growth.

In the growing medium, the presence of ammonium prevented the development of heterocyst (Singh, 1975; Anand and Karuppusamy, 1987). It has also been reported to inhibit the synthesis of nitrogenase in cultures that contain fully developed heterocysts (Rowell *et al.*, 1977; Ramos *et al.*, 1985). Ammonium at pH10 and concentration range of 0.5 mM^{-1} accelerates an inactivation of nitrogenase enzyme through a modification of the Fe-protein (Bohm *et al.*, 1992). Studies have shown that cyanobacterial tolerance limit for combined nitrogen was much higher for growth than for nitrogen fixation (Goyal, 1989).

Organic carbon:

Generally cyanobacteria do not depend upon exogenous carbon supply as long as the main metabolic event, the photosynthesis, persists in their living system and works actively (Stewart, 1969). Although most of the cyanobacteria are photoautotrophic in nature, several can live hetero/mixotrophically at the expense of various sugars, which provide both carbon and energy (Khoja and Whitton, 1975; Ladha and Kumar, 1977; Rippka *et al.*, 1979; Haury and Spiller, 1981; Smith, 1982; Sahu and Adhikary, 1988).

Carbohydrate constitutes the major fraction of the organic carbon in the soil. In the semiarid tropical soils, the amount of organic carbon in the soil is less than 1% (Virmani *et al.*, 1982). The major source of organic carbon is the decomposed crop residue and the dead remains of soil flora and fauna. The level of soil organic carbon determines the soil health and production potentials. Hence organic carbon forms one of the most important fertility criteria that determine the quality of the soil.

There is a close functional relationship between photosynthesis and nitrogen fixation in the cells, as evidenced by a higher rate of nitrogen fixation in light than in darkness (Fay, 1976). However, heterotrophic growth and nitrogen fixation has been reported in many cyanobacteria in darkness in presence of certain organic carbon source/s (Ramos *et al.*, 1985). The rate of nitrogen fixation was found to be very low due to incomplete TCA cycle reactions (Smith *et al.*, 1967; Pearce *et al.*, 1969). A considerable increase in the nitrogen fixation has been reported (De and Sulaiman, 1950) by cyanobacteria, by

passing CO_2 rich air over the surface of water in the field. Goldman *et al.* (1972) observed that cyanobacteria during their growth excrete aqueous CO_2 resulting in the increasing pH level that can affect the growth of these organisms. King (1970) found out that cyanobacteria under normal conditions take up only CO_2 and not carbonate and this was confirmed *in situ* by manipulating pH, nutrient concentrations and amount of free CO_2 in isolated lake zones. The CO_2 concentration is proportional to the pH values and this correlation between pH and CO_2 concentration makes the cyanobacteria predominate.

Other elements:

All cyanobacteria require **Molybdenum (**Mo) since it is one of the most important constituents of the N_2- fixing enzymes of cyanobacteria - the nitrogenase and the nitrate reductase (Apte and Thomas, 1980; Thomas and Apte, 1984). Many of the enzymatic processes of nitrogen fixation take place at a site on Mo-Fe protein of nitrogenase (Burgerson and Turner, 1973), showing the essentiality of Mo for these reactions (Peinkoss *et al.*, 1981; Misra and Kumar, 1984b). Field application of Mo did not show any significant effect on growth and nitrogen fixation of cyanobacteria. This may be due to the abundance of adequate amount of Mo in the Indian rice soils (Chopra and Dube, 1971). Nevertheless, it sometimes acts as a limiting factor for N_2 fixation, especially during dry period, when, N_2-fixing activity is more vigorous. In such cases, the beneficial effect of Mo application had been reported in certain cyanobacterial trials (Subramaniyan, 1972). Mo deficiency results in nitrogen starvation, apparently leading to a decrease in the level of heterocysts inhibitors (Kumar and Kumar, 1989). They observed that absence of Mo enhanced heterocyst differentiation by 13% over control with 5.6% heterocyst frequency in the cyanobacterium *Nostoc linckia*. There have been several reports revealing the necessity of **Sodium** (Na) in many cyanobacteria (Emerson and Lewis, 1942; Kratz and Myers, 1955; Thomas and Apte, 1984). It has been reported that Na and Mo are essential for the expression regulation of nitrogenase activity (Kumar and Kumar, 1989). The role of sodium chloride (NaCl) for maintaining higher nitrogenase activity of isolated heterocysts has been studied (Kumar *et al.*, 1982). Though the occurrence of *Spirulina, Oscillatoria* and *Synechococcus* have been reported in **Sulphur** hot springs, the requirement or

role of sulphur compounds in cyanobacterial augmentation in rice fields has not been studied in detail.

Though cyanobacteria require **Calcium** (Ca) for better growth and performance, their application in the form of lime and gypsum are mainly meant more for pH manipulation in the fields than for Ca availability (Roger and Reynaud, 1979). In cyanobacteria several physiological and biochemical processes such as growth (Bonilla *et al.*, 1995; Singh *et al.*, 1997a), heterocyst differentiation and nitrogen fixation (Cohen *et al.*, 1954; Smith *et al.*, 1987a,b; Rodriguez *et al.*, 1990; Singh *et al.*, 1997a), PS II activity (Piccioni and Mauzerall, 1978; England and Evans, 1983; Becker and Brand, 1985) and phosphate uptake (Kerson *et al.*, 1984) are known to be regulated by Ca^{2+}. Potassium (K) is an essential element in the transport mechanism within the plasma membrane (Reed *et al.*, 1986). It has been reported that the photosynthetic recovery of *Nostoc flagelliformae* during rehydration is a potassium dependent process (Qiu *et al.*, 2004).

Magnesium (Mg) is another important element that forms a prosthetic group of enzymes nitrogenase and glutamine synthetase (Stewart *et al.*, 1979). When $MgSO_4$ was used in the absence of phosphate, cyanobacterial growth was found to be suppressed (De and Sulaiman, 1950). Of all the micronutrients tested for cyanobacterial growth Manganese (Mn) was observed to be least toxic (Jha *et al.*, 2004). **Ferric iron** (Fe^{3+}) is the common form of "biologically available iron" utilized by microorganisms, but some meet their iron requirements by Fe^{2+} species (Anderson and Morel, 1980; Finden *et al.*, 1984). A stimulation of growth of cyanobacteria in a P-amended alkaline flooded soil by the application of iron has been reported (Stewart *et al.*, 1979). Under low Fe^{3+} conditions, it is reported that, the microbes excrete siderophores (low molecular weight water soluble compounds) (Kerry *et al.*, 1988; Hutchins *et al.*, 1991; Trick *et al.*, 1995) that render the iron soluble by binding the Fe^{3+} in a complex that can be transported. The cyanobacterial siderophores are catechol and hydroxamate that bind iron with very high specificity and affinity (Brown and Trick, 1992; Wilhelm and Trick, 1995). Deficiency of iron (Fe^{3+}) caused special shift of chlorophyll content and decreased photosynthetic capacity (Oquist, 1971, 1974a,b). It also resulted in the accumulation of intracellular

polysaccharides (Hardie *et al.*, 1983a,b). Iron starvation in several cyanobacteria resulted in a decrease in thylakoids membrane, phycobilisomes and carboxysomes (Sherman and Sherman, 1983). Alteration in the thylakoids protein (Guikema and Sherman, 1984), a blue-shift in chlorophyll absorption peak (Pakrasi *et al.*, 1985), flavodoxin replacement in photosynthetic apparatus (Laudenbach *et al.*, 1988; Bottin and Lagoutte, 1992), changes in the outer membrane protein profile (Scanlan *et al.*, 1989) are the other major cellular responses of several cyanobacteria to iron stress. It has been observed that toxic effect of iron was very prominent at the acidic pH and in presence of NaCl in the culture (Naik, 2000). Growth was found to be maximum in 50 µM Fe^{3+} and found to be lethal at 500 µM (Saxena *et al.*, 2002).

The role of **Zinc** (Zn) on the growth of *Synechococcus* sp. (6031) has been studied by Chintamani and Mohanty (1988) and found that the cyanobacterium grows well at increasing levels of $ZnSO_4$ containing medium. It has also been reported that in the cyanobacterium *Synechocystis aquatilis* f. *aquatilis* (de Magalhaes *et al.,* 2004), the photosynthetic performance of cells cultured in the presence of high Zinc levels showed a decline in both the apparent photosynthetic efficiency and photosynthetic maximal rate. Light microscopic observation of Zinc affected cells of this species revealed the presence of thick mucilaginous layer which could retain high amounts of metal ions from the medium, thus providing the cells of the species a mechanism to circumvent high toxic levels of Zinc (de Magalhaes *et al.,* 2004). **Boron** (B) is found to be essential in the absence of combined nitrogen in heterocystous cyanobacteria (Martinez *et al.*, 1986). Application of 5 kg Zn ha^{-1}or 0.04 % boron as boric acid spray was the ideal dose for enhancing nitrogen-fixing ability of autochthonous cyanobacteria (Jha *et al.*, 2004). **Copper** (Cu) acts as an algicide at higher concentrations (Gupta and Arora, 1978; Maheswari and Anand, 2000; Jha *et al.*, 2004), but it also functions as a trace element for the life activities of certain cyanobacteria (Walker, 1953). Tolerance to micronutrients is known to be strain specific, however, multiple tolerance phenomenon is not rare (Jha *et al.*, 2004). These reports clearly bring out the essentiality of various elements essential for cyanobacterial growth in paddy fields.

Agrochemicals:

Pesticides have become more essential in modern agriculture, but they are also a potential source of chemical degradation of soils. The 1992-1993 data shows that India used 47.62 million tonnes of insecticides (Pesticide Information, 2001).

In tropical countries rice has been identified as one of the crops that is particularly susceptible to the negative impacts of pesticide use. In addition to the physico-chemical nature of the pesticide, tropical climatic conditions and agricultural practices play important roles in determining the fate and distribution of pesticides in the tropical paddy field ecosystem (Abdullah *et al.*, 1997).

The tropical water- logged rice field is generally considered to be a fertile agro eco-system, but frequent crop intensification may affect its fertility. It is necessary to understand and predict how factors associated with crop intensification, especially agro-chemical use may affect the soil microbial biomass either directly through toxic effects or indirectly by decreasing the productivity of the photosynthetic aquatic biomass and inhibiting invertebrate population responsible for nutrient recycling and translocation.

A review of more than 200 published works on pesticidal effect of rice field microorganisms (Roger, 1990) showed that except eight papers all the other works were restricted to laboratory conditions. Of the eight studies, five studies reported no effect of pesticide application on microbial population or activities. Others showed a transitory drop of population followed by a recovery within 2-3 weeks. A few studies indicated that repeated application of a pesticide might enhance the growth of related specific decomposing microorganism and cause its rapid inactivation. This was observed with gamma-BHC, diazinon, aldicarbe and nitro phenols but not with carbofuran and benthiocarb. Repeated usage of pesticide may change the metabolic patterns of their decomposition (Moon and Kuwatsuka, 1984). Pesticides have been shown to bring about morphological, physiological, cytological and mutagenic changes in the microorganisms (Singh, 1973; Das and Singh, 1977; Ruptal and Saxena, 1980; Padhy, 1985; Zargar and Dar, 1990). Higher concentrations of the weedicide Benthiocarb have been shown to affect growth and nitrogen fixation by cyanobacteria (Kolte and Goyal, 1990). But, the influence of carbofuran on

Aulosira fertilissima was studied in relation to growth, nitrogen fixation and its forms of accumulation in culture medium and in 2 soils (alluvial and lateritic) under water-logged conditions and found that nitrogen fixation was encouraged at all levels of insecticide application up to 200 µg/ g soil during incubation for 1.5 months, but the magnitude of response varied between soils (Ghosh and Saha, 1988). Uma and Kannaiyan (1998b) observed that the insecticide carbofuran at 5 ppm stimulated ammonia excretion by the cyanobacterial cultures immobilized in PU foam. Increase was also noticed in the chlorophyll *a*, protein, carotenoid, phycobilin and polysaccharide contents. The increased production of these contents may be due to continuous photosynthetic activity in presence of carbofuran. Kannaiyan *et al.* (1993) have reported 5-10 fold increase in ammonia production in cyanobacterial cultures treated with benlate (Methyl-1-butyl-carbamoyl-2-benzimidazole carbamate), a systemic fungicide. Another systemic fungicide bavistin at 5 ppm level enhanced ammonia excretion and overall growth of immobilized cyanobacterial cultures suggesting that this fungicide effect could be better exploited in wet land rice field ecosystem for increasing the rice production in addition to its primary role as a fungicide (Uma and Kannaiyan, 1998a; Maheshwari and Anand, 2004).

Pesticides affect cyanobacteria under laboratory conditions differently (Gangawane and Saler, 1979; Anand and Veerappan, 1980; Kannaiyan, 1985; Anand, 1990b; Maheswari and Anand, 2004) and their effects with regards to the growth of these organisms has been classified as stimulatory, inhibitory and ineffective (Metting, 1985).

Pesticides have three major effects on rice field cyanobacteria. 1) A selective toxicity which affects preferentially green algae and there by promoting cyanobacterial growth. 2) A short term promoting effect of insecticides on micro algae due to temporary decrease of invertebrates those graze on algae. 3) A selective effect of insecticides on cyanobacterial flora by causing a recruitment of algal grazers which results in the dominance of strains forming mucilaginous macro colonies resistant to grazing such as *Nostoc* sp.

Since no field-studies have been undertaken over several crop cycles, information on the long-term effects of pesticide use on wet land soil micro flora

and fauna is inadequate. Agrochemical use did not bring any change in the primary production but by favouring algivorous arthropods, it also favoured nutrient recycling (Roger and Simpson, 1991). Pesticides applied on soil at recommended levels rarely had a detrimental effect on microbial populations or their activities (Roger *et al.,* 1994).

In Indian agriculture, the use of neem as biological pesticide is not less common. Neem has caught the attention of research field due to its nitrification inhibition property (Khandelwal *et al.*, 1977; Chhonkar and Mishra, 1978; Devkumar and Mukherjee, 1985) and increasing the nitrogen utilizing efficiency of the crops. Many a bio-reactive compounds have been isolated from various parts of the neem tree, some notable being meliantiole and azadirachtin (Botterworth and Morgan, 1968). Neem cake can control many pests of crop plants. Neem cake of 500 Kg/ha significantly reduced the pest(s) problem due to *Pyralis* sp. in *Azolla pinnata* (Nandabalan and Kannaiyan, 1984). Observation has made that use of different forms of neem (neem cake, neemax or the aqueous extract of neem leaves) even up to 0.5% (w/v), will not affect adversely the nitrogen-fixing cyanobacteria of rice fields. (Mishra and Adhikary, 1997). Neemcake is found to be less expensive and is safe to use as a nutrient as well as pesticide (Kannaiyan *et al.*, 1983; Kannaiyan and Sundaravarathan, 1998). Application of biopesticides like Neem products and *Bacillus thuringiensis* Kurstaki in crop production is advantageous in view of their ecosafety, specificity, reduced number of applications, no resistance to pests, increased yields and quality improvement of crops and suitability in rural areas (Vivek, 2005).

Fertilizers

The cyanobacterial flora and its better performance in water logged rice field are greatly influenced by quality, type, quantity and mode of application of nitrogen fertilizer. Bunt (1961) reported the absence of nitrogen fixing cyanobacteria in experimental plots in Australia and he thought that it might be due to high dose of nitrogen fertilizer application in rice field soil. The absence of nitrogen fixing cyanobacteria and abundance of non-nitrogen fixing forms has been reported in paddy fields at Madras (Anand, 1990a). The N_2 fertilizer has

differential effect on growth and N_2-fixation by cyanobacteria (Goyal and Marwaha, 1985).

Urea is good nitrogen source for many cyanobacteria belonging to different taxonomic groups (Kratz and Myers, 1955; Neilson and Larsson, 1980; Rawson, 1985). It is taken up actively by the cyanobacterial cells and metabolized intracellular (Healey, 1977) by urease releasing CO_2 (1 molecule) and Ammonia (2 molecules) (Berns *et al.*, 1966; Carvajal *et al.*, 1982; Mackerras and Smith, 1986).

There are several reports on the effect of nitrogenous fertilizer on heterocyst differentiation (Fogg, 1949; Michelson *et al.*, 1967; Ogawa and Carr, 1969). It has been reported that a reduction in heterocyst frequency was observed in *Anabaena oryzae* at 30 ppm of urea-N and at 60 ppm filaments were completely devoid of heterocysts and nitrogenase activity was completely arrested (Mekonnen *et al.,* 2002). Cyanobacteria inoculated in media containing 100 mg nitrate nitrogen/litre continued to reduce acetylene, but nitogenase activity was reduced as the cyanobacteria grow and this was accompanied by a reduction of heterocysts (Stewart *et al.*, 1968). The concentration of 0.1 mg/ml of ammonium sulphate and urea did not affect growth and heterocyst differentiation of certain taxa of cyanobacteria. These reports suggest that the cyanobacterial strains that are capable of fixing nitrogen even in the presence of available sources of nitrogen have immense value in biofertilizer programme for improving N-budget in rice cultivation (Mekonnen *et al.,* 2002).

Biotic factors:

Of the various factors, grazers form the major factor for the flourishing of the cyanobacteria (Roger and Reynaud, 1979). As there is a strong relationship between cyanobacteria and grazers early in the rice cultivation cycle, to encourage the growth of nitrogen fixing cyanobacteria, one has to reduce the grazing pressure on cyanobacteria. Copepods, cladocerans and rotifers are the various zooplanktonic grazers, which filter the phytoplankton and cyanobacteria from flooded water. At the floodwater/soil interphase, ostracods, chironomid larvae and molluscs browse the cyanobacterial growth, which luxuriate early in the rice cropping cycle (Grant *et al.*, 1986). Several reports have been attributed to the disappearance of inoculated cyanobacteria in the field due to grazing

(Singh, 1985; Chinnaswamy and Patel, 1990). *Cypris* species, an ostracod has been reported as a cyanobacterial predator (Wilson *et al.*, 1980; Grant and Alexander, 1981). Ghabbour *et al.* (1980) observed that protozoa and nematodes retarded the growth of cyanobacteria. Roger and Reynaud (1982) observed a species of *Limnaea*, a snail as a predator of cyanobacteria in the rice fields of Philippines. *Canthydrus cantabiles,* a coleoptera and *Idiopoma dissimilis*, a snail repeatedly deteriorated the growth and nitrogen yield of cyanobacteria in CRRI rice fields, at Cuttack (Singh and Bisoyi, 1989). Grant *et al.* (1983) observed that all species of inoculated cyanobacteria were readily consumed by an ostracod *Heterocypris luzonensis* Neale, which completely dominated the ostracod population established during both rice crops. They consume non-mucilaginous cyanobacteria as do the molluscs *Limnaea*, but neither group successfully consume the mucilaginous colonies of *Gloeotrichia, Nostoc and Aphanothece* (Grant *et al.*, 1985). Therefore grazing has a selective effect on cyanobacteria.

Plants having pesticidal properties are frequently cheap and may be safe to use if grown by farmers. Plants such as neem (*Azardirachta indica*) and those listed with molluscicidal properties are often available in rice growing areas (Grant *et al.*, 1986) to check the grazers.

Apart from grazers, various pathogenic and antagonistic organisms also limit cyanobacterial growth in fields. Certain bacteria, fungi, viruses, *etc.* have been shown to be pathogenic to them (Singh, 1973; Roger and Reynaud, 1979; Grant *et al.*, 1983). Various lytic bacteria may cause the lysis of vegetative cells of cyanobacteria (Roger and Reynaud, 1979).

Land preparation and management:

The various agronomic practices adopted during cultivation cycle influence the growth and establishment of cyanobacteria. Tillage has a disturbed effect on cyanobacteria, mainly because of the incorporation of cyanobacteria in the soil and dispersion of clay particles in water which decreases the available light (Roger and Kulasooriya, 1980). Moreover, it enhances the growth of eukaryotic algae due to soil ammonification. The motile *Oscillatoria* and *Pseudanabaena* are more adaptable to recolonization of the submersion water (Roger and Reynaud, 1979).

Cyanobacteria are generally sciophilous in nature (Reynaud and Roger, 1979). Hence, transplanting of rice enhanced cyanobacterial growth due to increased shade in rice fields in early cultivation cycle itself. Weeding has a negative effect on the cyanobacterial density and activity. This is because of the existence of epiphytic cyanobacteria on weeds of flooded paddy fields, which play an important role in inoculum's conservation and N_2 output. (Kulasooriya *et al.*, 1980a,b).

Water management is yet another factor that determine cyanobacterial quantity and quality. Periodic drying and wetting of rice fields, usage of minimum irrigation water, *etc.* can favour cyanobacterial growth and check eukaryotic algae and grazers that feed on cyanobacteria and other phytoplankton (Roger and Kulasooriya, 1980)

MATERIALS AND METHODS

Umamaheswari N. A. "Isolation and Characterisation of Nostocales (Cyanobacteria) of the Paddy fields of Kerala " Thesis. Department of Botany , University of Calicut, 2005

Materials & Methods

MATERIALS AND METHODS

The cyanobacterial species belonging to the order Nostocales, collected from the rice fields of different soil types of Kerala distributed in different districts were isolated and considered in the present investigation.

Systematic treatment of Nostocales:

Cyanobacteria grow together with the rice plants under waterlogged conditions and form patches of slimy bluish, blue-green, yellowish or brownish mass on the surface of water (Fig. 2). Sometimes they are seen attached to the paddy plants as mucilage balls. Samples of such visible cyanobacteria were collected from the paddy fields and adjoining areas of different localities of Kerala in plastic vials. The soil type, nature of habitat and habit of the thallus were noted for each sample. A part of the sample was fixed in 4% formaldehyde solution and the remaining was used for isolation and subsequent culturing of cyanobacterial species using the standard isolation techniques. The taxa were identfied up to their species/variety/forma levels from the preserved samples with the help of keys and classical monographs of Geitler (1932), Desikachary (1959) and Anand (1989) on cyanobacteria. Camera lucida diagrams were drawn and microphotographs of some of the species were taken and presented in the text.

Culture studies:

The fresh field samples were subjected to enrichment cultures in culture tubes and agar petriplates in sterile medium. The visible cyanobacterial masses were collected and washed in sterile water. A small quantity of the cyanobacterial aggregate was vortexed in 5 ml of the sterile culture medium and was used as initial inoculum. The isolation was carried out by the streak and spread plate methods (Stein, 1973; Kaushik, 1987). Sub culturing was done repeatedly, whereby pure cultures of some species (unicyanobacterial isolates) were obtained. By exposing these species to low concentrations of antibiotics like Streptomycin, axenic cultures of few species were raised. Cycloheximide 20 ppm/l was added so as to eliminate the growth of eukaryotic algae. The cultures were periodically examined under research microscope for assessing their pure

nature. Liquid cultures in conical flasks and solid cultures on agar plates/slants were done.

Culture methods:

Borosil glass tubes and conical flasks (250 ml) were used for isolation and purification of cyanobacteria. The glassware were cleaned in liquid detergent and dried in a hot air oven before use. After cooling, they were immersed in chromic acid [Potassium dichromate in conc. H_2SO_4 1% w/v] overnight followed by repeated washing in tap water and finally with distilled water.

The culture medium selected for the study was BG-11 medium with a nitrogen source for non-heterocystous cyanobacteria and without a nitrogen source (nitrogen free medium) for heterocystous types. The composition of the medium is given below.

BG-11 Nitrogen free medium- Composition (Rippka *et al.*, 1979).

Chemicals	Concentration (g/l)
$K_2HPO_4.3H_2O$	0.040
$MgSO_4.7H_2O$	0.075
$CaCl_2.2H_2O$	0.036
Citric acid	0.006
Ferric Ammonium Citrate	0.006
EDTA(Di sodium magnesium salt)	0.001
Na_2CO_3	0.02
A_6 Micronutrient solution	1mg/l

For non-heterocystous cyanobacteria, 1.5 g $NaNO_3$ (Sodium nitrate) is added to the BG-11 medium as nitrogen source (pH of the medium was adjusted to 7.4).

Composition of trace metal solution (After Rippka *et al.*, 1979).

Chemicals	Concentration (g/l)
H_3BO_3	2.86
$MnCl_2.4H_2O$	1.81
$ZnSO_4.7H_2O$	0.222
$Na_2MoO_4.2H_2O$	0.390
$CuSO_4.5H_2O$	0.079
$Co(NO_3)_2.6H_2O$	0.0494

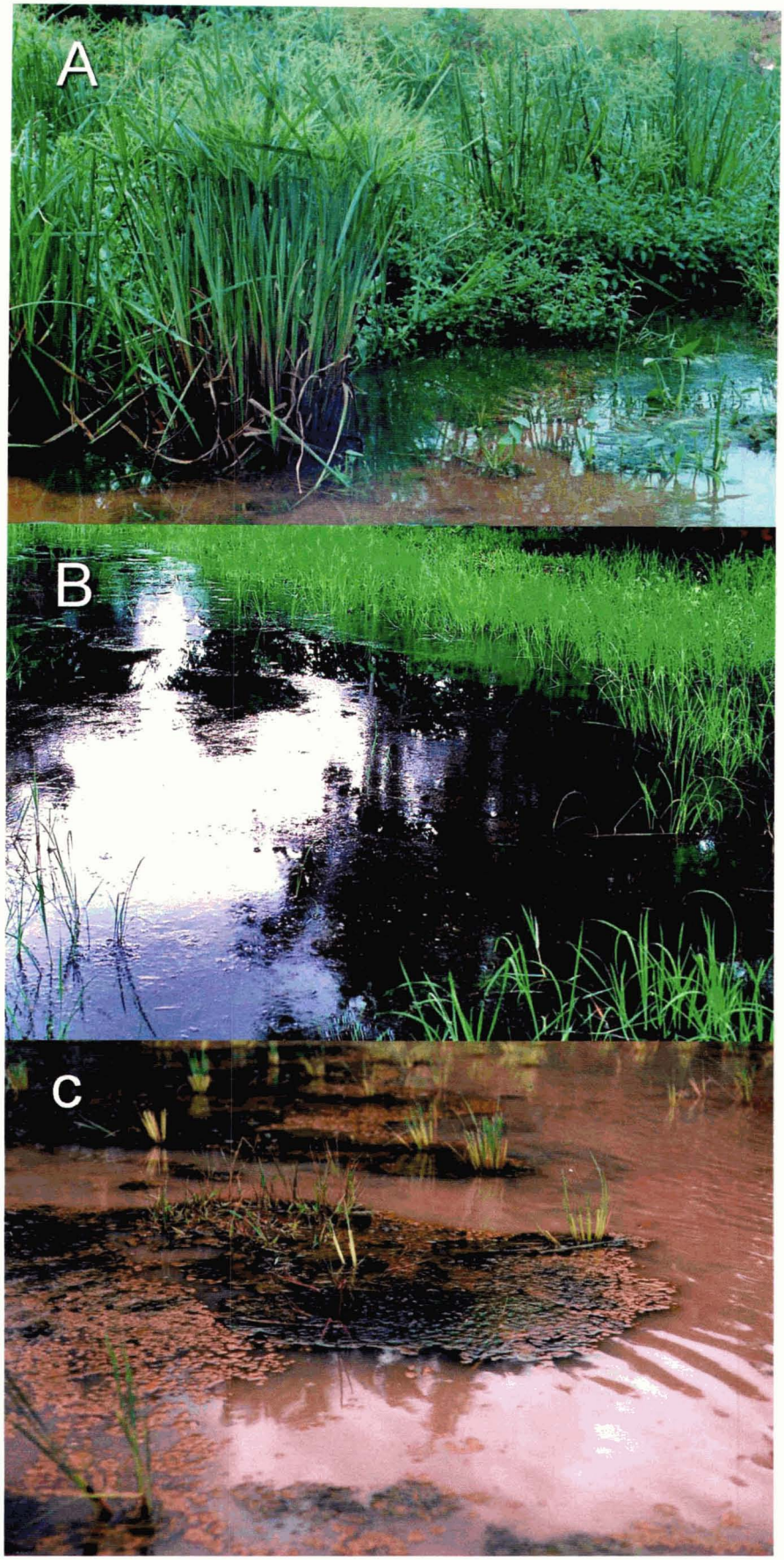


Fig. 2. Visible cyanobacterial mass in various rice fields and associated areas in Kerala. A. Kayal fields, Kottayam Dt., B. Kole fields, Thrissur dt., c. Poonthalpadom fields, Palakkad Dt.

Sterilization:

The glassware and culture medium used for the experiment were sterilized properly for 20 minutes in a pressure cooker. They were irradiated in Laminar airflow chamber for 2 hrs before using them in the experiments.

Culture conditions:

The cultures were maintained in culture room at 26±2°C under illumination of 2000-3000 lux of cool white fluorescent light programmed for 14/10 hrs L/D cycle. The cultures in the liquid medium were hand shaken daily to prevent sticking of the cyanobacteria to the wall of the container. Inoculation was carried out in a Laminar airflow chamber. The volume of the medium has been maintained less than half of the culture vessel volume in order to provide adequate air supply and eliminate contamination due to slashing during shaking.

For experiments, homogenized suspensions of each of the cyanobacterial species were prepared using a sterile glass tissue homogenizer. 10-12 days old cultures were used as inoculum for the experiments. Equal volume of cyanobacterial suspension was pipetted into each experimental tube/vessel containing the sterile medium.

Maintenance of culture:

The stock cultures of cyanobacteria were maintained in agar slants and petriplates. For use in the experiment, each organism was transferred into a conical flask (250 ml) containing 100 ml fresh sterile medium from the agar slants/ petriplates and these cultures were used in their exponential growth phase.

Characterisation under Laboratory conditions:

Selection of growth medium:

In order to select a suitable growth medium for carrying out various investigations, equal amounts of ten unicyanobacterial isolates (heterocystous) collected from rice fields were grown in two different nitrogen free growth media namely, BG-11 and Fogg's media for 20 days and their growth in absorbance at

760 nm and chlorophyll *a* content in either media were compared and the medium which showed better cyanobacterial growth was selected and adopted as the suitable culture medium in all experiments.

Nature of cyanobacterial species in solid and liquid medium – A comparative study:

The above ten unicyanobacterial cultures belonging to the genera *Nostoc, Cylindrospermum, Calothrix and Scytonema* were studied in solid and liquid nitrogen free BG-11 medium based on their morphological features. The various morphological parameters, such as, nature and colour of the thallus, pattern of growth in the medium were applied and visible morphological changes in the medium were considered.

Furtehr characterization of some selected species:

Of the above 10 unicyanobacterial cultures selected for studying their morphological behaviour in culture medium, three species *viz.*, *Nostoc linckia, Nostoc muscorum* and *Calothrix marchica* var. *intermedia* were selected for further characterization, *viz.,* ecological, nutritional and agrochemical responses.

Ecological responses:

Ecological responses of the cyanobacterial species were studied in terms of pH and salinity.

For pH studies, the selected cyanobacterial forms were homogenized aseptically with the help of a micro tissue homogenizer: 1 ml of each of the cyanobacterial suspension thus prepared was inoculated in 10 ml of BG-11 (N_2 free) medium contained in 15 mm × 150 cm test tubes, amended with varying levels of pH [4.0, 5.0, 6.0, 7.0, 7.4 (control), 8.0 and 9.0] and incubated under culture conditions for a period of 25 days. All set ups were made aseptically. The acid tolerance of the above cyanobacterial species was also determined by this study.

In order to study the salinity tolerance, 1 ml of the cyanobacterial isolates after homogenization was introduced in test tubes containing 10 ml BG-11 (N_2 free) medium amended aseptically with desirable concentrations of NaCl (0.1M,

0.2M, 0.3M and 0.4M) and incubated under culture condtions for a period of 25 days. One set with medium and inoculum was kept as control.

Nutritional responses:

The nutritional responses of the selected cyanobacterial species were studied by observing their growth in presence of exogenous carbon compounds and by studying their autotrophic, mixotrophic, and heterotrophic growth capacity.

For studying the response of cyanobacteria to various exogenous carbon compounds five species of cyanobacteria, namely, *Nostoc linckia, Nostoc muscorum, Nostoc spongiaeforme, Calothrix elenkinii* and *Calothrix marchica* var. *intermedia* were selected and 1 ml of each of the cyanobacterial isolates after homogenization was introduced in test tubes containing BG11 (N_2 free) medium supplemented with 2 sugars, *viz.,* glucose and sucrose at a concentration of 15 mM each in the culture and incubated under light with 12 hr L/D cycle for a period of 15 days.

For studying the autotrophic, mixotrophic, heterotrophic growth capacity of the above five cyanobacterial species, 2 sugars *viz,* sucrose and glucose were supplemented to BG11 (nitrogen free) medium at a concentration of 15 mM in the culture. Cultures were incubated under light with 12 hr L/D cycle and in the dark for a period of 15 days. Since these sugars (glucose and sucrose) are simple and important energizers belonging to mono and disaccharides respectively, they were selected in the study as exogenous carbon compounds.

The nutritional responses were also studied by making amendments in the nutrient media in the following ways.

For studying the role of phosphate the three selected cyanobacteria were grown in the presence and absence of K_2HPO_4. For this, 1 ml of homogenized suspension of each of the cyanobacterial species at the exponential stage was inoculated in 10 ml of BG-11 N_2-free medium with or without K_2HPO_4 (40 mgl^{-1}) and incubated under culture conditions for a period of 16 days. The growth performance of these species by supplementing the medium with and without

combined inorganic nitrogen source (10 mM NO_3^- medium) in the presence and absence of phosphate source, K_2HPO_4 (40 mgl^{-1}) was also studied.

Agrochemical responses:

The selected three species of cyanobacteria, *viz.*, *Nostoc linckia, N. muscorum* and *Calothrix marchica* var. *intermedia* were tested for their response to various agro-substances like neem cake, furadan (carbofuran) and NPK fertilizers (urea, super phosphate and potash).

The effect of neem cake (a natural pesticide) on the above mentioned cyanobacteria were studied. For this neem cake were ground with sterile distilled water at room temperature, filtered through cellulose membrane filter asceptically and the resultant clear extract was added to BG-11 nitrogen free medium to obtain the desired concentrations (0.2%, 0.5%, 1%, 2%, 3%, 4%). One ml of the homogenized culture suspension was inoculated into the experimental culture tubes and incubated under culture conditions for a period of 25 days.

For studying the response of furadan, the three cyanobacterial species were grown in BG-11 N_2 free medium amended with desired concentration of furadan ranging from 2 ppm - 500 ppm (2,5,10,20,50,100, 200 and 500 ppm) for a period of 25 days under culture conditions.

Studies were made on the response of the three cyanobacterial species - to various fertilizers like urea, potash and super phosphate. For this 1 ml of the homogenized suspension of either strain was grown in 10ml of BG –11 N_2 free medium and media amended with either type of fertilizers in desirable concentrations, *viz.*, (0.01, 0.05, 0.1, 0.5, 1.0, 2.0, 3.0 mg/ml) for a period of 15 days.

Growth measurements in the above experiments were measured in terms of the following methods - absorbance of culture suspension at 760 nm, by calculating growth constant (K), chlorophyll *a* content (McKinney, 1941) and protein content (Lowry *et al.*, 1951).

Specific growth constant (K) was calculated using the formula:

$K = \ln (N_1 / N_0) (1.443/t)$

Where N_1 and N_0 are cell concentrations at the end and beginning of a period of time 't' days.

By observing the cyanobacterial species under microsope heterocyst frequency was also done using the formula:

$$\text{Heterocyst frequency} = \frac{\text{Number of heterocysts}}{\text{Total number of vegetative cells}} \times 100$$

Triplicates were set up for each set of the experiments and the results were presented either graphically or as mean values of the three readings ± S.D.

RESULTS AND DISCUSSION

Umamaheswari N. A. "Isolation and Characterisation of Nostocales (Cyanobacteria) of the Paddy fields of Kerala " Thesis. Department of Botany , University of Calicut, 2005

Results & Discussion

TAXONOMIC TREATMENT

Diagnostic key to the various orders of cyanobacteria

1. Forms unicellular.....2
1. Forms filamentous.....3
2. Forms attached. .....Chamaesiphonales
2. Forms not attached..... .....Chroococcales
3. Pseudoparenchymatous growth present.....Pleurocapsales
3. Pseudoparenchymatous growth absent.....4
4. Filaments unbranched or showing false branching.....**Nostocales**
4. Filaments showing true branching.....Stigonematales

NOSTOCALES Geitler

Synopt. darst. Cyano. Beih. bot. Cbl. 41: 252, 1925.

Plants filamentous, hormogones present, heterocyst and akinetes present, true branching absent, false branching present.

Diagnostic key to the various families of Nostocales of Cyanobacteria

1. Filaments heterocystous with spores..... 2
1. Filaments non heterocystous without spores.....Oscillatoriaceae
2. Filament showing uniform width.....3
2. Filaments showing base-apex polarity.....Rivulariaceae
3. Filaments unbranched. .....Nostocaceae
3. Filaments showing false branching.....Scytonemataceae

OSCILLATORIACEAE Kirchner

Nat. Pflanzenfam, Ed., Ia., 61,1898.

Trichome with a single row of similar and uniformly broader cells, sometimes tapering at the extreme ends; not branched, with or without diffluent mucilage or a homogeneous or more or less lamellated firm sheath; occasionally branched in genera with a firm sheath; trichome straight or bent at the apex, or, regularly or irregularly spirally coiled, single or many within a sheath; heterocysts and spores absent; hormogones present; majority show a spiral movement by rotation along the longitudinal axis.

Key to the genera

1. Trichome with a sheath.....2
1. Trichome without a sheath.....4
2. Trichome many within a sheath.....*Microcoleus*
2. Trichome single within a sheath.....3
3. Sheath firm.....*Lyngbya*
3. Sheath mucilaginous.....*Phormidium*
4. Trichome spirally coiled.....*Spirulina*
4. Trichome straight or bent slightly at the apex.....5
5. Cells mostly longer than broad.....*Geitlerinema*
5. Cells mostly shorter than broad.....*Oscillatoria*

SPIRULINA Turpin em. Gardn.

Dic. Sci. nat. de Levrault, 50: 309, 1827.

Trichome multicellular, septa between cells are not clear giving the whole trichome a unicellular appearance; sheath absent, trichome loosely or tightly coiled, coils regular; apex not attenuated, terminal cell rounded, without calyptra.

Key to the species

1. Spirals close and compact.....4
1. Spirals distantly placed. 2
2. Spirals large 12-20 µm distant.....*S. laxissima*
2. Spirals closer.....3
3. Spirals 1.5-2 µm distant.....*S. subtilissima*
3. Spirals 4-5 µm distant.....*S. major*
4. Spirals 2-3 µm broad.....*S.labyrinthiformis*
4. Spirals 4-5 µm broad.....*S. subsalsa*

Spirulina labyrinthiformis (Menegh.) Gom., Monogr. Oscillariees, 255,1892; Geitler, Kryptogamenflora, 928, 1932; Desikachary, Cyanophyta, 195, 1959. (Fig. 3: a)

Trichome 1 µm broad, blue-green; spirals very close to each other; 2-3 µm broad.

Observed as epiphytic on other algae in Kayal and Sandy alluvial soils.

Specimen examined: CU 81355.

Spirulina laxissima West, G. S., J. Linn. Soc. (Lond.) Bot., 38: 78, 1907; Geitler, Kryptogamenflora, 929, 1932; Desikachary, Cyanophyta, 196, 1959. (Fig.3: b& 7 A)

Trichome 0.7-0.8 µm broad, blue-green; spirals very loose, but regular 4.2-5 µm broad, 12-20 µm distant from each other; end cells rounded or obtuse apex.

Seen in slow running canals near Laterite and Kole paddy fields.

Specimen examined: CU 81407.

Spirulina major Kutz. ex Gom., Phyc. gene., 183, 1843; Geitler, Kryptogamenflora, 930, 1932; Desikachary, Cyanophyta, 196, 1959. (Fig. 3: d)

Trichomes free, growing among other algae, 1.5 µm broad, blue-green; regularly and spirally coiled, spirals 2.5-4.7 µm broad, 4-5 µm distant.

Collected as epiphytic & edaphic populations in the Laterite, Kole and poonthalpadom fields.

Specimen examined: CU 81552

Spirulina subsalsa Oerst. ex. Gom., Nat. Tidskr., 17, 1842; Geitler, Kryptogamenflora, 927, 1932; Desikachary, Cyanophyta, 193, 1959. (Fig. 3: e)

Trichome 1.5-1.7 µm broad, densely and irregularly spirally coiled, blue-green or yellowish green; spirals 4-4.5 µm broad, very closely arranged.

Epiphytic, collected from the Kari and Laterite fields.

Specimen examined: CU 81340

Spirulina subtilissima Kutz. ex Gom., Phyc. gene., 183, 1843; Gomont, Monogr. Oscillariees, 252, 1892; Geitler, Kryptogamenflora, 929, 1932; Desikachary, Cyanophyta, 196, 1959. (Fig. 3: c)

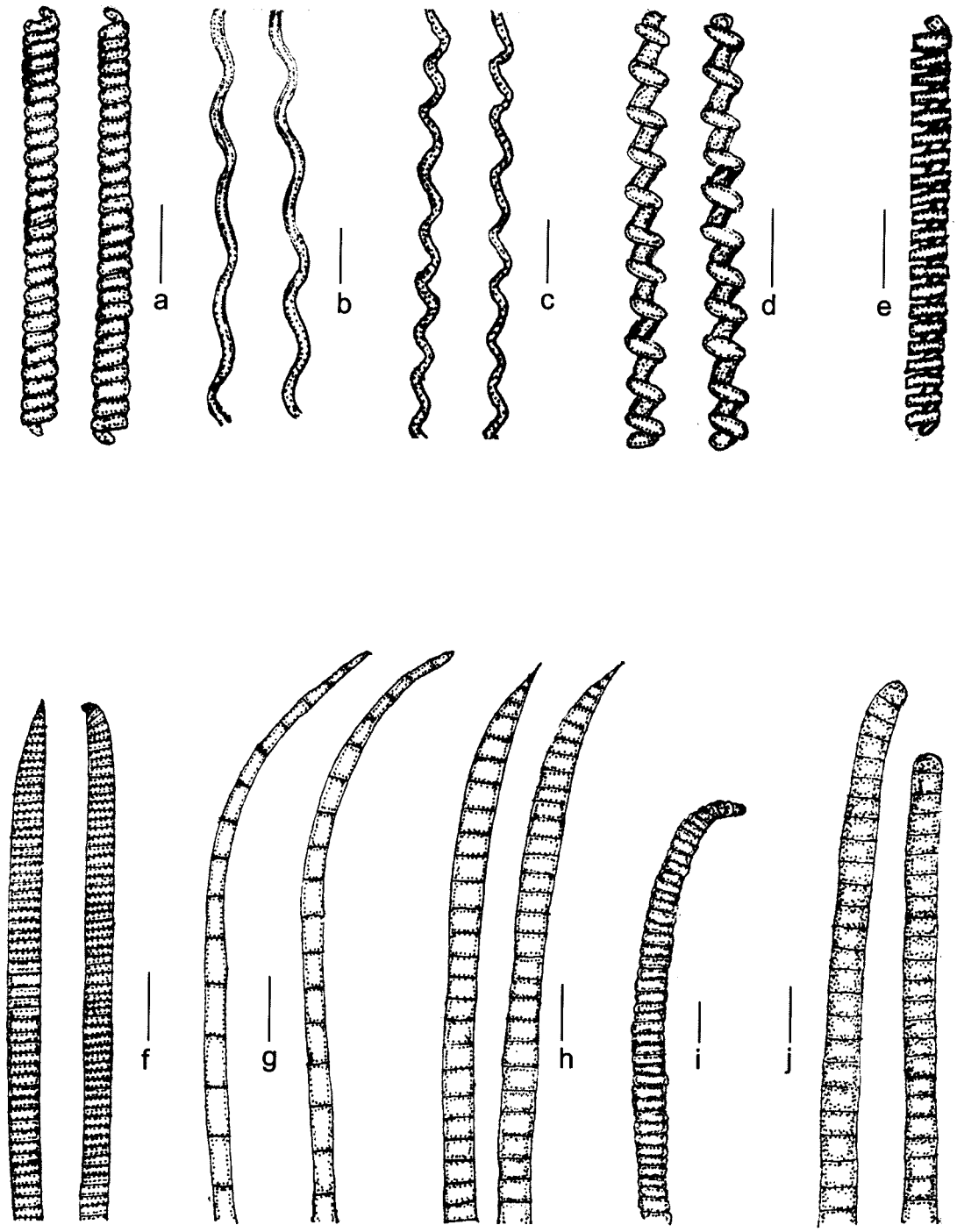


Fig. 3: **a**: *Spirulina labyrinthiformis*, **b**: *S. laxissima*, **c**: *S. subtilissima*, **d**: *S. major*, **e**: *S. subsalsa*, **f**: *Oscillatoria acuta*, **g**: *O. acuminata*, **h**: *O. animalis*, **i**: *O. chalybea*, **j**: *O. chlorina* (Bar 10 µm)

Trichomes 0.6-0.9 μm broad, regularly and spirally coiled, bright blue-green, or yellowish blue-green; spirals 1.5-2.5 μm broad, distance between the spirals, 1.5-2 μm.

Seen in paddy fields with Lateritic soil, along with other cyanobacteria. *Specimen examined*: CU 81409.

OSCILLATORIA Vaucher

Hist. conf., 165,1803.

Trichome solitary and scattered or in groups forming an expanded mass; sheath absent or thin, motile either by oscillation or creeping; end cell of the trichome pointed or otherwise, with or without calyptra; hormogones present.

Key to the species

1. Trichome distinctly attenuated.....16
1. Trichome slightly or not attenuated.....2
2. Cells shorter than broad.....3
2. Cells longer than broad.....15
3. Trichome constricted at the cross walls.....4
3. Trichome slightly/not constricted at the cross walls.....7
4. Trichome up to 11 μm broad.....5
4. Trichome 11-16 μm broad.....6
5. Trichome abruptly bent aside, end cell rounded.....*O. ornata* var. *crassa*
5. Trichome straight, end cell conical.....*O. okeni*
6. Apices capitate with a thickened membrane.....*O. sancta*
6. Trichome not capitate.....*O. perornata*
7. Trichome up to 14 μm broad.....8
7. Trichome 14-50 μm broad.....14
8. Trichome with a thickened outer wall.....11
8. Trichome with out a thickened outer wall.....9
9. Trichome 4-6 μm broad.....*O. chlorina*
9. Trichome 5-11 μm broad.....10
10. Trichome 5-6 μm broad.....*O. subbrevis*
10. Trichome 8.5-11 μm broad.....*O. subbrevis* f. *major*

11. Cross-walls granulated.....12
11. Cross walls not granulated.....*O. irrigua*
12. Trichome 5-6.5 μm broad.....*O. tenuis*
12. Trichome 8-14 μm broad.....13
13. Trichome 8-10 μm broad.....*O. vizagapatensis*
13. Trichome 13-14 μm broad.....*O. limosa*
14. End cell rounded, 14-16 μm broad.....*O. curviceps*
14. End cell flatly rounded, capitate, 16-50 μm broad.....*O. princeps*
15. End cell rounded, cells 2½-6ts as long as broad.....*O. limnetica*
15. End cell obtuse rounded, cells 1¼-2ts as long as broad..... .... O. *pseudogeminata* var. *unigranulata*
16. Trichome capitate.....17
16. Trichome not capitate.....18
17. Trichome slightly capitate and septa granulated.....*O. willei*
17. Trichome prominently capitate and septa not granulated.....*O. proboscidea*
18. Trichome broad and distinctly constricted.....*O. chalybea*
18. Trichome up to 5 μm broad and unconstricted.....19
19. Cell shorter than broad, end cell acute conical.....*O. acuta*
19. Cells quadrate/longer.....20
20. Cells quadrate, end cell acute pointed.....*O. animalis*
20. Cells longer than broad end acuminating.....O. *acuminata*

Oscillatoria acuminata Gom., Monogr. Oscillariees, 227, 1892; Geitler, Kryptogamenflora, 978, 1932; Desikachary, Cyanophyta, 240, 1959. (Fig. 3: g)

Thallus blue-green; trichome straight, not constricted at the cross walls, 3-5 μm broad, at the ends briefly tapering, sharply pointed, bent; cells longer than broad, 5-7.5 μm long, end cell acuminate, without calyptra.

Observed as planktonic in Laterite and Kayal fields of Malappuram and Alappuzha districts.

Specimen examined: CU 81432.

Oscillatoria acuta Bruhl *et* Biswas, J. Dept. Sci., Calcutta Univ., 5: 3, 1922; Geitler, Kryptogamenflora, 978, 1932; Desikachary, Cyanophyta, 240, 1959. (Fig. 3: f & 4 D)

Trichome solitary, filaments blue-green, long, not constricted at the cross walls, 4-5 μm broad; terminal end acute, conical, non capitate, non calyptrate, apex straight, but more often abruptly bent aside; cells 2.5-4 μm long, contents finely granular, sometimes with large granules.

Observed as planktonic, throughout the major rice fields of Kerala.

Specimen examined: CU 81362

Oscillatoria animalis Ag. ex. Gom., Aufzahlung, Flora, 10: 632, 1827; Geitler, Kryptogamenflora, 978, 1932; Desikachary, Cyanophyta, 239, 1959. (Fig. 3: h)

Thallus dark blue-green; trichome straight, not constricted at the cross walls, briefly attenuated at the ends, slightly bent; 4-5 μm broad, non granulated at the septa, cells mostly shorter or as long as broad, 3-5 μm long; end cell pointed, acute, not capitate, not calyptrate.

Observed as planktonic, along with other algae forming a definite stratum in ponds, ditches and in paddy fields throughout Kerala.

Specimen examined: CU 81318

Oscillatoria chalybea (Mertens) Gom., Monogr. Oscillariees, 232, 1892; Geitler, Kryptogamenflora, 956, 1932; Desikachary, Cyanophyta, 218, 1959. (Fig. 3:i & 4C)

Thallus dark blue-green; trichome straight, slightly bent and attenuated at the apex; constricted at the septa, 7-8 μm broad, 4-6 μm long, septa not granulated; end cell obtuse, not capitate.

Observed as planktonic, forming a definite stratum in ponds and ditches in Karappadom, Kari and Pokkali paddy fields of Kerala.

Specimen examined: CU 81325.

Oscillatoria chlorina Gom., Phyc. gene., 185, 1843; Geitler, Kryptogamenflora, 951, 1932; Desikachary, Cyanophyta, 215, 1959. Fig. 3: j

Thallus dark blue-green; trichome straight, slightly bent at the ends, not constricted at the cross walls, not granulated, 4-6 μm broad, 3.5-5.8 μm long; end cell rounded, without calyptra.

Observed as planktonic in Pokkali and Poonthalpadom fields.

Specimen examined: CU 81481

Oscillatoria curviceps Ag. ex. Gom., Syst. Alg., 68, 1824; Geitler, Kryptogamenflora, 947, 1932; Desikachary, Cyanophyta, 209, 1959. (Fig.5: a & 4 A)

Thallus dull blue-green; trichome straight, slightly bent at the end, not attenuated, not constricted at the cross walls, 14-16 μm broad, 2-4.5 μm long, septa slightly granulated, end cell rounded, not capitate.

Observed as planktonic, forming a definite stratum in ponds, ditches and Laterite, Pokkali, Poonthalpadom and Kole paddy fields.

Specimen examined: CU 81515

Oscillatoria irrigua (Kutz.) Gom., Monogr. Oscillariees, 218, 1892; Desikachary, Cyanophyta, 224, 1959. (Fig. 5: b)

Thallus blackish blue-green; trichome light bluish purple, straight, 6-9 μm broad, cells quadrate; septa not constricted, not granulated; apical cell with a thickened outer membrane.

Observed as planktonic in Laterite rice fields of Malappuram and Thrissur districts.

Specimen examined: CU 81544

Oscillatoria limnetica Lemm., Ber. dtsch. bot. Ges., 18: 310, 1900; Geitler, Kryptogamenflora, 963, 1932; Desikachary, Cyanophyta, 226, 1959. (Fig. 5: c)

Trichome solitary, floating, straight, slightly constricted at the cross walls, pale blue-green; 1.5 μm broad, 4-7-9 μm long, end cell flat, rounded, without calyptra.

Observed as planktonic in Laterite, Poonthalpadom and Pokkali rice fields.

Specimen examined: CU 81483

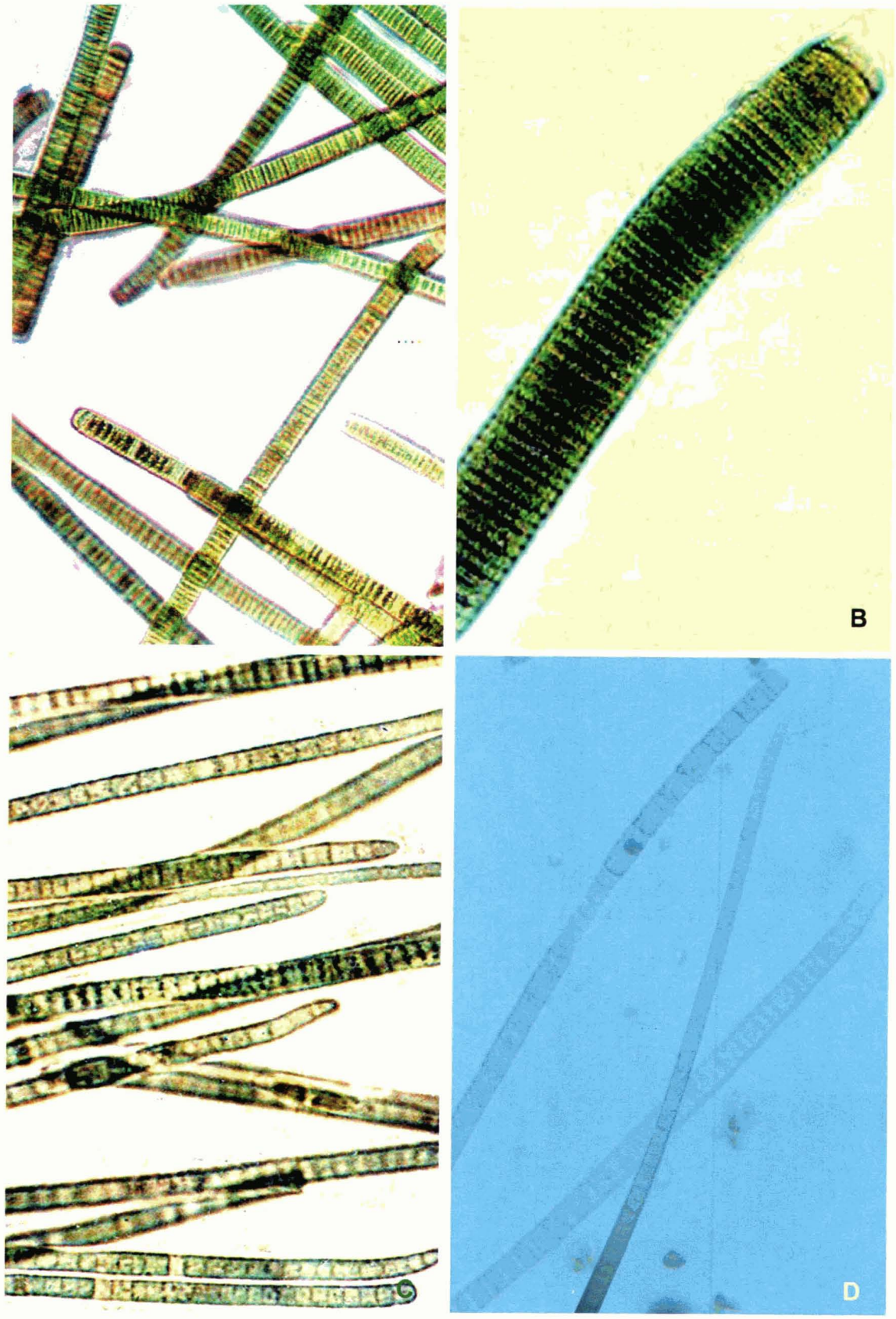


Fig. 4 A: *Oscillatoria curviceps*, **B:** *O. princeps*, **C:** *O. chalybea*, **D:** *O. acuta*

Oscillatoria limosa Ag. ex. Gom., Dispositio Algar. Sueciae., 35, 1812; Geitler, Kryptogamenflora, 944, 1932; Desikachary, Cyanophyta, 206, 1959. (Fig. 5: d)

Thallus dark blue-green; trichome straight, dull blue-green, not constricted at the cross walls; 13-14 μm broad, 2-5 μm long; cross walls granulated sometimes; end cell flatly rounded, with slightly thickened outer membrane.

Observed as planktonic in Sandy alluvial fields and Pokkali fields of Thrissur and Ernakulam districts and Poonthalpadom fields.

Specimen examined: CU 81335, CU 81563

Oscillatoria okeni Ag. ex Gom., Aufzahlung, Flora, 10: 633, 1827; Geitler, Kryptogamenflora, 969, 1932; Desikachary, Cyanophyta, 231, 1959. (Fig. 5: e)

Thallus dull blue-green; trichome straight, constricted at the cross walls, 5.5-8 μm broad, and 2.5-5 μm long; at the ends up to 7 μm long; end cell conical, not capitate.

Observed as planktonic in Kari, Pokkali and Laterite rice fields.

Specimen examined: CU 81497

Oscillatoria ornata var. ***crassa*** Rao, Proc. Indian. Acad. Sci., 8: 165, 1938; Desikachary, Cyanophyta, 206, 1959. (Fig. 5: f)

Thallus dark blue-green to brownish green; trichome straight or bent aside at the ends, constricted at the cross walls, 8.5-11 μm broad, 2.5-5 μm long; cross walls granulated, apex slightly attenuated, end cell rounded, not capitate.

Observed as planktonic in rice fields Laterite, Kayal and Kole fields.

Specimen examined: CU 81433

Oscillatoria perornata Skuja, Zur Susswasseralgenflora Burmas, Nov. Acta Reg. Soc. Uppsal. 14: 47, 1949; Desikachary, Cyanophyta 205, 1959. (Fig. 5: g)

Trichomes blue-green; erect, apices briefly and slightly attenuated and bent, distinctly constricted at the cross walls; 13-15 μm broad, 2.5-6 μm long; septa sometimes granulated; end cell rounded or hemispherical, not capitate.

Observed as planktonic in Laterite, Sandy alluvial, Kayal and Kole fields.

Specimen examined: CU 81542.

Oscillatoria princeps Vaucher ex Gom., Hist. conf. 190, 1803; Geitler, Kryptogamenflora 947, 1932; Desikachary, Cyanophyta 210, 1959. (Fig. 5: h & 4B)

Trichomes more or less brownish blue-green, straight, not constricted at the cross walls, 16-50 μm broad, 3.5-6 μm long; end cells flatly rounded, slightly capitate.

Observed as planktonic in Laterite rice fields of Kozhikkode, Kole and Pokkali fields of Thrissur, Sandy alluvial of Kollam and Poonthalpadom fields of Palakkad districts.

Specimen examined: CU 81504

Oscillatoria proboscidea Gom., Monogr. Oscillariees, 209, 1892; Geitler, Kryptogamenflora, 948, 1932; Desikachary, Cyanophyta, 211, 1959. (Fig. 5: I)

Thallus dull green; trichome straight, not constricted at the cross walls, 12-15 μm broad, at the ends distinctly attenuated, slightly curved, blue-green; cells 2-4 μm long, not granulated at the end walls, end cell prominently capitate.

Observed as planktonic in Karappadom and Kole rice fields in standing water or on moist soil.

Specimen examined: CU 81572

Oscillatoria pseudogeminata var. ***unigranulata*** Biswas, J. Fed. Malay. Stat. Mus., 14: 409, 1929; Geitler, Kryptogamenflora, 966, 1932; Desikachary, Cyanophyta, 29, 1959. (Fig. 5 j)

Thallus dirty blue-green; trichome straight, curved at ends, not constricted at the cross walls, not attenuated at the apices, end cell obtuse round, not capitate; cells 2-3 μm broad, 2.5-4 μm long; cell wall thick, distinct with one large granule situated at the centre of the partition walls on either side; cell contents uniformly granular.

Observed as planktonic in Laterite, Sandy alluvial, Kayal, Poonthalpadom and Pokkali rice fields.

Specimen examined: CU 81484

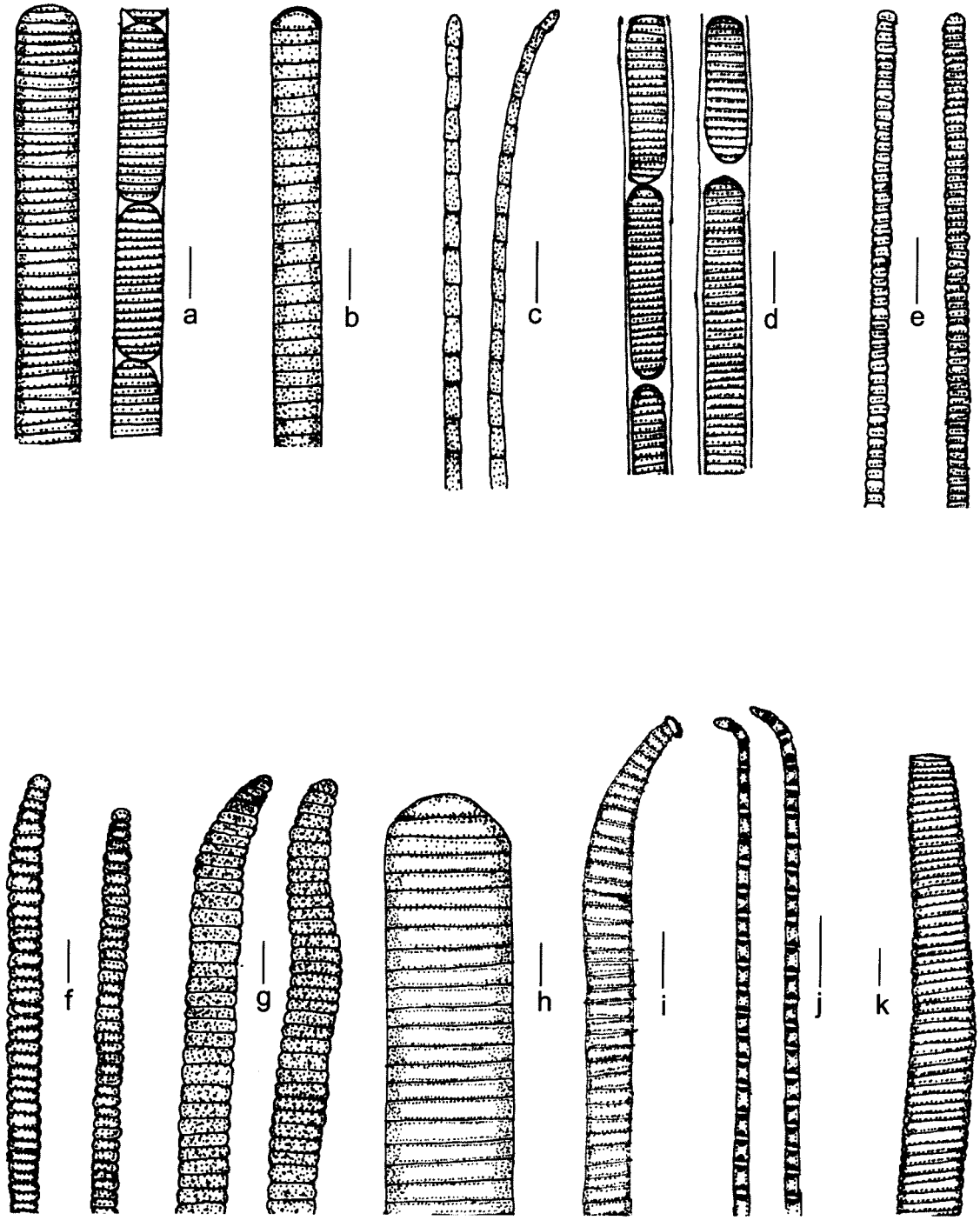


Fig. 5: **a**: *Oscillatoria curviceps*, **b**: *O. irrigua*, **c**: *O. limnetica*, **d**: *O. limosa*, **e**: *O. okeni*, **f**: *O. ornata*, **g**: *O. perornata*, **h**: *O. princeps*, **i**: *O. proboscidea*, **j**: *O. pseudogeminata* var. *unigranulata*, **k**: *O. sancta* (Bar 10 µm)

Oscillatoria sancta (Kutz.) Gom., Monogr. Oscillariees, 209, 1892; Geitler, Kryptogamenflora, 943, 1932; Desikachary, Cyanophyta, 203, 1959. (Fig. 5: k)

Thallus dark blue-green; shining, thin, gelatinous; trichome, straight, bent, distinctly constricted at the cross walls, 11-16 μm broad, 2.5-5 μm long; dull blue-green; granulated at the cross walls, end cell rounded, hemispherical, capitate, with a thickened membrane.

Observed as planktonic, forming a definite stratum in ponds and ditches in paddy fields and nearby areas of Laterite soils of Malappuram district.
Specimen examined: CU 81516

Oscillatoria subbrevis Schmidle Engler's Bot. Jahrb., 30: 243, 1901; Geitler, Kryptogamenflora, 949, 1932; Desikachary, Cyanophyta, 207, 1959. (Fig. 6: a)

Trichome single or in small groups, blue-green; nearly straight, 5-6 μm broad, 1-2 μm long; not granulated at the septa; not attenuated at the apex, end cell rounded, not capitate, not calyptrate.

Observed as planktonic in ponds, ditches and in almost all fields of Kerala.
Specimen examined: CU 81342

Oscillatoria subbrevis f. ***major*** West, J. Linn. Soc. (Lond.) Bot., 38: 78, 1907; Desikachary, Cyanophyta, 209,1959. (Fig. 6:b)

Trichome blue-green, straight, single or in groups; 8.5-11 μm broad, not attenuated, not constricted at the septa, not granulated at the septa; cells 2-3 μm long, end cell rounded.

Planktonic and epiphytic in all rice fields of Kerala.
Specimens examined: CU 81403, CU 81480

Oscillatoria tenuis Ag. ex. Gom., Alg. Dec. 2: 25, 1813; Geitler, Kryptogamenflora, 959, 1932; Desikachary, Cyanophyta, 222, 1959. (Fig. 6: c)

Thallus thin, blue-green, slimy; trichome straight, slightly constricted and granulated at the cross walls, 5-6.5 μm broad, 2.5-3.5 μm long; sometimes bent at the ends, not attenuated at the apex, not capitate; end cell hemispherical, with thickened outer membrane.

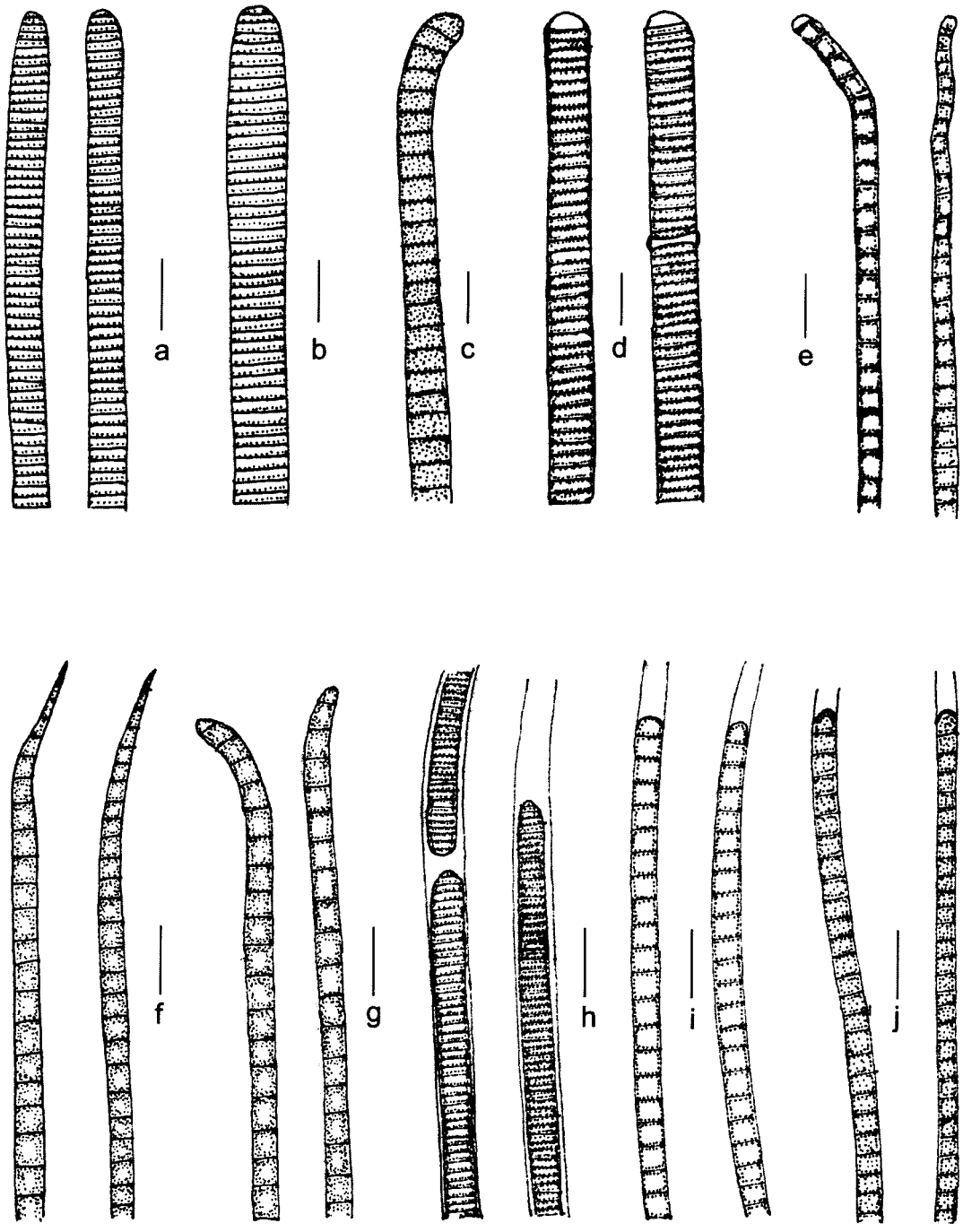


Fig. 6: **a**: *Oscillatoria subbrevis*, **b**: *O. subbrevis f. major*, **c**: *O. tenuis*, **d**: *O. vizagapatensis*, **e**: *O. willei*, **f**: *Geitlerinema earlei*, **g**: *G. jasorvense*, **h**: *Phormidium ambiguum*, **i**: *P. corium*, **j**: *P. corium* var. *capitatum* (Bar 10 µm)

Observed as planktonic in Kole and Poonthalpadom rice fields.

Specimen examined: CU 81567

Oscillatoria vizagapatensis Rao, J. Indian bot. Soc., 17: 89, 1938; Desikachary, Cyanophyta, 205, 1959. (Fig. 6: d)

Thallus blue-green; trichome straight, bent at the ends, pale blue-green, uniformly broad except at the apices; 8-10 μm broad, 1.8-2.2 μm long; contents granular, not constricted at the cross walls; end cell broadly rounded, with a slightly thickened outer wall.

Observed as planktonic in Sandy alluvial, Laterite and Pokkali rice fields.

Specimen examined: CU 81481, CU 81521

Oscillatoria willei Gardn. em. Drouet, Amer. J. Bot., 24: 606, 1937; Geitler, Kryptogamenflora, 954, 1932; Desikachary, Cyanophyta, 217, 1959. (Fig. 6: e)

Trichome pale blue-green, bent at the ends, cells 2.4-3.8- 4 μm broad, 1.5-5 μm long; septa granulated, not constricted at the cross walls; ends somewhat attenuated, end cell rounded slightly capitate, without a thickened membrane.

Observed as planktonic in Laterite, Poonthalpadom, Pokkali and Kole rice fields of Malappuram, Palakkad and Thrissur districts respectively.

Specimen examined: CU 81573

GEITLERINEMA (Anagn. *et* Kom.) Anagn.

Pl. Syst. Evol., 164: 33,1989.

Thallus thin, blue-green and delicate; trichome cylindrical, straight orcurved; sheath absent, trichome attenuated at the ends, terminal cell conical, hooked; cells longer than broad, granular.

Key to the species

1. Apical cell rounded.....*G. jasorvense*
1. Apical cell acuminate.....*G. earlei*

Geitlerinema earlei (Gardn.) Anagn., Pl. Syst. Evol., 164: 33, 1989; *Oscillatoria earlei* Gardn., Mem. N. Y. bot. Gard., 7: 36, 1927; Geitler, Kryptogamenflora, 976, 1932; Desikachary, Cyanophyta, 238, 1959. (Fig. 6: f)

Trichome 2-2.5 μm broad, straight, attenuated, bent at the ends, not constricted at cross walls; cells quadrate or slightly longer, end cell acuminate.

Truly planktonic and epipelic in the Laterite and Sandy alluvium rice fields of Kerala. Slight distribution noticed in Poonthalpadom, Kole and Pokkali fields.

Specimen examined: CU 81491

Geitlerinema jasorvense (Vouk.) Anagn., Pl. Syst. Evol. 164: 33, 1989; *Oscillatoria Jasorvensis* Vouk, Jugosl. Akad. Zagreb. 14: 133, 1919; Geitler, Kryptogamenflora, 962, 1932; Desikachary, Cyanophyta, 221, 1959. (Fig. 6: g)

Trichome 3-5 μm broad, straight, bent at the ends, not constricted at cross walls; cells quadrate or slightly longer, apical cell rounded.

Planktonic in the fields of Palakkad district (Poonthalpadom fields).

Specimen examined: CU 81461

PHORMIDIUM Kutz.

Phyc. gene., 190, 1843

Filaments blue-green, coiled or entangled or parallel; many forming a leathery stratum; sheath thin, colourless, often diffluent; trichome cylindrical; trichome apices attenuated, straight or apex slightly bent, with or without calyptra.

Key to the species

1. Trichome constricted at the cross walls.....2
1. Trichome not or slightly constricted at the cross walls.....5
2. Trichome below 3.5 μm broad.....3
2. Trichome 4-6 μm broad.....*P. jadinianum*
3. Trichome up to 2 μm broad.....*P. foveolarum*
3. Trichome broader.....4
4. End cells rounded, not attenuated..... *P. molle*

4. End cell more conical.....*P. fragile*

5. Trichome up to 4 μm broad.....6

5. Trichome broader.....9

6. End cell conical more or less pointed.....7

6. End cell rounded.....8

7. Cross walls granulated, ends bent.....*P. laminosum*

7. Cross walls not granulated, ends straight.....*P. tenue*

8. Cells 1.7-2 μm broad, 1-1½ times longer than broad..... .*P. luridum*

8. Cells 2.5-4 μm broad, 2-2½ times longer than broad..... *P. mucosum*

9. Trichome up to 6 μm broad. 10

9. Trichome broader.....12

10. Trichome without a thick membrane.....11

10. Trichome with a thick membrane .....*P.corium* var. *capitatum*

11. End cell obtuse conical.....*P. corium*

11. End cell rounded.....*P. ambiguum*

12. Trichome capitate.....13

12. Trichome not capitate.....*P. retzii*

13. Trichome apices straight.....*P. lucidum*

13. Trichome apices bent.....*P.uncinatum*

Phormidium ambiguum Gom., Monogr. Oscillariees, 178, 1892; Geitler, Kryptogamenflora, 1015, 1932; Desikachary, Cyanophyta, 266, 1959. (Fig. 6: h)

Thallus expanded, bright blue-green; filaments flexuous, variously entangled; sheath thin, firm; trichome slightly constricted at the cross walls, not attenuated, not capitate; cells 4.5-6 μm broad, 2.5-3.2 μm long; end cell rounded, calyptra absent.

Observed as submerged edaphic and epipelic forms in Kole rice field soils in Thrissur district.

Specimen examined: CU 81379

Phormidium corium (Ag.) Gom., J. de Bot. Fr., 4: 355, 1890; Geitler, Kryptogamenflora, 1018, 1932; Desikachary, Cyanophyta, 269, 1959. (Fig. 6: I)

Thallus expanded, membranous, leathery, brownish green; filaments long, flexuous, densely entangled; sheath thin, diffluent; trichome straight, ends

bent, blue-green; cells 4-4.5 μm broad, 3.5-7.5 μm long, not constricted at cross walls; end cell obtuse conical, calyptra absent.

Observed as planktonic in the Laterite rice fields in almost all districts and also in other rice fields.

Specimen examined: CU 81366, CU 81514

Phormidium corium var. ***capitatum*** Gardn., Univ. Calif. Publ. Bot., 14: 1927; Geitler, Kryptogamenflora, 1019, 1932; Desikachary, Cyanophyta, 271, 1959. (Fig. 6: j)

Sheath thin, firm, and smooth; trichome 4.5-5.5 μm broad, not constricted at the cross walls, pale blue-green; end cell obtuse conical, with distinctly thickened outer membrane.

Observed in Laterite rice fields in Malappuram and Thrissur districts.

Specimen examined: CU 81506

Phormidium foveolarum (Mont.) Gom., Monogr. Oscillariees, 164, 1892; Geitler, Kryptogamenflora, 999, 1932; Desikachary, Cyanophyta, 254, 1959. (Fig. 8: a & 7 D)

Thallus thin, dark blue-green; sheath colourless, diffluent; trichome flexuous, constricted at the cross walls, septa not granulated, not attenuated at the ends; cells 1.5-1.8-2 μm broad, 1.5-2.3 μm long; end cell rounded, calyptra absent.

Planktonic and epiphytic in all rice fields.

Specimen examined: CU 81334, CU 81558

Phormidium fragile (Menegh.) Gom., Monogr. Oscillariees, 163, 1892; Geitler, Kryptogamenflora, 999, 1932; Desikachary, Cyanophyta, 253, 1959. (Fig. 8: b & 7 C)

Thallus mucilaginous, lamellated, pale brownish blue-green; sheath thin, diffluent; trichome flexuous, entangled, distinctly constricted at the cross walls, septa not granulated, attenuated at the ends; cells 1.5-2.5 μm broad, 1.5-3 μm long; end cell more conical, calyptra absent.

Observed in Laterite fields in Malappuram and Kole and Pokkali rice fields in Thrissur districts.

Specimen examined: CU 81477

Phormidium jadinianum Gom., Bull. Soc. Bot. Fr., 40: 161, 1893; Geitler, Kryptogamenflora, 1002, 1932; Desikachary, Cyanophyta, 256, 1959. (Fig. 8: c)

Thallus dark green, thin; filaments parallel, sheath thin, diffluent; trichome olive green, straight, distinctly constricted and not granulated at the cross walls; cells 4-6 μm broad, 2-3.6 μm long or quadrate, contents granulated with a hyaline area in the centre; end cell acute conical, calyptra absent.

Observed on submerged objects in Pokkali and Kole rice fields.

Specimen examined: CU 81394

Phormidium laminosum Gom., Monogr. Oscillariees, 167, 1892; Geitler, Kryptogamenflora, 1005, 1932; Desikachary, Cyanophyta, 259, 1959. (Fig. 8: d)

Thallus pale blue-green or yellowish, expanded, membranous; filaments flexuous, densely entangled; sheath thin, diffluent; trichome not constricted at the cross walls, pale blue-green, cross walls inconspicuous with granules; cells 1.2-1.5 μm broad, 2-3.9 μm long; ends bent, end cell conical, pointed, calyptra absent.

Observed on submerged objects in Laterite rice field soils.

Specimen examined: CU 81313, CU 81471

Phormidium lucidum Kutz. ex. Gom., Phyc. gene., 194, 1843; Monogr. Oscillariees, 179, 1892; Geitler, Kryptogamenflora, 1025, 1932; Desikachary, Cyanophyta, 275, 1959. (Fig. 8: e)

Thallus dark blue-green, expanded; trichome generally without sheath, flexuous, straight; not constricted or slightly constricted at the septa, granulated at the cross walls; cells 6.5-8 μm broad, 2.2-2.5 μm long; end cell capitate, obtuse, with a subconical calyptra.

Observed in Kari and Kayal Paddy fields of Alappuzha district.

Specimen examined: CU 81472

Phormidium luridum (Kutz.) Gom., Monogr. Oscillariees, 165, 1892; Geitler, Kryptogamenflora, 1009, 1932; Desikachary, Cyanophyta, 263, 1959. (Fig. 8: f)

Thallus membranous, dark blue-green; sheath thin, diffluent; trichome flexuous, ends not bent, not attenuated, slightly or not constricted at the end walls; cells 1.7-2 μm broad, 1.8-3 μm long, septa not granulated; end cell rounded, calyptra absent.

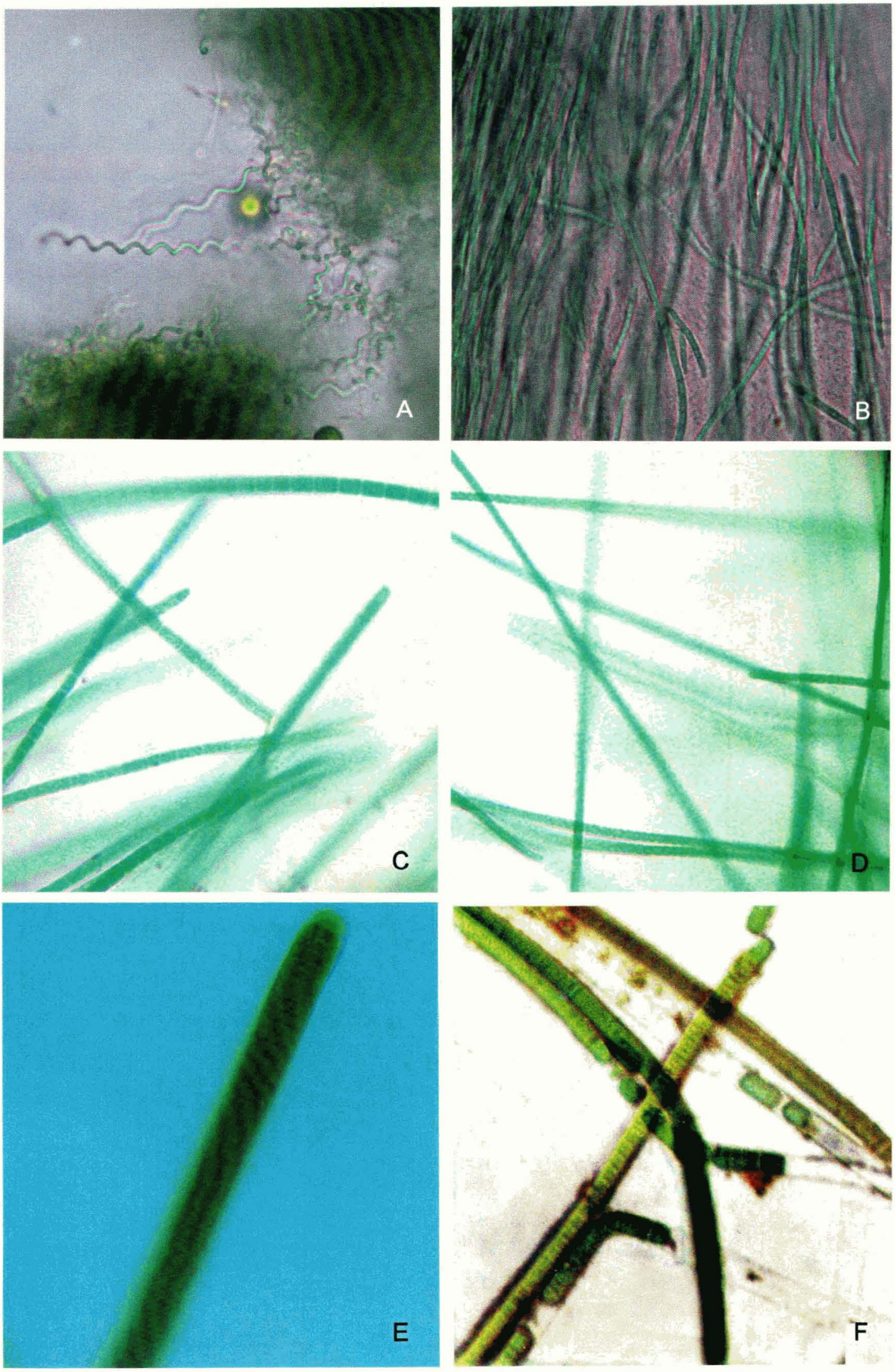


Fig. 7 **A**: *Spirulina laxissima*, **B**: *Phormidium tenue*, **C**: *P. fragile*, **D**: *P. fovaeolarum*, **E**: *P. uncinatum*, **F**: *Lyngbya stagnina*

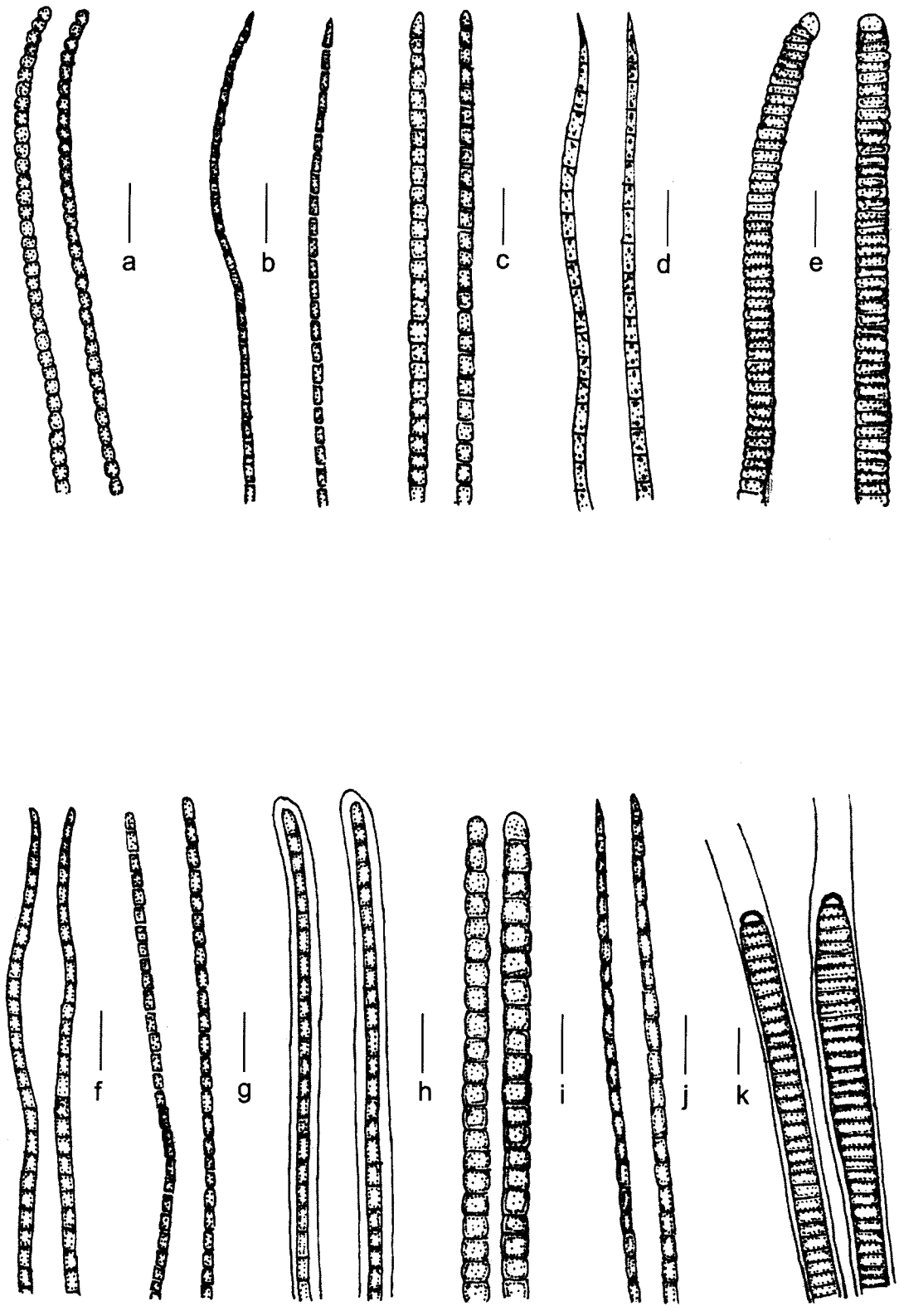


Fig. 8: **a**: *Phormidium foveolarum*, **b**: *P. fragile*, **c**: *P. jadinianum*, **d**: *P. laminosum*, **e**: *P. lucidum*, **f**: *P. luridum*, **g**: *P. molle*, **h**: *P. mucosum*, **i**: *P. retzii*, **j**: *P. tenue*, **k**: *P. uncinatum* (Bar 10 µm)

Observed as planktonic and epiphytic forms in the Pokkali, Kole and also in the Laterite soils.

Specimens examined: CU 81398, CU 81473

Phormidium molle (Kutz.) Gom., Monogr. Oscillariees, 163, 1892; Geitler, Kryptogamenflora, 1000, 1932; Desikachary, Cyanophyta, 255, 1959. (Fig. 8: g)

Thallus mucilaginous, thin, light blue-green; sheath diffluent, colourless; trichome straight, distinctly constricted and not granulated at the septa; cells 2.6-3.5 μm broad, 3-5-6.5 μm long; end cell rounded, not attenuated, calyptra absent.

Observed in Sandy alluvium fields.

Specimen examined: CU 81326

Phormidium mucosum Gardn., Mem. N. Y. bot. Gdn. 7: 43, 1927; Geitler, Kryptogamenflora, 1012, 1932; Desikachary, Cyanophyta, 265, 1959. (Fig. 8: h)

Filaments 7-8 μm broad, long, straight, bent at the ends; sheath thick, colourless, unlamellated; trichome not constricted at the cross walls, 2.5-4 μm broad, cells 2-2½ times as long as broad; end cell rounded.

In stagnant water of Kayal, Karappadom and Kari fields

Specimen examined: CU 81422

Phormidium retzii (Ag.) Gom., Monogr. Oscillariees, 175, 1892; Geitler, Kryptogamenflora, 1012, 1932; Desikachary, Cyanophyta, 268, 1959. (Fig. 8: I)

Thallus soft, bright blue-green, compact; filaments straight, unconstricted at the end walls, not attenuated, 6.5-10.2 μm broad; sheath hyaline, thin, firm, diffluent; cells shorter or longer than broad, 4-9 μm long, septa not granulated; end cell broadly rounded or truncated, not capitate.

In stagnant water and submerged plant parts of Kole and Laterite fields.

Specimen examined: CU 81525

Phormidium tenue (Menegh.) Gom., Monogr. Oscillariees, 169, 1892; Geitler, Kryptogamenflora, 1004, 1932; Desikachary, Cyanophyta, 259, 1959. (Fig. 8: j & 7 B)

Thallus pale blue-green, thin, expanded, membranous; filaments flexuous, densely entangled; sheath thin, diffluent; trichome straight, not constricted and not granulated at the cross walls, cross walls not visible; cells

1.5-2.5 μm broad, 3.5-7 μm long; ends straight, end cell acute conical, calyptra absent.

In stagnant water of Kole, Pokkali, Poonthalpadom, Sandy alluvial and Laterite fields.

Specimen examined: CU 81320, CU 81331

Phormidium uncinatum (Ag.) Gom., Morot's J. de Bot., 4: 355, 1890; Geitler, Kryptogamenflora, 1025, 1932; Desikachary, Cyanophyta, 276, 1959. (Fig. 8: k & 7 E)

Thallus expanded, dark green to brownish black, thin, firm; filaments straight or slightly bent, sheath mucilaginous, distinct; trichome blue-green, not constricted at the cross walls; cells 6.5-9 μm broad, 2.5-4 μm long; cross walls granulated; apices briefly attenuated, bent, capitate, with a round conical calyptra.

In stagnant waters of Laterite and Kole fields.

Specimen examined: CU 81452

LYNGBYA Ag

Syst. Alg., 25, 1824.

Trichome solitary or free in a thin or massive thalli; sheath firm, sometimes lamellated, hyaline or coloured; filaments straight, sometimes flexuous; hormogones present; end cell rounded or flat.

Key to the species

1. Trichome not constricted at the cross walls.....2
1. Trichome constricted at the cross walls.....*L. putealis*
2. Trichome free floating.....3
2. Trichome seen among other algae.....5
3. Sheath thick, filaments 20 - 26 μm broad.....*L. birgei*
3. Sheath thin, filaments narrower.....4
4. Filaments 1-1.5 μm broad, free-floating.....*L.limnetica*
4. Filaments 5.5-7 μm broad.....*L. aerugineo-coerulea*
5. Sheath thick.....6

5. Sheath thin, diffluent.....9
6. End cell flat, with outer membrane.....*L. major*
6. End cell rounded.....7
7. Trichome 4.5-8 μm broad.....*L. palmarum*
7. Trichome 6-12 μm broad.....8
8. Trichome 6-10 μm broad.....*L. martensiana*
8. Trichome 9.5-12 μm broad.....*L. stagnina*
9. Filaments up to 7 μm broad.....10
9. Filaments 10-14 μm broad.....*L. ceylanica*
10. Cells 1½-2½ times as long as broad.....*L. rubida*
10. Cells quadrate to 1½ times as long as broad.....*L. allorgei*

Lyngbya aerugineo-coerulea (Kutz.) Gom., Monogr. Oscillariees, 146, 1892; Geitler, Kryptogamenflora, 1062, 1932; Desikachary, Cyanophyta, 315, 1959. (Fig. 9: b)

Filaments forming dull blue-green, expanded, flexuous thallus; sheath thin, firm; cells 5.5-6.8-7 μm broad, 2.8-3.5 μm long, not constricted at the cross walls, granulated; end cell flattened, conical, with a slightly thickened membrane.

In stagnant water of Laterite, Sandy alluvial and Kole fields.

Specimen examined: CU 81396

Lyngbya allorgei Fremy, Myxo. d'Afr. equat. franc., 189, 1929; Geitler, Kryptogamenflora, 1059, 1932; Desikachary, Cyanophyta, 313, 1959. (Fig. 9: a)

Filaments solitary, or in small groups; sheath thin, firm, colourless; trichome pale violet, not constricted at the cross walls; 4.5-5.5 μm broad, cells quadrate or slightly longer; cross walls not granulated, end cell rounded, calyptra absent.

In stagnant water of Poonthalpadom fields of Palakkad district.

Specimen examined: CU 81540

Lyngbya birgei Smith, Bull. Torrey bot. 43: 482, 1916; Geitler, Kryptogamenflora, 1048, 1932; Desikachary, Cyanophyta, 296, 1959. (Fig. 9: c)

Filaments straight, free floating, 20-26 μm broad; sheath thick, firm, colourless, unlamellated; trichome not constricted at the cross walls; pale blue-green; cells 15-20 μm broad, 3-4 μm long, granulated with gas vacuoles; ends rounded, not attenuated, not capitate.

Observed as epiphytic on the submerged leaf bases of paddy plants of Palakkad district.

Specimen examined: CU 81460

Lyngbya ceylanica Wille, Denkschr. Akad. Wiss. Wien. Math. nat., 91: 161, 1914; Geitler, Kryptogamenflora, 1054, 1932; Desikachary, Cyanophyta, 299, 1959. (Fig. 9: d)

Thallus blue-green; filaments 10-14 μm broad, straight; sheath thin, colourless; trichome unconstricted and not granulated at the cross walls, not attenuated at the ends, 8-12 μm broad, cells quadrate or shorter than broad; end cell rotund, without calyptra.

Observed as epiphytic on the submerged leaf bases of Kole paddy fields of Thrissur district.

Specimen examined: CU 81558

Lyngbya limnetica Lemm., Beitrage z. Kenntnis d. Plankt. Alg., II, Bot. Centralbl., 76: 154, 1898; Geitler, Kryptogamenflora, 1046, 1932; Desikachary, Cyanophyta, 294, 1959. (Fig. 9: e)

Filaments straight, single, free floating; sheath firm, thin, colourless; cells 1-1.5 μm broad, 1-3 μm long; not constricted at the cross walls; pale blue-green; end cell rounded.

In stagnant water of Laterite fields.

Specimen examined: CU 81485

Lyngbya major Menegh. ex, Gom., Conspectus Algol. eugan., 12, 1837; Geitler, Kryptogamenflora, 1066, 1932; Desikachary, Cyanophyta, 320, 1959. (Fig. 9:g)

Filaments long, straight, blue-green; sheath thick, colourless, and lamellated; cells 14.5-19.5 μm broad, 3-6 μm long, dull blue-green; granulated at the septa, not constricted at the cross walls; end cell flat with a slightly thickened membrane.

Collected from Pokkali (Thrissur), Poonthalpadom (Palakkad) and Laterite (Malappuram, Kozhikkode, Wayanad and Idukki districts) paddy fields as planktonic and epipsammic forms.

Specimen examined: CU 81574

Lyngbya martensiana Menegh. ex, Gom., Conspectus Algol. eugan., 12, 1837; Geitler, Kryptogamenflora, 1064, 1932; Desikachary, Cyanophyta, 318, 1959. (Fig. 9: f)

Thallus caespitose, blue-green; filaments long flexible; sheath colourless, thick; trichome 6-10 μm broad, cells 2-3 μm long; not constricted at the cross walls, apices not attenuated, pale blue-green; end cell round, calyptra absent.

Collected from Laterite, Pokkali and Poonthalpadom fields.

Specimen examined: CU 81306, CU 81428

Lyngbya palmarum (Martens) Bruhl *et* Biswas, J. Dept. Sci. Calcutta Univ. 11, 1923; Desikachary, Cyanophyta, 301, 1959. (Fig. 9: h)

Thallus extensively tomentose, filaments flexuous, loosely entangled, 6-9 μm broad; sheath colourless or brown, trichome not constricted at the cross walls, cells 4.5-8 μm broad, 4.5-8 μm long, septa inconspicuous due to granules; end cell rounded, with or without thickened membrane.

In stagnant waters of Pokkali and Kole fields.

Specimen examined: CU 81427

Lyngbya putealis Mont. ex Gom., Ann. Sci. Nat. Bot. 13: 200, 1840; Geitler, Kryptogamenflora, 1063, 1932; Desikachary, Cyanophyta, 317, 1959. (Fig. 9: I).

Thallus caespitose, expanded, dull blue-green; filaments 8-11.5 μm broad, slightly curved, sheath thick, colourless; cells 5-9.5 μm broad, cells 3-8 μm long, constricted at the end walls, cross walls sometimes granulated; not attenuated, not capitate; end cell rounded.

In stagnant waters of Sandy alluvial soils of Kollam and Karappadom fields of Alappuzha districts.

Specimen examined: CU 81587

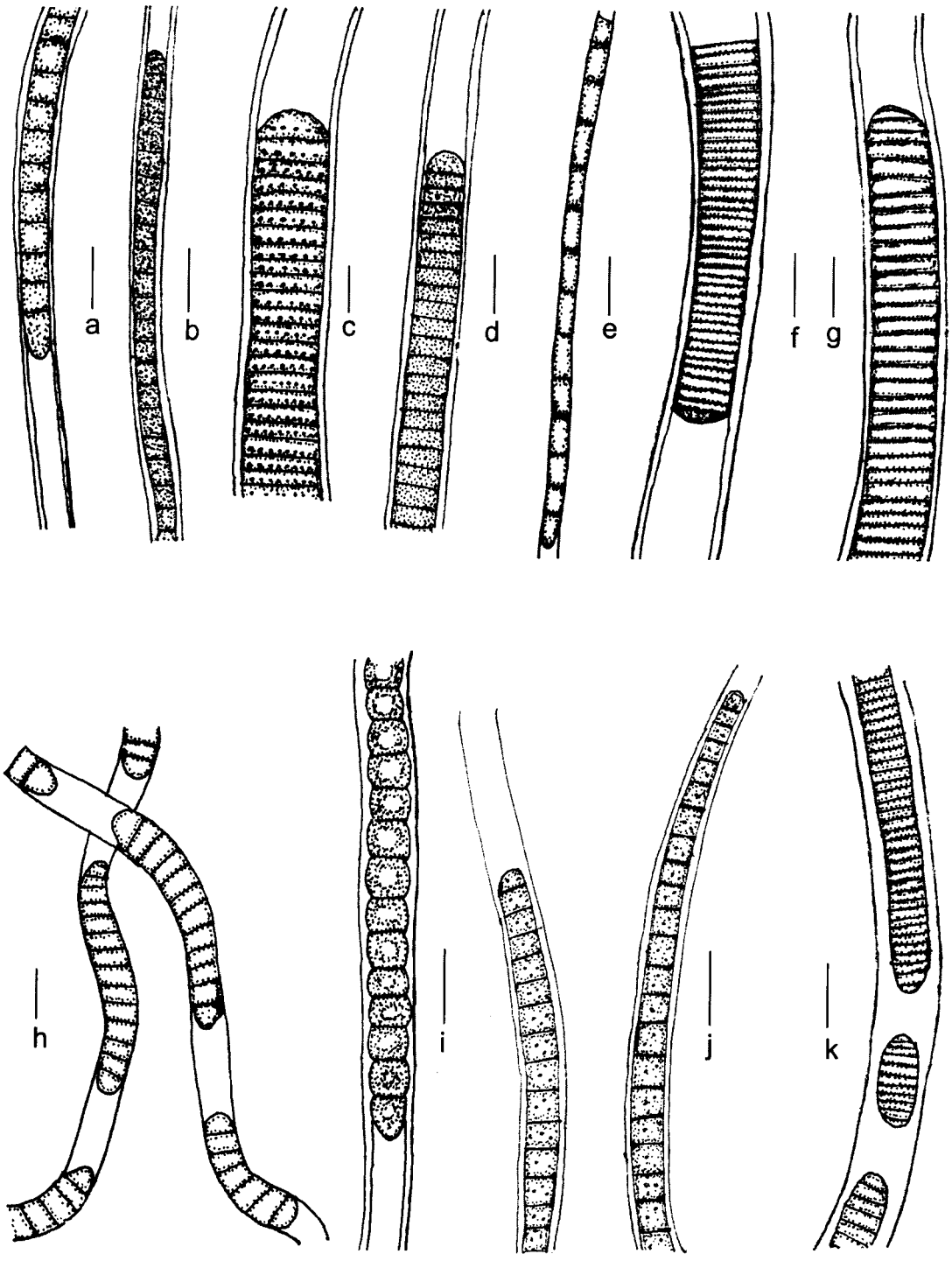


Fig. 9: **a**: *Lyngbya allorgei*, **b**: *L. aerugineo-coerulea*, **c**: *L. birgei*, **d**: *L. ceylanica*, **e**: *L. limnetica*, **f**: *L. martensiana*, **g**: *L. major*, **h**: *L. palmarum*, **i**: *L. putealis*, **j**: *L. rubida*, **k**: *L. stagnina* (Bar 10 µm)

Lyngbya rubida Fremy, Myxo d'Afr. equat. franc., 187, 1929; Geitler, Kryptogamenflora, 1054, 1932; Desikachary, Cyanophyta, 298, 1959. (Fig. 9: j)

Thallus floccose, expanded, blue-green/brownish purple; filaments straight, 6-7 μm broad, loosely entangled; sheath, firm, thick, unlamellated, reddish when old; trichome brownish green, not constricted at the cross walls, granulated, gas vacuoles present; cells 4-5 μm broad, 6-9.5 μm long; end cell rounded, not attenuated, not capitate.

Collected from Laterite and Poonthalpadom paddy fields.

Specimen examined: CU 81462

Lyngbya stagnina Kutz., Species Alg., 281, 1849; Geitler, Kryptogamenflora, 1066, 1932; Desikachary, Cyanophyta, 317, 1959. (Fig. 9: k & 7 F)

Thallus dull green; filaments flexuous, 11-15 μm broad, sheath distinct, colourless or yellowish; trichome 9.5-12 μm broad, cells 2-4 μm long, not constricted at the cross walls, granulated at the cross walls; end cell broadly rounded, not attenuated.

Observed in stagnant waters of Karappadom, Pokkali and Kole fields in edaphic form.

Specimen examined: CU 81437

MICROCOLEUS Desmaz.

Catal. pl. Botanagr. belg., 7, 1823.

Filaments unbranched or sparsely branched; sheath mostly colourless, not lamellated, sometimes gelatinizing when old; trichome many in each sheath; densely aggregated; often coiled like a rope; ends straight, end cells usually conical, without calyptra.

Key to the species

1. Trichome constricted at the septa.....2
1. Trichome unconstricted at the septa.....3
2. Filaments 14-20 μm broad, end cell pointed, conical.....*M. chthonoplastes*
2. Filaments 25-45 μm broad, end cell rounded, conical.....*M. lacustris*
3. Trichome 2 μm broad.....*M. acutissimus*
3. Trichome 4.7-6.5 μm broad.....*M. paludosus*

Microcoleus acutissimus Gardn., Mem. N. Y. Bot. Gard. 7: 55, 1927; Geitler, Kryptogamenflora, 1138, 1932; Desikachary, Cyanophyta, 344, 1959. (Fig. 11:d)

Filaments straight, blue-green, with many trichomes; sheath colourless, uneven, gelatinous; trichomes almost parallel, not constricted, ends long, attenuated, pointed; cells 2 μm broad, 3.5-8 μm long, end cell acutely conical.

Observed as planktonic and epipsammic in water bodies of Kerala associated with Kole and Laterite paddy fields.

Specimen examined: CU 81501, CU 81571

Microcoleus chthonoplastes Thuret ex Gom., Ann. Sci. nat. Bot. 1: 378, 1875; Geitler, Kryptogamenflora, 1133, 1932; Desikachary, Cyanophyta, 343, 1959. (Fig. 11: a & 10 B)

Filaments dirty blue-green/dark blue-green to blackish, 14-20 μm broad; sheath uneven, gelatinizing and colourless having many closely arranged trichomes, rope like; cells constricted at the cross walls, 2.5-5 μm broad, 1½ times to 3 times as long as broad, 3.5-9.5 μm long, not granulated at the septal walls; end cells pointed and conical.

Observed as planktonic and epipsammic in many water bodies of Kerala associated with paddy fields.

Specimen examined: CU 81319

Microcoleus lacustris (Rabenh.) Far., Alg. Am. bor., 227, 1877; Geitler, Krypto-gamenflora, 1142, 1932; Desikachary, Cyanophyta, 345, 1959. (Fig. 11: b & 10 A)

Thallus dark blue-green, filaments contorted, 25-45 μm broad, sheath colourless and slimy; trichomes many together, parallel, distinctly separated at the septa, 3-4 μm broad; cells cylindrical, 1-3 times as long as broad, 5-10 μm long; end walls rounded, conical, not capitate.

Observed in Laterite, Kari, Karappadom and Poonthalpadom paddy fields.

Specimen examined: CU 81312, CU 81343

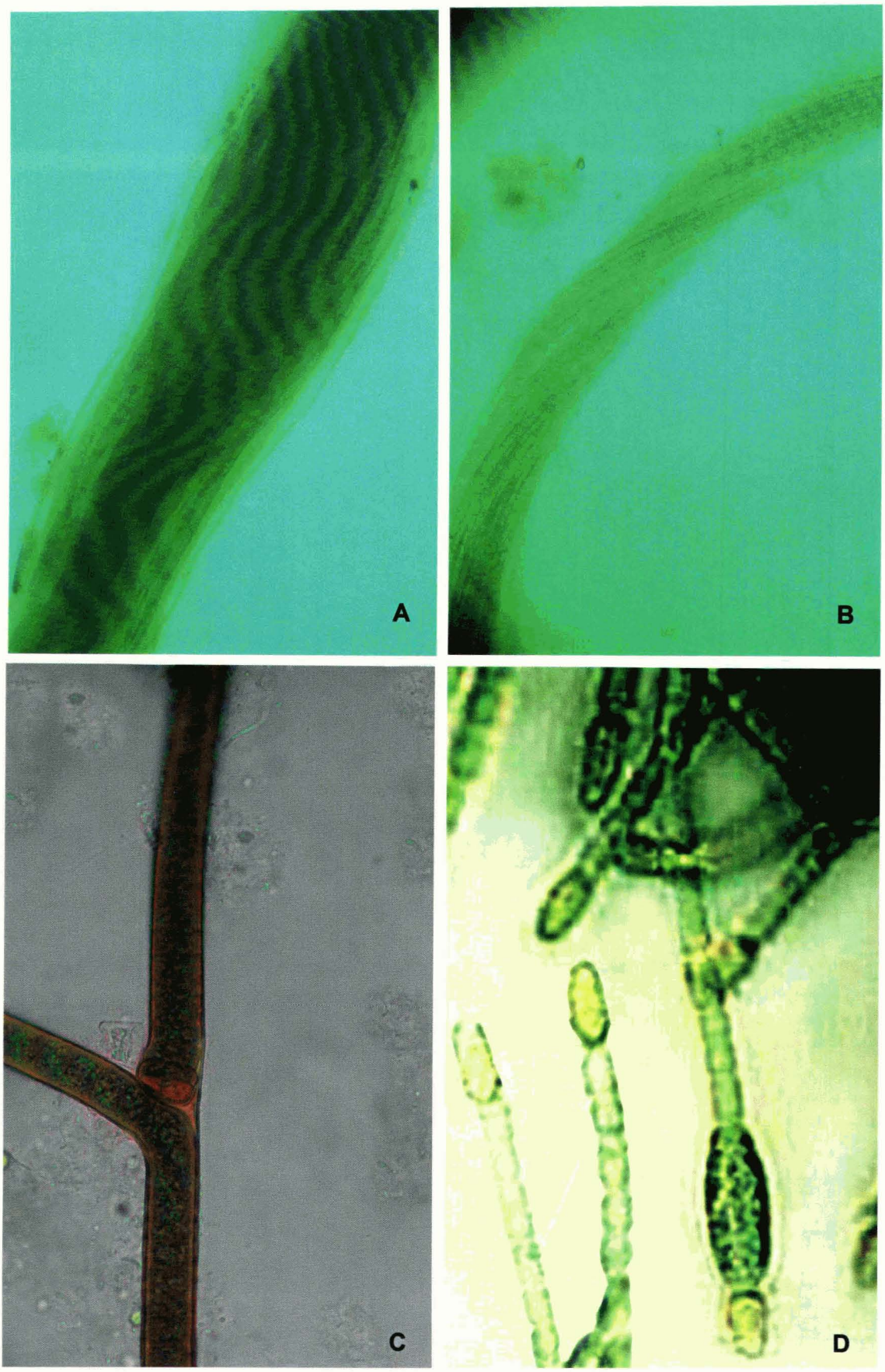


Fig. 10 A: *Microcoleus lacustris*, **B:** *M. chthonoplastes*, **C:** *Tolypothrix tenuis*, ***D*:** *Cylindrospermum muscicola*

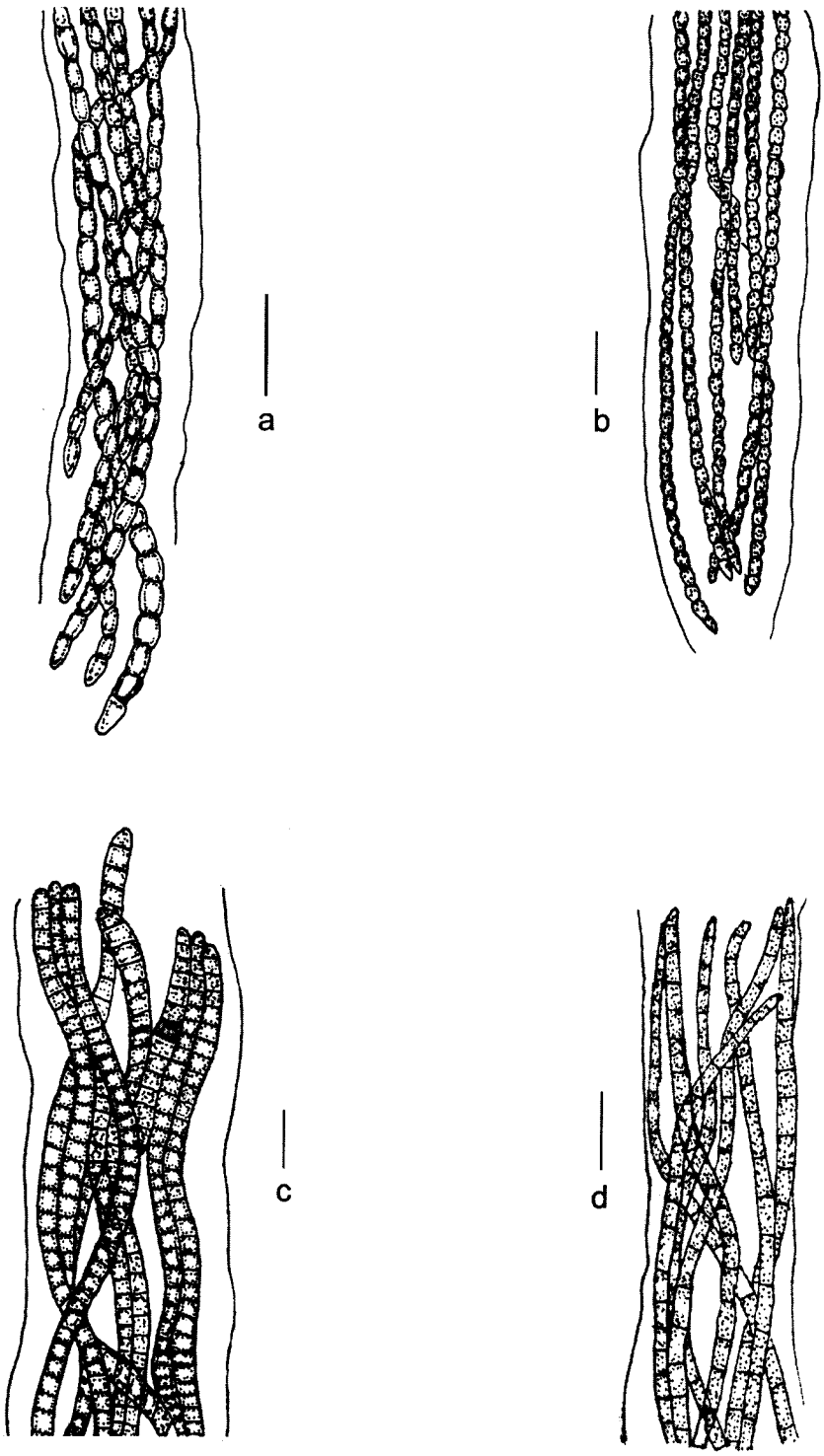


fig. 11: **a**: *Microcoleus chthonoplastes*, **b**: *M. lacustris*, **c**: *M. paludosus*, **d**: *M. acutissimus* (Bar 10 µm)

Microcoleus paludosus (Kutz.) Gom., Monogr. Oscillariees, 358, 1892; Geitler, Kryptogamenflora, 1144, 1932; Desikachary, Cyanophyta, 344, 1959. (Fig. 11: c)

Filaments single or form a dark blue-green entangled thready masses among other algae; sheath gelatinous with many straight or rope like trichomes; trichomes are not granulated, not constricted at the cross walls; cells 4.7-6.5 μm broad, 4-10 μm long, bright blue- green; apical cell conical.

Planktonic in Kari, Kayal and Pokkali fields.

Specimen examined: CU 81420, CU 81391

NOSTOCACEAE kutz.

Phyc. gene., 203, 1843.

Trichome free or in a common mucilage to form colonies of various shapes; filamentous, cells generally similar throughout, end cells attenuated; sheath thick or thin, mucilaginous or firm; hormogones present, heterocyst present or absent, if present intercalary or terminal, single or in chains of 2 or 3; spores present or absent, single or in series beginning from near the heterocyst or in between two of them.

Key to the genera

1. Trichome with a definite sheath.....*Aulosira*
1. Trichome without a definite sheath.....2
2. Heterocyst always terminal with spore adjoining.....*Cylindrospermum*
2. Heterocyst mostly intercalary, spores adjoining/away.....3
3. Filaments free or in a formless mucilaginous mass.....*Anabaena*
3. Filaments entwined in a definite colony.....*Nostoc*

CYLINDROSPERMUM Kutz.

Phyc. gene., 211, 1843.

Thallus mucilaginous, mostly dull blue-green; trichome uniformly broad, short, without sheath, but in a common, mostly very delicate and often invisible or faint mucilage of thin consistency; cells cylindrical, constricted at the cross walls; heterocysts terminal, at both ends or at one end only, sometimes Intercalary; spores single, rarely in series, next to the heterocyst on one side, much bigger than the vegetative cells.

Key to the species

1. Spores with sculptured epispore with papillae.....*C. majus*
1. Spores with smooth epispore without papillae.....2
2. Spores cylindrical.....*C. stagnale*
2. Spores ellipsoidal.....3
3. Heterocyst oblong.....4
3. Heterocyst oval or nearly spherical.....5
4. Spores 9-12 µm broad, 10-20 µm long.....*C. muscicola*
4. Spores 8.5-12 µm broad, 20-28 µm long.....*C. muscicola* var. *longispora*
5. Spores 8-12 µm broad, 12-25 µm long..... *C. michailovskoense*
5. Spores 8.5-9 µm broad, 15-18.5 µm long.....*C. indicum*

Cylindrospermum indicum Rao, Proc. Indian Acad. Sci., 3: 169, 1936; Desikachary, Cyanophyta, 369, 1959. (Fig. 12: d)

Trichomes single with deep constrictions at the cross walls, 3.6-3.9 µm broad, dark blue-green; cells quadrate, 3-4.5 µm long; heterocyst spherical or sub conical, one at each end of the trichome, 2.8-6 µm broad, 3-7.5 µm long; spores ellipsoidal, sub terminal, at either end of the trichome with a thick yellow membrane, 8.5-9 µm broad, 15-18.5 µm long, without membrane.

Planktonic as well as epiphytic on Laterite soils of Malappuram and Thrissur.

Specimen examined: CU 81538

Cylindrospermum majus Kutz. ex. Born. *et* Flah., Phyc. gene., 212, 1843; Geitler, Kryptogamenflora, 815, 1932; Desikachary, Cyanophyta, 360, 1959. (Fig. 12: a)

Thallus mucilaginous, expanded, blackish-green; trichome 4-5 µm broad, constricted at the cross walls, pale blue-green; cells cylindrical, 5-6 µm long; heterocysts oblong, broader than the trichome, 5-6 µm broad, 8-10 µm long; spores ellipsoidal, sub-terminal, 10-15 µm broad, 20-26 µm long, epispore brownish with distinct papillae.

In moist shady soils as epipsammic populations in all paddy fields.

Specimens examined: CU 81340, CU 81429

Cylindrospermum michailovskoense Elen., Bull. Jardin. Imp. Pierre le Gr., 11: 162, 1911; Desikachary, Cyanophyta, 368, 1959. (Fig. 12: e & 19 A)

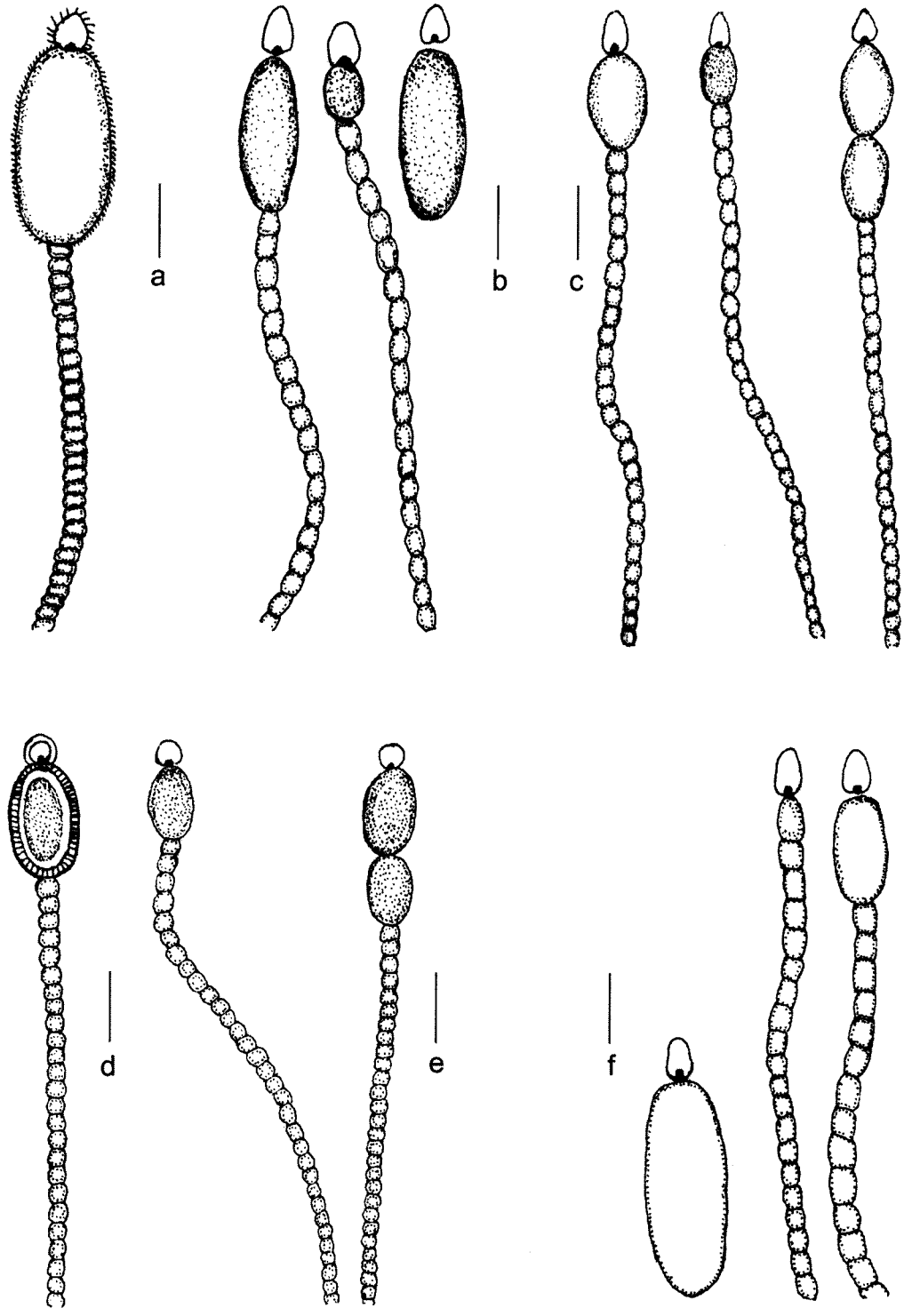


Fig. 12: **a**: *Cylindrospermum majus*, **b**: *C. muscicola* var. *longispora*, **c**: *C. muscicola* var. *muscicola*, **d**: *C. indicum*, **e**: *C. michailovskoense*, **f**: *C. stagnale* (Bar 10 µm)

Thallus expanded, mucilaginous, blue-green; trichome pale blue-green, bent, aggregated; cells quadrate or cylindrical, constricted at the cross walls, 3.5- 5 µm broad, 4-6 µm long; heterocyst ob-spherical, 5-6 µm broad, 6-7 µm long; spores single or two to many, ellipsoidal, 8-12 µm broad, 12-25 µm long, epispore smooth.

Observed as epiphytic and epipsammic populations in the Kole and Poonthalpadom fields of Palakkad districts.

Specimen examined: CU 81532

Cylindrospermum muscicola (Kutz.) ex Born. *et* Flah., Phyc. gene., 173, 1845; Geitler, Kryptogamenflora, 822, 1932; Desikachary, Cyano-phyta, 366, 1959. (Fig. 12: c)

Thallus expanded, mucilaginous, blackish-green; trichomes 3-5 µm broad, 4-5 µm long, cylindrical; heterocyst oblong, 4-5 µm broad, 5-7 µm long; spores elipsoidal, 9-12 µm broad, 10-20 µm long, epispore smooth, yellowish brown.

In moist shady Laterite soils and Sandy alluvial paddy fields of almost all parts of Kerala as epiphytic communities.

Specimens examined: CU 81413, CU 81474

Cylindrospermum muscicola var. ***longispora*** Dixit, Proc. Indian Acad. Sci., 3: 100, 1936; Desikachary, Cyanophyta, 367, 1959. (Fig. 12: b & 10 D)

Trichome 3-5 µm broad; heterocyst 5-6 µm broad, 7-9.5 µm long; spores 8.5-12 µm broad, 20-28 µm long, epispore smooth, yellowish brown.

On water logged Pokkali, Kole, Poonthalpadom and Laterite rice fields of Kerala in epiphytic form.

Specimen examined: CU 81522

Cylindrospermum stagnale (Kutz.) Born. *et* Flah., Revision des Nostoc. heteroc., 250, 1888; Geitler, Kryptogamenflora, 819, 1932; Desikachary, Cyanophyta, 363, 1959. (Fig.12: f & 19 B)

Thallus floccose, expanded, blue-green; trichomes 4-4.8 µm broad, constricted at the cross walls, 4-6.5 µm long, cylindrical; heterocyst oblong, 4-6 µm broad, 6-9 µm long; spores cylindrical with rounded ends, 9-15 µm broad, 18-30 µm long, epispore smooth, yellowish brown.

Observed as planktonic, epiphytic and edaphic groups in all rice fields and adjoining areas.

Specimens examined: CU 81354, CU 81435

NOSTOC Vaucher

Hist. des Confer. d' eau douce, 203,1803.

Thallus mucilaginous, globose, foliose or bullose, solid or hollow, free or attached, the periphery dense and darkly coloured; filament curved or entangled, unbranched, torulose; sheath generally diffluent, sometimes distinct, cells barrel shaped, cylindrical or spherical; heterocyst intercalary and/or terminal, spores spherical or oblong, formed centrifugally in series in between the heterocysts.

Key to the species

1. Thallus with a firm outer layer.....2
1. Thallus without a firm layer, soft and formless.....4
2. Heterocyst generally intercalary.....3
2. Heterocyst terminal.....*N. commune*
3. Trichome 4-5 µm broad.....*N. sphaericum*
3. Trichome 2-3 µm broad.....*N. amplissimum*
4. Trichome densely coiled, zigzag, hardly seen.....*N. punctiforme*
4. Trichome less densely coiled, cells clearly visible.....5
5. Thallus microscopically small.....6
5. Thallus macroscopic 7
6. Trichome densely entangled.....*N. entophytum*
6. Trichome loosely arranged.....*N. paludosum*
7. Cells cylindrical.....8
7. Cells barrel shaped/spherical.....10
8. Trichome 3-4 µm broad.....9
8. Trichome 4-4.5 µm broad.....*N. spongiaeforme*
9. Heterocysts 6 µm broad..... *N. carneum*
9. Heterocysts 5-5.5 µm broad..... *N. carneum* f. *minor*
10. Trichome densely arranged coiled catenate.....*N. linckia*
10. Trichome less densely arranged not catenate.....11

11. Spores spherical.....*N. piscinale*
11. Spores oblong/subspherical.....12
12. Cells 2.5-4 µm broad.....13
12. Cells 3.5-5 µm broad.....*N. muscorum*
13. Spores oblong.....*N. humifusum*
13. Spores sub spherical.....*N. calcicola*

Nostoc amplissimum Setch., Erythea, 7: 50, 1899; Desikachary, Cyanophyta, 390, 1959. (Fig. 14: a)

Colonies spherical, later becoming irregular; solid initially later becoming hollow with mucilaginous outer layer; filaments variously arranged, sheath of the peripheral filaments distinct and colourless; trichome 2-3 µm broad, 2.5-5 µm long, densely entangled, cells sub spherical to short cylindrical; heterocyst 3-4 µm broad, nearly spherical; spores not observed.

Observed as planktonic in Pokkali, Kayal and Karappadom fields.

Specimen examined: CU 81430

Nostoc calcicola Born. *et* Flah., Monogr. Nostoc. Italicarum, 121, 1843; Geitler, Kryptogamenflora, 842, 1932; Desikachary, Cyanophyta, 384, 1959. (Fig. 14: b)

Thallus mucilaginous, slightly diffluent, irregular, expanded, dull olive green to brown; filaments loosely entangled, sheath distinct only at the periphery of the thallus; trichome 2.5-4 µm broad, pale blue green, cells barrel shaped sub spherical, rarely longer than broad; heterocysts terminal or intercalary, sub spherical, 4-5-5.6 µm broad; spores sub spherical, 4.5-5.5 µm broad, epispore smooth.

Planktonic as well as epiphytic on Laterite soils.

Specimens examined: CU 81324, CU 81440

Nostoc carneum Ag.ex. Born *et* Flah.,Syst. Alg., 22, 1824; Geitler, Kryptogamen-flora, 839, 1932; Desikachary, Cyanophyta, 381, 1959. (Fig. 13A)

Thallus globose, brown or flesh coloured; Filaments loosely entangled; trichome 3-4 µm broad, cells cylindrical with rounded ends, cells longer than broad, heterocysts oblong, 6 µm broad, spore oval to ellipsoidal, 6 µm broad.

Observed as epiphytic, epipelic and edaphic groups in various paddy fields.

Specimen examined: CU 81412

Nostoc carneum f. minor Bharad., Proc. Indian Acad. Sci., 2: 102, 1935; Desikachary, Cyanophyta, 382, 1959. (Fig. 14: c, 16 A)

Thallus mucilaginous, brown; trichome 3.2-3.4 µm broad, cells 3.2-6.5 µm long; heterocyst 5-5.5 µm broad, and 6.8-8.5 µm long; spores ellipsoidal, 5-6 µm broad, 9-10 µm long, epispore smooth.

Observed as epiphytic, epipelic and edaphic populations in various paddy fields especially of Laterite and Poonthalpadom soils

Specimen examined: CU 81337

Nostoc commune Vaucher ex. Born. *et* Flah., Hist. des Confer. d'eau douce, 222, 1803; Geitler, Kryptogamenflora, 845, 1932; Desikachary, Cyanophyta, 387, 1959. (Fig.15: d & 13 B)

Thallus firm, gelatinous, at first globose, later irregularly expanding, membranous, blue-green to brown; filaments flexuous and entangled; sheath mostly distinct only at the periphery, thick, yellowish brown; trichome 4.5-6 µm broad, cells short, barrel shaped, 5-5.5 µm long; heterocysts nearly spherical, 5.5- 7 µm broad, terminal; spores not observed.

Observed in Laterite fields of Kannur and Malappuram, Sandy alluvium of Kollam and Poonthalpadom fields of Palakkad districts on moist soils and submerged leaves.

Specimen examined: CU 81308

Nostoc entophytum Born. *et* Flah., Revision des Nostoc. heteroc., 190, 1888; Geitler, Kryptogamenflora, 836,1932; Desikachary, Cyanophyta, 375, 1959. (Fig. 15: e)

Thallus inconspicuous, blue-green; filaments distinct, densely entangled; sheath distinct at the periphery; trichome 2.5-3 µm broad, cells short, barrel shaped; heterocysts broader than the vegetative cells, 3.5-4 µm broad; spores spherical, 5-6 µm broad, 5-8 µm long.

Observed in Laterite and Poonthalpadom rice fields on moist soils and submerged leaves.

Specimen examined: CU 81450

Nostoc humifusum Carmi. ex Born. *et* Flah., Hooker's British Flora 2: 399, 1833; Geitler, Kryptogamenflora, 842, 1932; Desikachary, Cyanophyta, 384, 1959. (Fig. 15: f)

Thallus mucilaginous, irregular with various sizes, punctiforme, attached, brownish-green; filaments twisted and flexuous, densely entangled; sheath slightly distinct; trichome 2.5-3.8 µm broad, blue-green, cells sub spherical; heterocysts 3.3-4 µm broad, sub spherical; spores oblong, 4.5-6.2 µm broad, 6 µm long.

Observed in Poonthalpadom and Sandy alluvial fields on moist soils and submerged leaves.

Specimen examined: CU 81463

Nostoc linckia (Roth) ex. Born. *et* Flah., Notes Algolo., 86, 1880; Geitler, Kryptogamenflora, 838, 1932; Desikachary, Cyanophyta, 377, 1959 (Fig. 14: e & 13 D)

Thallus varying in size, punctiforme, globose later irregularly expanding, blue-green to blackish-green; filaments densely entangled, flexuous; sheath diffluent, and colourless; trichome 3.5-4.5 µm broad, catenate, pale blue-green; cells quadrate or long, barrel shaped; heterocyst sub spherical, 4.5-5.5 µm broad, 4.8-5.6 µm long; spores sub spherical, 4-5 µm broad, 5.5-7.5 µm long.

Observed as epiphytic and edaphic populations in all rice fields.

Specimens examined: CU 81305, CU 81344

Nostoc muscorum Ag. ex Born. *et* Flah., Dispositio Algar. Sueciae, 44, 1812; Geitler, Kryptogamenflora, 844, 1932; Desikachary, Cyanophyta, 385, 1959. (Fig. 14: f & 13 C)

Thallus irregularly lobed and expanded, attached, dull olive to brown; filaments densely entangled, sheath distinct only at the periphery; trichome 3.5-5 µm broad, cells short, barrel to cylindrical, 4-6.5 µm long; heterocysts spherical or long triangular, 5.6-7 µm broad, 5-7.5 µm long; spores oblong, many in series, 5-8 µm broad, 7-12 µm long, epispore smooth and yellowish.

Observed in many rice fields and adjoining areas of Kerala especially in the Kole and Sandy alluvial fields as epiphytic communities.

Specimens examined: CU 81341, CU 81364, CU 81479

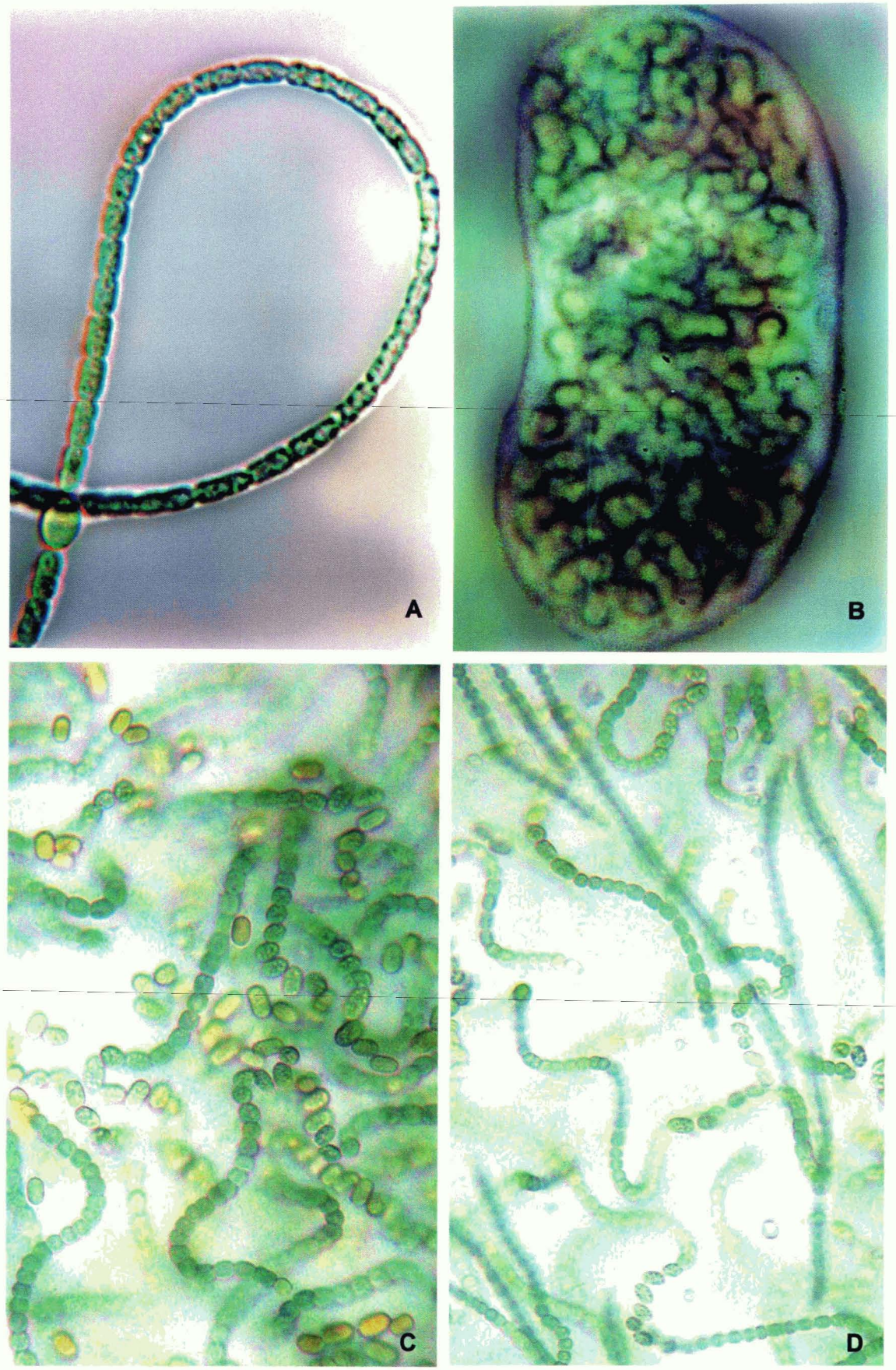


Fig 13 **A**: *Nostoc carneum*, **B**: *N. commune*, **C**: *N. muscorum*, **D**: *N. linckia*

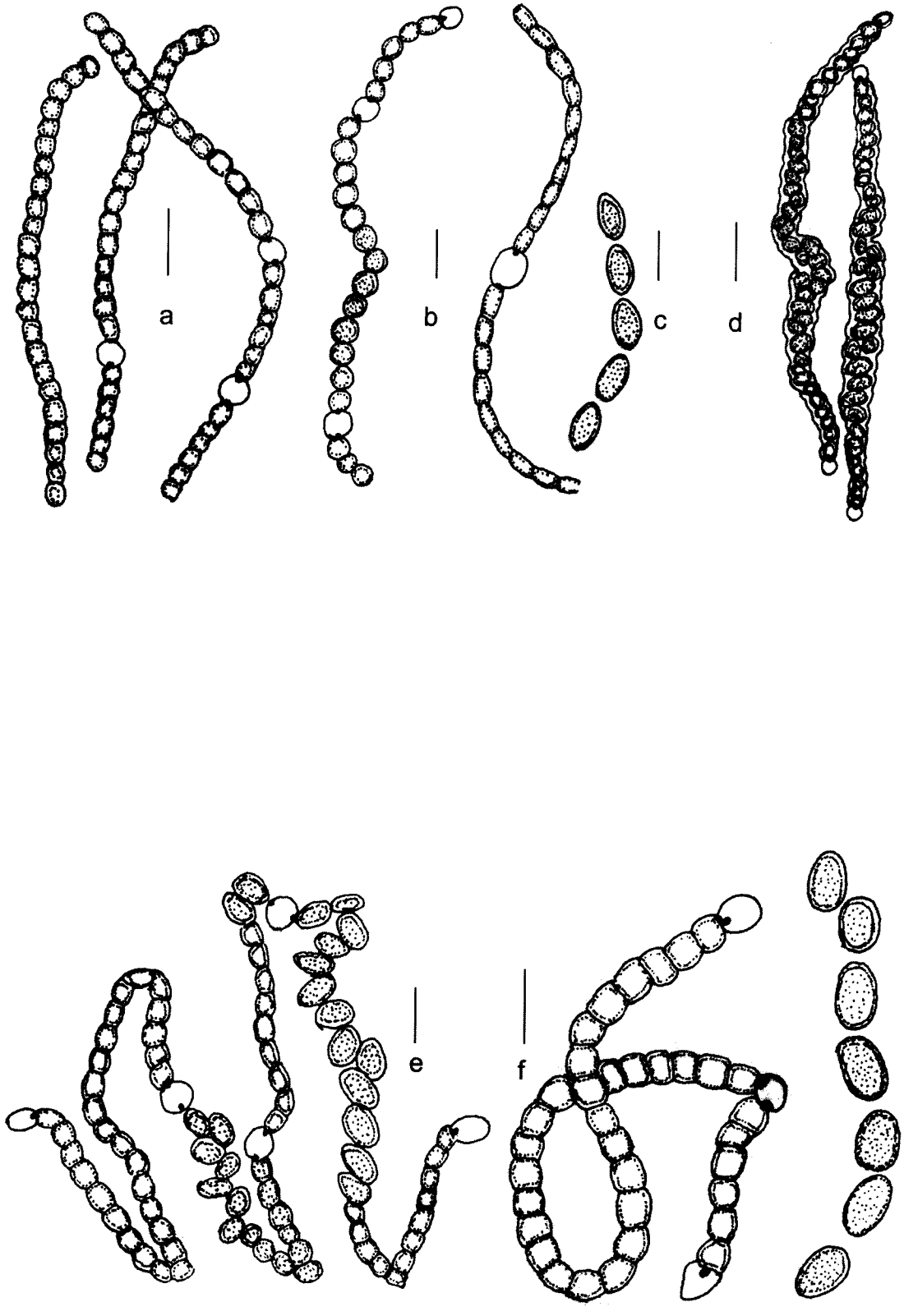


Fig. 14: **a**: *Nostoc amplissimum*, **b**: *N. calcicola*, **c**: *N. carneum f. minor*, **d**: *N. punctiforme*, **e**: *N. linckia*, **f**: *N. muscorum* (Bar 10 µm)

Nostoc paludosum Kutz. ex Born. *et* Flah., Tab. Phycolog., 2: 1, 1850; Geitler, Kryptogamenflora, 836, 1932; Desikachary, Cyanophyta, 375, 1959. (Fig. 15: g)

Thallus microscopically not visible, punctiforme, gelatinous; sheath broad, colourless; trichome 3-4 µm broad, loosely arranged, cells as long as broad, barrel shaped, pale blue-green; heterocysts broader than the vegetative cells; spores oval, 4-4.5 µm broad, 6-7.8 µm long, with smooth hyaline membrane.

Observed in Laterite rice fields and adjoining areas of Malappuram District.

Specimens examined: CU 81302, CU 81495

Nostoc piscinale (Kutz.) ex Born. *et* Flah., Phyc. gene., 208, 1843; Geitler, Kryptogamenflora, 838, 1932; Desikachary, Cyanophyta, 377, 1959. (Fig. 15: b & 16 C)

Thallus at first globose, later variously tuberculate, mucilaginous, pale blue-green or brown; filaments flexuous, loosely entangled; trichome 4-5.5 µm broad, cells shorter or longer than broad; heterocyst sub spherical, 4.5-6.8 µm broad; spores globose, 6-7 µm broad, in long chains, epispore hyaline and smooth with a sheath.

Paddy field soil cultures in various fields especially Laterite fields of Kozhikkode and Palakkad District.

Specimens examined: CU 81478, CU 81494

Nostoc punctiforme (Kutz.) Hariot J. de Bot., 5: 31, 1891; Geitler, Kryptogamenflora, 834, 1932; Desikachary, Cyanophyta, 374, 1959. (Fig. 14: d)

Thallus sub globose, scattered, dark blue-green; filaments flexuous, densely entangled; sheath delicate, hyaline; trichome 4-5.5 µm broad, zigzag; densely coiled, cells short, barrel shaped; heterocysts 4.5-6.5 µm broad, 6-7.5 µm long, terminal, hemispherical; spores oblong, 5-6 µm broad, 5-7.8 µm long, epispore thick and smooth.

In almost all paddy field soils of Kerala as edaphic communities.

Specimen examined: CU 81369

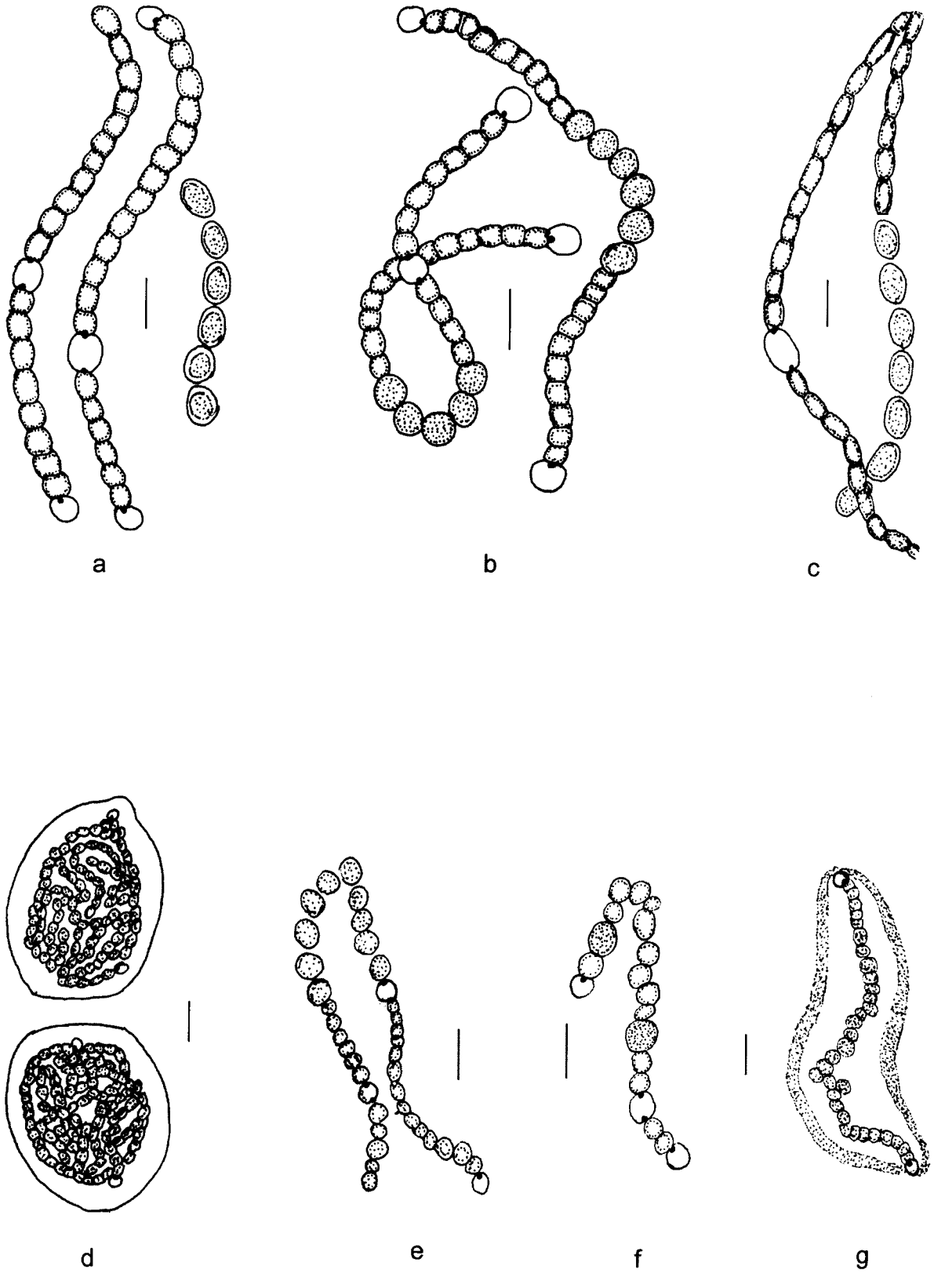


Fig. 15: **a**. *Nostoc sphaericum*, **b**: *N. piscinale*, **c**: *N. spongiaeforme*, **d**: *N. commune*, **e**: *N. entophytum*, **f**: *N. humifusum*, **g**: *N. paludosum* (Bar: 10 µm)

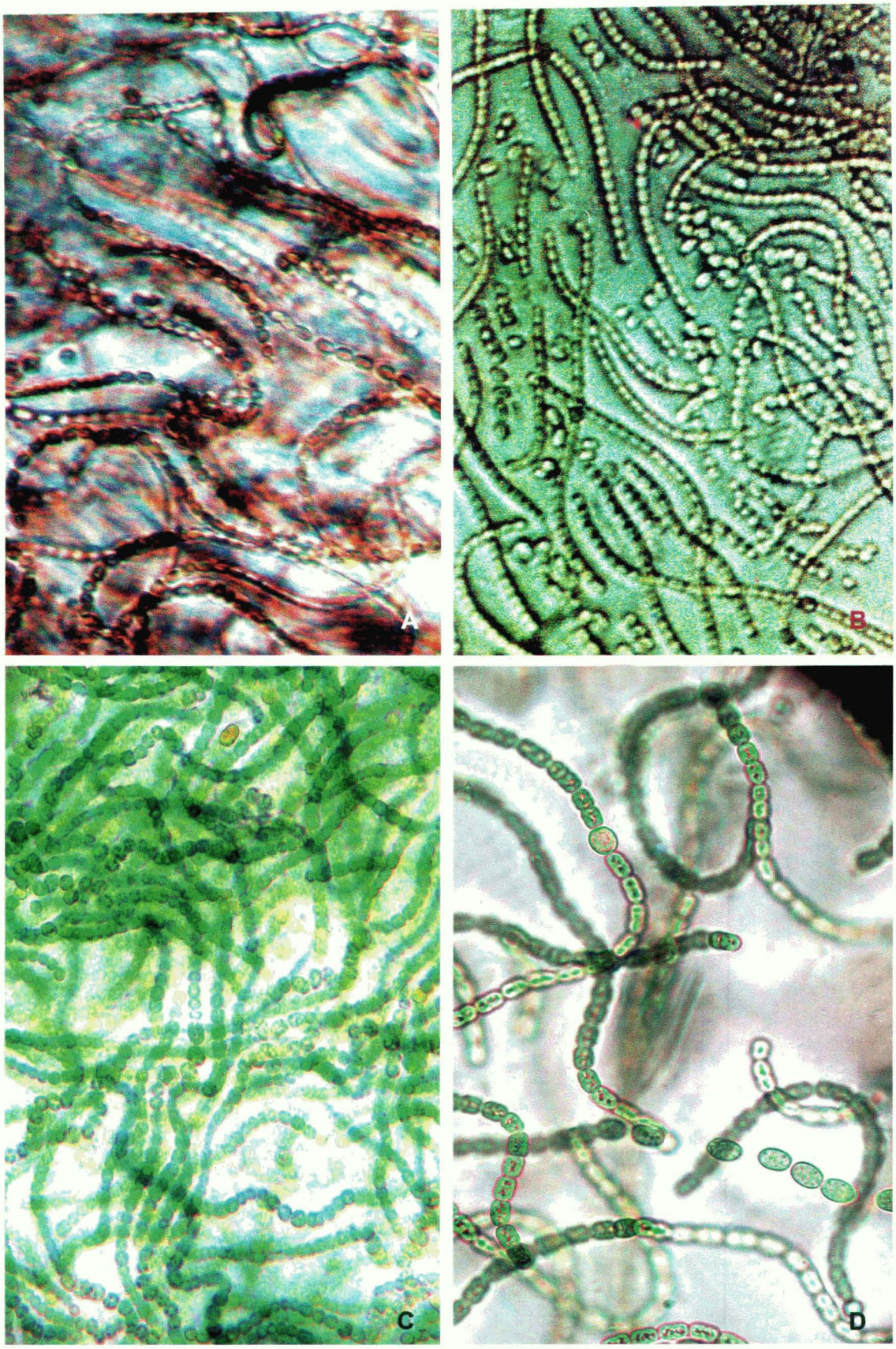


Fig. 16 **A**: *Nostoc carneum* f. *minor*, **B**: *N. sphaericum*, **C**: *N. piscinale*, **D**: *N. spongiaeforme*

Nostoc sphaericum Vaucher ex Born. *et* Flah., Hist. des Confer. d'eau douce, 223, 1803; Geitler, Kryptogamenflora, 850, 1932; Desikachary, Cyanophyta, 390, 1959. (Fig. 15: a & 16 B)

Thallus free, globose, later irregularly tuberculate, thick, olive-green or brown, with firm outer layer; filaments flexuous, densely entangled; trichome 4-5 µm broad, cells short, compressed, spherical or barrel shaped; heterocyst 5-6 µm broad, generallay intercalary, sub spherical; spores 5.5-6.5 µm broad, 6-9.5 µm long.

In paddy field soils of Pokkali and Poonthalpadom fields.

Specimens examined: CU 81373, CU 81418

Nostoc spongiaeforme Ag. ex Born. *et* Flah., Revision des Nostoc. heteroc., 197, 1888; Geitler, Kryptogamenflora, 839, 1932; Desikachary, Cyanophyta, 380, 1959. (Fig. 15: c & 16 D).

Thallus first globose, later expanding, light blue green or brownish; filaments flexuous, loosely entangled; sheath diffluent and distinct towards the periphery; trichomes 4-4.5 µm broad, blue-green; cells elongated, cylindrical, 6-9 µm long or short, barrel shaped; heterocysts sub spherical or oblong, 4.5-5.5 µm broad, 7-8 µm long; spores oblong, in chains, 5-6 µm broad, 7.5-11 µm long, epispore smooth, yellowish.

Observed in Laterite and Poonthalpadom rice fields and adjoining areas as epiphytic and edaphic form.

Specimens examined: CU 81329, CU 81580

ANABAENA Bory

Dict. class. d'hist. nat., 1: 307, 1822.

Trichomes solitary or in small clusters, uniformly broader throughout or apices slightly acuminated, sheath absent or diffluent, forming a free, torn or soft mucilaginous thallus; cells torulose, cylindrical or barrel shaped; heterocysts generally intercalary, spherical to cylindrical; spores single or in series, formed from near the heterocysts or in between the heterocysts, spherical, oval, cylindrical or ellipsoidal.

Key to the species

1. Spores spherical or sub spherical.....2
1. Spores ellipsoidal/cylindrical.....4
2. Spores not contiguous to heterocyst.....3
2. Spores usually contiguous to heterocyst.....*A. sphaerica*
3. Spores not contiguous with intercalary heterocyst but occasionally with terminal heterocyst.....*A. oryzae*
3. Spores remote from the heterocyst.....*A. fertilissima*
4. Spores ellipsoidal, remote/ contiguous to heterocysts 5
4. Spores cylindrical, seen on either sides of the heterocyst.....8
5. Spores with rounded ends, contiguous to heterocysts 6
5. Spores barrel shaped with flattened ends, remote from the heterocysts .... *A. variabilis*
6. Spores many in chains, contiguous to the heterocysts.....*A. doliolum*
6. Spores limited, contiguous to heterocyst.....7
7. Spores on one side of the heterocyst only.....*A. volzii*
7. Spores on both sides of the heterocyst.....*A. iyengarii*
8. Spores twice as long as broad with flattened ends, end cells acute, conical .... *A. torulosa*
8. Spores up to 3-4 times as long as broad with rounded ends, end cells rounded .... *A. oscillarioides*

Anabaena doliolum Rao, Proc. Indian Acad. Sci., B, 2: 105, 1935; Desikachary, Cyanophyta, 410, 1959. (Fig. 17: h)

Thallus mucilaginous, blue-green; trichome single, free swimming, straight, slightly curved, 3.9-4.3 µm broad, end cell conical, with pointed apex; cells barrel shaped, as broad as or slightly longer or shorter than broad; heterocysts 5-6.5 µm broad, 6-9.5 µm long, barrel shaped, mostly intercalary; spores ellipsoidal with rounded ends, in short or long chains, adjoining the heterocyst in a centrifugal fashion, epispore thick, hyaline, smooth.

Observed in Kole rice fields among other cyanobacteria.

Specimen examined: CU 81577

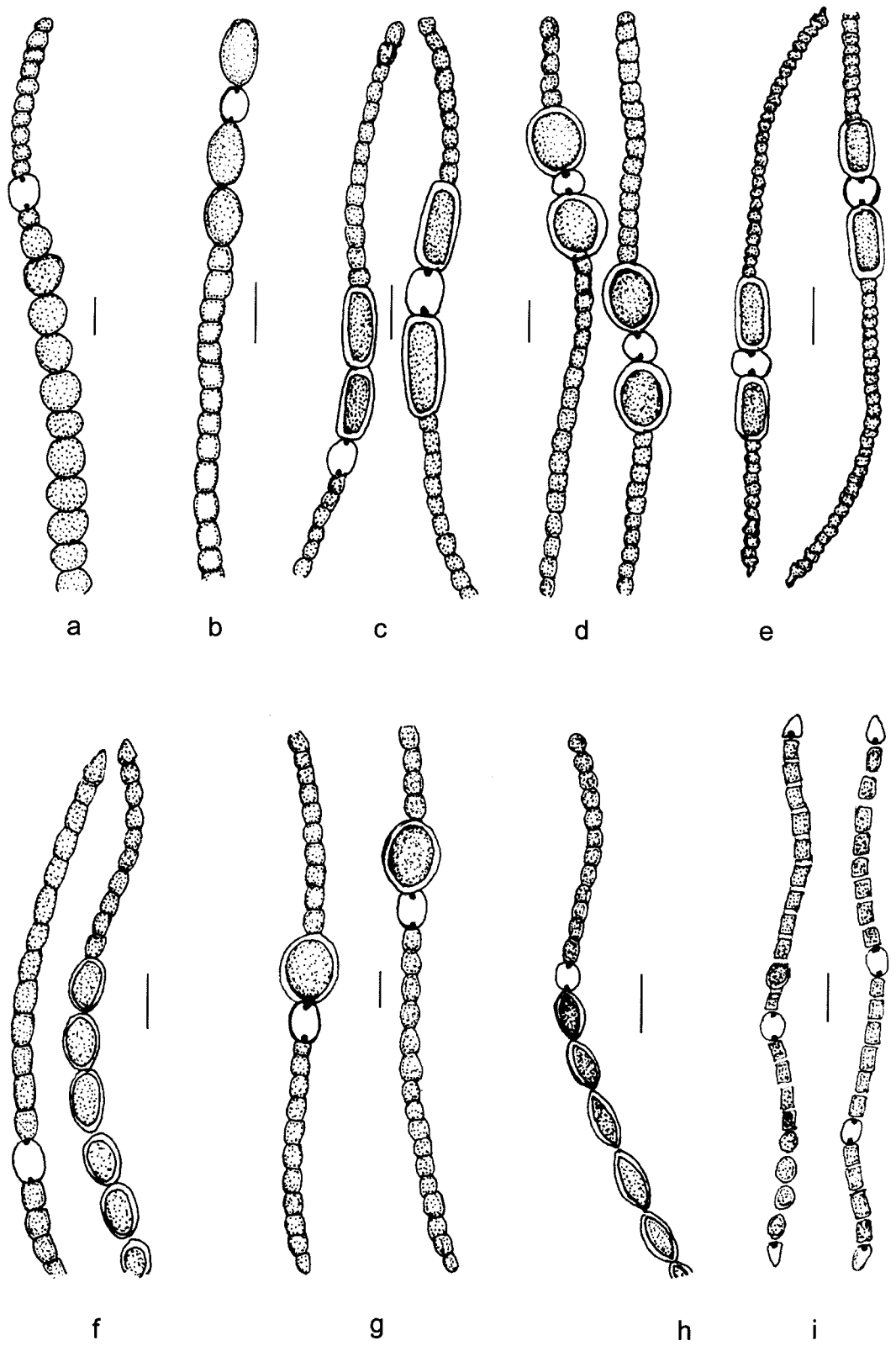


Fig. 17: **a**: *Anabaena fertilissima*, **b**: *A. iyengarii*, **c**: *A. oscillarioides*, **d**. *A. sphaerica*, **e**: *A. torulosa*, **f**: *A. variabilis*, **g**: *A. volzii*, **h**: *A. doliolum*, **i**: *A. oryzae* (Bar: 10 µm)

Anabaena fertilissima Rao, Proc. Indian Acad. Sci., 6: 363, 1937; Desikachary, Cyanophyta, 398, 1959. (Fig. 17: a)

Trichome single, straight or bent, with rounded end cells, blue-green; trichome 5-6.5 µm broad, at the apex 4 µm broad, cells barrel shaped, 5-7.5 µm long; heterocyst spherical, or slightly elongated, 6.5-8.2 µm broad; spores in long chains, later making the entire trichome sporogenous, formed centrifugally remote from the heterocysts, 5-7.8 µm broad, 4.5-8 µm long, epispore smooth and hyaline.

Observed in Poonthalpadom and Sandy alluvial fields of Kerala as planktonic populations.

Specimen examined: CU 81328

Anabaena iyengarii Bharad., Proc. Indian Acad. Sci., 2: 105, 1935; Desikachary, Cyanophyta, 406, 1959. (Fig. 17: b)

Trichome mostly single, 4-5.5 µm broad; cells barrel shaped or slightly longer than broad, end cell rounded; heterocyst barrel shaped, 6-7 µm broad, 7.5-8.5 µm long; spores ellipsoidal, on either side of heterocysts.

Collected from the Pokkali fields of Thrissur and Ernakulam districts.

Specimen examined: CU 81545

Anabaena oryzae Fritsch, J. Indian bot. Soc., 28: 135, 1949; Desikachary, Cyanophyta, 396, 1959. (Fig. 17: l)

Thallus soft, pale blue-green, gelatinous, membranous; trichome short, straight, densely aggregated, 2.5-3.2 µm broad, barrel shaped, cells 4-4.5 µm long; heterocysts terminal or intercalary, terminal ones conical twice longer than broad, intercalary ones barrel shaped, single or 2-3 in series; spores single/in series, next to the terminal heterocyst, or, away from the intercalary heterocysts, sub spherical, 5-5.5 µm broad; epispore yellowish brown.

Observed in many Laterite and Sandy alluvial rice fields and adjoining areas.

Specimen examined: CU 81385

Anabaena oscillarioides Born. *et* Flah., Revision des Nostoc. heteroc., 233, 1888; Geitler, Kryptogamenflora, 886,1932; Desikachary, Cyanophyta, 417, 1959. (Fig. 17: c & 19 D)

Thallus soft, gelatinous, dark green; trichome 4.5-6 µm broad, cells barrel shaped, as long as broad or longer, 5-6.5 µm long, end cell conical or rounded; heterocysts spherical to oval, 6-6.5 µm broad, 6.5-7.6 µm long; spores on either sides of the heterocysts, single or 2-3, initially oval later cylindrical, 6-8 µm broad; 17.5-23 µm long, epispore smooth, pale brown.

Observed in Poonthalpadom rice fields and adjoining areas of Kerala as epiphytic communities.

Specimens examined: CU 81365, CU 81442

Anabaena sphaerica Born. *et* Flah., Revision des Nostoc. heteroc., 228, 1888; Geitler, Kryptogamenflora, 878, 1932; Desikachary, Cyanophyta, 393, 1959. (Fig. 17: d)

Thallus floccose, blue-green; trichomes moniliform, straight, 5-6 µm broad; cells spherical to barrel shaped, end cell rounded; heterocysts sub spherical, 6-6.8 µm broad, spores on both sides of the heterocysts, sub spherical to oval, 10-12.2 µm broad, 12-16.8 µm long, epispore smooth, yellowish brown.

Observed in Karappadom, Kari and Kayal fields of Alappuzha district.

Specimen examined: CU 81588

Anabaena torulosa (Carm.) Lagerh. ex Born. *et* Flah., Revision des Nostoc. heteroc., 236, 1888; Geitler, Kryptogamenflora, 887, 1932; Desikachary, Cyanophyta, 415, 1959. (Fig. 17: e)

Thallus mucilaginous, thin, blue-green; trichome 4-5 µm broad, end cell acutely conical; cells barrel shaped, as long as or slightly shorter than broad; heterocysts, sub spherical ovoid, 5.5-6.2 µm broad, 6-8 µm long; spores on both sides of the heterocysts, single/ pairs; sub cylindrical with flattened ends, 6.5-8 µm broad, up to twice as long as broad, epispore smooth, pale brown.

Observed as planktonic and edaphic forms in Kole fields and Poonthalpadom fields.

Specimen examined: CU 81386, CU 81560

Anabaena variabilis (Bharad.) Fritsch, J. Indian bot. Soc., 28: 155, 1949; Desikachary, Cyanophyta, 413,1959. (Fig. 17: f & 19 C)

Thallus gelatinous, dark-green; trichome sheath-less, flexuous, 4-5 µm broad, slightly constricted at the cross walls, end cells conical; cells barrel shaped, with gas vacuoles, 3.8-6 µm long; heterocysts cylindrical, 4.5-5.5 µm broad, 6-7 µm long; spores formed centrifugally, away from the heterocyst, barrel shaped, in series, 6-8 µm broad, 8-12 µm long, epispore smooth, yellowish brown.

Observed in all rice fields and adjoining areas of Kerala as planktonic, epipsammic and edaphic populations.

Specimen examined: CU 81345, CU 81417

Anabaena volzii Lemm., Abh. Nat. Ver. Bremen, 18: 153, 1906; Geitler, Kryptogamenflora, 901,1932; Desikachary, Cyanophyta, 403,1959. (Fig. 17: g)

Thallus single, mucilaginous, blue-green; trichome 4-5.3 µm broad, constricted at the cross walls; cells 6.4-7.5 µm long, cylindrical, end cells rounded or conical; heterocyst single, intercalary, cylindrical, 5-6 µm broad, 7-9.5 µm long; spores single, ellipsoidal or oval, on one side of the heterocyst, 10.5-16 µm broad, 20-24 µm long, epispore smooth.

Planktonic and epipsammic in Kole and pokkali fields.

Specimen examined: CU 81548

AULOSIRA Kirch.

Kryptogamenflora, von Schlesien, 238, 1878.

Filaments free or in fascicles, uniformly broad, no base-apex differentiation, trichomes within a definite sheath; cells rectangular or short cylindrical; heterocysts intercalary, round or ovate; spores often in chains, formed near or away from heterocyst, cylindrical.

Key to the species

1. Filaments 5-6 µm broad.....*A. prolifica*
1. Filaments broader.....2
2. Filaments with false branching, spores absent.....*A. pseudoramosa*
2. Filaments without false branching, spores present.....*A. fertilissima*

Aulosira fertilissima Ghose, J. Linn. Soc. Bot., 46: 342, 1924; Geitler, Kryptogamenflora, 675, 1932; Desikachary, Cyanophyta, 431, 1959. (Fig. 18: a)

Thallus expanded, dark blue-green; trichomes straight, false branching absent, flexuous, cells 6-10.5 µm broad, 6-9 µm long; cylindrical or barrel shaped, contents granular sheath hyaline, firm and pale brown, 3.5-5 µm thick; heterocysts intercalary, oblong, 8-8.6 µm broad, 10-12 µm long; spores in series, generally alternating with dead cells, oblong or elliptical, 8-12 µm broad, 15.5-17.5 µm long.

Observed in Kari and Poonthalpadom rice fields and adjoining areas of Kerala.

Specimen examined: CU 81349

Aulosira prolifica Bharad., Ann. Bot., 47: 131, 1933; Desikachary, Cyanophyta, 426, 1959. (Fig. 18: b)

Thallus forming a dense mucilaginous scum, blue-green or green; Filaments 5-6 µm broad, straight or slightly curved; sheath thin, firm; trichome distinctly constricted at the septa, apical cell rounded or acute conical, cells cylindrical, longer than broad, 3.1-5 µm broad, 6-10 µm long; heterocyst intercalary, broader than trichome, ellipsoidal, 5.5-7 µm broad, 12-13 µm long; spores absent.

Observed in Poonthalpadom rice fields and adjoining areas.

Specimen examined: CU 81589

Aulosira pseudoramosa Bharad., Ann. Bot., 47: 137, 1933; Desikachary, Cyanophyta, 430, 1959. (Fig. 18: c)

Thallus a flat compact system with an uneven surface ordinarily made of unbranched and pseudobranched filaments, 7-10 µm broad, generally irregularly bent, densely entangled; sheath thick, hyaline, firm; trichome constricted at the septa, cells cylindrical slightly longer than broad, 6-9 µm broad, blue-green; heterocyst absent in young condition, intercalary, occurring single, as broad as trichomes, 6-9.5 µm broad, 6.5-15 µm long, spores absent.

In cultures of Laterite paddy fields of Malappuram and Thrissur districts.

Specimen examined: CU 81358

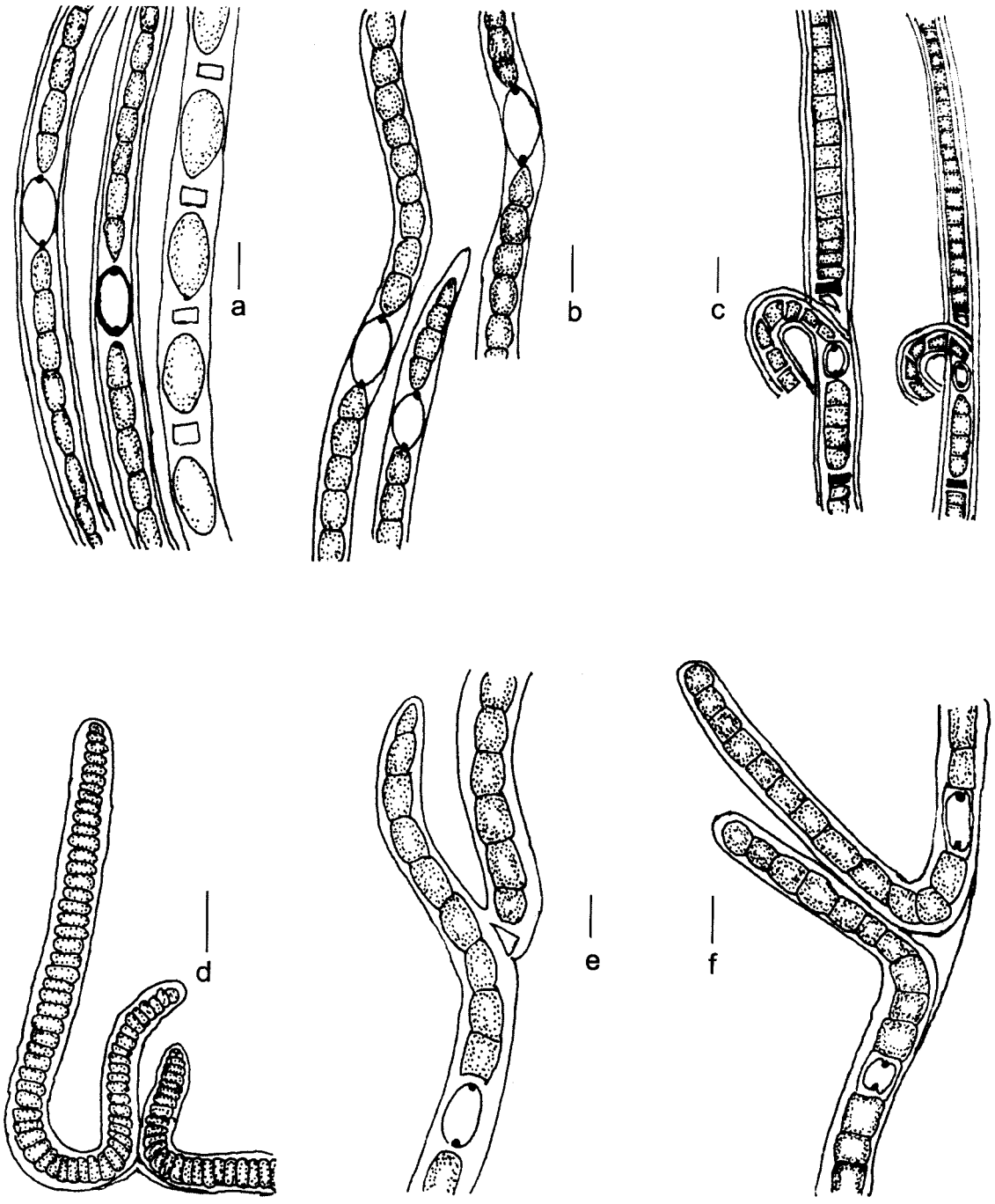


Fig. 18: **a**: *Aulosira fertilissima*, **b**: *A. prolifica*, **c**: *A. pseudoramosa*, **d**: *Plectonema radiosum*, **e**: *Scytonematopsis kashyapi*, **f**: *Scytonema bohneri* (Bar 10 µm)

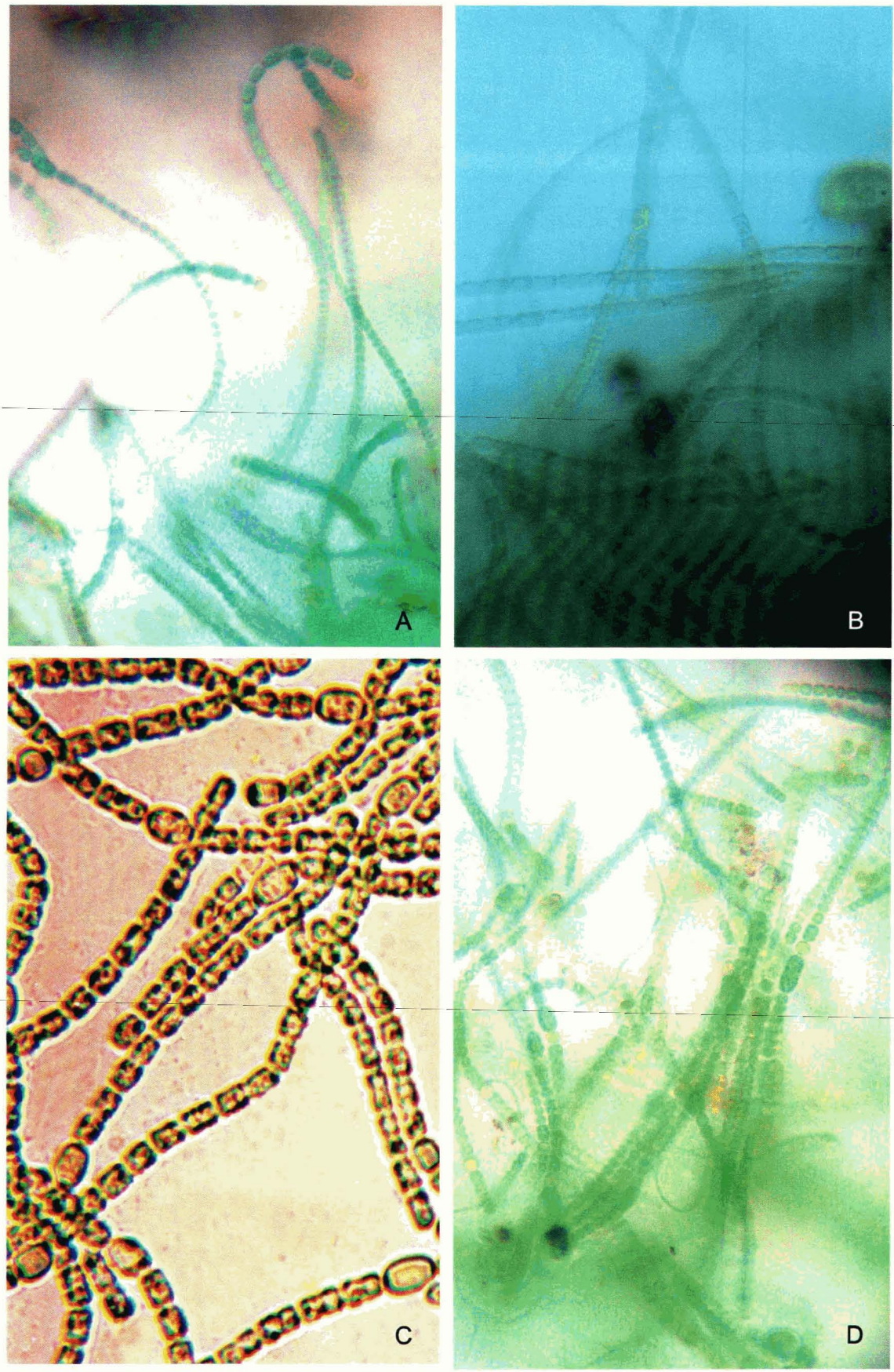


Fig. 19 **A**: *Cylindrospermum michailovskoense*, **B**: *C. stagnale*, **C**: *Anabaena variabilis*, **D**: *A. oscillarioides*

SCYTONEMATACEAE Rabenhorst
Fl. Eur. Alg., 2: 246, 1865.

Filaments with a thick, firm, lamellated or divergent sheath; false branched, branches single or geminate; trichome filamentous, heterocysts intercalary, situated next to a single false branch, hormogones present, spores seen in some.

Key to the genera

1. Heterocyst present.....2
1. Heterocyst absent.....*Plectonema*
2. Apex tapering.....*Scytonematopsis*
2. Apex not tapering.....3
3. False branches single, arise next to the heterocyst.....*Tolypothrix*
3. False branches mostly geminate, not next to the heterocyst.....*Scytonema*

PLECTONEMA Thuret

Ann.Sci. nat. Bot. 1: 375, 1875.

Plectonema radiosum (Sch.) Gom., Monogr. Oscillariees, 100, 1892; Geitler, Kryptogamenflora, 687, 1932; Desikachary, Cyanophyta, 437, 1959. Fig. 18: d

Filaments curved, abundantly false branched; sheath thin, unlamellated, brownish; trichome 5-7 µm broad, cells 1-2 µm long, distinctly constricted at the septa; slightly attenuated at the apex, end cell rounded, hormogones present.

Observed as edaphic community in the Laterite soils of Malappuram and Wayanad districts.

Specimen examined: CU 81466

SCYTONEMATOPSIS Kiss.

J. Soc. Bot. Russ., 15: 174, 1930.

Scytonematopsis kashyapi (Bharad.), Geitler, Arch. Hydrobiol. Suppl., 14: 445, 1935; Desikachary, Cyanophyta, 448, 1959. (Fig. 18: e)

Thallus of blue green wooly tufts; filaments straight or curved slightly; sheath hyaline; trichome 5-6 µm broad, slightly constricted at the septa, false branched; cells 2-3 times as long as broad; heterocysts ellipsoidal, 6-7 µm broad, 10-12 µm long, apex tapering.

Observed as edaphic populations in Poonthalpadom fields.

Specimen examined: CU 81465

SCYTONEMA Ag.

Syst. Alg., 26, 1824.

Filaments forming wirely and wooly mats, false branched, branches single or geminate, formed laterally in between heterocysts; trichome solitary in each sheath, straight, hormogones terminal, solitary; cells quadrate, short, cylindrical; heterocyst quadrate or cylindrical, with rounded ends, sometimes compound; spores not observed.

Key to the species

1. Trichome constricted.....2
1. Trichome not constricted.....3
2. Cells 3-5 times broader than long.....*S. cincinnatum*
2. Cells 1-2 times broader than long.....*S. stuposum*
3 Cells as long as broad.....4
3. Cells quadrate or longer than broad.....5
4. Filaments 15-21 µm broad, trichomes10-15 µm broad.....*S. guyanense*
4. Filaments 15-20 µm broad, trichomes 6-11 µm broad.....*S. mirabile*
5. Filaments more than 12 µm broad.....6
5. Filaments up to 12 µm broad.....8
6. Filaments lamellated.....*S. tolypothrichoides*
6. Filaments non-lamellated.....7
7. Filaments up to 16 µm broad.....*S. simplex*
7. Filaments 16-19 µm broad.....*S. coactile*
8. Filaments up to 7-12 µm broad; false branches in fascicles .....*S. hofmanni*
8. Filaments up to 10-12 µm broad; false branches simple .....*S. bohneri*

Scytonema bohneri Schmid., Engler's. Bot. Jahrb., 30: 60, 1901; Desikachary, Cyanophyta, 457, 1959. (Fig. 18: f)

Stratum filamentous, blackish brown; filaments partly creeping, partly ascending; filaments 10-12 µm broad, false branched, branches mostly simple; sheath colourless, homogeneous; trichome bluish-green, 5-8 µm broad; cells rectangular, short at the apex, 1-1½ times as long as broad in the rest; heterocyst compressed, rectangular, longer than broad, wall hyaline.

Epiphytic and edaphic in Laterite paddy fields of Malappuram district.

Specimen examined: CU 81357

Scytonema cincinnatum Born. *et* Flah., Revision des Nostoc. heteroc., 89, 1887; Geitler, Kryptogamenflora, 748, 1932; Desikachary, Cyanophyta, 453, 1959. (Fig. 20: a & 21 C)

Stratum caespitose, wooly, brownish-green; filaments 16-25 µm broad, false branches mostly geminate; sheath firm, hyaline, sometimes brownish; trichome 10-18 µm broad, cells 4-7 µm long, 3-5 times broader than long, distinctly or slightly constricted at the septa; heterocyst depressed, quadrate or short, cylindrical.

Epiphytic and edaphic in Poonthalpadom fields.

Specimen examined: CU 81307

Scytonema coactile Born. *et* Flah. Revision des Nostoc. heteroc., 90, 1887; Geitler, Kryptogamenflora, 753, 1932; Desikachary, Cyanophyta, 455, 1959. (Fig. 20: d)

Thallus expanded, wooly, greenish blue; filaments 16-19 µm broad, false branches long, erect; sheath firm, yellowish; trichome 10-14.6 µm broad, cells quadrate to longer than broad, heterocysts sparse, sub quadrate.

Seen attached at the edges of rice fields and adjoining areas in Kole fields.

Specimen examined: CU 81431

Scytonema guyanense (Mont.) Born. *et* Flah., Revision des Nostoc. heteroc., 94, 1887; Geitler, Kryptogamenflora,770, 1932; Desikachary, Cyanophyta, 469, 1959. (Fig. 20: b & 21 D)

Thallus expanded like a cushion, blackish green; filaments 15-21 µm broad, trichomes 10-15 µm broad, false branches long, bent, united at the base; sheath firm, lamellated, yellowish brown; cells quadrate, not constricted at the septa, heterocyst quadrate, as broad as vegetative cells.

Planktonic and edaphic in Sandy alluvial, Kayal and Kole fields

Specimen examined: CU 81582

Scytonema hofmanni *Ag.* ex. Born. *et.* Flah., Syn. Algar. Sueciee, 117, 1817; Geitler, Kryptogamenflora, 772, 1932; Desikachary, Cyanophyta, 476, 1959. (Fig. 20: e)

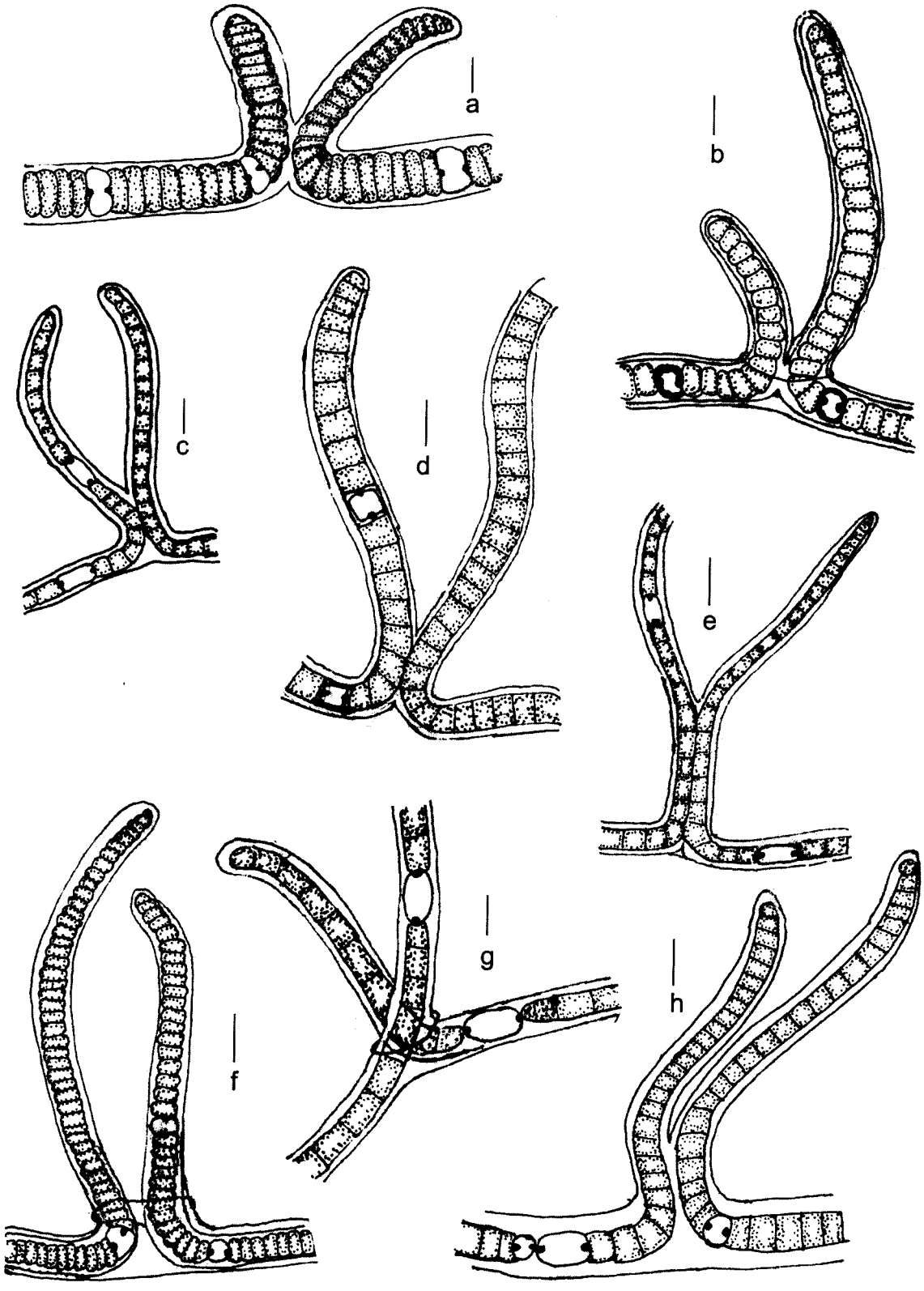


Fig. 20: **a**: *Scytonema cincinnatum*, **b**: *S. guyanense*, **c**: *S. mirabile*, **d**: *S. coactile*, **e**: *S. hofmanii*, **f**: *S. stuposum*, **g**: *S. simplex*, **h**: *S. tolypothrichoides* (Bar 10 µm)

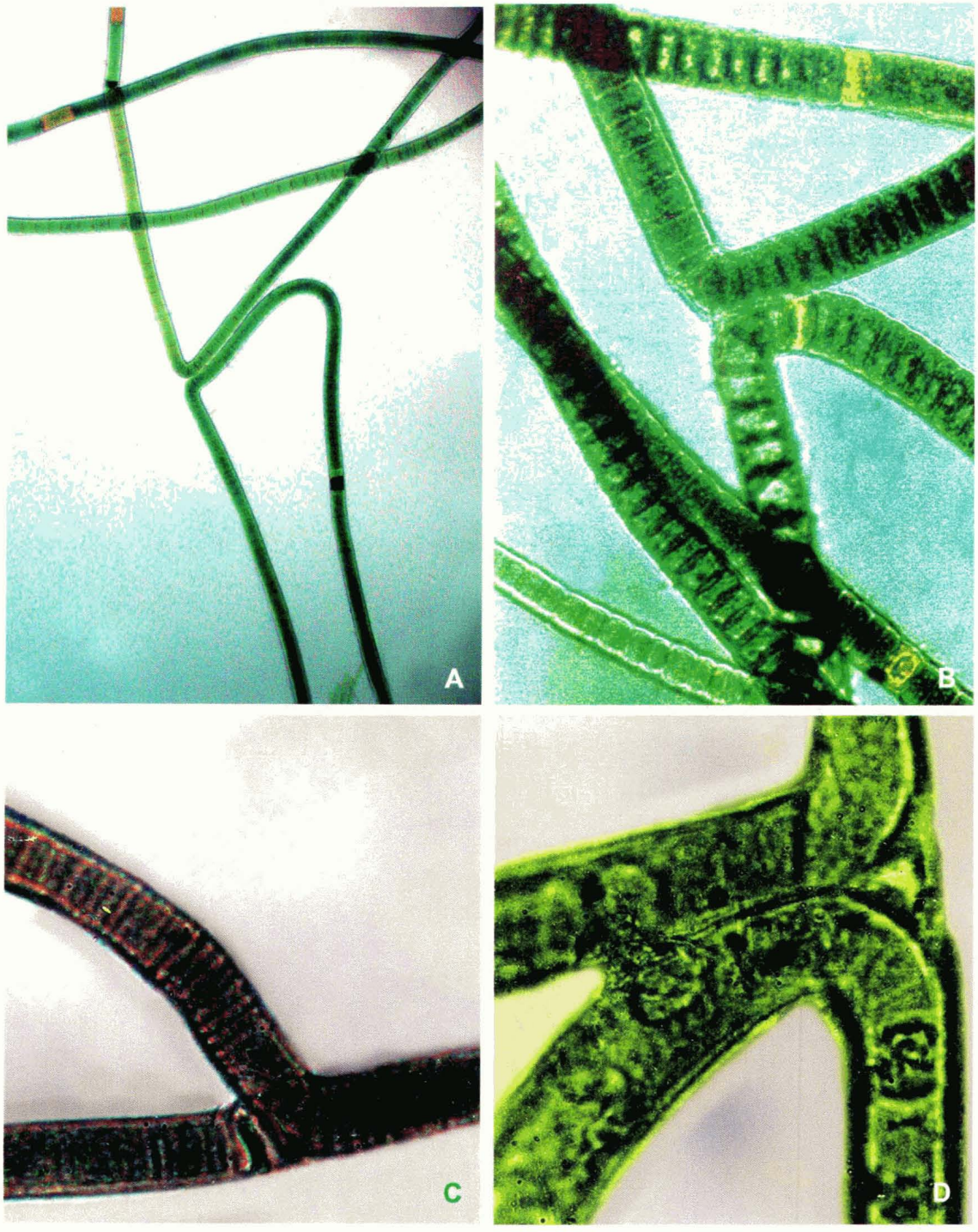


Fig. 21 A: *Scytonema tolypothrichoides*, **B:** *S. stuposum*, **C:** *S. cincinnatum*, **D:** *S. guyanense*

Thallus cushion like, expanded, blackish blue-green to bluish grey; filaments 7-12 µm broad, aggregated to vertical fascicles; false branches aggregated, sheath firm; trichome 5-10 µm broad, blue-green, cells unequal in length; heterocyst oblong.

Seen as Epiphytic and edaphic in Laterite fields of Kozhikkode District.

Specimen examined: CU 81513.

Scytonema mirabile (Dillw.) Born., Bull. Soc. bot. Fr., 36: 155, 1889; Geitler, Kryptogamenflora, 775, 1932; Desikachary, Cyanophyta, 483, 1959. (Fig. 20: c)

Thallus expanded, cushion like, tomentose; filaments brownish black or dark green wooly mats; 15-20 µm broad, false branches long; sheath slightly lamellated, yellowish brown; trichome 6-11 µm broad, yellow to olive green or blue-green; cells quadrate, cylindrical or barrel shaped; heterocysts quadrate or longer than broad, brownish, usually broader than the trichome.

Epiphytic and edaphic in Laterite fields

Specimen examined: CU 81454

Scytonema simplex Bharad., Rev. Algol. 7:157, 1934; Desikachary, Cyanophyta, 455, 1959. (Fig. 20: g).

Thallus thick, dark, dirty blue-green; filaments up to 16 µm broad, loosely entangled, irregularly bent; false branches common, geminate and single in equal numbers; sheath thick, firm, non-lamellated, hyaline or yellow; trichome 8-10 µm broad, cells not constricted at the septa, cells usually elongated up to 4 times as long as broad, sometimes quadrate or cylindrical; heterocyst single or in pairs as broad as trichome, 9.4-11.5 µm broad, 11.5-16.2 µm long.

Epiphytic and edaphic in Poonthalpadom fields.

Specimen examined: CU 81323

Scytonema stuposum (Kutz.) Born. *et.* Flah., Revision des Nostoc. heteroc., 92, 1887; Geitler, Kryptogamenflora, 756, 1932; Desikachary, Cyanophyta, 459, 1959. (Fig. 20: f & 21 B)

Thallus broadly expanded, wooly, tomentose, greenish brown filaments, 16.8-21 µm broad; sheath firm, thick, false branches single or geminate;

trichome 12-16 µm broad, constricted at the septa; cells 1-2 times broader than long, heterocyst as broad as the vegetative cells.

Epiphytic and edaphic in Laterite fields of Kannur District.

Specimen examined: CU 81315

Scytonema tolypothrichoides Born. *et* Flah., Revision des Nostoc. heteroc., 100, 1887; Geitler, Kryptogamenflora, 779, 1932; Desikachary, Cyanophyta, 479, 1959. (Fig. 20: h & 21 A)

Thallus greenish brown, filaments 12-14 µm broad; false branches common, sheath 3-6 µm broad, lamellations parallel; trichome 6.5-9 µm broad, not constricted at the septa; cells quadrate or slightly longer, 7.5-9 µm long; heterocyst quadrate-cylindrical, 7-9 µm broad, 12.5-17 µm long; apical cell rounded, without calyptra.

Observed as edaphic communities in Pokkali and Kole fields

Specimen examined: CU 81547

TOLYPOTHRIX Kutz.

Phyc. gen., 227, 1843.

Filaments with firm, thin or thick sheath with a single trichome within; false branched, free, prostrate or erect; false branching single mostly subtending a heterocyst; occasionally geminate, hormogones formed from the tips, trichome with apical growth, apex often broader with shorter cells; spores present in some species.

Key to the species

1. Trichome 5-13 µm broad, cells quadrate/long.....*T. tenuis*

1. Trichome 9-11µm broad, cells long as broad.....*T. byssoidea*

Tolypothrix byssoidea (Berk.) Kirch., Naturlichen Pflanzenfam., I: 80, 1900; Geitler, Kryptogamenflora, 728, 1932; Desikachary, Cyanophyta, 502, 1959. (Fig. 22: a)

Thallus wooly, cushion like, brownish; Filaments 10-15 µm broad, irregularly false branched, false branches short, curved; sheath thin, close to trichome, yellowish brown; trichome 9-11 µm broad, torulose; cells barrel

shaped, as long as broad, not constricted at the end walls; heterocyst basal or intercalary, cylindrical/rounded, single/2 in a row.

Epiphytic and edaphic in Laterite fields.

Specimen examined: CU 81330

Tolypothrix tenuis (Kutz.) Johs, Schmidt em. Bot. Tidsskr., 22: 383, 1899; Desikachary, Cyanophyta, 494, 1959. (Fig. 22: b & 10 C)

Thallus caespitose, blue-green/brown; filaments 6-15 µm broad, repeatedly branched; sheath thin, initially colourless, later yellow; cells 5-13 µm broad, quadrate or slightly longer than broad, not constricted at the cross walls; heterocyst rounded to discoid, 5.5-14 µm broad, 3-7 µm long, yellowish, solitary or in chains.

Epiphytic and edaphic in Laterite fields.

Specimen examined: CU 81537

RIVULARIACEAE Rabenhorst

Fl. Eur. Alg., 2: 200, 1868.

Trichome uniseriate, ends attenuated or tapering in a hair; unbranched or false branched; sometimes with a distinct intercalary growth region; heterocyst present or absent, hormogones present, spores present or absent, when present, single or in series.

Key to the genera

1. Filaments single, false branches free.....*Calothrix*
1. Filaments in a spherical thallus, Sheath gelatinous.....2
2. Spores commonly formed, large.....*Gloeotrichia*
2. Spores absent.....*Rivularia*

CALOTHRIX Ag.

Syst. Alg., 24, 1824.

Filaments single or in small groups, caespitose, pulvinate, unbranched, sometimes with false branches from the mid region; filaments arranged more or less parallel; sheath firm, sometimes seen only at the base; vegetative cells shorter than wide below and longer than wide above; heterocysts mostly basal, seldom intercalary, subglobose, spherical, hemispherical; spores not observed.

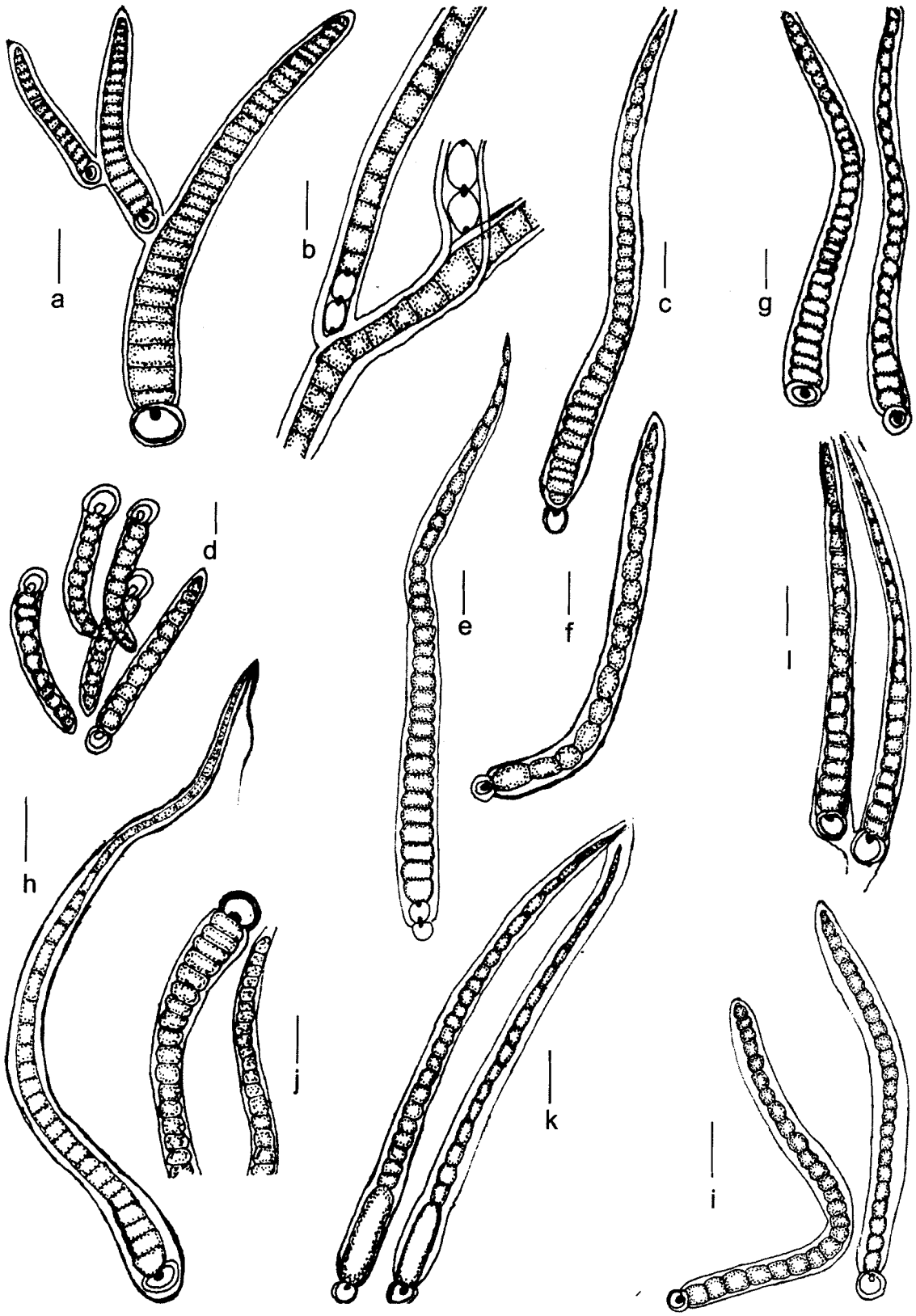


Fig. 22: **a**: *Tolypothrix byssoidea*, **b**: *T. tenuis*, **c**: *Calothrix braunii*, **d**: *C. brevissima*, **e**: *C. castellii*, **f**: *C. clavata*, **g**: *C. elenkini*, **h**: *C. fusca*, **i**: *C. marchica* var. *intermedia*, **j**: *C. marchica* var. *crassa*, **k**: *Gleotrichia intermedia*, **l**: *Rivularia globiceps* (Bar 10 µm)

Key to the species

1. Trichomes swollen at the base.....2
1. Trichomes not so swollen at the base.....6
2. Trichomes more than 5 µm broad at the base.....3
2. Trichome 4-4.5 µm broad.....*C.brevissima*
3. Trichome gradually ending in a hair.....4
3. Trichome long and attenuated gradually without a hair.....*C. elenkinii*
4. Trichome more than 9 µm broad at the base .... *C. castellii* var. *somastipurense*
4. Trichome less than 9 µm broad at the base.....5
5. Trichome slightly bent at the base, 5.5-6.7 µm broad at the base...*C. braunii*
5. Trichome bent at the base, 6.2-8.5 µm broad at the base.....*C.fusca*
6. Trichome with a short terminal hair.....*C. clavata*
6. Trichome free of hair.....7
7. Trichome 5.8-6.8 µm broad at the base .....*C. marchica* var. *intermedia*
7. Trichome 7.5-12 µm broad at the base.....*C. marchica* var. *crassa*

Calothrix braunii (A. Br.) Born. *et* Flah., Revision des Nostoc. heteroc., 368, 1886; Geitler, Kryptogamenflora, 606, 1932; Desikachary, Cyanophyta, 535, 1959. (Fig. 22: c)

Thallus caespitose, brownish; trichome swollen at the base and slightly bent, cells 5.5-6.7 µm broad, shorter than broad, constricted at the cross walls, ending in a long hair; sheath thin, close to the trichome, colourless; heterocyst basal, hemispherical.

Observed as epiphytic populations in Kayal, Karappadom and Poonthalpadom fields.

Specimen examined: CU 81509

Calothrix brevissima West, J. Linn. Soc., 38: 180, 1907; Geitler, Kryptogamenflora, 624, 1932; Desikachary, Cyanophyta, 533, 1959. (Fig. 22 D)

Filaments epiphytic, many together, very short, 4.5-5 µm broad, slightly attenuated with rounded end cells; trichome 4-4.5 µm broad, slightly constricted

at the septa, cells at the base equal to or slightly shorter than broad, brownish green or olive green; heterocyst single, broad, hemispherical, 4-4.5 µm across.

Collected from the Laterite and Poonthalpadom rice fields in epiphytic and planktonic form.

Specimen examined: CU 81575

Calothrix castellii var. ***somastipurense*** Rao, Proc. Indian Acad. Sci. 9: 145, 1939; Desikachary, Cyanophyta, 531, 1959. (Fig. 22: e)

Filaments densely entangled, 12.5-16 µm broad at the base; trichome swollen, 9-12.2 µm broad at the base, 2 µm broad at the apex; cells shorter than broad at the base and becoming longer towards the apex ending in a hair; heterocyst basal, hemispherical.

Edaphic communities in the Laterite fields of Malappuram District.

Specimen examined: CU 81590

Calothrix clavata West, Voyage d'explor. Colombie, 1019,1914; Geitler, Krypto-gamenflora, 609, 1932; Desikachary, Cyanophyta, 542, 1959. (Fig. 22: f & 23 A)

Filaments single or in small groups, straight/slightly bent, not swollen at the base; filaments 6.5-7 µm broad, with slight attenuation, with a short small terminal hair; sheath thin, firm, hyaline, close to the trichome; trichome 6-6.5 µm broad at the base, slightly constricted at the septa, cells discoid /slightly longer at the base,

2-5 µm broad in the middle, narrower towards the apex and 2-3 times as long as broad; heterocysts basal single spherical/hemispherical.

Edaphic communities in the Laterite fields.

Specimen examined: CU 81593

Calothrix elenkinii Koss., Not. Syst. Crypt. Inst. Horti. Bot. Petropol. 3: 11, 1924; Geitler, Kryptogamenflora, 609, 1932; Desikachary, Cyanophyta, 531, 1959. (Fig. 22 g) .

Filaments seen in tufts, bent and swollen at the base, false branches present; trichome 6-6.5 µm broad at the base, not constricted at the septa in the lower regions, slightly constricted at the upper regions, attenuated gradually,

apical hair absent; cells quadrate, shorter than broad at the base; heterocyst basal, single, 5-6.5 µm broad, hemispherical.

Collected from Karappadom (Kuttanad) and Poonthalpadom fields (Palakkad District) as edaphic communities.

Specimen examined: CU 81464

Calothrix fusca (Kutz.) Born. *et* Flah., Revision des Nostoc. hetero. 364, 1886; Geitler, Kryptogamenflora, 610, 1932; Desikachary, Cyanophyta, 527, 1959. (Fig. 22: h & 23 C)

Filaments single, or in small groups, curved or straight, attached to the mucilage of other algae; 9.5-12.5 µm broad, sheath broad, colourless, diffluent at the apices; trichome 6.5-8.5 µm broad and bent at the base, ending in a long hair; cells often shorter than broad; heterocyst basal, hemispherical, smaller than the basal cell of the trichome.

Planktonic, epiphytic and edaphic in all rice fields of Kerala.

Specimens examined: CU 81310, CU 81411

Calothrix marchica Lemm. var. ***crassa*** Rao, proc. Indian Acad. Sci. 6: 349, 1937; Desikachary, Cyanophyta, 543, 1959. (Fig. 22: j & 23 D)

Filaments in groups, 8.5-12.5 µm broad, sheath thin, firm, yellow/hyaline; trichomes 7.5-12 µm broad, constricted at the septa, cells shorter or as long as broad; trichome end tapering without a hair; end cell conical with a round apex; heterocysts single, basal, spherical, slightly shorter.

Edaphic in Laterite fields.

Specimen examined: CU 81596

Calothrix marchica Lemm. var. ***intermedia*** Rao, proc. Indian Acad. Sci. 6: 350, 1937; Desikachary, Cyanophyta, 544, 1959. (Fig. 22: i & 23 B)

Thallus brown to reddish brown, epiphytic on other algae; single or in small groups, filaments 6-7 µm broad, with slight attenuation and without a terminal hair; sheath thin, firm, hyaline; trichome 5.8-6.8 µm broad, slightly constricted at the septa; cells quadrate, slightly longer or shorter than broad, 4.5-7 µm long, end cell rounded; heterocyst single, basal / intercalary, spherical, 5.5-6.8 µm broad.

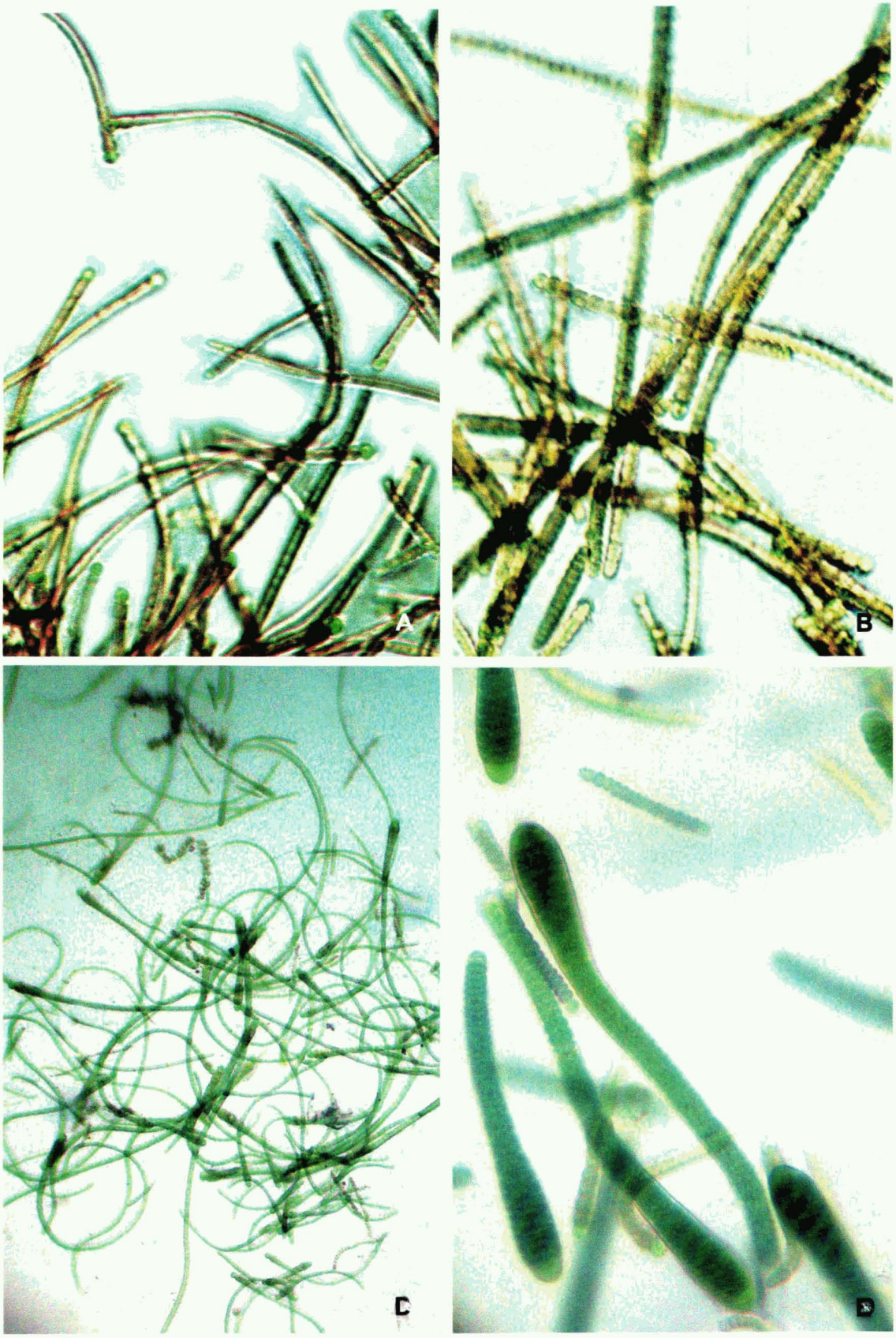


Fig. 23 **A**: *Calothrix clavata,* **B**: *C. marchica* var. *intermedia,* **C**: *C. fusca,* **D**: *C. marchica* var. *crassa*

Planktonic, epiphytic and edaphic in all rice fields and adjoining areas of Kerala especially in Poonthalpadom and Laterite fields.

Specimen examined: CU 81381, CU 81327, CU 81346

GLOEOTRICHIA Ag.

Alg. Maris et Adriati., 8, 1842.

Gloeotrichia intermedia var, ***kanwaensis*** Rao, Proc. Indian Acad. Sci. 3: 168, 1936; Desikachary, Cyanophyta, 560, 1959. (Fig. 22: k)

Thallus spherical, soft, trichome constricted at the septa at the base; ending in a long hair towards the apex; cells 5-9.5 µm broad, 3.5-5 µm long at the base and becoming less broad and more long at the tail region; heterocyst hemispherical, single or in series; spores sub terminal to heterocyst, cylindrical, 8-9 µm broad, 40-50 µm long.

Observed as planktonic community in the Kole fields of Thrissur District.

Specimen examined: CU 81469

RIVULARIA (Roth) Ag.

Syst. Alg., 19, 1824.

Rivularia globiceps West, J. Linn. Soc. Bot. Lond., 38: 182, 1907; Geitler, Kryptogamenflora, 652, 1932; Desikachary, Cyanophyta, 552, 1959. (Fig. 22: l)

Thallus hemispherical to spherical, filaments slightly densely packed together; sheath thin, colourless, unlamellated, gradually attenuated at the ends; trichome 5-6 µm broad, cells longer than broad, ending in a long thin hair; heterocyst spherical.

Observed as epipsammic populations in the Sandy alluvial soils of Kasargode district.

Specimen examined: CU 81317

Floristic Analysis:

The present investigation reports the occurrence of 111 spp. of cyanobacteria spread over 17 genera and 4 families of Nostocales from the rice fields and adjoining areas of Kerala (Fig. 24 & 25). The study is exclusively based on the morphological features available in the field materials. Differential and disributional pattern of cyanobacterial flora were observed in the rice fields in various soil types of Kerala.

13 spp. *viz.*, *Oscillatoria acuta, O. acuminata, O. animalis, O. subbrevis* f. *major, Phormidium foveolarum, Microcoleus chthonoplastes, Cylindrospermum majus, C. stagnale, Nostoc linckia, N. punctiforme, Anabaena variabilis, Calothrix fusca* and *C. marchica* var. *intermedia* are found in all types of rice fields of Kerala irrespective of the soil types or pH or other soil or climatic conditions.

The investigation revealed distribution of 7 spp. *viz.*, *Oscillatoria pseudogeminata, O. subbrevis, Geitlerinema earlei, Phormidium corium, P. tenue, Nostoc carneum f. minor,* and *N. muscorum* in 60-75% of rice field soils *i.e.*, found in 5-6 soil types out of the total eight soil types of Kerala.

An interesting feature noticed was the abundance of cyanobacterial flora in the Laterite soils of Kerala, which occupied the major bulk of Kerala. About 82% of generic and 61% of species diversity was observed in this type of soil (Table 1, Figs. 26 & 27). Moreover, these species were distributed uniformly in the fields. Some of the species in addition to the common spp. collected from these areas include, *Spirulina laxissima, S. major, S. subsalsa, Oscillatoria acuminatata, O. curviceps, O. limnetica, O. limosa, O. okeni, O. ornata, O. princeps, O. pseudogeminata, O. sancta, O. subbrevis, Geitlerinema earlei, Phormidium corium, P. luridum, P. tenue, P. uncinatum, Lyngbya major, L. martensiana, L. palmarum, L. rubida, Cylindrospermum michailovskoense, C. muscicola, C. muscicola var. longispora, Nostoc calcicola, N. carneum* f. *major N. commune, N. muscorum, N. spongiaeforme, Aulosira pseudoramosa, Plectonema radiosum, Scytonema bohneri, S. cincinnatum, S. coactile, S. mirabile, S. stuposum, Tolypothrix tenuis, Calothrix brevissima* and *C. castellii.*

Fig. 24. Cyanobacterial (Nostocales) diversity in the Paddy fields of Kerala at species, genera and family levels

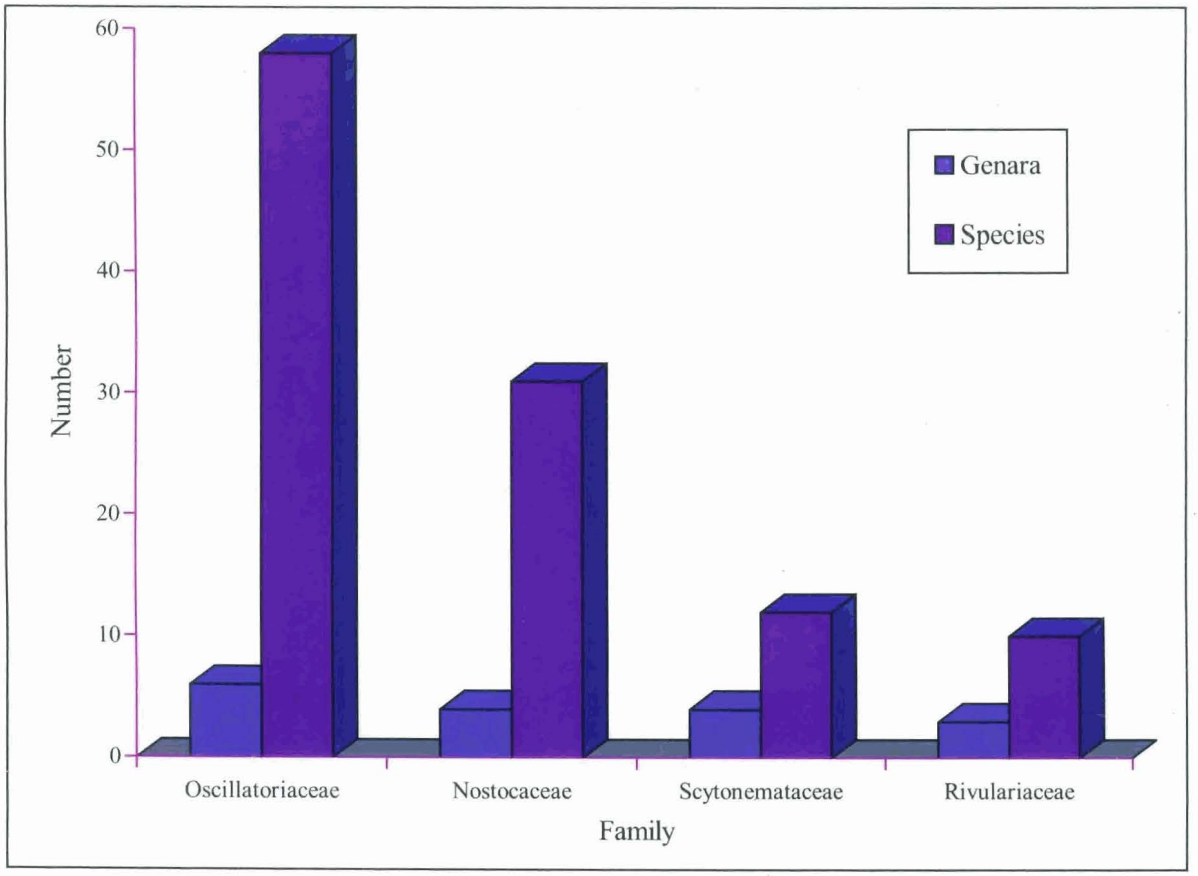


Fig. 25. Cyanobacterial (Nostocales) diversity in the paddy fields of Kerala at generic and species levels

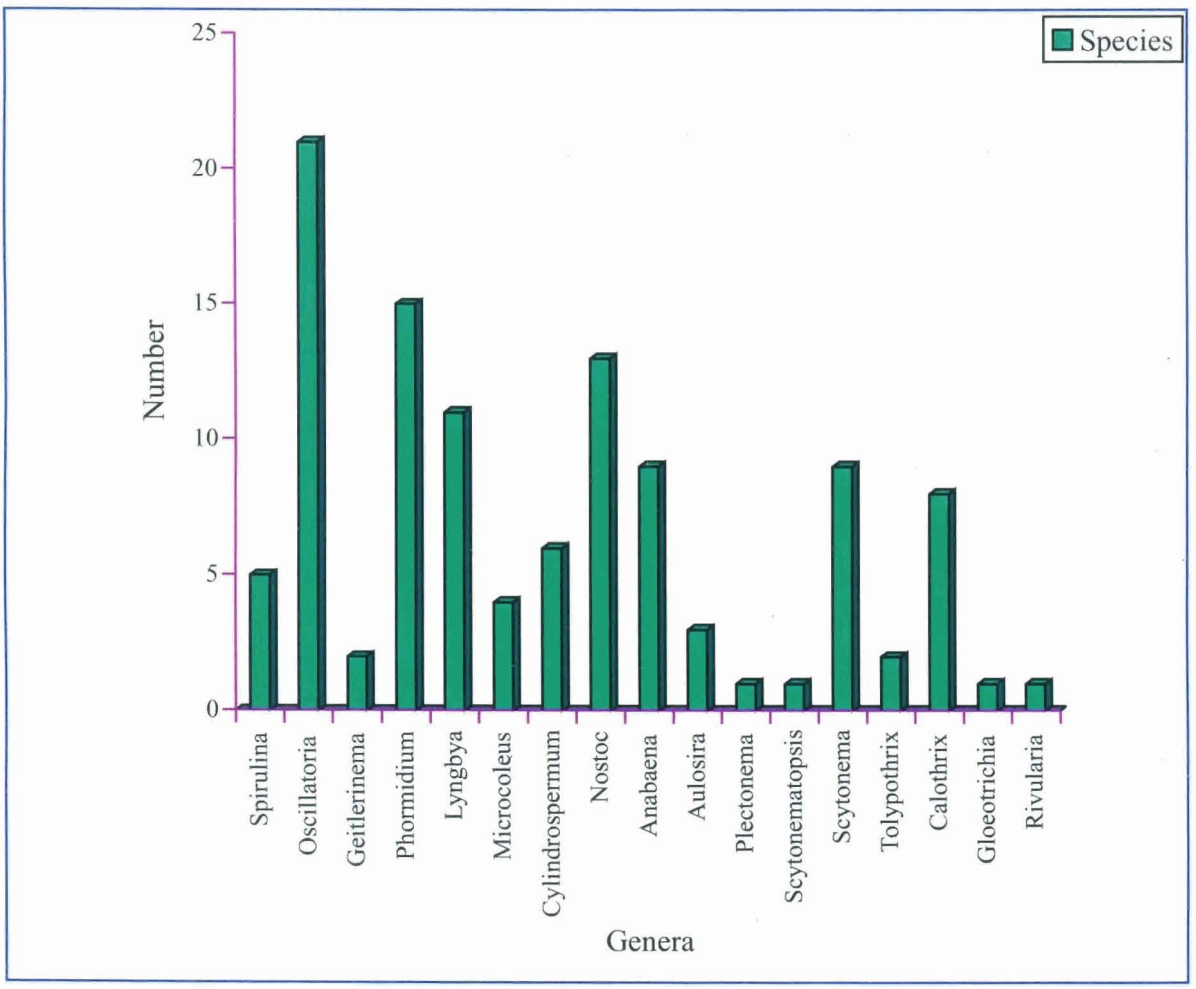


Fig. 26. Cyanobacterial (Nostocales) diversity in different soil types of Kerala

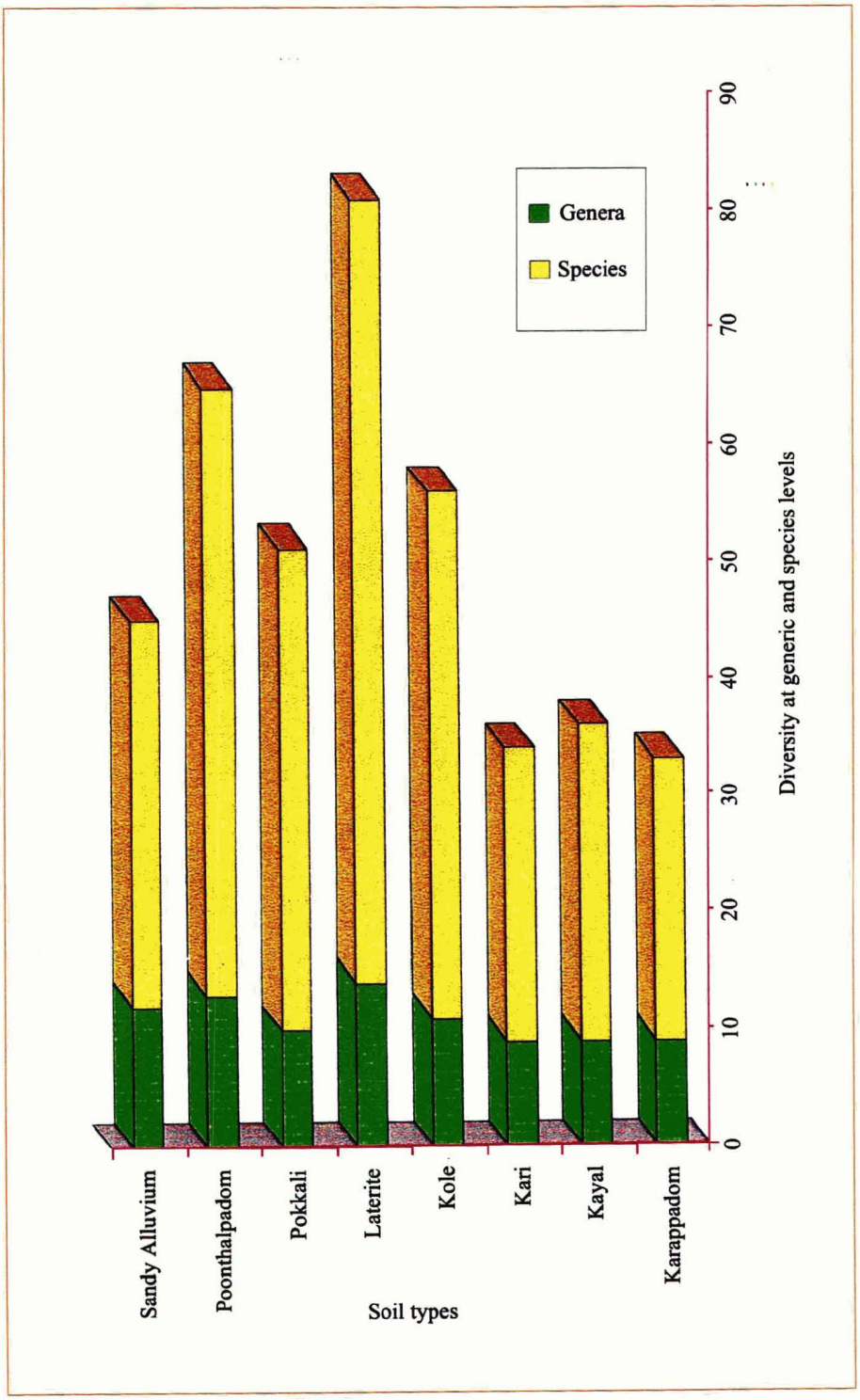


Table 1: pH and Cyanobacterial (Nostocales) diversity in different soil types of Kerala with references to rice fields

Sl. No.	Types of soils	pH	No. of genera	No. of species	% distribution (in nearest whole number)	
					Genera	Species
1.	Karappadom	4.35	9	23	53	21
2.	Kari	3.61	9	23	53	21
3.	Kayal	4.18	9	25	53	23
4.	Kole	4.56	11	45	65	42
5.	Laterite	5.17	14	67	82	61
6.	Pokkali	3.51	10	41	59	37
7.	Poonthalpadom	7.74	13	50	76	45
8.	Sandy alluvium	5.04	12	33	70	30

The Poonthalpadom fields of Palakkad Districts showed the next higher rate of generic (76%) and species (45%) diversity (Fig. 26,28 & Table-1). Some important species collected from this field other than the the common types include -*Spirulina major, Oscillatoria chlorina, O. limnetica, O. limosa, O. princeps, O. tenuis, O. pseudogeminata, O. subbrevis, Geitlerinema jasorvense, Lyngbya allorgei, L. birgei, L. rubida, Cylindrospermum michailovskoense, Nostoc commune, N. sphaericum, N. spongiaeforme, Aulosira fertilissima, A. prolifica, Scytonematopsis kashyapi, Scytonema cincinnatum, S. simplex, Calothrix braunii, C. brevissima* and *C. elenkinii.*

The cyanobacterial diversity of Kole and Pokkali fields were more or less same at the species level. The genera and species distribution in these two fields were respectively 65% and 59% at generic and 42% and 37% at species level (Figs. 29 & 30). In Sandy alluvial soil the generic and species distribution observed were 70% and 30%respectively (Figs. 31). In Kayal, Kari, and Karappadom fields the generic (53%) and species (20-23%) distribution was more or less uniform, but, less when compared to other fields (Table 1, Fig. 26 and Figs. 32-34).

Of the 111 Nostocalean spp. of cyanobacteria collected from the rice fields of Kerala, 57 species that spread over 6 genera were non-heterocystous and 54 species distributed among 11 genera were heterocystous. The dominant flora of the former category was *Oscillatoria* and that of heterocystous type was

Nostoc. It has been reported that non-heterocystous are the dominant forms in Indian rice fields (Anand, 1989). The findings of this study also agree with the previous reports (Fig. 35).

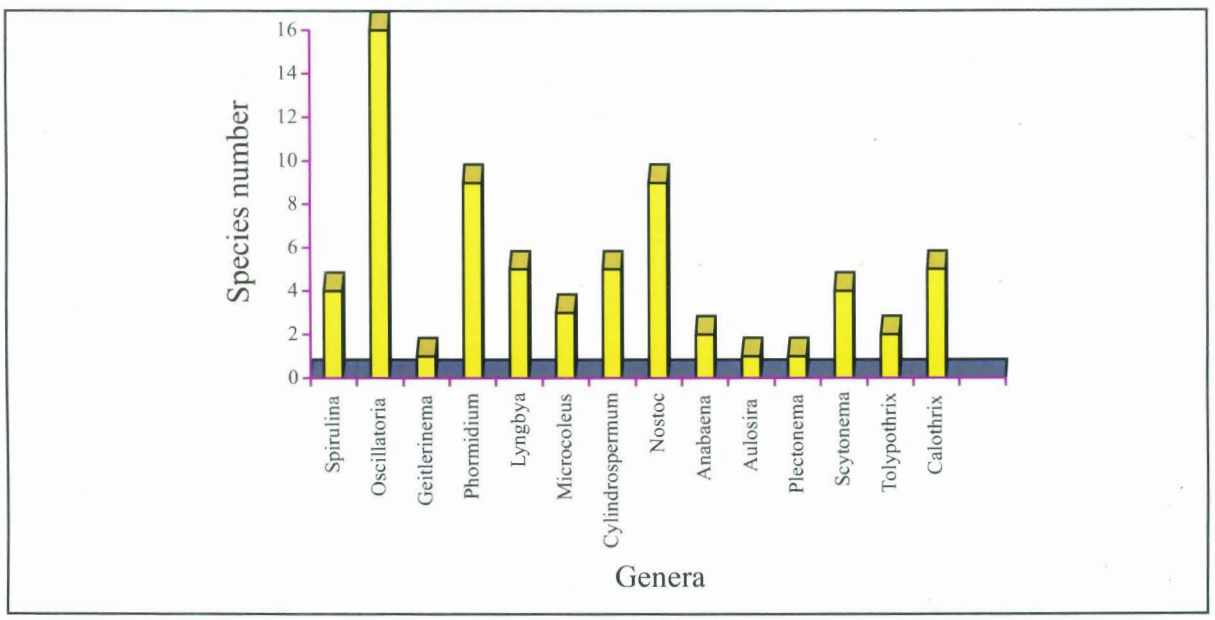
When the abundance of heterocystous and non-heterocystous cyanobacteria of individual soil types was taken into consideration except for Poonthalpadom and Karappadom fields all the other fields were found to be dominated by nonheterocystous cyanobacteria. In Poonthalpadom fields the distribution of hetero and non-heterocystous cyanobacteria were equal (50% each). In Karappadom soil type, the heterocystous cyanobacteria dominated over the non-heterocystous members.

In all the soil types, in the present study, the genus *Oscillatoria* remained to be the dominant non-heterocystous cyanobacterium. In heterocystous group the genus *Nostoc* represented the dominant cyanobacterium in all the fields but for Kole and Karappadom fields where the genera *Anabaena* and *Calothrix* dominated respectively (Table 1, Figs. 27-34).

Discussion:

The works on the cyanobacterial flora of Kerala dates back to 1940 with the work of Parukutty. Later works were that of Aiyer (1965) and Amma *et al.* (1966). But these reports were from a few fields with high acidic pH. The reports of Aiyer stated the occurrence of 19 spp. with ubiquitous distribution of *Aulosira fertilissima* throughout the acidic soils of Kuttanad. Anand and Hopper (1987) gave a preliminary report on 30 taxa of cyanobacterial flora representing various districts of Kerala. In 1995, they made a detailed survey of the cyanobacterial flora of Kerala comprising 158 taxa included under 33 genera. They observed a 5 fold increase in the cyanobacterial diversity than the preliminary findings. But Shaji and Panikkar (1994), Dominic (1997) recognized only less species. While Shaji and Panikkar observed 32 taxa belonging to 3 genera, Dominic after a complete survey on cyanobacterial flora of paddy fields of Kerala observed 92 spp. assigned to 31 genera. This type of diverse observations in the cyanobacterial abundance of rice fields of Kerala necessitates retrospection to the species concept of cyanobacteria.

Fig. 27. Cyanobacterial (Nostocales) diversity in the Laterite fields of Kerala



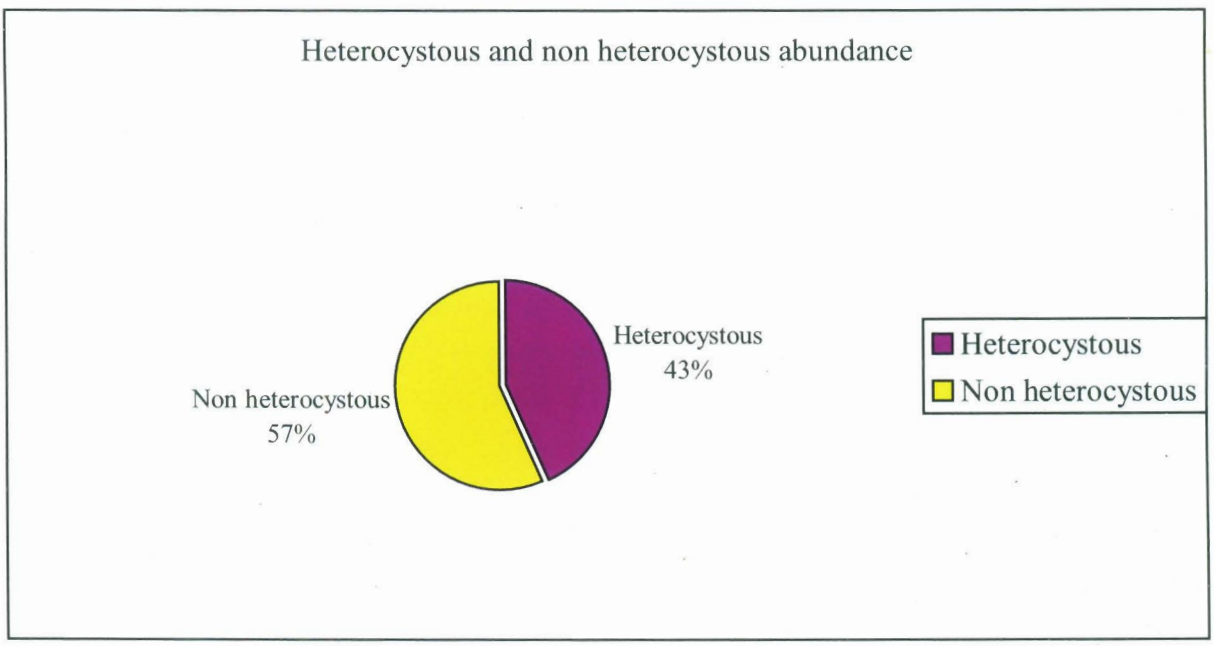
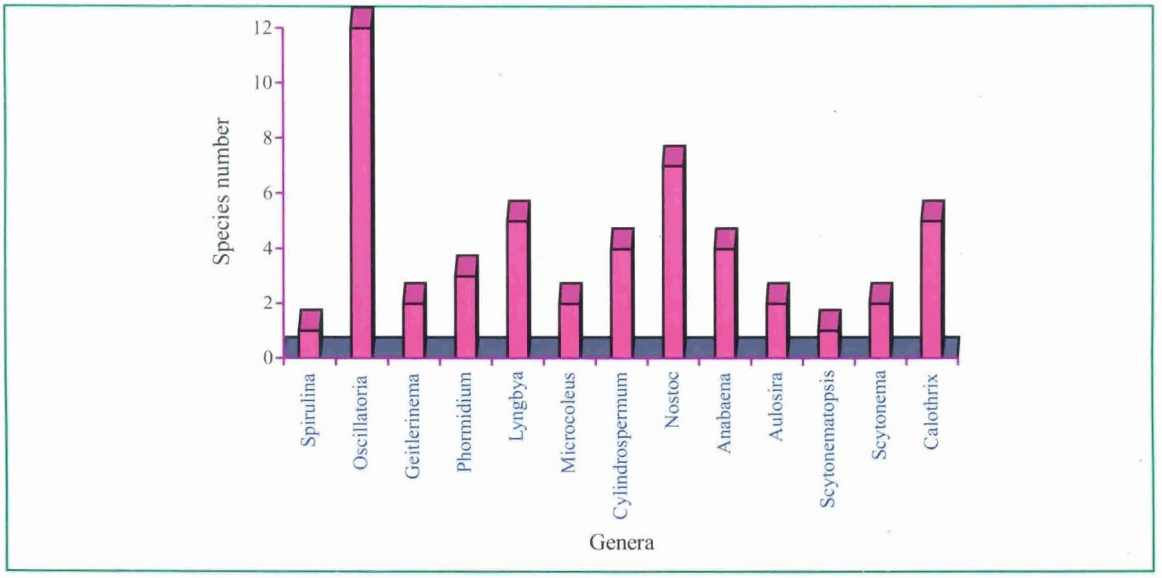


Fig. 28. Cyanobacterial (Nostocales) diversity in Poonthalpadom fields of Kerala



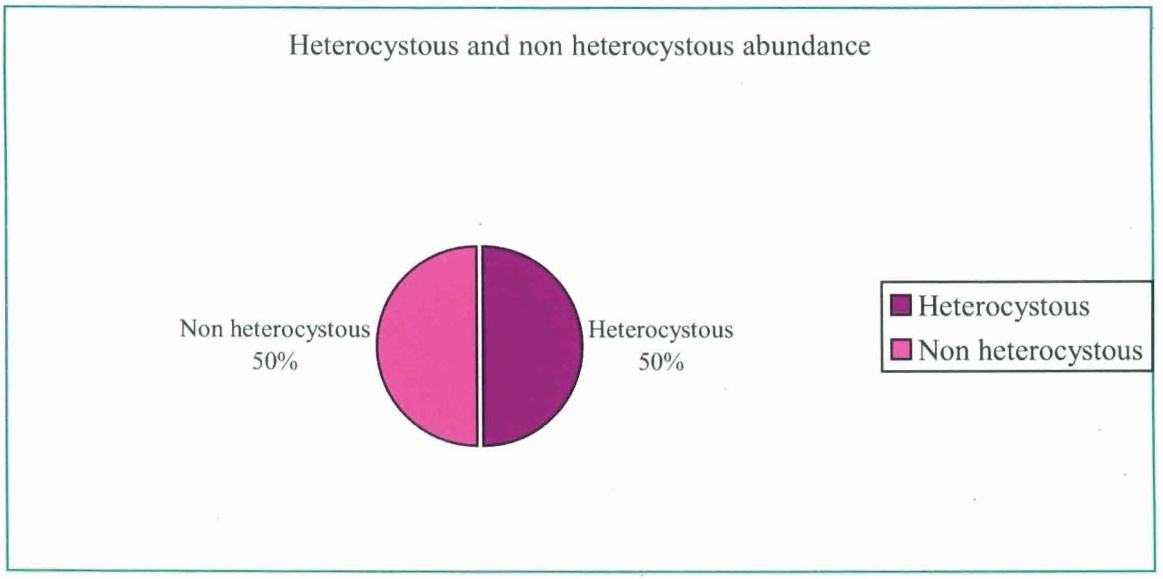
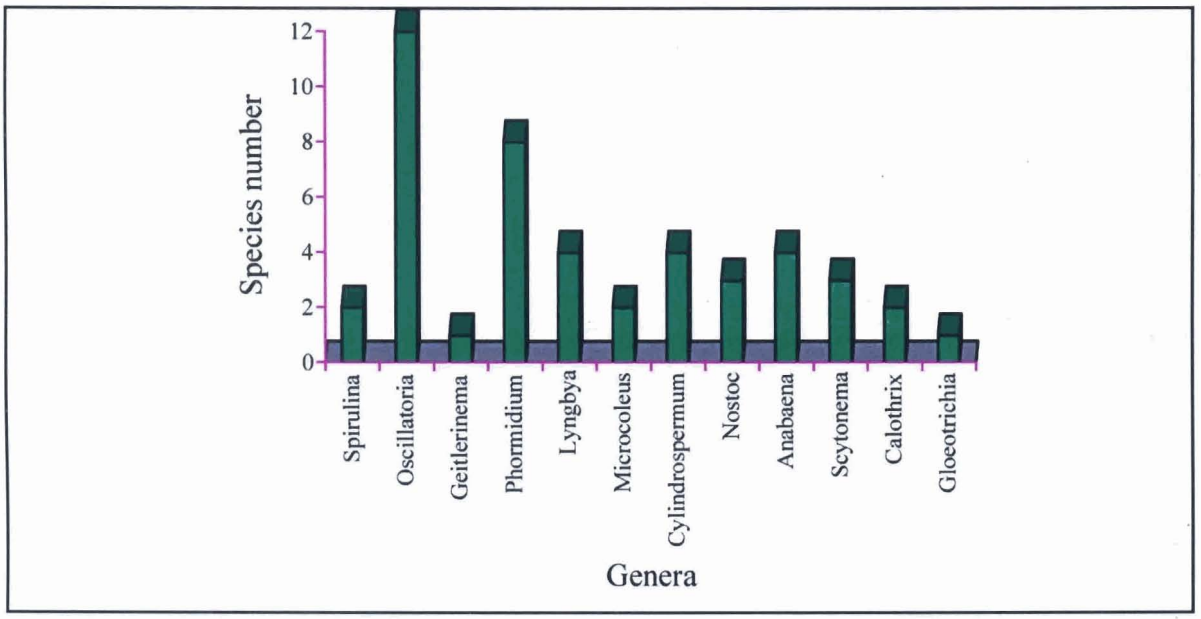


Fig. 29. Cyanobacterial (Nostocales) diversity in the Kole fields of Kerala



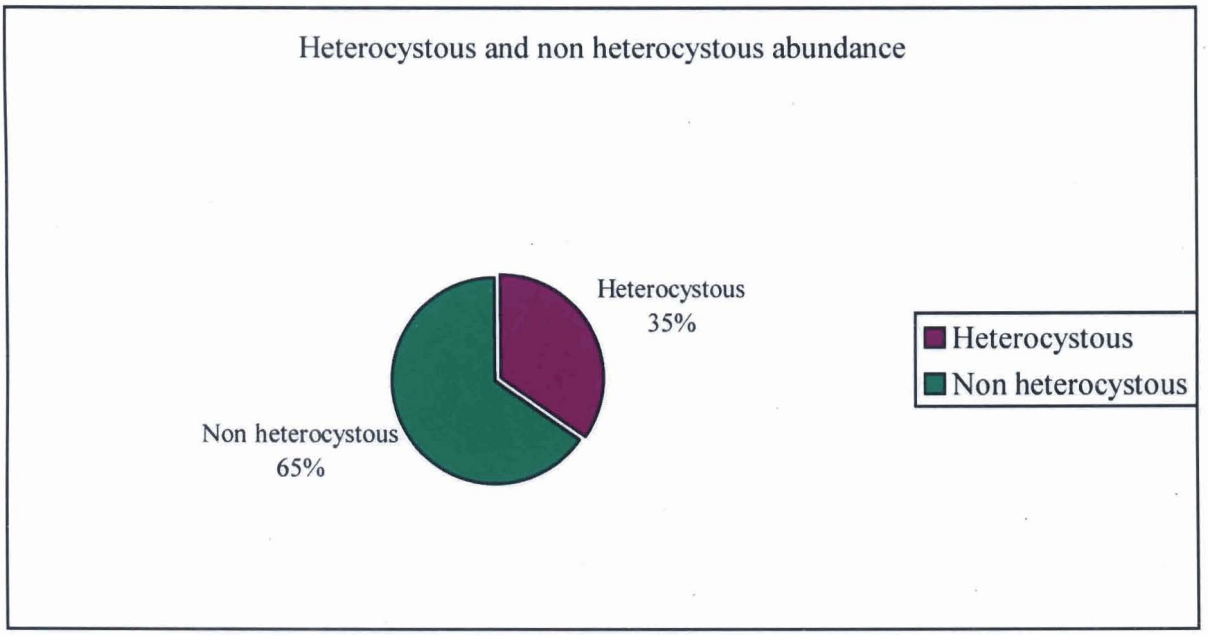
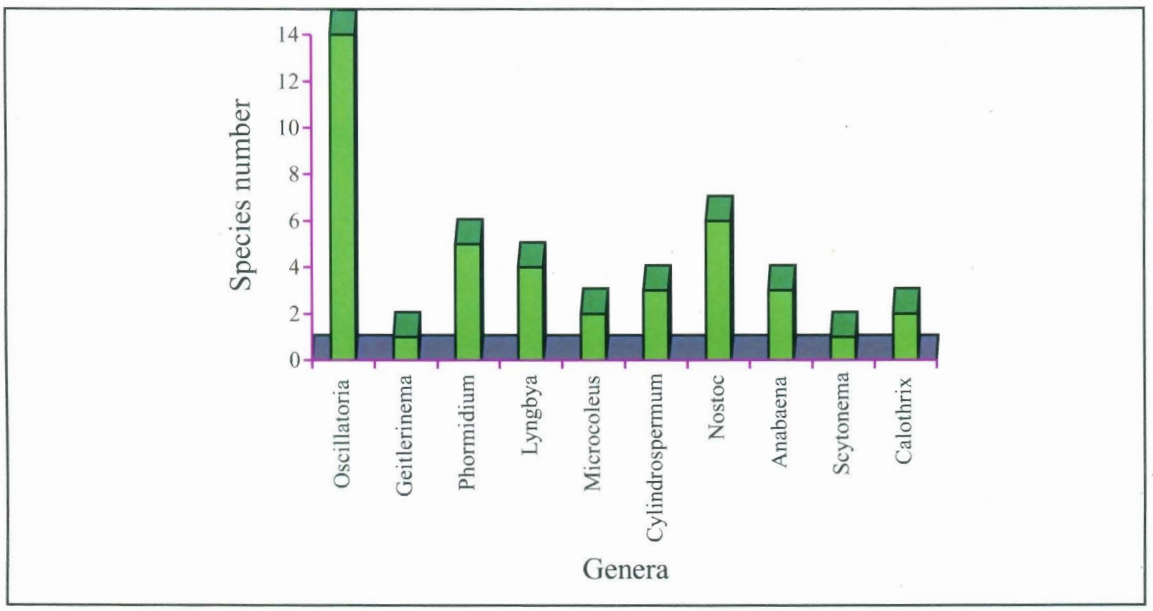


Fig. 30. Cyanobacterial (Nostocales) diversity in the Pokkali fields of Kerala



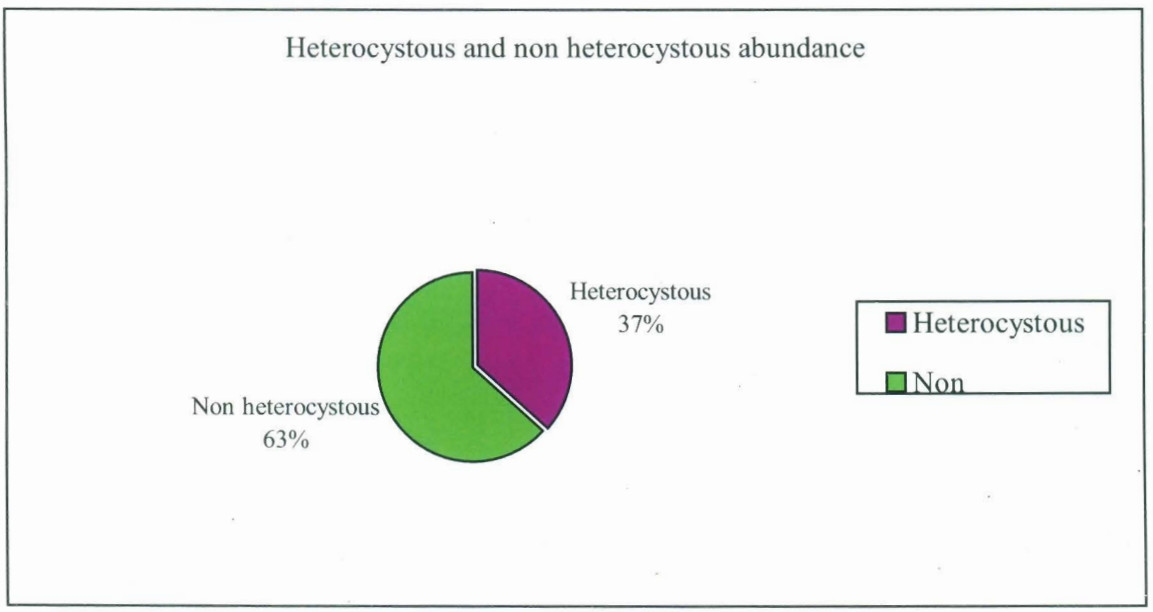
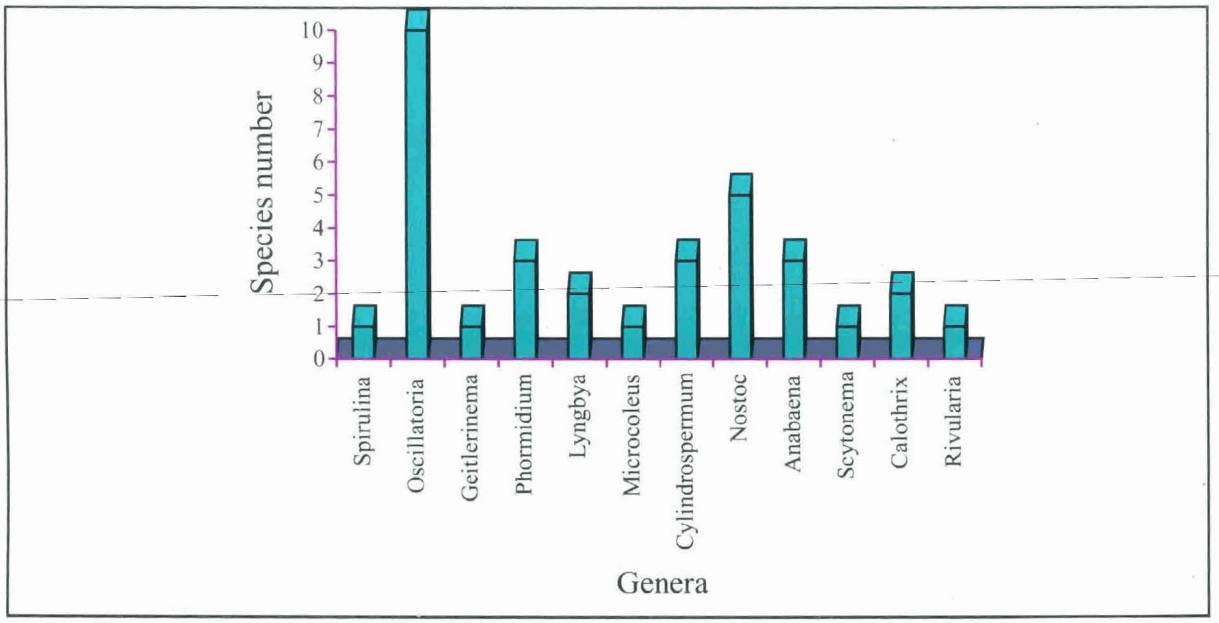


Fig. 31 Cyanobacterial (Nostocales) diversity in the Sandy Alluvium fields of Kerala



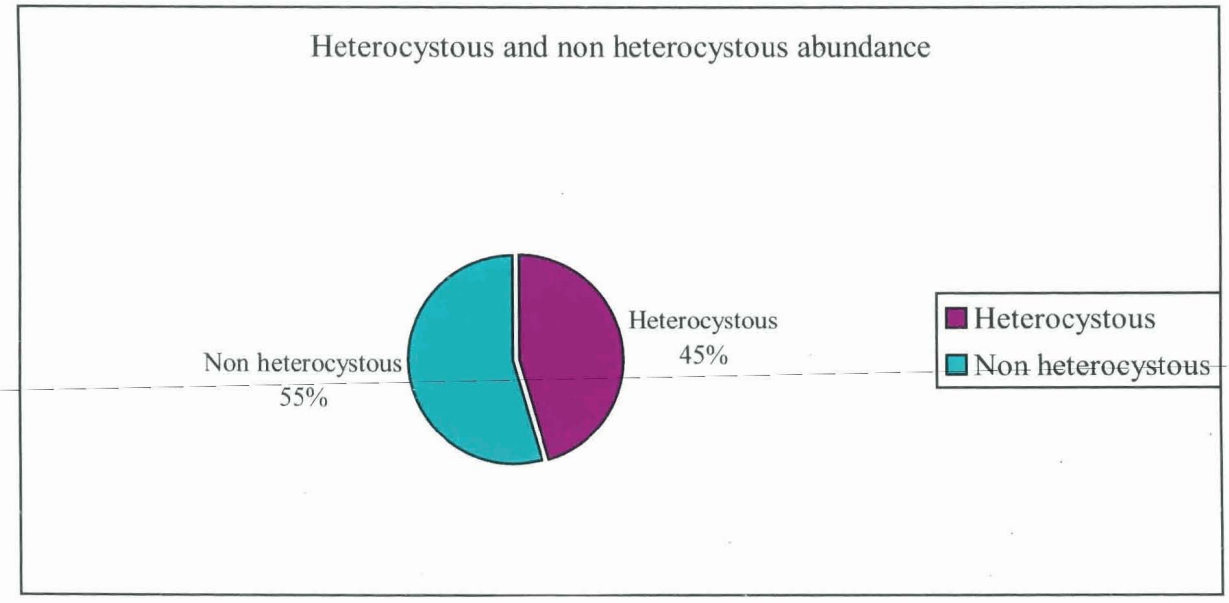
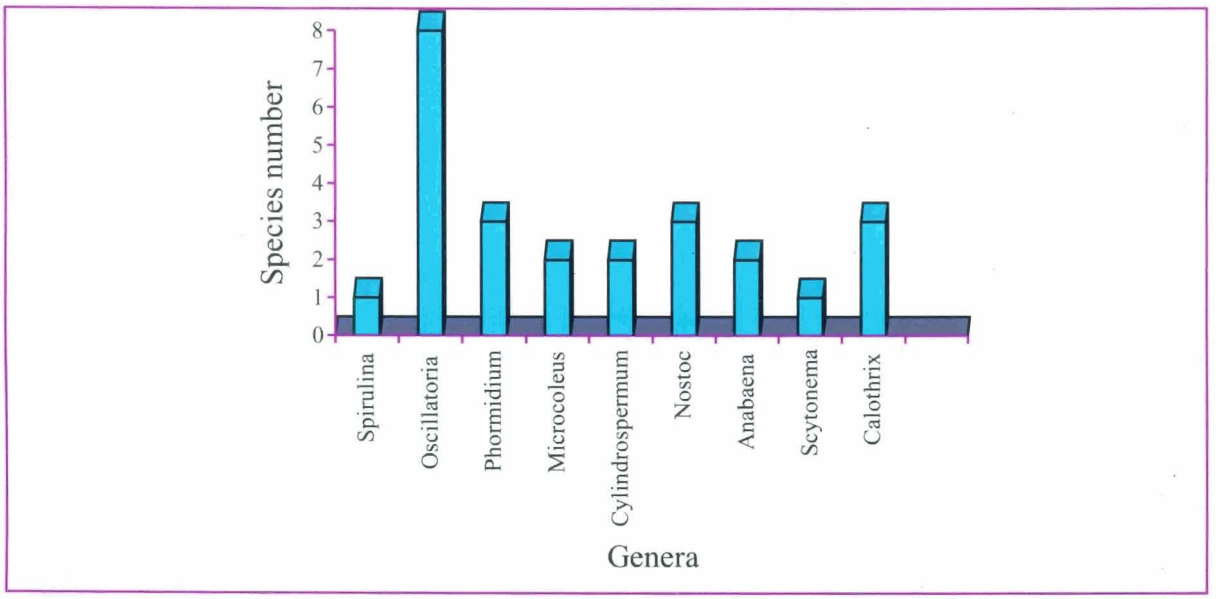


Fig. 32. Cyanobacterial (Nostocales) diversity in the Kayal fields of Kerala



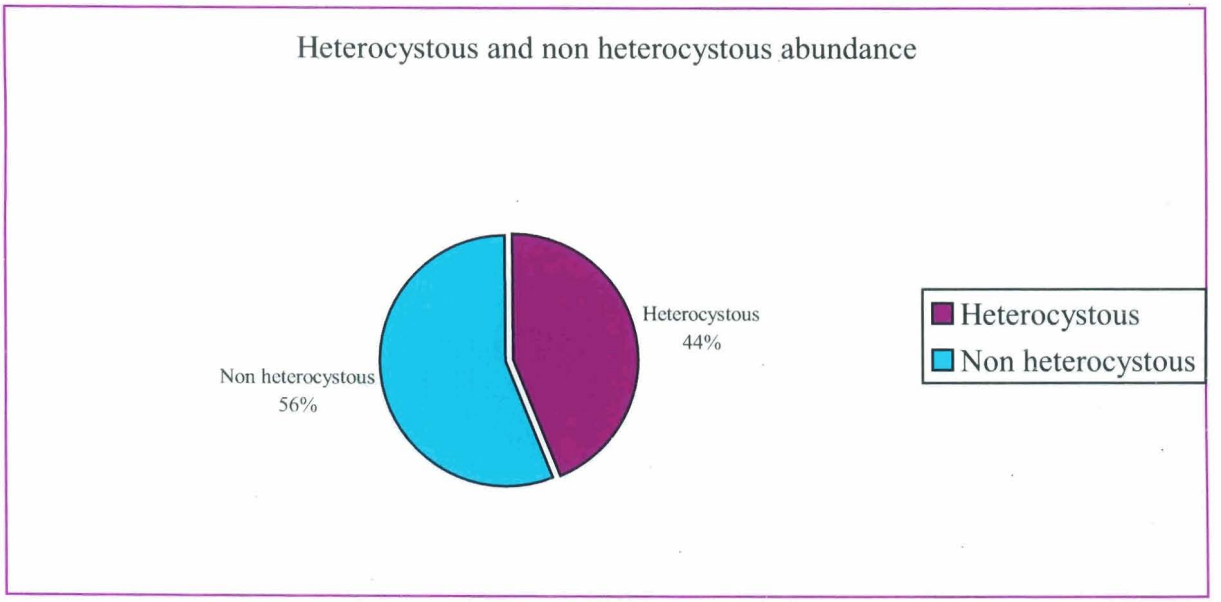
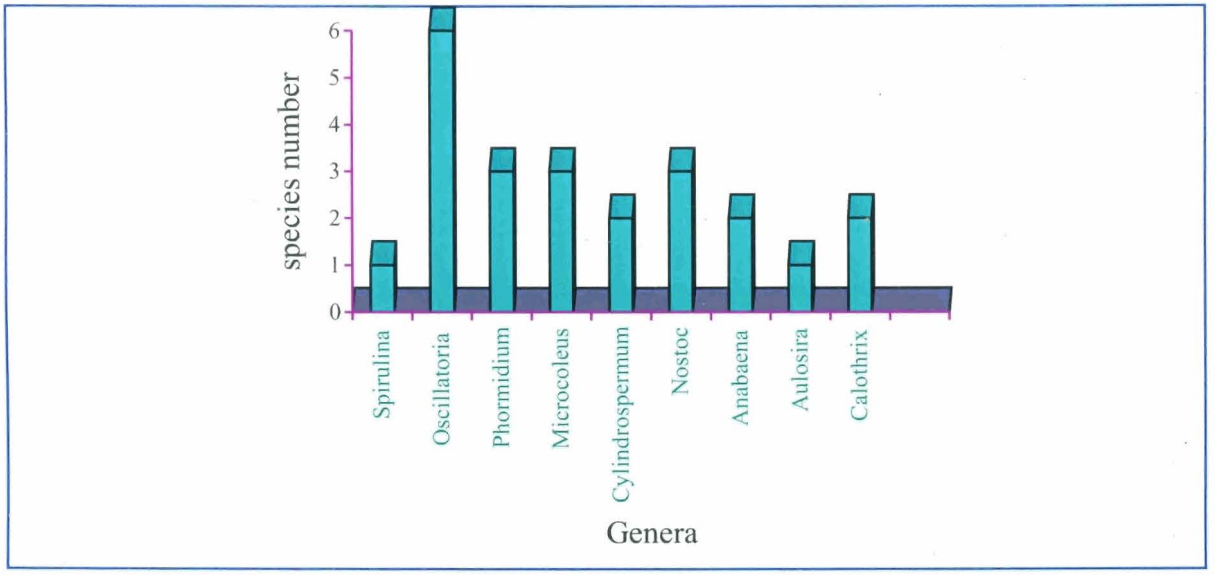


Fig. 33. Cyanobacterial (Nostocales) diversity in the Kari fields of Kerala



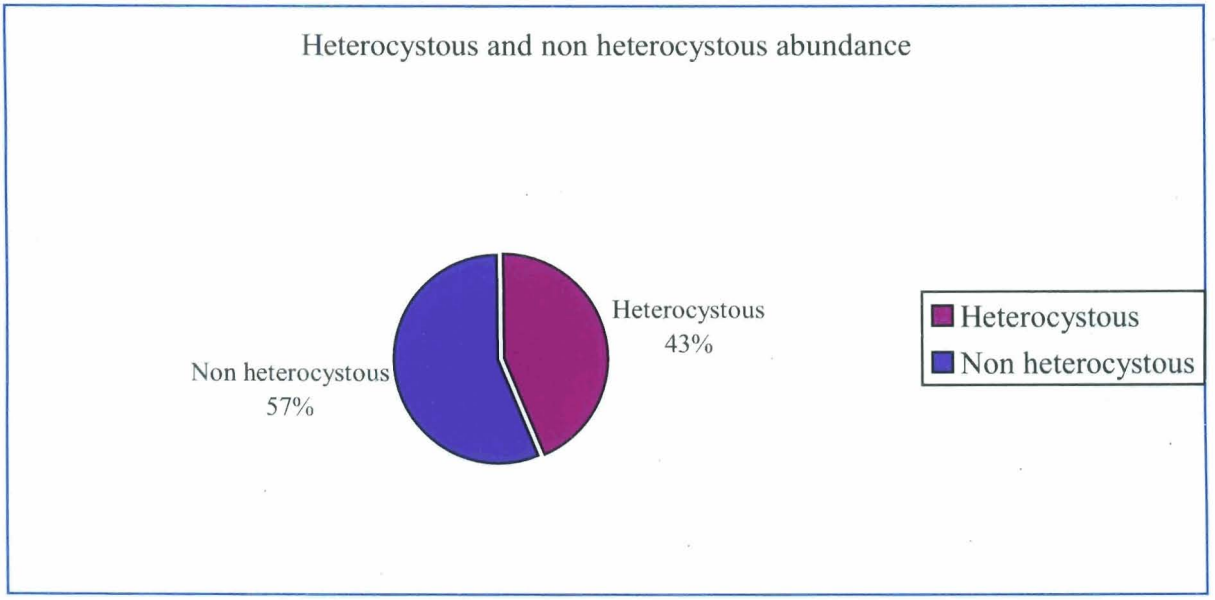
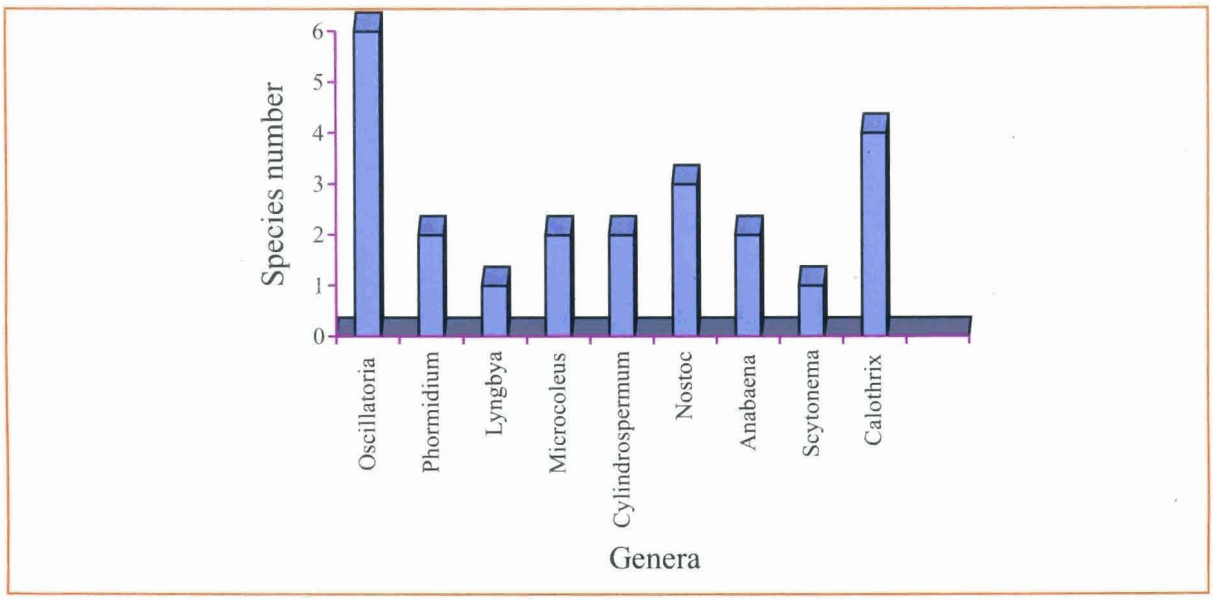


Fig. 34. Cyanobacterial (Nostocales) diversity in the Karappadom fields of Kerala



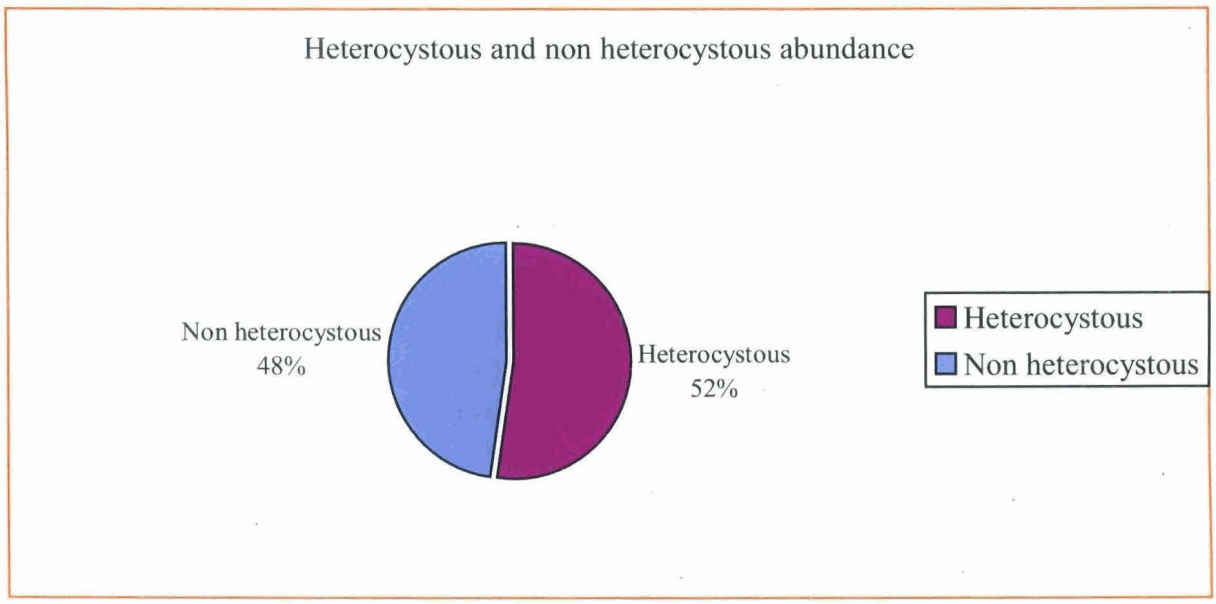
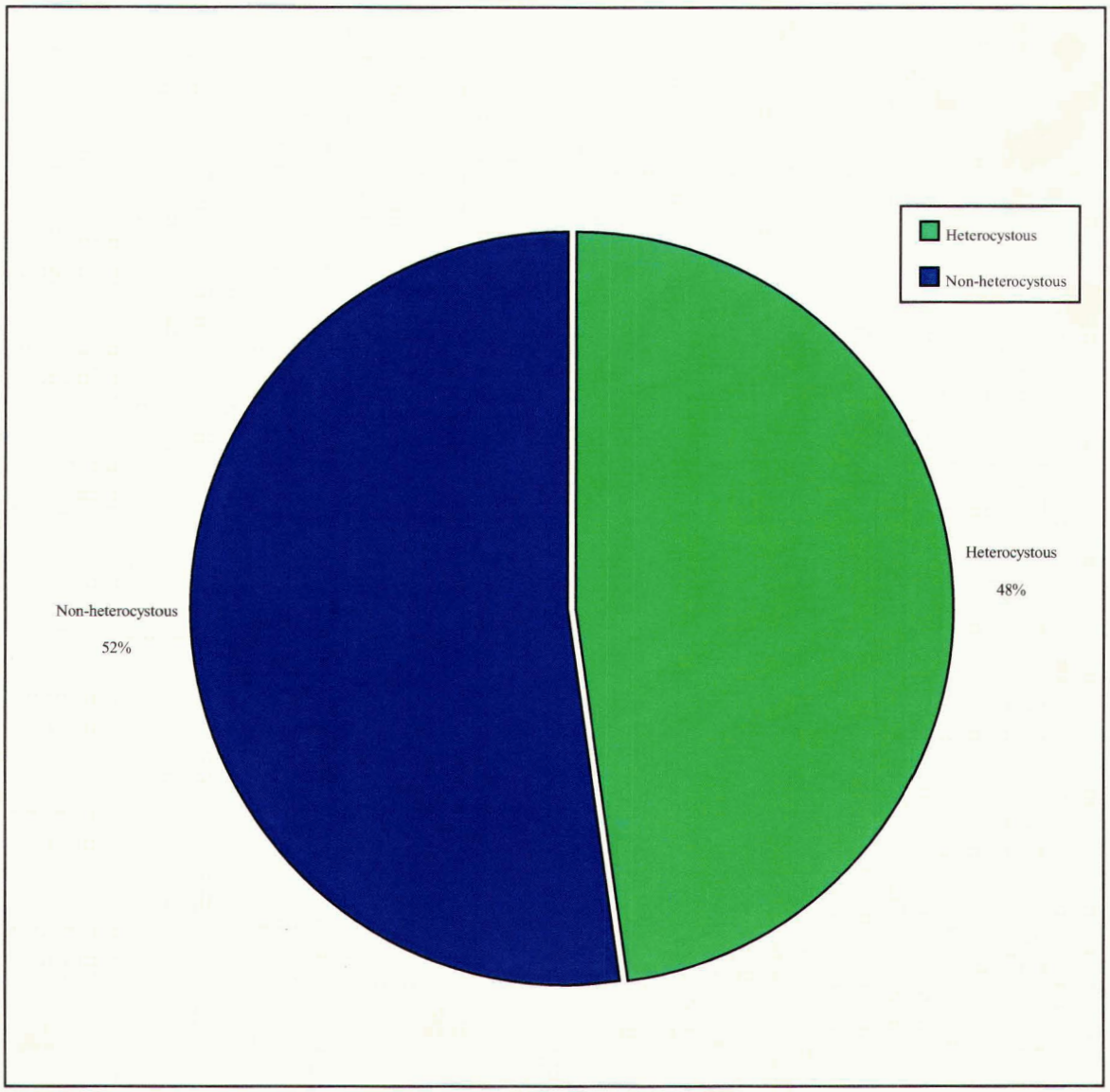


Fig. 35. Heterocystous and non-heterocystous cyanobacterial (Nostocales) abundance in the rice fields of Kerala



The concept of species as far as cyanobacteria are concerned is a controversial subject (South and Whittick, 1987). Rippka and Cohen-Bazire (1983) and Anand (1989) critically analyzed the cyanobacterial taxonomy and commented that the present systems of identification of cyanobacteria at species level are often leading to confusion. Roger (1991) opined that the existing classification of cyanobacteria is inadequate to delimit the species and hence a field worker on cyanobacteria has no proper literature to identify the taxa. More over, the simple organization of the thallus of cyanobacterial members, except for the presence of certain unique structures like heterocyst and akinete, based on which the cyanobacterial classification has been designed is basically acting as a limiting factor in cyanobacterial taxonomy. The classic works on cyanobacterial taxonomy by Geitler (1932), Desikachary (1959), Starmach (1966), Anand (1989) are the compilations of many a new species erected by different workers on trivial grounds. Hence taxonomic work in cyanobacteria has become a problematical and frustrating task resulting in doubtful and uncertain identification.

Recent sophisticated and reliable techniques like chromosome and RAPD analysis, *etc.* help in the identification of cyanobacterial species. But, majority of the institutions have no facility for this and such techniques are quite expensive. Moreover, those who prefer conventional methods attempt to identify species based on morphological and sometimes, physiological characters. Since cyanobacteria are very simple in organization morphological characters especially thallus nature and sometimes position, size, shape and number of heterocyst and akinete if any, alone can be considered for taxonomic characterization.

The present study reveals that a wide range of cyanobacterial diversity exists within the order Nostocales in the rice fields of Kerala. Reports of Anand and Hopper (1987, 1995) and Dominic (1997) revealed the wide range of the diversity. The diversity at generic level is more easily distinguishable than at species level. This is because the characters at higher ranks are well demarcated, but as it goes down to the species level the delimitation is very feeble and rather impractical due to the lack of precise qualitative characters. In this study the species were delimited only when conspicuous morphological

characters were found. Accordingly only 111 species could be recognized in the present study.

Earlier, studies were made considering the cyanobacterial flora wholly. But in the present study a special stress has been given to the order Nostocales of cyanobacteria. When one goes through the cyanobacterial flora of paddy field of various states, it will be very clear that the members of Nostocalean order dominates more than any other orders of cyanobacteria in the paddy fields of each state (Table 2). They comprise both heterocystous and non-heterocystous types- unbranched or pseudo branched. Instead of studying repeatedly on the taxonomy of whole cyanobacterial flora of paddy field, emphasis has been made in the present study for the taxonomic revision of the order Nostocales. Since cyanobacteria are prokaryotes simple in organization, in addition to their morphological features based on field study, their characters in cultures have also been taken into account. The characterization of these organisms in cultures helps the researchers to suggest a natural classification. They also help to define a new species (Hoffmann, 1988) and allow to characterize a strain by a variety of eco-physiological or biochemical characters (Stanier *et al.*, 1971; Rippka *et al.*, 1979). Anagnostdis and Komarek (1985) suggest and portrait the future cyanobacterial taxonomy, "as a blend of classical botanical taste with molecular interference using herbarium specimens of cyanobacterial populations in both nature and culture". The result obtained from the present study can be useful to assess and identify the various taxa of cyanobacteria (Nostocales) in a classical pattern till, a suitable, better, readily identifiable natural classification takes its origin, that could be a boon for every researchers in the field of cyanobacterial taxonomy.

Table 2: Nostocalean status in the paddy fields and associated areas of some states of India.

Sl. No.	State	Author	Year	Total number of Cyanobacterial species	Species under Nostocales
1.	Assam	Saikia and Bardoloi	1994	28	26
2.	Arunachal Pradesh	Singh *et al.*	1997	83	62
3.	Karnataka	Singh and Bongale	1990	45	38
4.	Kerala	Shaji and Panikkar Anand and Hopper Dominic	1994 1995 1997	32 158 92	29 122 75
5.	Madhya Pradesh	Sangita and Naik	1996	32	28
6.	Mizorum	Singh *et al.*	1996	48	26
7.	Nagaland	Singh *et al.*	1997	64	34
8.	Orissa	Sahu *et al.*	1996	143	133
9.	Tamil Nadu	Anand and Revathi	1987	35	28
10.	Tripura	Singh *et al.*	1997	79	46

CHARACTERIZATION UNDER LABORATORY CONDITIONS

Selection of growth media:

In order to study the response of cyanobacteria to various nutrient media and to choose a common and suitable culture medium for further experimentation, ten unicyanobacterial species *viz.*, *Nostoc linckia* (CU 81305), *Nostoc carneum f. minor* (CU 81337), *Nostoc muscorum* (CU 81341), *Nostoc spongiaeforme* (CU 81329), *Cylindrospermum muscicola* (CU 81522), *Calothrix brevissima* (CU 81575), *Calothrix elenkinii* (CU 81464), *Calothrix fusca* (CU 81411), *Calothrix marchica* var. *intermedia* (CU 81327) and *Scytonema stuposum* (CU 81315) were grown in BG11 and Fogg's media (nitrogen free) for a period of 20 days. Their growth performance in terms of absorbance at 760 nm and chlorophyll *a* content was compared during the period.

The study showed that all the species grew well in both the media. But, it was also found that except *Cylindrospermum muscicola* and *Calothrix marchica* var. *intermedia* all the other spp. preferred BG-11 N_2 free medium to Fogg's medium for their growth. *Cylindrospermum muscicola* showed a better result in Fogg's medium and *Calothrix marchica* var. *intermedia* exhibited equal growth rate in both media (Table 3).

Discussion:

Analysis of the above findings showed that various species of cyanobacteria require varying types of nutrient media for the efficient growth. The media composition significantly influenced the growth and chlorophyll *a* content of all the above ten cyanobacterial species. Except *Cylindrospermum muscicola,* all the other species grew well in BG-11 (N_2 free) medium. Preference for BG-11 (N_2 free) medium by majority of the cyanobacterial species in the experiment might be due to the presence of appropriate nutrient elements in the medium in right proportion. Though growth and chlorophyll *a* content were more in BG-11 (N_2 free) medium, there was not much difference in growth rates in either media and this might be due to the N_2 free nature of the either media.

Table 3: Growth of cyanobacterial species in different culture media.

Cyanobacterial Species	Collection number	Growth in Absorbance (O.D at 760nm)		Chlorophyll *a* content (µg/ml)	
		BG -11 medium	Fogg's medium	BG -11 medium	Fogg's medium
Nostoc linckia	CU 81305	0.60±0.08	0.58±0.05	8.95±0.28	8.70±0.19
Nostoc muscorum	CU 81341	0.59±0.05	0.56±0.04	8.85±0.21	8.55±0.14
Nostoc carneum f. *minor*	CU 81337	0.57±0.04	0.545±0.01	8.70±0.18	8.35±0.16
Nostoc spongiaeforme	CU 81329	0.58±0.05	0.57±0.03	8.87±0.19	8.74±0.18
Cylindrospermum muscicola	CU 81522	0.58±0.04	0.59±0.06	8.82±0.20	8.9±0.25
Calothrix brevissima	CU 81575	0.53±0.05	0.52±0.05	8.55±0.28	8.25±0.20
Calothrix elenkinii	CU 81464	0.54±0.04	0.53±0.02	8.59±0.17	8.47±0.09
Calothrix fusca	CU 81411	0.55±0.01	0.535±0.04	8.65±0.19	8.5±15
Calothrix marchica var.*intermedia*	CU 81327	0.57±0.06	0.57±0.05	8.93±0.24	8.95±0.21
Scytonema stuposum	CU 81315	0.52±0.02	0.51±0.04	8.25±0.27	8.04±0.23

BG-11 and Fogg's culture media free of combined inorganic nitrogen source were used. Cultures grown under culture conditions for 20 days were evaluated for growth in terms of Absorbance at 760nm and chlorophyll *a* content.

Response of cyanobacterial species to various growth media has been studied earlier (Sureshbabu and Kannaiyan, 1998; Meenakshee, 2001). In order to study the quality and nature of establishment of cyanobacterial inoculum that gives the desired response at field level, knowledge on their response to various factors at laboratory conditions is essential. So they have to be cultured in suitable growth medium that aids their growth and establishment.

Growth and thallus characteristics of cyanobacterial species in culture medium (BG 11 (N_2 free) - both liquid and solid):

The time taken for colony initiation and spreading of colonies in the agar plates in all the above ten cyanobacterial species in BG11 (nitrogen free) medium was compared. Except *Calothrix and Scytonema* spp. all the other species grew at a faster rate in the medium. Visible colony initiation in *Nostoc* spp. and *Cylindrospermum muscicola* were noticed 6-8 days after inoculation. The *Nostoc* species filled the agar plates with their colonies within 30 days after colony initiation. But in *Cylindrospermum muscicola* the spreading time of colonies in the agar plates was between 30-35 days. Here the growth after formation of colonies was slow and steady when compared to *Nostoc* spp. *Calothrix* and *Scytonema* spp. took 1-1½ weeks more for colony initiation when compared to *Nostoc* spp. The visible colony initiation appeared 12-16 days after inoculation in these spp. and about 40-55 days were needed for their complete growth in the agar plate. Colony initiation and growth were very slow in *Scytonema* spp. when compared to all the cyanobacterial species considered in the experiment.

The thallus characteristics of all the above cyanobacterial species in both liquid and solid medium were analysed (Table 4). The colour of the thallus varied from species to species. The thalli of *Nostoc* spp. and *Cylindrospermum muscicola* appeared blue-green except for *Nostoc carneum* and *N. spongiaeforme*, which were brown to brownish green, while that of *Calothrix and Scytonema* spp, were brown, blue-green / brownish green.

In liquid medium, all the species were growing in a luxuriant manner with different growth nature (table 4). The thallus of most of the species especially that of *Calothrix and Scytonema*, showed a great tendency to stick on to the wall of the container and such a tendency was least exhibited by *Cylindrospermum muscicola.* Some species changed the pH and colour of the medium as their growth progressed.

Table 4: Culture characteristics of different cyanobacterial species in BG 11 (nitrogen free) Culture medium –both solid and liquid

Sl. No	Cyanobacterial species	Collection number	Time taken for the following processes after inoculation in petripates (solid medium) (In days)		Day of spore Initiation, nature, position, shape, *etc.*	Thallus characteristics in both solid and liquid media
			Visible colony initiation	Complete spreading on the agar plate		
1.	*Nostoc linckia*	CU 81305	6-8	30	20-22, in chains, between heterocysts, oval.	Solid medium: Blue-green, colony globose, soon irregular and spreading the agar surface. Liquid medium: Dense blue green masses, turning the medium brown at later stages. Seen sticking to the wall of the container.
2.	*Nostoc carneum* f. *minor*	CU 81337	6-8	30	20-22, in chains between heterocysts, cylindrical	Solid medium: Brown, globose, localized masses spreading later. Liquid medium: Brown aggregates which turn the medium also yellowish brown.
3.	*Nostoc muscorum*	CU 81341	6-8	30	20-22, in chains between heterocysts, cylindrical	Solid medium: Olive-green, globose colony, soon spreading the entire agar plate. Liquid medium: Dense aggregates of olive green filaments.

Table 4: (ctd..)

Sl. No.	Cyanobacterial species	Collection number	Time taken for the following processes after inoculation in petripates (solid medium) (In days)		Day of spore Initiation, nature, position, shape, *etc.*	Thallus characteristics in both solid and liquid media
			Visible colony initiation	Complete spreading on the agar plate		
4.	*Nostoc spongiaeforme*	CU 81329	6-8	30	20-22, in chains, between heterocysts, cylindrical	Solid medium: Brownish green, globose colonies spreading soon. Liquid medium: Mucilaginous brownish green masses. Medium changed to light brown.
5.	*Cylindrospermum muscicola*	CU81522	6-8	30-35	25-58, single, large, ellipsoidal, next to terminal heterocyst	Solid medium: Blue-green, ring like colonies, later coalesce. Liquid medium: Pale blue green masses, floating and suspended in the medium.
6.	*Calothrix brevissima*	CU 81575	12-14	40-45	" "	Solid medium: Light brown, thin, membraneous rings that coalesce later. Liquid medium: Seen as a feeble pale brown aggregates suspended in the medium.

Table 4: (ctd..)

Sl. No.	Cyanobacterial species	Collection number	Time taken for the following processes after inoculation in petripates (solid medium) (In days)		Day of spore Initiation, nature, position, shape, *etc.*	Thallus characteristics in both solid and liquid media
7.	*Calothrix elenkinii*	CU 81464	12-14	40-45	" "	Solid medium: Pale brown, large ring like colonies, later coalesce. Liquid medium: Appearing as brown tufts in and on the surface of the medium. Medium changed to pale brown gradually.
8.	*Calothrix fusca*	CU 81411	12-14	40-45	" "	Solid medium: Yellowish to pale brownish green cushion like rings, coalesce and spreading soon. Liquid medium: Dense masses of pale Yellowish green filaments, turns the medium pale yellow.
9.	*Calothrix marchica* var. *intermedia*	CU 81327	12-14	40-45	No sporulation	Solid medium: Dark brown, tough, large ring like colonies, later coalesces. Liquid medium: Brown aggregates of luxuriant growth, turns the medium pale brown.
10.	*Scytonema stuposum*	CU 81315	14-16	Beyond 45 days	" "	Solid medium: greenish brown, wooly, rings, becoming large, slowly coalesce. Liquid medium: Greenish brown, wooly, tomentose, expanded masses, sticking to the wall of the container later.

In solid medium, *Nostoc* spp. formed small globose colonies initially, which coalesced and sprawled the entire dish in due course. *Cylindrospermum muscicola* and spp. of *Calothrix and Scytonema* formed circular rings of colonies, which gradually became bigger and finally coalesced. The thallus was very soft, flimsy and shiny in *Cylindrospermum muscicola*; smooth, mucilaginous and shiny in *Nostoc* spp.; rough and thick in *Calothrix* spp. and wooly/cushion-like in *Scytonema stuposum*. Thus varying culture characteristics were found exhibited by different types of cyanobacterial species.

Discussion:

The time taken for colony initiation and spreading of colonies in the agar plate in all the above ten cyanobacterial species in the medium was different. The different species grew differently, though the medium was quite favourable for their growth. The different growth timing might be attributable to the time needed for the initial establishment of each strain in the medium in which they grow and their pattern of growth. The study on thallus characteristics in culture medum may aid identification of the taxa, by noting and comparing their nature (thallus structure) in fields and cultures to certain extent (Anand, 1988).

Based on the present findings, the cyanobacterial species can be categorized into two groups–the fast growing species like *Nostoc* and *Cylindrospermum,* the slow growing like *Calothrix* and *Scytonema* (Naik, 2000). The fast growing species were found producing large number of spores whereas the slow growing the hormogones. The tendency to stick to the wall of the container by *Calothrix* spp. and other members suggests their epiphytic habit under field conditions (Kulasooriya *et al.*, 1980; Dominic, 1997). The gregarious growth nature of cyanobacteria makes them an excellent biofertilizer in paddy fields.

Further Characterizations:

Of the above ten nitrogen fixing cyanobacterial species considered for the study of culture characteristics in the BG11 (nitrogen free) medium, three species, which are very frequent and easily cultivable *viz.*, *Nostoc linckia* (CU 81305), *Nostoc muscorum* (CU 81341) and *Calothrix marchica* var. *intermedia* (CU 81327) were selected for studying their response to pH, salinity, nutritional

habits and nutritional preference, pesticide, insecticide and fertilizers. These studies are considered essential in order to explore their suitability in the biofertilizer technology.

pH tolerance:

Cyanobacterial species Nostoc linckia (CU 81305), Nostoc muscorum (CU 81341) and *Calothrix marchica* var. *intermedia* (CU 81327) were cultured in aseptic conditions in BG11 (N_2 free) medium (Rippka *et al.*, 1979). 1ml of the homogenized suspension of each of the cyanobacterial species at exponential growth period was introduced in tubes containing 10 ml of nitrogen free BG11 medium amended with different pH levels (4.0, 5.0, 6.0, 7.0, 7.4 (control), 8.0, 9.0). The cultures were incubated at 26 ±2° C under 14/10 hrs L/D cycle with an illumination of 2000-3000 lux light in the culture room for a period of 25 days. Growth was determined by measuring the absorbance of homogenized suspension at 760 nm every 5 days. Chlorophyll a (McKinney, 1941) and Protein estimation (Lowry *et al.*, 1951) were also done.

Of the three different species considered for the experiment, Nostoc linckia recorded maximum growth and high chlorophyll a content, protein content and heterocysts frequency in the alkaline pH (8.0). However, it tolerated a wider pH range also. Two peaks were noticed in the graph in this species, one in acidic pH and the other in the alkaline pH. Nostoc muscorum preferred an alkaline pH range to acidic for enhanced growth. The growth in terms of chlorophyll a and protein content were systematically increasing from acidic to alkaline range and the cyanobacterium recorded its maximum growth and heterocysts frequency at pH 9.0. Calothrx marchica, on the other hand, exhibited maximum growth rate at acidic to neutral pH (from 6.0 to 7.0) after 7.4, the growth activity was found to be declining (Figs. 36, 37,38 & table 5).

Fig. 36. pH tolerance
Growth in terms of Absorbance at 760nm

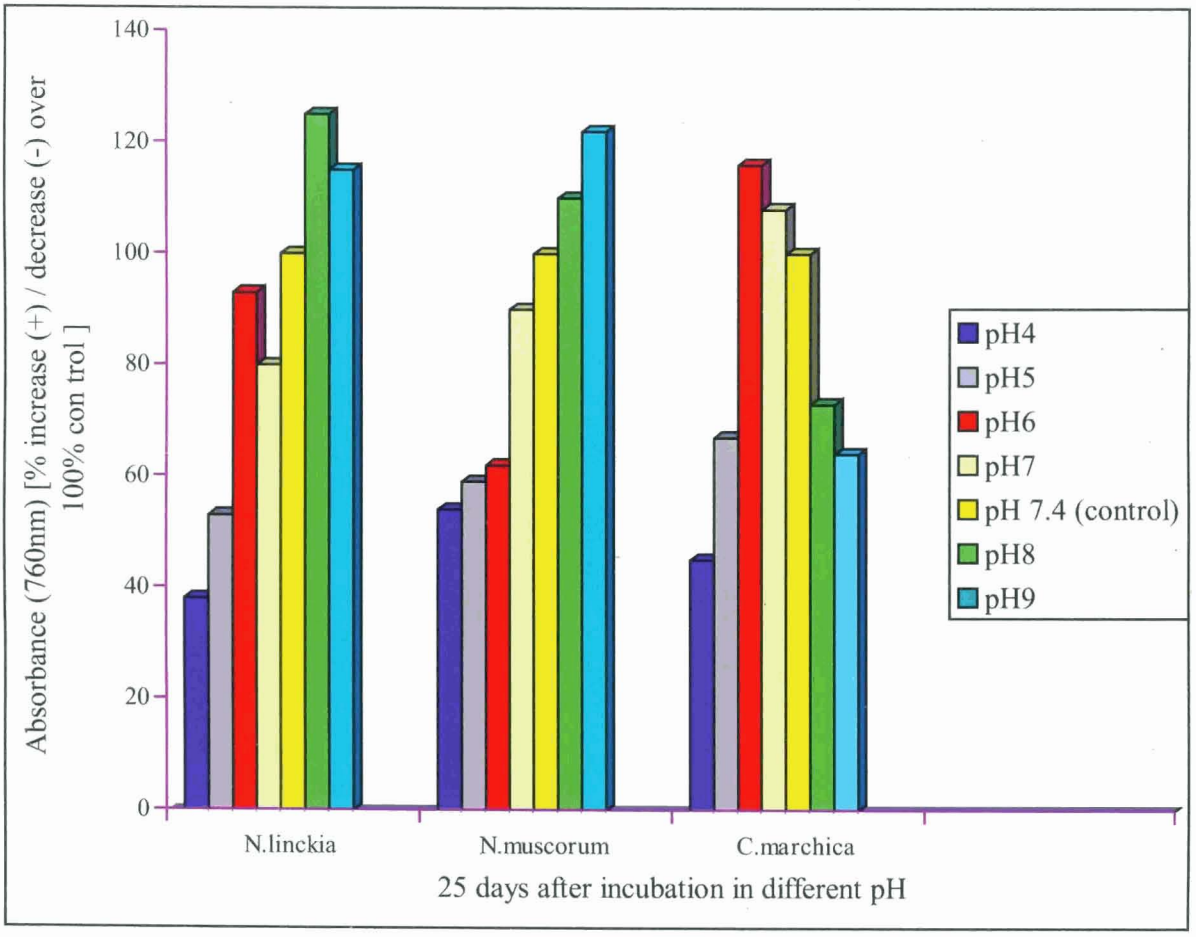


Fig. 37. pH tolerance
Growth in terms of Chlorophyll a content

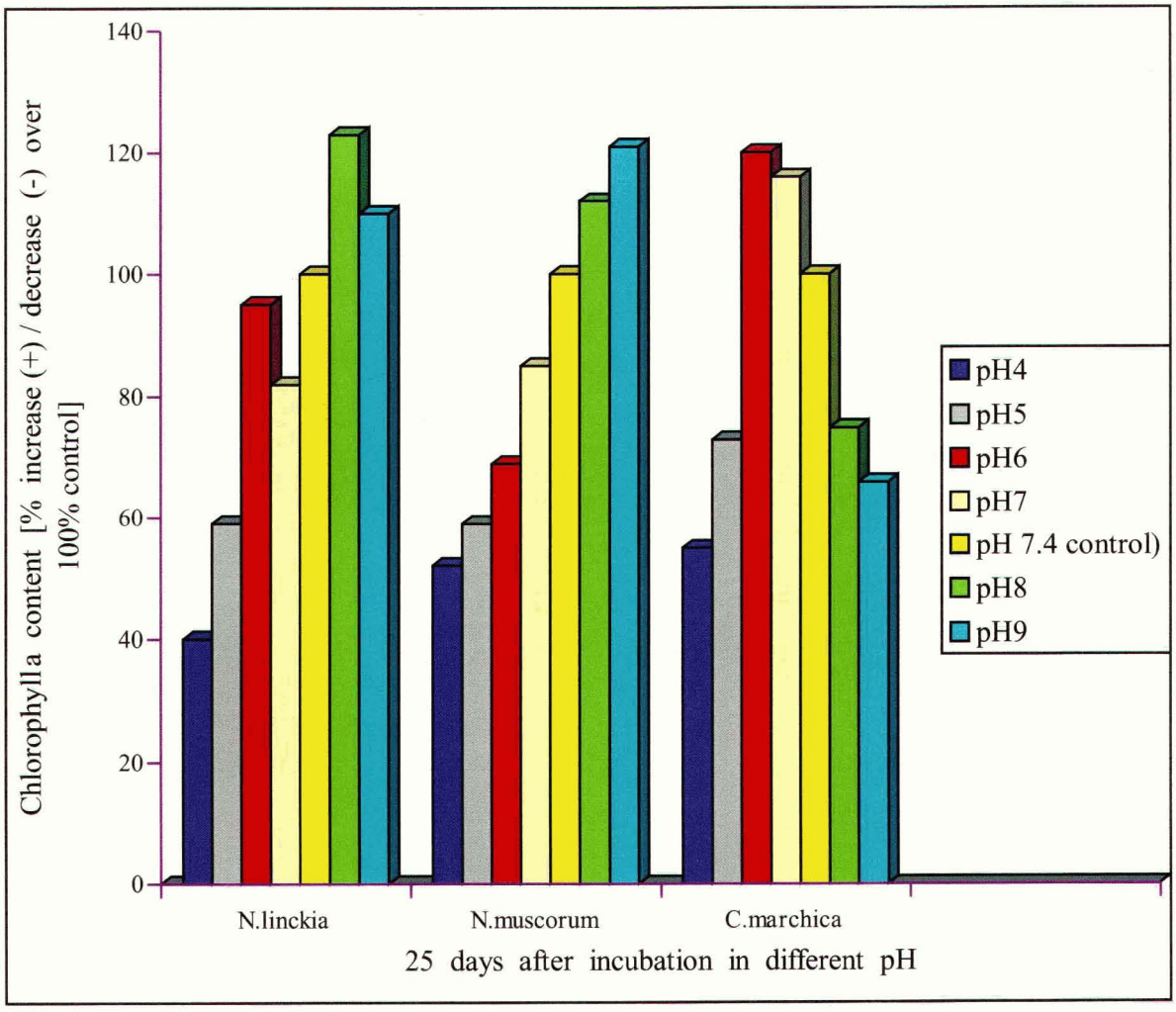


Fig. 38. pH tolerance
Growth in terms of Protein content

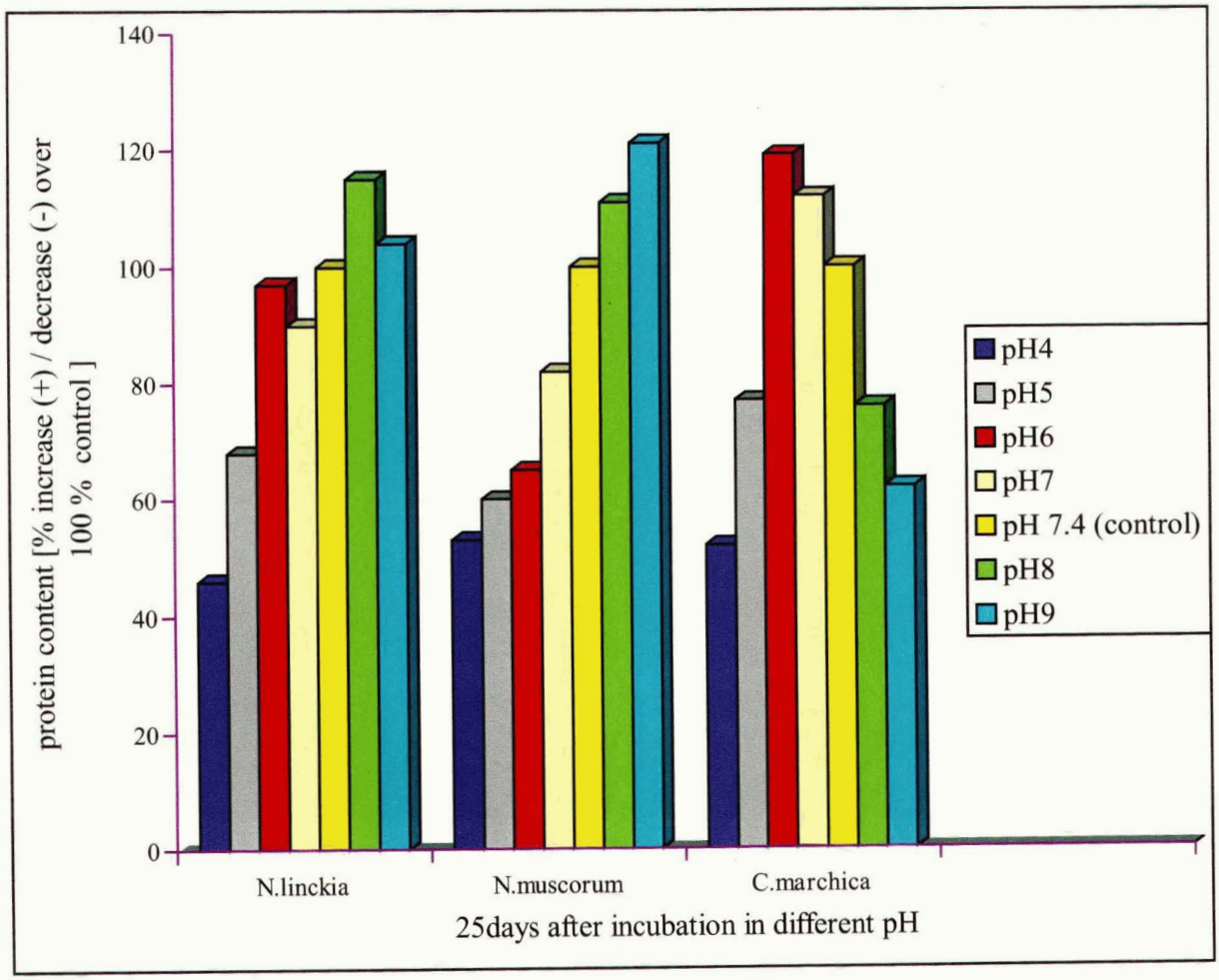


Table 5: Effect of different pH on the heterocysts frequency of two *Nostoc* species

Cyanobacteria	Heterocysts frequency at different pH levels after 20 days of incubation						
	pH 4	pH 5	pH6	pH 7	pH 7.4 (Control)	pH 8	pH 9
Nostoc linckia	4.8±0.12	5.0±0.17	5.5±0.20	5.45±0.19	5.65±0.35	6.5±0.43	6.38±0.32
Nostoc muscorum	4.5±0.1	4.7±0.14	5.1±0.17	5.41±0.15	5.8±0.29	6.4±0.39	6.8±0.48

As far as the morphological changes are concerned, production of high mucilage content accompanied by breakage of trichome, release of various cellular contents, production of large number of akinetes, release of heterocysts into the medium, etc. were observed at low pH values in the initial stage in both the Nostoc spp., when viewed through the microscope. Variation in the shape and size of the cells of the filaments were also observed. Some are enlarged while some small; some are distorted and disorganized. Soon a recovery from pH stress was attained by the species whereby, normal nature and growth of the thallus was regained. Under favourable pH levels the filaments were long, with abundant heterocysts and healthy vegetative cells. Akinete induction was observed at the post-exponential stages in both *Nostoc* spp. In *Calothrix marchica* var. *intermedia*, akinete production was not observed. At low pH (pH 4.0 and pH 5.0) disruption of the thallus and production of many hormogones were observed in the initial stages. In favourable pHs (pH 6.0-7.4) hormogones were observed at later stages of growth. Though growth was lesser in alkaline pHs (pH 8.0-9.0) the species survived. Neutral and control pH (pH 7.0-7.4) induced false branches in this cyanobacterium.

Discussion:

Analysis of the above data showed dominance of *Nostoc linckia* for their tolerance to wider pH ranges without any appreciable decrease in their growth activity. Thus, the above three cyanobacteria are graded in three categories based on the results, with regard to their response to pH. When *Nostoc linckia* falls under the wide range pH-tolerating category, (acidic, neutral and basic) the cyanobacterium *Nostoc muscorum* is restricted to alkalinity. Experiments have

proved that an acidic pH supports the growth of *Calothrix marchica* var. *intermedia* rather than an alkaline one.

Previous studies have shown that among the edaphic factors, pH is the most important in determining the cyanobacterial floral composition in a given ecosystem. Several workers studied the positive correlation between the pH, occurrence and establishment of cyanobacteria earlier (Roger and Reynaud, 1976; Reynaud and Roger, 1977; Roger *et al.*, 1987). In the culture media, the optimal range of pH for cyanobacterial growth varied from 7.5 to 10.0 in the upper limit and from 6.0 to 7.0 in the lower limit (Holm-Hansen, 1968). Usually, most cyanobacteria prefer a neutral to slightly alkaline soil for better growth. Many reports have shown that cyanobacteria thrive well in acidic [5.0-6.0 (Prasad *et al.*, 1978), < 5.0 (Aiyer, 1965; Amma *et al.*, 1966; Sardeshpande and Goyal, 1981b; Roger *et al.*, 1987; Dominic, and Madhusoodanan, 1996] as well as in alkaline pH.

Reports have shown that some alkalophilic cyanobacteria adapted to pH increase by synthesizing more polysaccharides (Saxena and Kaushik, 1992; Singh *et al.*, 1995) Similarly, production of high amount of mucilage, breakage of trichome, release of cellular contents and heterocysts and production of akinetes observed here at low/unfavorable pH might be viewed as adaptations to circumvent pH stress. The branched cyanobacteria *Mastigocladus laminosus* exhibited maximum branching at pH 9.5 and growth at 8.5 (Sahu and Adhikary, 1987). Similar false branching was seen in *Calothrix marchica* var. *intermedia* at pH values at 6.0 to 7.5. According to Shivaprakash and Shetty (1998), neutral to slightly acidic range induced akinete differentiation in *Anabaena* sp. for survival. Similar akinetes induction was noticed in *Nostoc linckia* and *Nostoc muscorum* at pH 4.0 and 5.0.

The results show that various species of cyanobacteria prefer various range of pH for their optimum growth, establishment and N_2 fixing potential. Hence it is highly essential to screen and categorize them in order to utilize them suitably in biofertilizer technology.

Acid tolerance of cyanobacteria:

The response of cyanobacteria to varying levels of pH also explains their acid tolerance capacity. *Nostoc linckia* showed growth in acidic pH with a maximum growth at pH 6 (but it was less when compared to control) (Fig. 39). The species performed well in basic pH (Fig. 36). Thus it exhibited moderate growth at acidic and better growth at basic pHs. Hence it can be considered as acid tolerant and wide range species. *Calothrix marchica* var. *intermedia* was capable of surviving better at pH 6-7.4. Its growth at pH 6 was higher than that of control (Fig. 39). Hence, it can be included under acidophilic category. However, *Nostoc muscorum* responded less to acidic pH and performed better growth activity at alkaline pH. Though there was a gradual increase in growth from acidic to alkaline pH in this species, maximum growth output was brought out when the pH was beyond 7.5 (Fig. 36 & 39).

The response of the above three cyanobacerial species in terms of chlorophyll *a* content varies (Fig. 40). While *Nostoc linckia* showed an increasing chlorophyll *a* content from pH 4.0 - pH 6.0 with a maximum amount at pH 6.0 in acidic range, *Calothrix marchica* var. *intermedia* recorded maximum chlorophyll *a* content at a pH range 6.0. The chlorophyll *a* chart of *Nostoc muscorum* was very poor in acidic pH. However, it was steadily increasing from acidic to alkaline. Though there was a steady increase in chlorophyll *a* content in the acidic pH in *Nostoc muscorum* it was not comparable to that of the other two species due to its inadequate growth in acidic pH (Fig. 40).

The protein content of the above three cyanobacterial species in acid pH was estimated and it was found out that protein content was recorded maximum in *Nostoc linckia* at pH 6.0. The protein was high in *Calothrix marchica* var. *intermedia* at pH 6.0. and *Nostoc muscorum* recorded poor protein content in acidic range, though the amount was systematically increasing from acid to alkaline (Fig. 41). When the pH of the medium was examined 10 days after incubation there was an increase in the pH of the medium in all experimental tubes of the different cyanobacterial species, which was proportional to the growth rate of the species in the respective pH levels.

Fig. 39. Acid tolerance
Growth in terms of Absorbance at 760nm

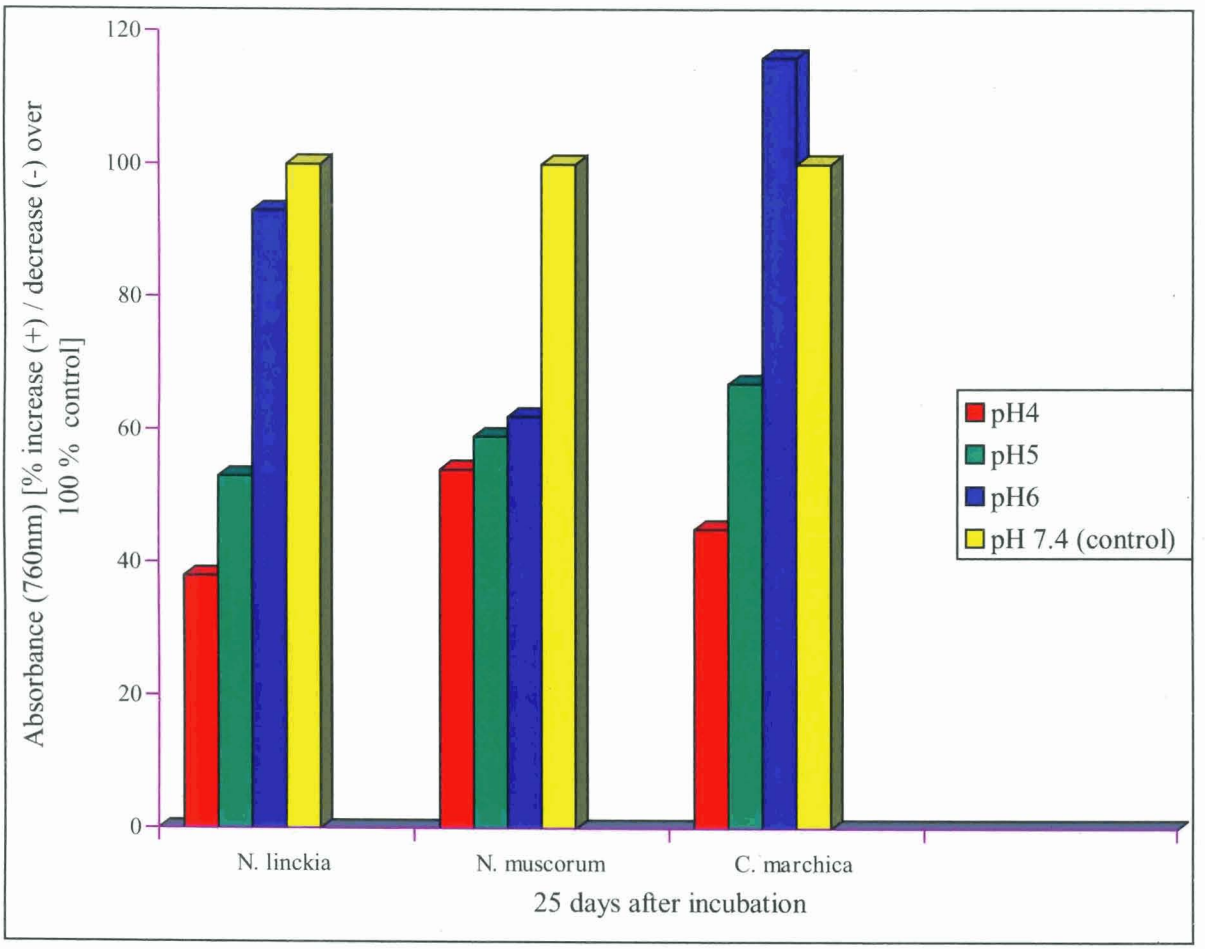


Fig. 40. Acid tolerance
Growth in terms of Chlorophyll a content

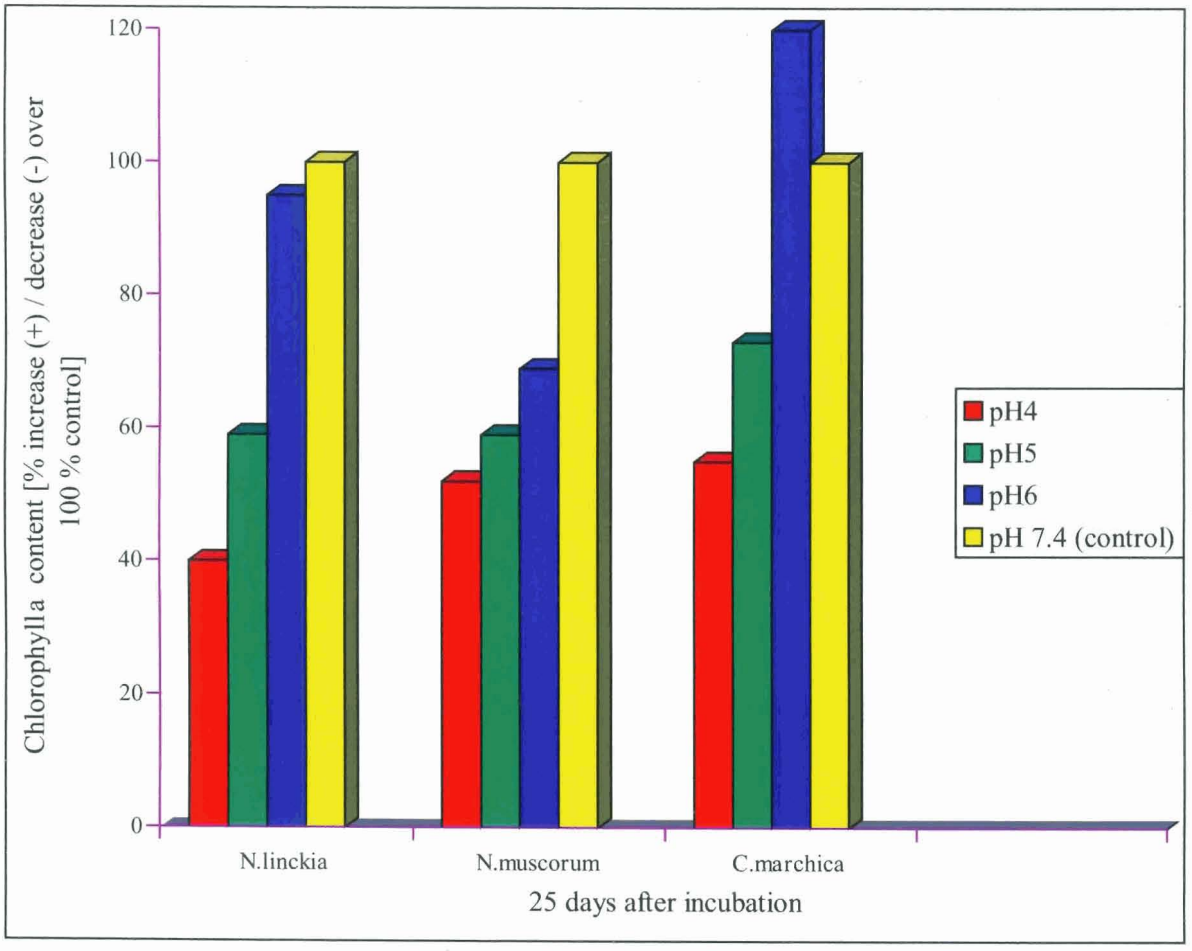
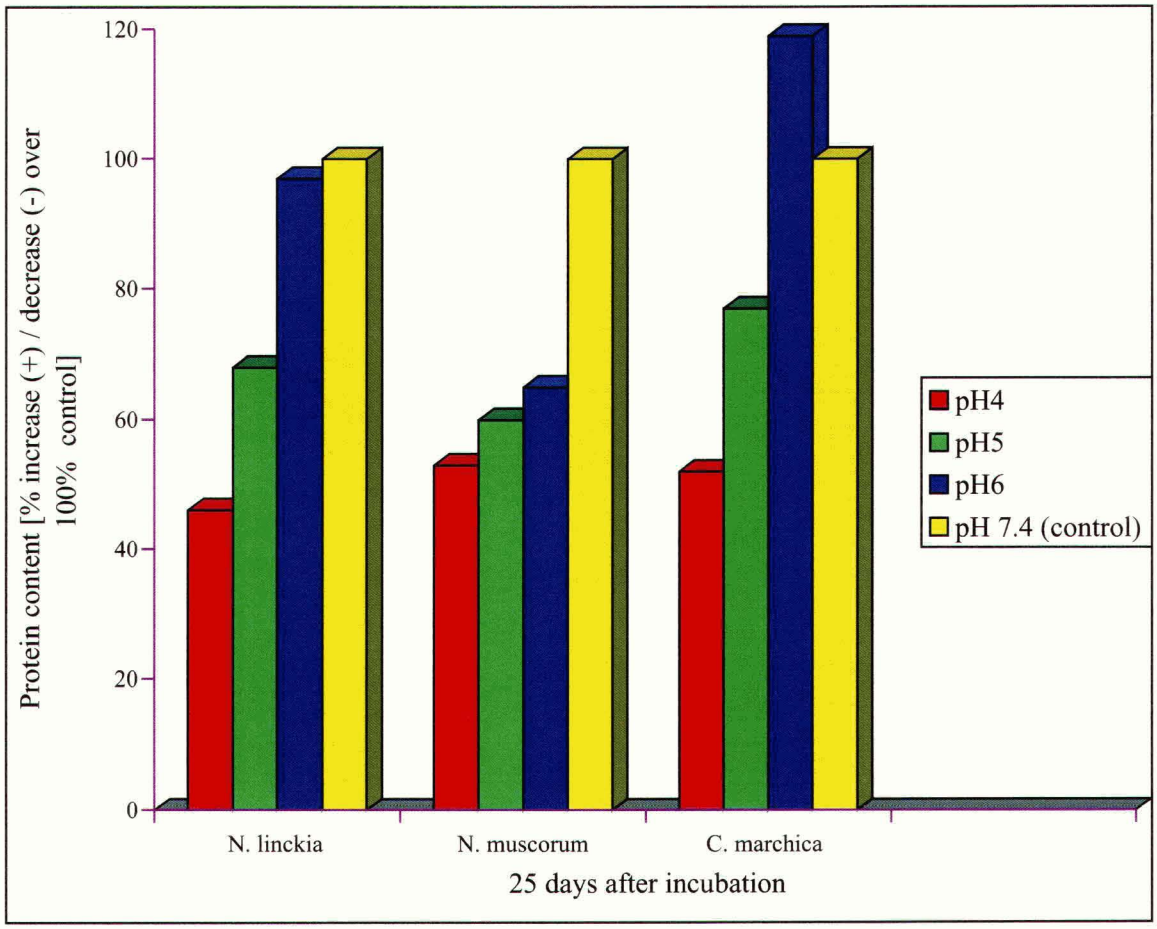


Fig. 41. Acid tolerance
Growth in terms of Protein content



Discussion:

Of the three different species considered for the experiment, *Nostoc linckia* showed better growth and higher chlorophyll *a* and protein content in acidic and alkaline pH. Two peaks were noticed in the graph in this species, one in acidic pH (6.0) and the other in the alkaline pH (8.0)(Fig. 36). Presence of two peaks one each in acidic and alkaline range suggested that the cyanobacterium *Nostoc linckia* could thrive in both ranges, but the results also showed that it preferred an alkaline medium for its better survival. At the same time, it tolerated acidic range as well. *Nostoc muscorum* preferred an alkaline pH range. The growth in terms of absorbance, chlorophyll *a* and protein content were systematically increasing from acidic to alkaline range and the cyanobacterium recorded its maximum growth at pH 9.0. Even if, there was a steady increase in the overall growth rate from acidic to alkaline range, the growth in acidic range was very poor when compared to that in the basic range. *Calothrix marchica* var. *intermedia* preferred an acidic neutral range to basic pH. It performed well between pH 6.0 to 7.4. In short, when the acid tolerance of all the above three species are considered, *Nostoc linckia and Calothrix marchica* var. *intermedia* are acid tolerant/ acidophilic, while *Nostoc muscorum* is an acid sensitive species.

Brock (1973) reported the absence of cyanobacteria from habitats in which the pH was less than 4.0-5.0. He speculated that this might be due to lack of proper protection of chlorophyll molecule in the cyanobacterial cells from H^+ toxicity, which is highly acid sensitive. Various reports (Durrell, 1964; Aiyer, 1965; Amma *et al.*, 1966; Granhall, 1970; Sardeshpande and Goyal, 1981b; Dominic and Madhusoodanan, 1996,1999; and Dominic, 1997) of cyanobacteria from acidic environments had the problem of H^+ toxicity, yet they were found resistant to acidic surroundings. A rapid transient growth of *Synechococcus* sp. was observed when they were transferred to a medium of low pH (Kallas and Castenholz, 1982). Eventhough cells were exposed to low pH, the cells maintained an intracellular pH. This strongly suggests that the inhibition of growth at low pH is not due to an acid pH of cytoplasm but due to some other reasons, which require further investigation.

Several species of cyanobacteria like *Microcystis* sp. (Swain and Adhikary, 1991) and *Phormidium* sp. BDU 30501 (Prabhakaran and Subramanian, 1990) can grow over wide range of initial pH levels (even as low as pH 4.0) in the medium with a subsequent increase in the pH of external medium, sometimes up to pH 9 within about 10 days. In the present study, also the pH value was found to be increasing in the external medium by cyanobacterial exudates. Though *Nostoc muscorum* was found to be an acid sensitive species, it was capable of survival at low pH range may be due to maintenance of intracellular pH. Thus, these cyanobacteria presumably have an efficient pH regulating mechanism in their body as has been reported by Fogg *et al.* (1973).

Several workers have reported that higher pH levels are conducive for the growth of cyanobacteria (Venkataraman, 1961; Sharma and Kumar, 1975). However, they are known to survive under acidic conditions also in field soils suggesting that they tolerate low pH levels (Juergensen and Davey, 1968). It is possible that under field conditions the soil components may act as a buffer system, thus permitting the cyanobacterial growth even under unfavourable pH conditions.

The toxicity of high H^+ concentration may cause a negative impact on cyanobacteria (Coleman and Colman, 1981). Low pH initially acts on cell membrane (Kallas and Castenholz, 1982). A subsequent failure in solute transport caused by changing the intracellular concentration of nutrients or cofactors may lower the consistency of DNA, RNA or protein synthesis resulting in the loss of viability.

Sardeshpande and Goyal (1981b) observed several strains isolated from acidic soils have been found to grow well at pH levels of their original habitat. Dominic (1997) reported that none of the strains collected from soils of pH 3.6 have shown growth optima at that pH. But, their best performance occurs at slightly higher pH, invariably in the acidic pH range only. Some strains though collected from acid soils grew equally well in neutral and even slightly alkaline pH. Hence these strains should be considered to be acid tolerant rather than acidophilic strain. Thus, it is possible to obtain ecologically well-adapted strains,

through proper screening of their sensitivity to soil reactions, from different agro climatic conditions.

Rice is one among the few crops that favours acidic pH (Konokhova, 1985) for optimum growth and crop yield. Most of the high yielding paddy fields in the world, especially that of Asia are either slightly or sometimes strongly acidic (Grist, 1960). So the acid tolerant strains found out in this study can be utilized as an excellent biofertilizer in the acid soils of Kerala or any similar acidic habitats elsewhere.

Fig. 42. Salinity tolerance
Growth in terms of Absorbance at 760nm

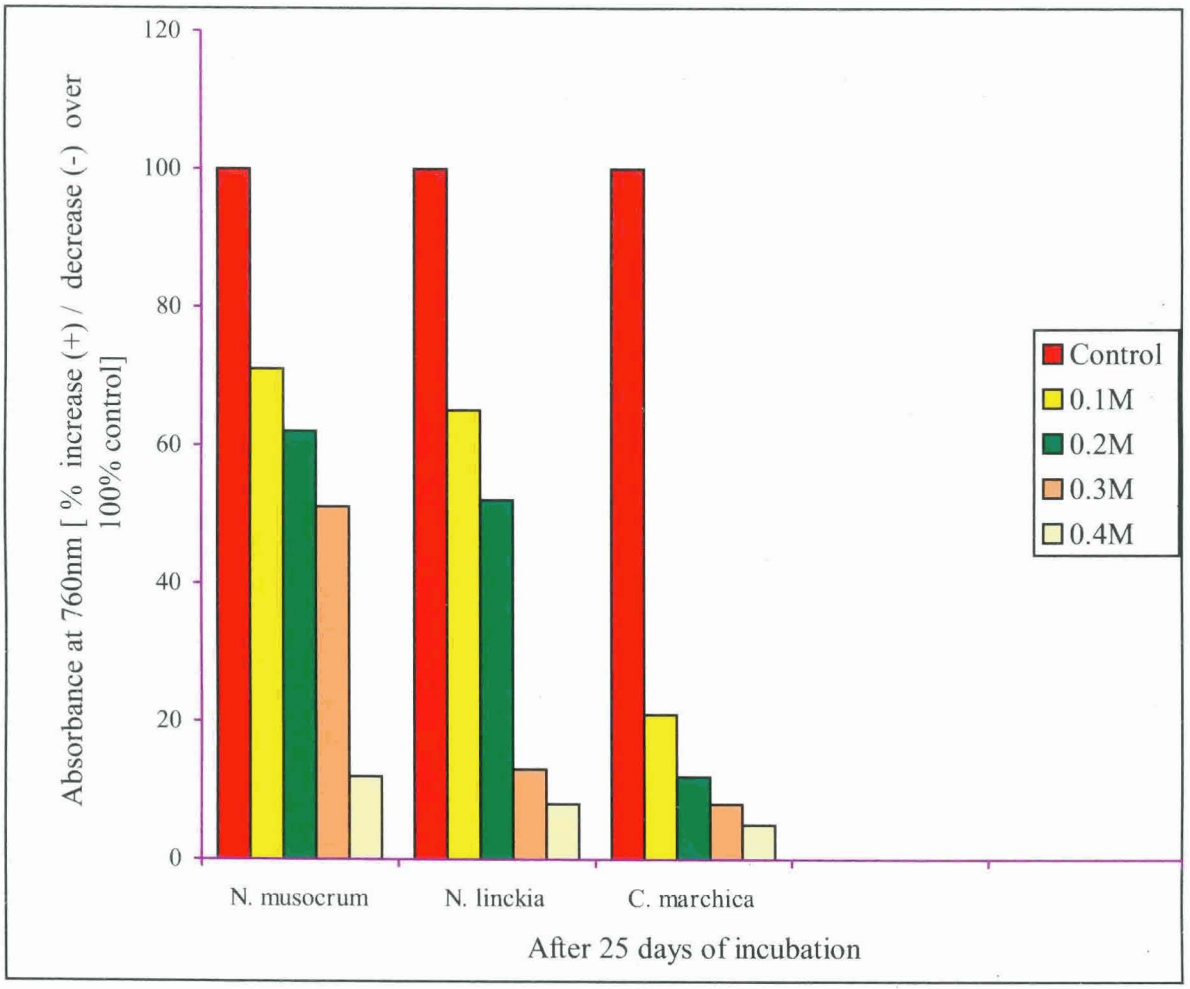


Fig. 43. Salinity tolerance
Growth in terms of Chlorophyll a content
(After 5 days of incubation)

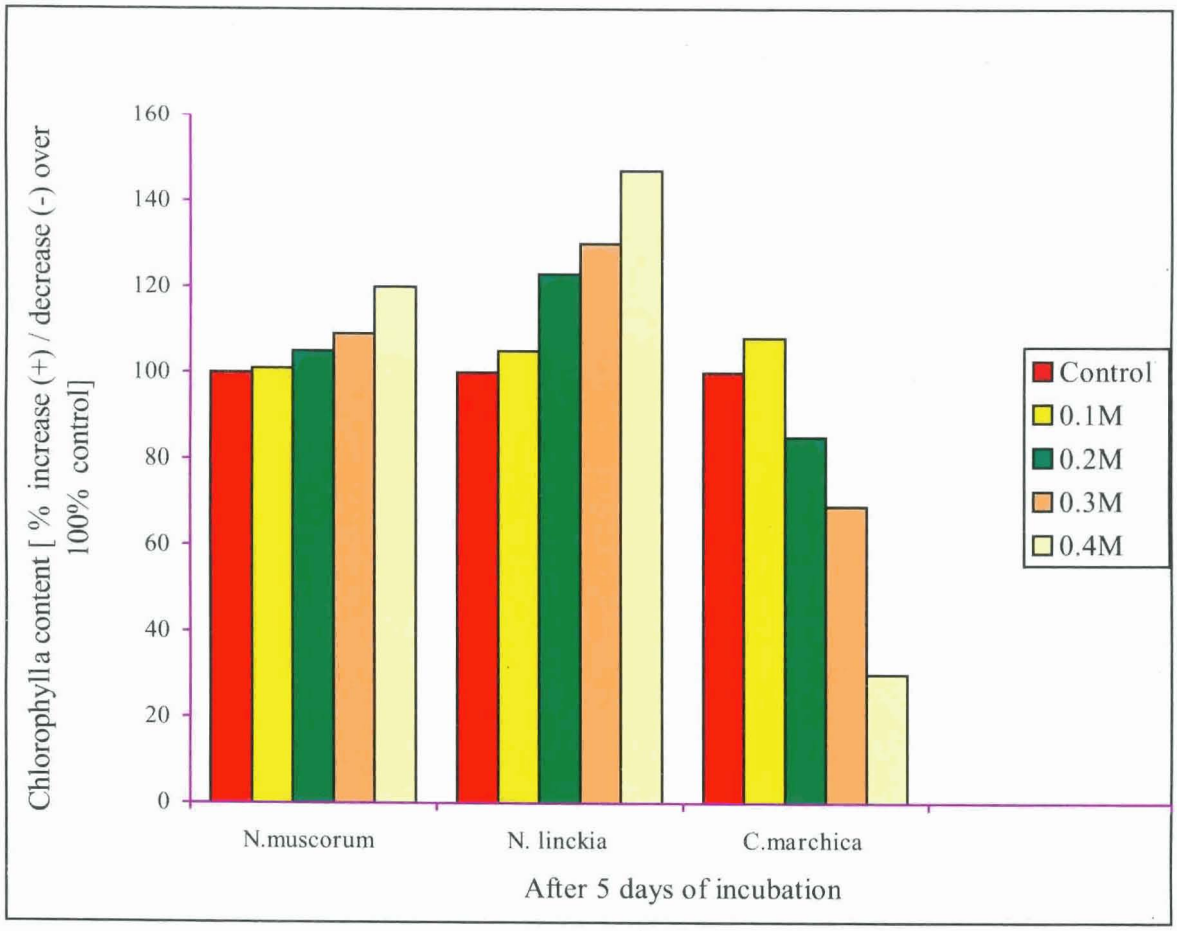


Fig. 44. Salinity tolerance
Growth in terms of Chlorophyll a content
(After 15 days of incubation)

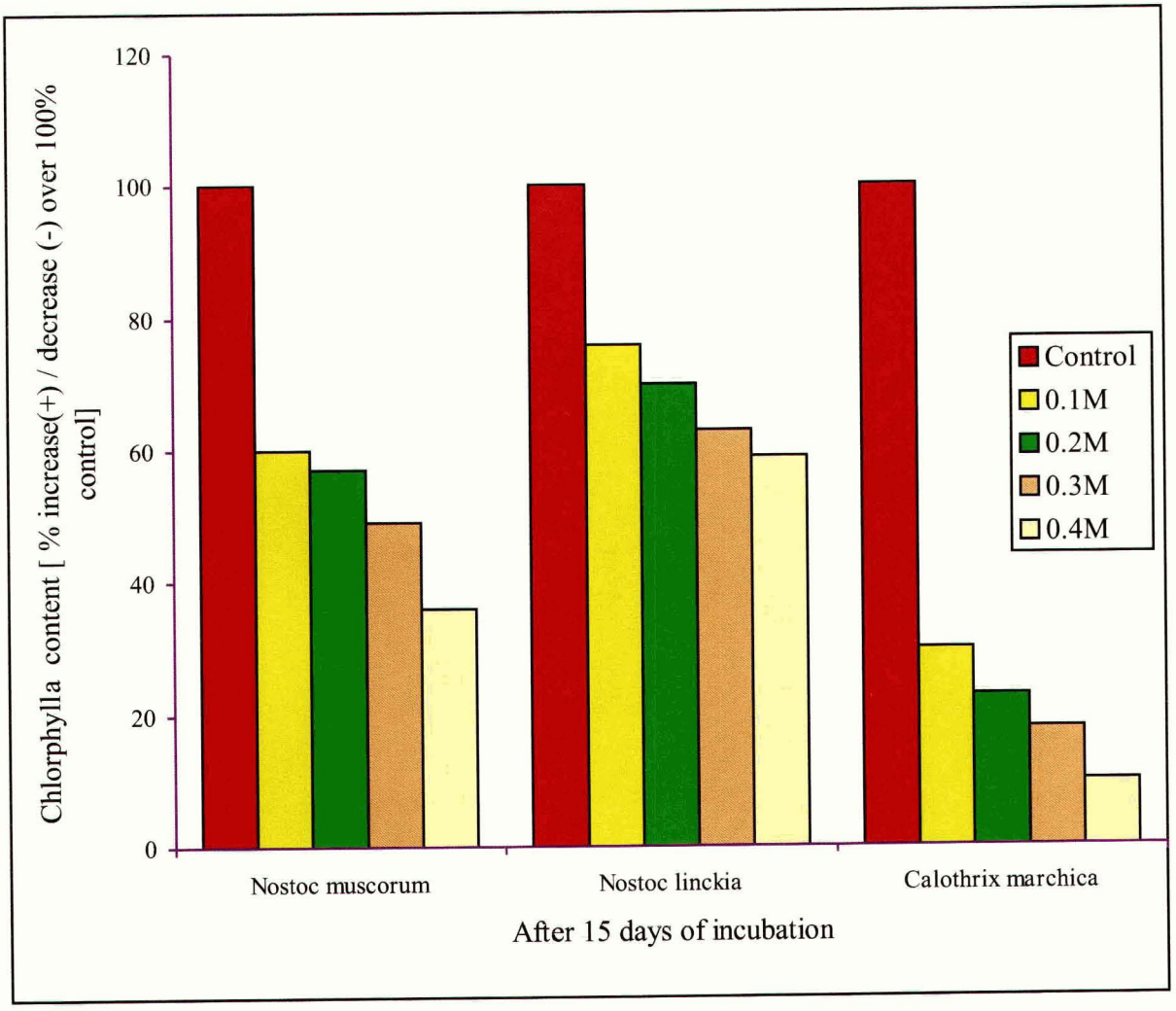


Fig. 45. Salinity tolerance
Growth in terms of Chlorophyll *a* content
(After 25 days of incubation)

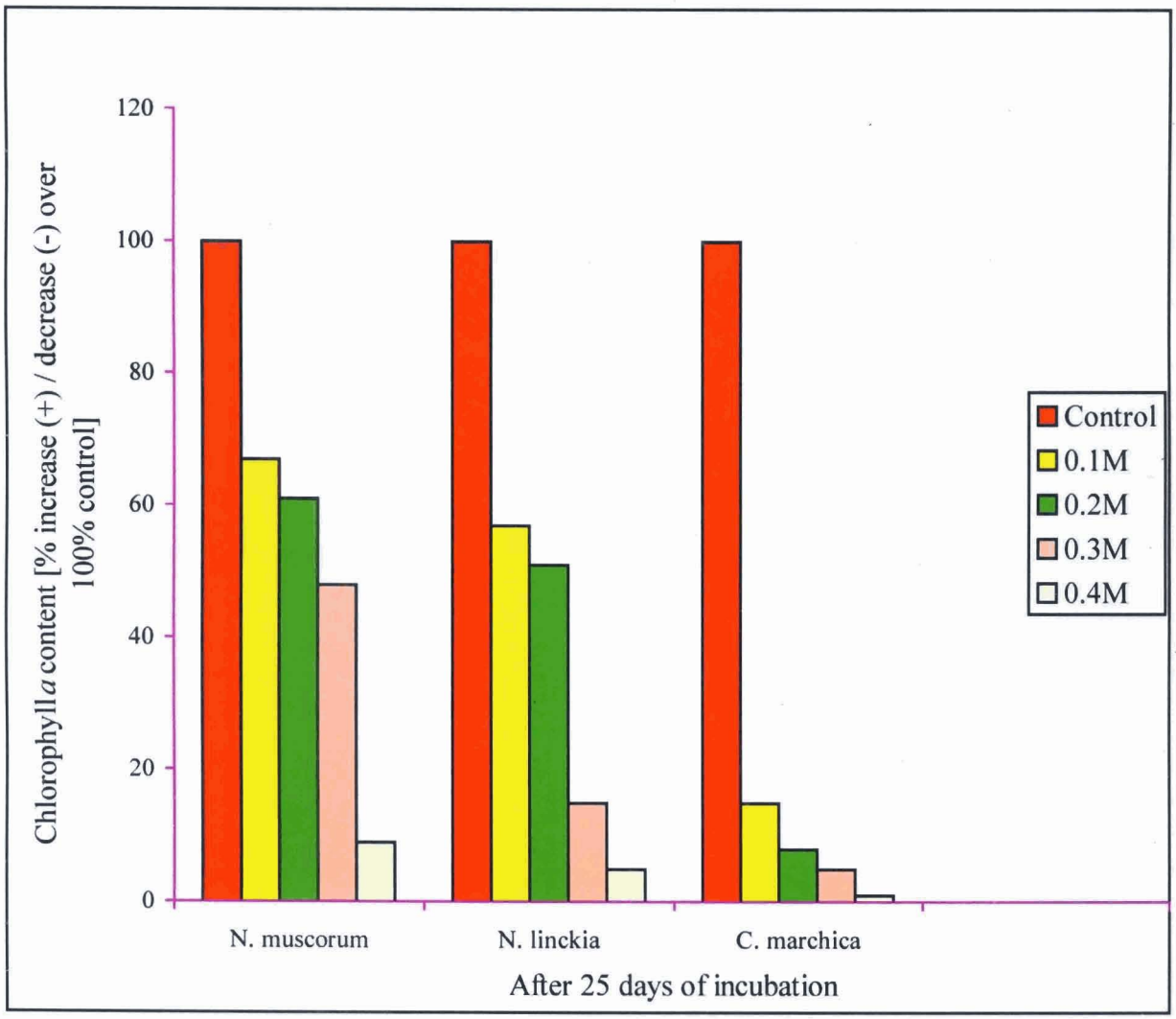
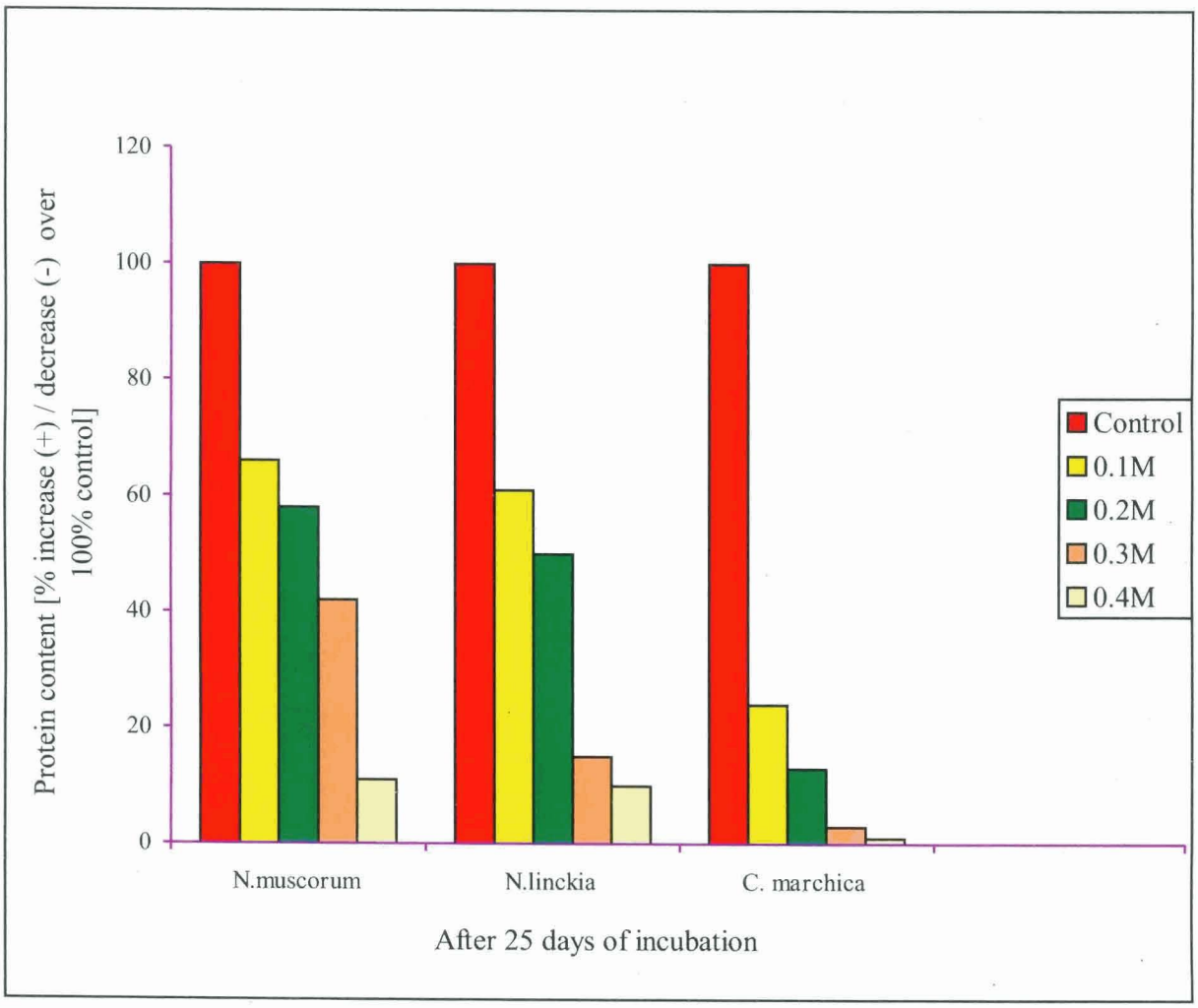


Fig. 46. Salinity tolerance
Growth in terms of Protein content



Salinity tolerance:

Relative tolerance of the three species of N_2 fixing cyanobacteria belonging to *Nostoc* and *Calothrix* (used in the earlier experiment) showed differential tolerance to NaCl ranging from zero tolerance to 0.3 M tolerances. The order of tolerance is as follows*: Nostoc muscorum* (up to 0.3 M) > *Nostoc linckia* (up to 0.2 M) > *Calothrix marchica* var. *intermedia* (nil).

The response of these cyanobacteria to salinity tolerance (varying from 0.1 -0.4 M) was studied by measuring the growth every 5 days in terms of absorbance (760 nm), chlorophyll-*a* content, and protein content and also by observing the thallus and other cellular characteristics frequently for a period of 25 days. *Nostoc muscorum* tolerated higher salinity levels than that of the other two species selected in the experiment (*Nostoc linckia* and *Calothrix marchica* var. *intermedia)* and it grew moderately up to 0.3 M NaCl and the growth declined therafter (fig: 42). So, this can be considered as a halo tolerant strain. The cyanobacterium *Nostoc linckia* tolerated 0.2 M salinity. However, *Calothrix marchica* var. *intermedia* was highly sensitive to NaCl and its thallus appeared to be getting gradually bleached even at 0.1M salinity and the signs of bleaching was observed from 2-3 days of incubation.

The chlorophyll-*a* content recorded was high in control sample in all the species. In other samples amended with varying levels of NaCl, the chlorophyll *a* content varied with species to species based on their degree of tolerance to NaCl. The chlorophyll *a* content of *Calothrix marchica* var. *intermedia* showed a marked decrease due to its severe loss of pigments due to salt stress. The chlorophyll-*a* content of *Nostoc linckia* increased in the initial stages in proportion to the concentration of the NaCl, followed by a decrease up to the last day of incubation. The decrease was more when salt concentration was higher. However, the chlorophyll *a* content of *Nostoc muscorum,* the most tolerant species of the three, showed an initial slight increase on exposure to different levels of salt. Soon a sudden decrease followed by a stabilization phase and then an increase in the chlorophyll *a* content was observed at the end. The increase was less or not observed, when concentration of salinity was

higher. The results showed that the chlorophyll *a* content has recovered after a 'salt shock' of 5-6 days (Fig. 43, 44, & 45).

The protein content of the three cyanobacteria in varying levels of NaCl was less in the initial stage due to sudden exposure to salt, but, the amount gradually increased depending upon the capacity of the species to tolerate salt stress, the period of incubation and the amount of protein resembled that of adapted cells. Control sample revealed maximum protein content in all the species (Fig. 46).

A fall in heterocysts frequency and thereby an impairment of nitogenase activity was noticed in both *Nostoc* spp. within a day or two of incubation in salt containing media. However, a recovery was marked after five days, suggesting a recovery of the heterocysts level after cellular adaptation to salt (Table 6). The cells of *Nostoc muscorum* and *Nostoc linckia* showed no cell damage on microscopic examination. In *Calothrix marchica* var. *intermedia,* severe disintegration of the thallus was observed which was measured by filamentous breakage, cell disruption and discolouraton of the thallus due to leaching out of the pigments.

Discussion:

Analysis of these results reveals that cyanobacteria of rice fields possess varying degree of tolerance to NaCl in culture. The degree of variation did not confine to specific genera or species. Earlier reports have revealed that supplementation of 0.4 M of NaCl decreased the biomass accumulation, chlorophyll *a* content and cell protein level of *Calothrix brevissima* isolated from saline sodic soils whereas *Anabaena* species of fresh water tolerated only up to 0.1 M NaCl (Kumar and Kaushik, 1994). When the unicellular cyanobacterium *Microcystis firma* tolerated up to 1 M NaCl another unicellular sp. *Synechocystis aquatilis* showed salt tolerance up to 0.3 M NaCl. Blumwald & Tel-Or (1982a) observed complete growth inhibition of *Nostoc muscorum* on exposure to 0.4 M NaCl. All these results show the prevalence of varying degree of tolerance to NaCl among the various species of cyanobacteria.

Table 6: Effect of different concentrations of NaCl on the heterocysts frequency of two species of *Nostoc*

NaCl concentrations	Heterocyst frequency in days									
	Nostoc muscorum					*Nostoc linckia*				
	5 days	10 days	15 days	20 days	25 days	5 days	10 days	15 days	20 days	25 days
0 M (control)	4.5±0.19	5.1±0.3	5.7±0.33	5.8±0.35	6.2±0.46	4.3±0.17	5.2±0.31	5.5±0.38	5.65±0.38	6.1±0.53
0.1M	4.1±0.13	4.3±0.22	5.0±0.23	5.2±0.27	5.2±0.32	4.0±0.13	4.3±0.27	4.4±0.29	4.9±0.38	5.1±0.44
0.2M	3.8±0.12	4.1±0.18	4.5±0.15	4.6±0.25	4.8±0.2	3.7±0.13	3.9±0.23	4.3±0.24	4.5±0.41	4.5±0.38
0.3M	3.6±0.08	3.8±0.11	4.1±0.14	4.2±0.1	4.2±0.11	3.0±0.11	2.2±0.10	-	-	-
0.4M	3.3±0.06	2.5±0	-	-	-	2.5±0.05	-	-	-	

The tolerance of *Nostoc* species may be due to the presence of high amount of mucilage around their cells / filaments which may collect and store the excess sodium from the surroundings. Apte and Thomas (1997) observed that more than 90% of the cell bound sodium remained extra-cellular in the slime layers of cyanobacteria. Nevertheless, even on possessing a definite sheath the strain *Calothrix marchica* var. *intermedia* exhibited high sensitivity to NaCl. It has been reported that cyanobacteria having distinct sheath/ copious mucilage around their cells were salt sensitive (Naik, 2000) revealing thereby the insignificant role of external envelopes in certain cases in osmo-protection.

A number of reports indicated several salt induced events: (i) the stimulation of photosynthesis (ii) accumulation of sugars (iii) the reorganization of photosynthetic apparatus (iv) modification of cell size, shape and membrane properties (v) reduction in the level of heterocyst frequency and (vi) leaching out of pigments. (Blumwald & Tel- Or, 1982a, 1982b & Blumwald *et al.*, 1983; Anand & Parameswaran, 1990)

In the present study a sudden reduction in the protein levels due to salt stress has been observed, followed by an increase in the level that resembled the control/ adapted cells. Similar observations were reported earlier also (Hagemann *et al.*, 1991). Reduction in protein levels can be due to 'Salt shock' or high pigment release as the phycobilin pigments constitute as much as 40% of the total soluble proteins in cyanobacteria (Fay, 1969). On the analogy of reports on the sensitivity of nitrogen fixing systems (a fall in heterocysts frequency level) of cyanobacteria to salinity, the heterocyst frequency and thereby the nitrogenase activity was greatly affected within few days of incubation in salt containing media.

The salt stress damage to *Calothrix marchica* var. *intermedia* was so marked that it neve recover from the shock. However, in *Nostoc* spp. a recovery was marked after subsequent period *i.e.*, after 5 days suggesting the recovery of the activity after their cellular adaptation to salt. Such an adaptation to an environmental stress like salinity requires large investment of energy for biosynthesis and reorganization. Previous reports suggest that cyanobacteria meet these energy requirements by enhancing photosynthesis and by changing

their PSI / PSII stoichiometry which are the immediate responses to salinity stress in cyanobacteria (Blumwald & Tel-Or, 1982a; Murakami *et al.*, 1997).

Sodium (Na^+) is essential for the nitrogenase activity and its deficiency leads to the impairment of photosynthesis due to nitrogen starvation (Apte & Thomas, 1983). Reports also reveal that the nitrogen fixing machinery of cyanobacteria is damaged by salt stress (high salinity level), which was not fully restored in subsequent periods in salt free media (Blumwald & Tel-Or, 1982b; Naik, 2000). Nevertheless, the pigment synthesis due to increased synthesis of thylakoids during adaptation was easily restored in subsequent periods of incubation.

Though halophilic cyanobacteria exist in hyper-saline environments, species are also reported from regions of fluctuating salinity. The salt tolerance in such cyanobacteria has been explained as due to various mechanisms. These include achievement of Na^+ influx (Apte *et al.*, 1987; Kumar and Kaushik, 1994; Senthil *et al.*, 1994), accumulation of K^+ (Miller *et al.*, 1984), production and storage of osmo-protective compounds (Reed *et al.*, 1986; Waar *et al.*, 1994) production of exogenous carbon compounds like saccharides, alcohols, etc. (Blumwald and Tel-Or, 1982a & b and Blumwald *et* al., 1983; Anand and Parameswaran, 1990) and aminoacids (Kumar and Kaushik,1994; Padhi *et al.*,1998).

About 7 million hectares of land are designated as saline sodic soils in India and many more areas are under threat of infiltration of salt water especially in coastal areas. These soils have to be reclaimed by using suitable cyanobacterial strains. For that proper screening is essential. The various extra-cellular compounds they release into the salt affected soils, change the soil pH, texture, conductivity, *etc.*, and thereby lower the salinity and make the soil suitable for paddy cultivation.

Though the rice fields of Kerala are acidic, alkaline soils are also found in Palakkad district and coastal areas. Sodium (Na^+) is the prominent soluble cation in most of the saline soils and water particularly in coastal areas. Here rice is cultivated and / or periodically flooded with salt water. The rice plant grown in such saline environments exhibit poor yield due to (i) Intra-cellular

accumulation of Na^+, which is toxic to the cellular metabolism. (ii) Water loss and subsequent growth inhibition due to osmotic component of salinity stress (Serrano & Glaxiola, 1994).

Hence, for paddy cultivation in saline environments, reclamation of the soil is the prime requisite. The conventional method is the application of gypsum followed by leaching out of excess of salts. In this process, the salinity may get reduced, but the application of gypsum has side effects. Singh (1961b) proposed a biological approach of amelioration of the saline/ sodic soil using cyanobacteria. The cyanobacterial uptake and immobilize Na^+ by intra-cellular manner that results in the decline of salinity of the soils (Kaushik *et al.*, 1981). However, Apte & Thomas (1997) observed that more than 90% of the cell bound sodium is removed by extra-cellular method in the slime/ mucilage layer of cyanobacteria. The topsoils containing cyanobacterial systems decrease the salinity of the soil by up to 38% (Apte and Thomas, 1997). All these findings strongly recommend the application of cyanobacteria for soil salinity reclamation. Species with abundant mucilage and tolerating high salinity level can be employed for the processes. Both the species of the *Nostoc* that grew well in the culture producing copious slime can be used as biofertilizer especially in those areas where the rice fields are facing fluctuating salinity.

Nutritional response:

Response of cyanobacteria to exogenous carbon compound / s:

For studying the response of cyanobacteria to various exogenous carbon compounds the selected species of cyanobacteria, namely, *Nostoc linckia, N. muscorum, N. spongiaeforme, Calothix elenkinii* and *Calothrix marchica* var. *intermedia* were grown in BG11 (N_2 free) medium supplemented with glucose and sucrose at a concentration of 15mM in the culture. Since these sugars (glucose and sucrose) are simple and important energizers belonging to mono and disaccharides respectively they were selected in the study as exogenous carbon compound/ s. Cultures were incubated under light with 12 hr L/D cycle for a period of 15 days and their growth were estimated in terms of growth in absorbance at 760nm and chlorophyll *a* content.

All the species except *Calothix elenkinii* grew well in presence of the exogenous carbon sugars glucose and sucrose than in control. Again, majority of the species grew better in glucose than in sucrose (Table 7). The growth in absorbance and the chlorophyll *a* content in the above species were higher than that of the control. *Calothix elenkinii* could not grow at higher rate than in control in both sugars in light and the growth was very poor in sucrose. Hence *Calothix elenkinii* can be described as obligate autotrophs. These results show that while some cyanobacteria are capable of utilizing the exogenous carbon sugars for their growth efficiencies, some like, *Calothix elenkinii* make less use of them.

Autotrophic, mixotrophic and heterotrophic growth capacity of cyanobacterial species.

A study on the autotrophic, mixotrophic and heterotrophic growth capacity of the above five cyanobacterial species isolated from the rice fields of Kerala was conducted. Two sugars, *viz.,* glucose and sucrose were supplemented to BG11 (nitrogen free) medium at a concentration of 15 mM in the culture. Cultures were incubated under light with 12 hr L/D cycle or in the darkness for a period of 15 days. Their growth was estimated in terms of growth in absorbance and chlorophyll *a* content.

Of the five isolates belonging to the genera *Nostoc* and *Calothrix, Calothix elenkinii* was found to be obligate autotrophs and it could not grow higher in any type of exogenous carbon compounds than in control in light. The other four species such as, *Nostoc linckia, Nostoc muscorum, Nostoc spongiaeforme* and *Calothrix marchica* var. *intermedia* grew both autotrophically and mixotrophically. Here growth was higher than that in control in presence of exogenous sugars, in light. While *Nostoc linckia, Nostoc muscorum* and *Nostoc spongiaeforme* exhibited high growth in glucose, *Calothrix marchica* var. *intermedia* recorded maximum growth in sucrose. Growth measurements were higher in the sugar glucose than in sucrose in majority of the species (Table 8). This fact reveals that glucose is mostly preferred to sucrose by cyanobacteria.

The growth of all these species in darkness was varying. When *Nostoc linckia, Nostoc spongiaeforme* and *Calothix elenkinii* could not grow in the darkness, the other two species *Nostoc muscorum* and *Calothrix marchica* var. *intermedia* produced a feeble growth in darkness (in the absence of exogenous

sugars). *Calothrix marchica* var. *intermedia* performed better growth in darkness than *Nostoc muscorum*. The heterotrophic growth in presence of exogenous sugars was comparatively better in *Calothrix marchica* var. *intermedia* than in *Nostoc linckia and Nostoc muscorum* and better growth responses were seen in the presence of glucose than in sucrose except for *Calothrix marchica* var. *intermedia* where the growth was slightly high in sucrose (Table 8). Thus *Nostoc linckia, N. muscorum* and *Calothrix marchica* var. *intermedia* grew both heterotrophically and mixotrophically utilizing any one or both sugar/s, *viz.*, glucose and sucrose as the carbon source. Heterotrophic growth was totally absent in *Nostoc spongiaeforme* and *Calothix elenkinii.*

When chlorophyll *a* content was measured in all the above species in light and in darkness, it was found to be increasing in proportion with their growth in absorbance in control and other exogenous carbon compounds (Table 9). The darkness grown cultures had high carotenoid and low chlorophyll *a* content. The naturally brown coloured cyanobacterium *Calothrix marchica* var. *intermedia* was found to be very pale in darkness.

Discussion:

The results showed that only a few of the cyanobacteria in rice field utilize exogenous carbon for their growth in light and in darkness, though a few others could use exogenous carbon sugars efficiently only mixotrophically (when cultured under light). Even though, these species utilize either of the substrate (sucrose/ glucose) for their growth in darkness, their heterotrophic growth was never comparable to the respective autotrophic growth rate suggesting that some of the cyanobacterial species (here, in this experiment, *Nostoc linckia, Nostoc muscorum* and *Calothrix marchica* var. *intermedia*) are capable of growing facultative heterotrophically utilizing specific exogenous carbon sugars and some other species like *Calothix elenkinii* are found to be purely autotrophic.

The growth of the above species was estimated in terms of absorbance (at 760 nm) and chlorophyll *a* content. The capability of using specific sugars varied between and within the species under each genus and this might be correlated to membrane properties. In general, glucose was preferred by the

cyanobacterial species to sucrose. This might be because of its simple monosaccharide nature and high diffusion capability through membranes. The glucose and sucrose supplementation in the medium supported mixotrophic growth as well as heterotrophic growth. Absence of growth in presence of exogenous sugars was also noticed in certain species like *Nostoc spongiaeforme* and *Calothix elenkinii* in darkness.

Of the five cyanobacteria tested in the experiment, growth in absorbance and chlorophyll *a* content of four species were increasing in mixotrophic cultures when supplemented with glucose or sucrose or both (Table 8,9). The growth under mixotrophic cultures was always higher than the autotrophic and heterotrophic cultures. However, the substrate specificity to support such mixotrophic growth varies from species to species. Further the substrate that supported the better growth of an organism in light, did not favour better growth in heterotrophic cultures. The exogenous sugars used were specific in each type of culture conditions, species, etc. This reveals that the selection and utilization of exogenous carbon sugars depend on the strain and growth conditions and this may be due to differential cell membrane permeability and transport properties for assimilation of organic substrate based on light and darkness treatments.

Many reports establish that most of the cyanobacteria are obligate autotrophs and do not grow heterotrophically in darkness (Hoare *et al.*, 1967; Smith *et al.*, 1967; Holm- Hansen, 1968). However, various other reports revealed that a great number of cyanobacteria grow heterotrophically at the expense of various exogenous carbon sugars (Wolk, 1973; Smith, 1973, 1982; Khoja and Whitton, 1975; Adhikary and Patnaik, 1979,1981; Sahu and Adhikary, 1981, 1982; Banerjee and Kumar, 1987; Bastia *et al.*, 1993; Naik, 2000). It is also emphasized that waste organic matter serves as the main nutrient influencing heterotrophic growth of certain cyanobacteria under natural environment (Fogg *et al.*, 1973)

In the present work, growth of three cyanobacteria (*Nostoc linckia, Nostoc muscorum* and *Nostoc spongiaeforme*) in mixotrophic culture conditions supplemented with 15 mM glucose was found higher than in sucrose and in

their respective controls. But, the growth was found to be more in sucrose in *Calothrix marchica* var. *intermedia* than in glucose and in control. This varied response to exogenous sugars by cyanobacterial strains is closely linked to their membrane properties. Microscopic examination of *Calothrix marchica* var. *intermedia* revealed that the filaments were provided with thicker sheath in layers than in control. The formation of such thick sheath layers in presence of exogenous sugars corresponding to metabolic apparatus of the organism in response to the new environmental conditions has been reported earlier (Adhikary, 1990). Mixotrophic growth of cyanobacterial species *Calothrix elenkinii* was less than the autotrophic (control) growth suggesting that for certain species presence of organic substrate in the medium decreased their metabolic activity possibly due to cell membrane permeability restrictions (Rippka, 1972; Smith, 1982). It has been reported that many cyanobacterial strains were capable of utilizing a wide spectrum of exogenous carbon compounds in both light and darkness. But, only a few mono and disaccharides (glucose, fructose and sucrose) can act as substrate for support of growth in the darkness in addition to their autotrophic mode of growth in light (Smith, 1973,1982; Mishra *et* al., 1985; Barnum and Gendel, 1987). This might be due to the absence of specific enzymes to initiate and activate the metabolic activity in the darkness. The findings of the present investigation that glucose and sucrose were efficient in permitting growth of certain cyanobacteria in light and/or darkness are agreeable with the earlier reports (Khoja and Whitton, 1975; Bottomley and Van Baalen, 1978; Adhikary and Pattnaik, 1979, 1981; Barnum and Gendel, 1987; Adhikary and Sahu, 1988; Adhikary, 1990; Bastia *et al.*, 1993; Naik, 2000). The cyanobacteria capable of growing mixotrophically showed increase in growth and chlorophyll *a* content within five days of incubation in light. But in darkness the growth and chlorophyll *a* content slowly increased with increase in the period of incubation.

Table 7: Effect of different exogenous sugars, Glucose and sucrose (15 mM each) on the Growth in absorbance (760nm) and chlorophyll *a* content (µg/ml) of five species of cyanobacteria in light.

Cyanobacterial species	Initial optical density (OD at 760 nm)	Growth in absorbance after 15 days (OD at 760 nm)			Initial chlorophyll *a* content (µg/ml)	Chlorophyll *a* content after 15 days (µg/ml)		
		Light				Light		
		Control	Glucose	Sucrose		Control	Glucose	Sucrose
Nostoc linckia	0.030	0.37 ± 0.01	0.39 ± 0.03	0.38 ± 0.01	0.069	4.46 ± 0.01	4.80 ± 0.04	4.65 ± 0.02
Nostoc muscorum	0.030	0.36 ± 0.02	0.38 ± 0.01	0.37 ± 0.01	0.068	4.30 ± 0.02	4.67 ± 0.01	4.48 ± 0.01
Nostoc spongiaeforme	0.030	0.35 ± 0.02	0.41 ± 0.03	0.37 ± 0.02	0.063	4.19 ± 0.01	5.05 ± 0.01	4.53 ± 0
Calothrix elenkinii	0.030	0.32 ± 0.03	0.21 ± 0.01	0.19 ± 0.01	0.060	4.05 ± 0.01	2.65 ± 0.03	2.35 ± 0.02
Calothrix marchica var. *intermedia*	0.030	0.38 ± 0.01	0.40 ± 0.03	0.43 ± 0.04	0.067	4.95 ± 0.02	5.15 ± 0.03	5.31 ± 0.05

Table 8: Effect of different exogenous sugars, Glucose and Sucrose (15 mM each) on the growth (OD at 760 nm) of five species of cyanobacteria in light and dark.

Cyanobacterial species	Initial absorbance (OD at 760 nm)	Growth in terms of absorbance after 15 days (OD at 760 nm) of incubation					
		Light			Dark		
		Control	Glucose	Sucrose	Control	Glucose	Sucrose
Nostoc linckia	0.030	0.37 ± 0.01	0.39 ±0.03	0.38 ±0.01	0	0.07 ±0.02	0.05 ±0.01
Nostoc muscorum	0.030	0.36 ± 0.02	0.38 ±0.01	0.37 ±0.01	0.02 ±0.01	0.06 ±0	0.04 ±0.01
Nostoc spongiaeforme	0.030	0.35 ±0.02	0.41 ±0.03	0.37 ±0.02	0	0	0
Calothrix elenkinii	0.030	0.32 ±0.03	0.21 ±0.01	0.19 ±0.01	0	0	0
Calothrix marchica var. *intermedia*	0.030	0.38 ±0.01	0.40 ±0.03	0.43 ±0.04	0.03 ±0.01	0.07 ±0.01	0.09 ±0.03

Table 9: Effect of different exogenous sugars, Glucose and Sucrose (15 mM each) on the chlorophyll *a* content of five species of cyanobacteria in light and dark.

Cyanobacterial species	Initial Chlorophyll *a* content (µg/ml)	Chlorophyll *a* content after 15 days (µg/ml) of incubation					
		Light			Dark		
		Control	Glucose	Sucrose	Control	Glucose	Sucrose
Nostoc linckia	0.069	4.46 ±0.01	4.80 ±0.04	4.65 ±0.02	0	0.12 ± 0.02	0.095 ±0.01
Nostoc muscorum	0.068	4.30 ±0.02	4.67 ±0.01	4.48 ±0.01	0.035 ±0.01	0.11 ±0	0.081 ±0.01
Nostoc spongiaeforme	0.063	4.19 ±0.01	5.05 ±0	4.53 ± 0.01	0	0	0
Calothrix elenkinii	0.060	4.0 5±0.01	2.65 ±0.03	2.35 ±0.02	0	0	0
Calothrix marchica var. *intermedia*	0.067	4.95 ±0.02	5.15 ±0.05	5.31 ±0.03	0.05 ±0	0.125 ±0.01	0.14 ±0.03

This might be due to partial block in the glucose metabolism resulting in the reduction of growth in the organic substrate supplemented medium in the darkness. The formation of low chlorophyll *a* and high carotenoid content in cultures kept in darkness has been reported earlier also (Satapathy *et al.*, 1992). The pale brown colour of *Calothrix marchica* var. *intermedia* might be due to lack of phycoerythrin synthesis in darkness in proportion to the growth of the cyanobacterium as reported by Satapathy *et al.* (1992). The observed failure of growth stimulation in light by carbon sources in some species may be due to a low entry of these carbon compounds into the cells and resultant failure in the metabolism of these compounds. (Haury and Spiller, 1981).

N_2 fixation in the heterotrophic culture of cyanobacterial strains has been reported earlier (Fay, 1965; Watanabe and Yamamoto, 1967; Sahu and Adhikary, 1981; Barnum and Gendel, 1987). Many cyanobacterial strains can fix N_2 in darkness in presence of sugars; although the rate of fixation is low (Hoare *et al.*, 1971; Fay, 1976). The increase in N_2 fixation is due to the production of ATP by respiration. Use of cyanobacteria that can assimilate exogenous carbon compounds (sugars) for increased growth and N_2 fixation are of great significance for use as biofertilizer in rice fields located in areas rich in organic content.

Phosphate response:

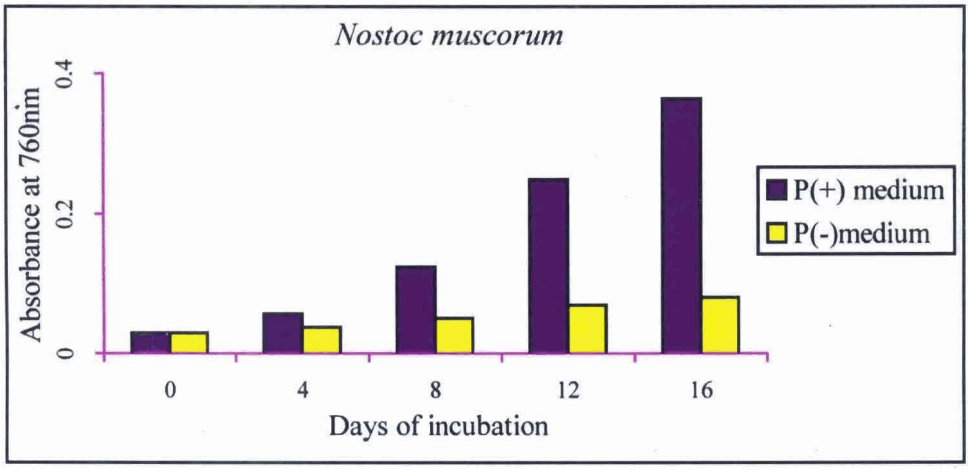
A study was also conducted on the performance of the above three species of cyanobacteria, *Nostoc linckia, Nostoc muscorum* and *Calothrix marchica* var. *intermedia* in the presence and absence of inorganic phosphates like K_2HPO_4. For this, 1 ml of homogenized suspension of each of the cyanobacterial strains at the exponential growth were inoculated in 10 ml of BG-11 N_2-free medium supplemented with and without (±) K_2HPO_4 (40 mgl^{-1}). The cultures were incubated at an illumination of 2000-3000 lux, L/D cycle of 14/10 hrs and temperature range of 26±2°C and were allowed to grow until they reached the post exponential or early stationary phase. The periodic growth rate was estimated in terms of growth in absorbance and chlorophyll *a* content.

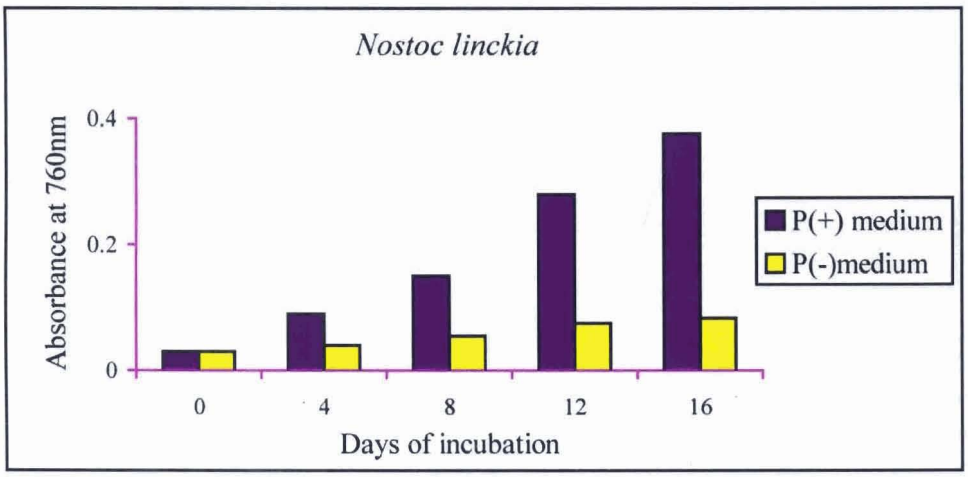
Results showed that all the three cyanobacterial species grew well in phosphate rich medium. That is, the growth was higher in K_2HPO_4 (40 mgl^{-1}) medium. Chlorophyll *a* content also showed a systematic increase in the phosphate rich (+) medium. *Calothrix marchica* var. *intermedia* (Fig. 47 C) grew better than *Nostoc* spp. (Fig. 47 A, B) with maximum recorded growth on the 16^{th} day of incubation.

In contrast, the growth was negligible in P depleted condition, where all the three species exhibited the 4 days lag period before a slight increase in growth. The growth was poor under P deficient condition and the cyanobacteria showed slight yellowish colouration during the incubation. The maximum growth yield under P depleted condition was observed in the order *Calothrix marchica* var. *intermedia* > *Nostoc linckia* > *Nostoc muscorum.* Growth in general was poor under P stress condition as it increased only 3 times of its initial inoculum absorbance value. Even this increase was due to the presence of the stored polyphosphate bodies in the cyanobacteria.

When the growth in terms of chlorophyll *a* content in P rich and P depleted conditions with respect to K_2HPO_4 (40 mgl^{-1}) was compared in all the three cyanobacterial species the growth yield observed on the day 16 in P rich condition was 4.8, 4.7 and 4.5 times higher than the P stress condition in *Calothrix marchica* var. *intermedia, Nostoc linckia* and *Nostoc muscorum* respectively (Fig. 48 A, B & C).

Fig. 47. Effect of K_2HPO_4 on the growth of different cyanobacterial species





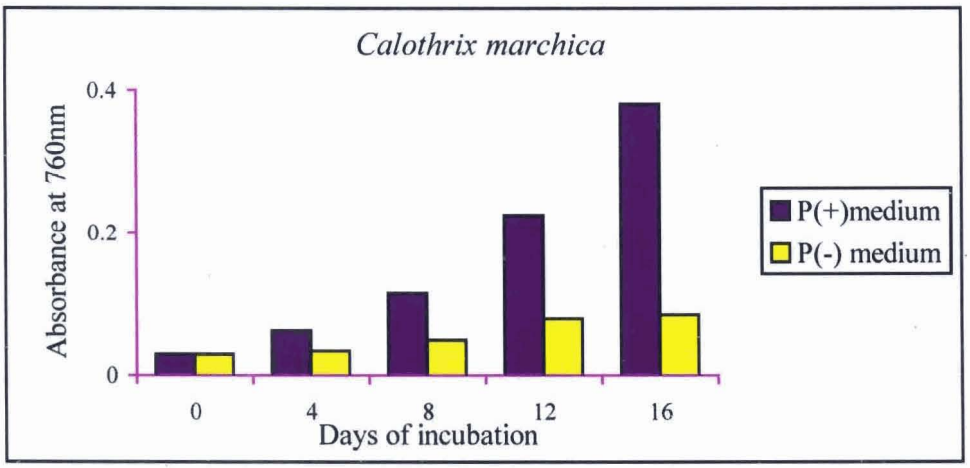
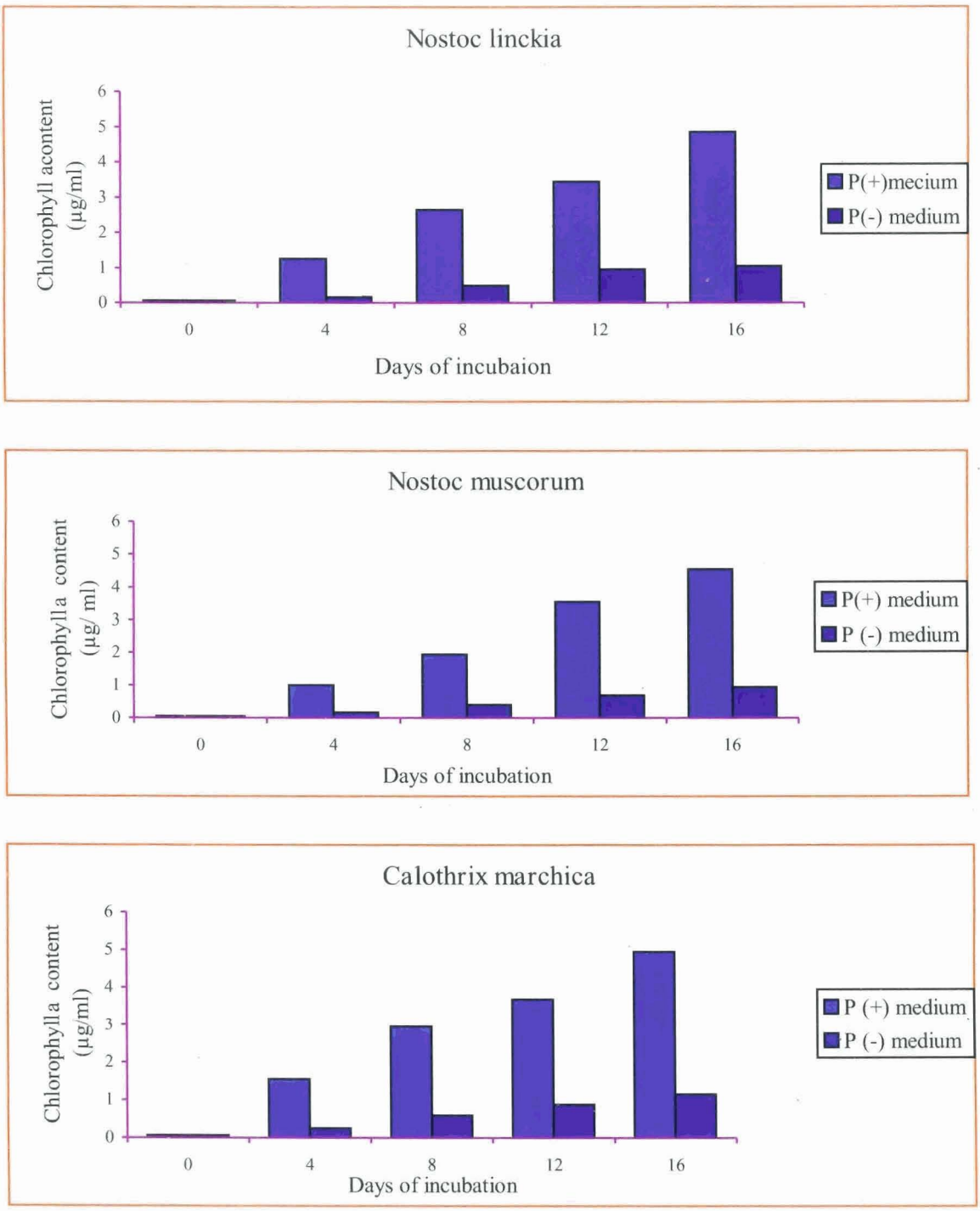


Fig. 48. Effect of KH_2PO_4 on the chlorophyll a content of different cyanobacterial strains



The P rich condition increased the heterocyst differentiation up to day 16 of incubation in *Nostoc* spp. Heterocyst differentiation was less in P depleted condition. While *Nostoc* spp. were able to form akinetes in their post exponential cultures, *Calothrix marchica* var. *intermedia* grew vegetatively without producing akinetes even on the day of 16 in P rich medium. An interesting feature noticed in P stress medium was that P deficiency, which checked the vegetative growth, was found to stimulate akinete formation in *Nostoc linckia* and *Nostoc muscorum.* The akinete produced in the cyanobacteria (*Nostoc* spp.) under P stress condition was numerous, less granulated with less potential to germination and subsequently disintegrated in cultures. This was in contrast to the akinete formed in P rich conditions in post-exponential cultures, which were found to be more granulated with high potential to germinate and liberate out healthy germlings.

Discussion:

Analysis of the above results shows that phosphorus is an essential macro element that is absolutely necessary for the growth and establishment of cyanobacteria. These photoautotrophs develop luxuriantly and rule on the water bodies with less N: P ratio. Several reports have revealed that phosphorus is an important factor affecting the growth and abundance of cyanobacteria in general (Sinclair and Whitton, 1977) and nitrogenase activity in particular (Rhee and Lederman, 1983; Gerber and Wickstorm, 1990; Oh *et al.*, 1991). It is the only macro element used along with cyanobacterial inoculum in biofertilizer technology (Roger and Kulasooriya, 1980). The findings of the present study are in agreement with the earlier reports (Meenakshee, 2001).

A reduction in growth, heterocysts production and chlorophyll *a* content that were noticed during P stress stage in the present investigation are agreeable with the previous reports on various cyanobacterial strains (Batterton and Van Baalen, 1968; Healey, 1973; Healey and Hendzel, 1975,1979; Marco and Orus, 1988). The P deficiency leads to a low level of ATP in the cell, which can be accounted for the retarded growth of the cyanobacterial strains (Carr and Whitton, 1982).

Cyanobacteria are unique in that they fix both CO_2 and N_2 at the expense of solar energy and reductant generated in the water splitting photochemical process. They are also capable of storing phosphate in the form of polyphosphate bodies. This ability of cyanobacteria to fix dinitrogen and store polyphosphate bodies is of great advantage for them under adverse or stress conditions (Fogg, 1975). Under N_2 fixing condition, the cyanobacteria appear to store P as sugar phosphates or poly phosphates (Kulaev and Vagabov, 1983; Cembella *et al.*, 1984) and also utilize P through different pathways under P rich and P limited conditions. Though growth was slow and poor in P stress conditions in all the three cyanobacterial species, the stored poly phosphate bodies present in these species, support some growth under P stress condition. The present findings agree with similar previous reports (Rhee, 1973,1980; Meenakshee, 2001). The growth performance of the three cyanobacteria in P depleted conditions, though generally poor, was varying. It was more in *Calothrix marchica* var. *intermedia* than in *Nostoc* spp. This is because the cyanobacterial species differed in their capacity to accumulate inorganic phosphate in the form of polyphosphate bodies and total cellular P content. The amount of chlorophyll *a* did not differ much in all the three cyanobacterial species under P depleted conditions indicating that the stored phosphate in the form of polyphosphate bodies may help the survival of cyanobacteria in water bodies like paddy fields, though, may not support a better growth.

Thus it can be concluded that the P supplementation in growth medium supported much better growth of cyanobacterial species showing the requirement of inorganic phosphate for their growth.

Effect of combined inorganic nitrogen source ($NaNO_3$):

Since the cultures showed a poor growth under P (-) condition, the growth performance of these strains by supplementing the medium with and without combined inorganic nitrogen source (NO_3^- medium) was studied. The three cyanobacteria were grown in BG 11 medium with 10 mM nitrate nitrogen ($NaNO_3$) in presence and absence of the inorganic phosphate source, K_2HPO_4 (40 mgl^{-1}). Nitrate supplemented BG-11 medium invariably supported better growth of all the three species of cyanobacteria under P rich condition. The

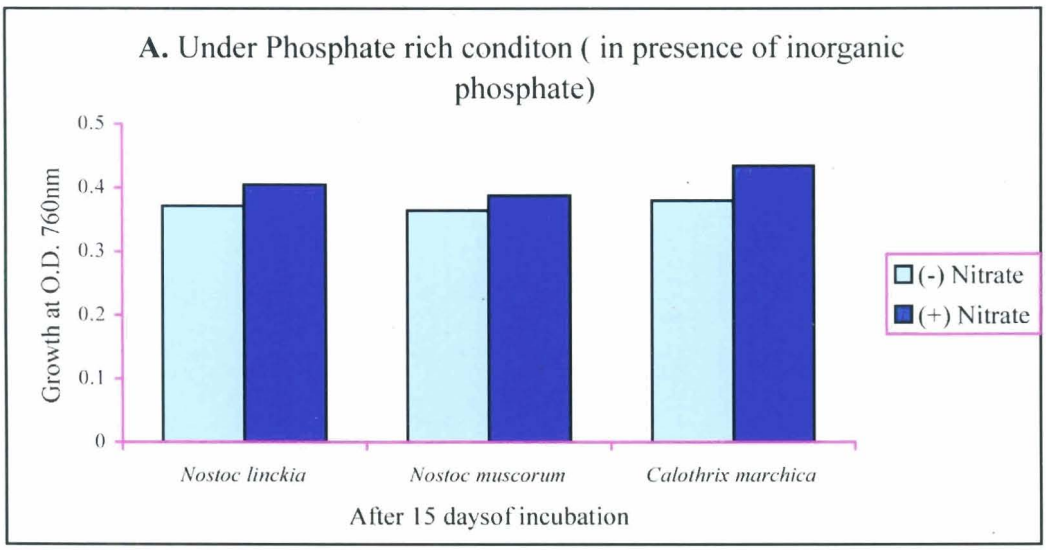
growth performance in terms of growth in absorbance (measured on the 16th day) of the three cyanobacteria were recorded in the order *Calothrix marchica* var. *intermedia* > *Nostoc linckia* > *Nostoc muscorum* (Fig. 49 A & B).

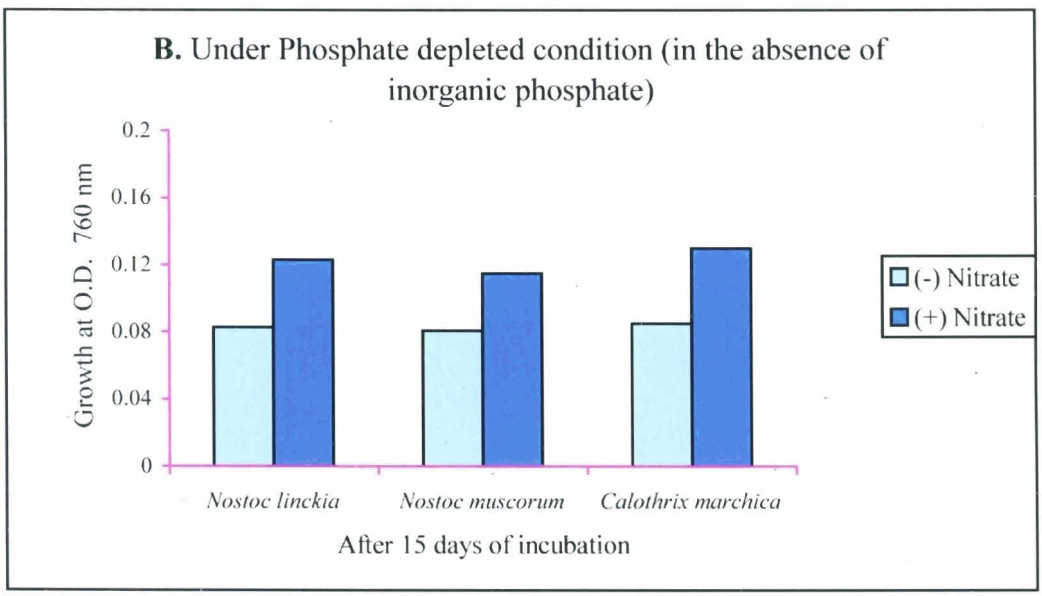
In P stress condition, the growth was, very much less than P rich condition. But, when supplemented with nitrate source, enhanced growth was shown. Though the performance of all the three cyanobacteria was generally poor, increased growth was exhibited by *Calothrix marchica* var. *intermedia* and minimum by *Nostoc muscorum.*

Discussion:

The results presented show that NO_3^- favoured the growth of all the diazo-trophic cyanobacterial species under both P rich and P depleted condition. Nitrate has been considered to be a preferred non-toxic source of combined nitrogen for most cyanobacteria (Stewart and Alexander, 1971; Meenakshee, 2001). The photosynthetic rate and nitrogenase activity decline with the reduction in the amount of phosphorus content. The addition of nitrogen helps the cyanobacterial species to compensate the loss of nitrogen due to reduced nitrogenase activity. The observed low-level development in cyanobacterial strains under P-deficient condition might be due to P-regulated task of the photosynthesis under diazotrophic conditions. Since the photosynthates under nitrogen-depleted condition supply the necessary energy (in the form of carbon sources) for nitrogenase activity, the overall growth is reduced further in P stress condition. In presence of combined nitrogen, the supply of energy to the heterocysts is reduced (combined nitrogen reduces heterocysts differentiation and nitrogenase activity) and thereby an increase in growth is noticed in both P rich and P stress conditions. Stewart and Alexander (1971) and Rhee and Lederman (1983) reported that there is a link between the nitrogenase activity and available phosphorus in cyanobacterial species. The increase in growth under P limited condition in presence of inorganic nitrogen source in the present findings also suggests the key process role of phosphorus in the diazotrophic mode of life of cyanobacteria.

Fig. 49. Effect of combined inorganic nitrogen on the growth of different cyanobacterial strains in presence and absence of inorganic phosphate, K_2HPO_4 (40mg/l^{-1}) in BG11 medium





Neemcake response:

The response of the above three cyanobacterial species to commercial neem cake was also studied. Varying concentrations of neemcake extract ranging from 0.2%-4% (0. 2%, 0.5%, 1%, 2%, 3%, 4%) were supplemented to BG11 (nitrogen free) medium in the culture and were incubated for a period of 25 days under culture conditions. Growth was estimated in terms of growth constant (K), chlorophyll *a* and protein content.

Table 10: Effect of Neemcake on the heterocysts frequency of two *Nostoc* species.

Cyanobacteria	Heterocysts frequency at different Neemcake concentrations after 20 days of incubation						
	0% Control)	0.2%	0.5%	1%	2%	3%	4%
Nostoc linckia	5.63 ± 0.33	6.3 ± 0.39	6.7 ± 0.31	7.1 ± 0.52	4.2 ± 0.14	2. 2 ± 0.1	-
Nostoc muscorum	5.8 ± 0.31	6.5 ± 0.41	7.2 ± 0.51	5.6 ± 0.35	4.0 ± 0.11	1.5 ± 0	-

Growth of *Nostoc linckia* increased with increase in the concentration of neem cake extract. In the first three weeks, maximum growth was recorded in culture medium amended with 0.2% - 0. 5% neem extract, but after that high growth rate, heterocyst frequency, chlorophyll *a* content and protein content were observed at 1% concentration. *Nostoc muscorum* exhibited maximum growth, heterocysts, chlorophyll *a* and protein content at 0.5% and after 0.5% a sharp decline was noticed and the growth was completely arrested after 2% (Table 10). *Calothrix marchica* var. *intermedia* on the other hand, showed increased growth rate up to 1% concentration. Further increase in concentration had an adverse effect. The cyanobacterium recorded high chlorophyll *a* content at 1% (w/v) concentration followed by a sharp decline. Algicidal dose of the extract was noticed from 2% concentration of neem extract. Growth was found

to be completely arrested at 2% and 3% concentration. When tested for cell protein at various life-supporting levels of neem cake extract (0. 2%- 2%), it showed increase in content in proportion to the growth at different concentration (Figs. 50,51 & 52).

Application of neem cake extract at varying concentrations showed distinct morphological changes in all the three cyanobacteria. The colour of the thallus at varying concentrations of the nutrient media amended with neem cake extract showed significant variations when compared with the control. In *Nostoc linckia* the colour of the thallus was pale blue-green in control, whereas, it became progressively intense (bright blue-green) in culture media having the neem cake concentrations up to 1% level and after 1% the colour became an unhealthy pale greenish yellow. *Nostoc muscorum* exhibited light olive green colour in control, bright green in varying levels of neem cake amended media up to 0.5 % and pale after 1%. In *Calothrix marchica* var. *intermedia* the colour of the thallus was found greyish-brown in control, dark brown in neem extract concentrations of the media up to 1% and yellowish in media having 2% and 3% neem cake extract. The change in colouration after 1% concentration of neem extract explains the harmful effect of neem cake extract on the growth of the cyanobacteria.

Filaments were found long and healthy with abundant heterocysts in both *Nostoc* spp. up to 1% neem cake extract and highest heterocyst frequency was observed in the media amended with neem extract of 1% and 0.5% respectively in *Nostoc linckia* and *Nostoc muscorum* . High mucilage content and clumping of the filaments were observed beyond 1% application of neem extract to the medium. In *Calothrix marchica* var. *intermedia* microscopic observation showed healthy growth, false branch and hormogone production at 1% concentration. Intercalary heterocysts were also noticed in 1% concentration of neem extract. The treatment of neem cake extract above 1% exhibited fatal plasmolysis of the filaments. The cells of the filaments were found withdrawn from their sheath and were highly shrunken with lumps of protoplast. The whole cyanobacterium had turned yellow.

Fig. 50. Neemcake response
Growth in terms of Growth constant (K)

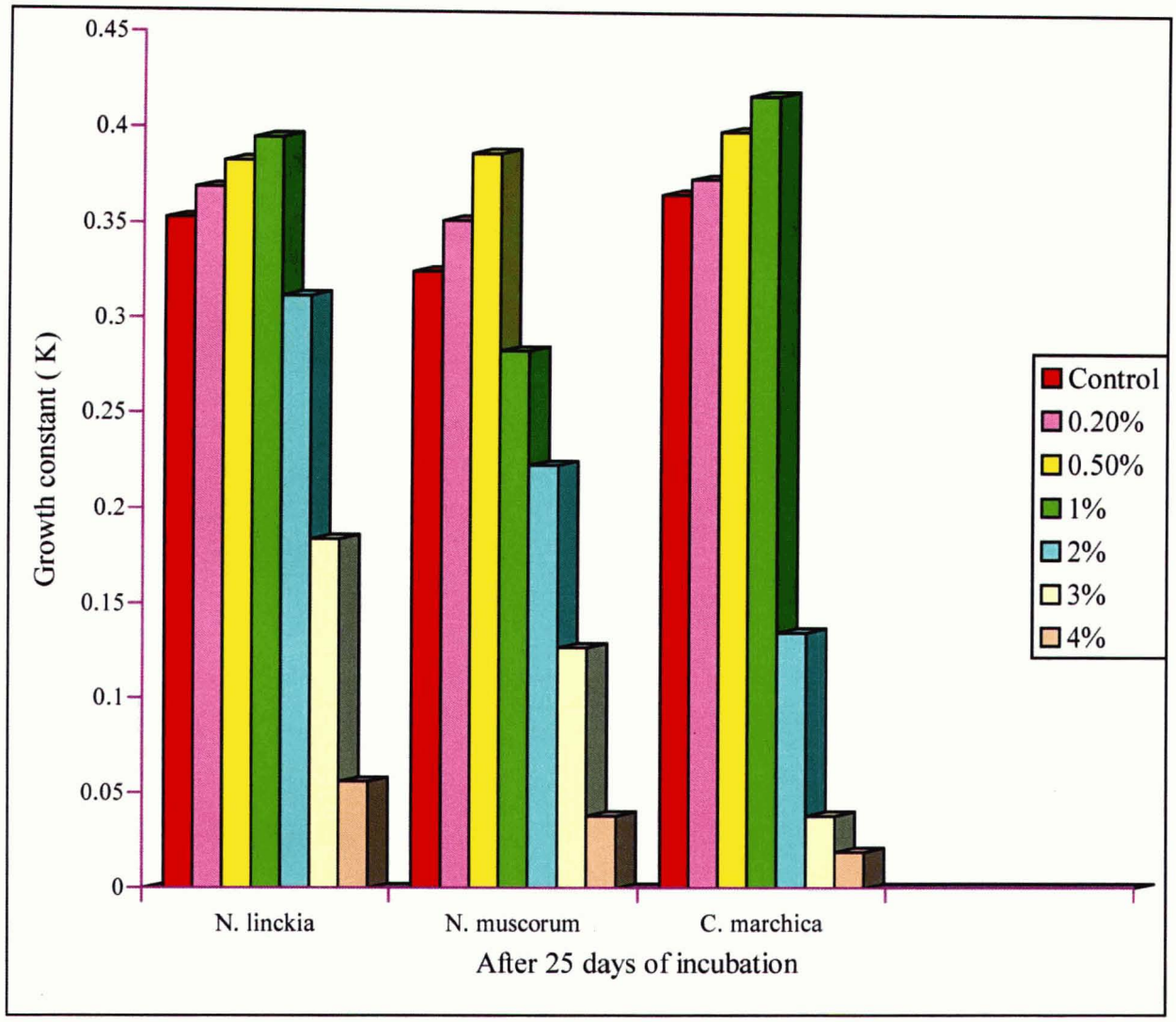


Fig. 51. Neem cake response
Growth in terms of Chlorophyll a content

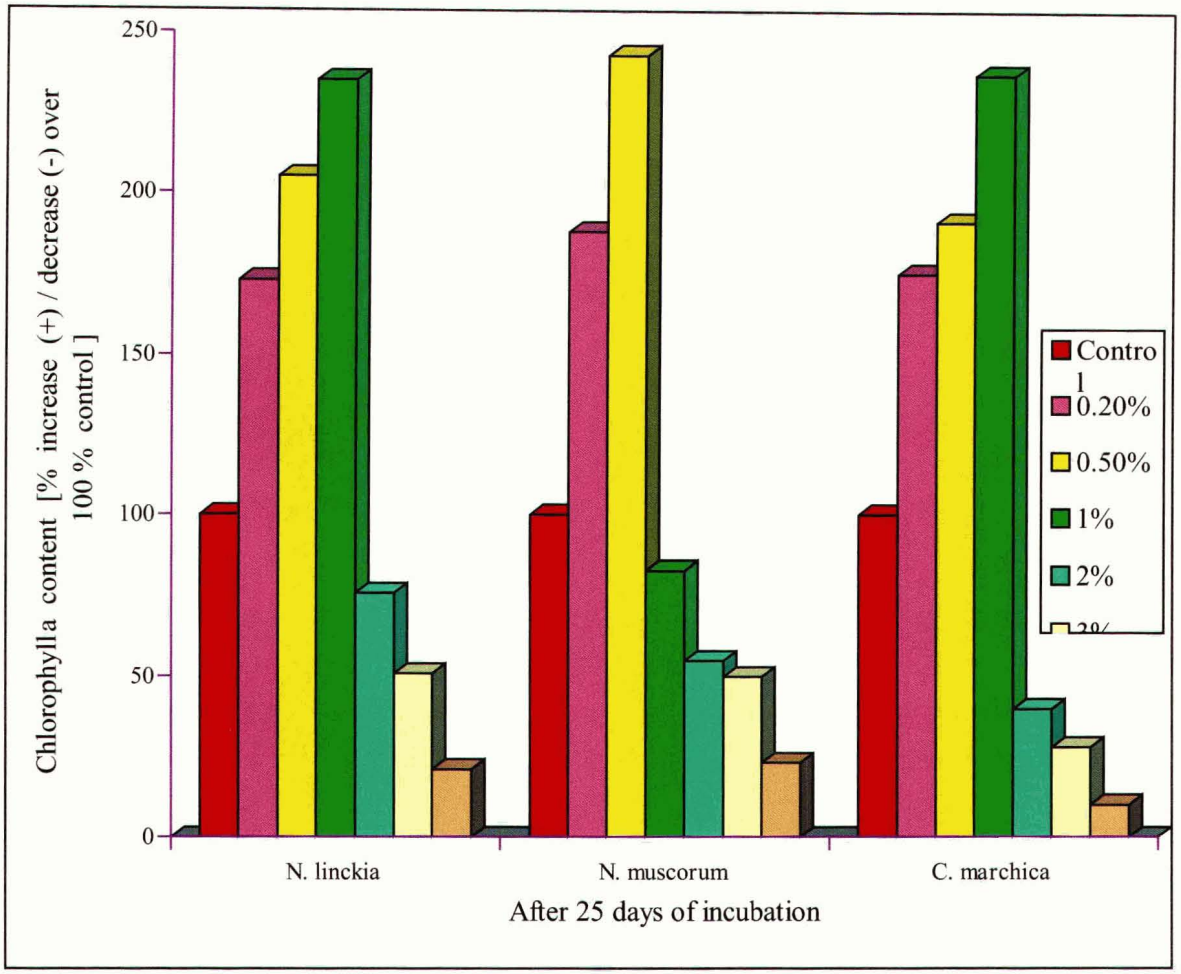
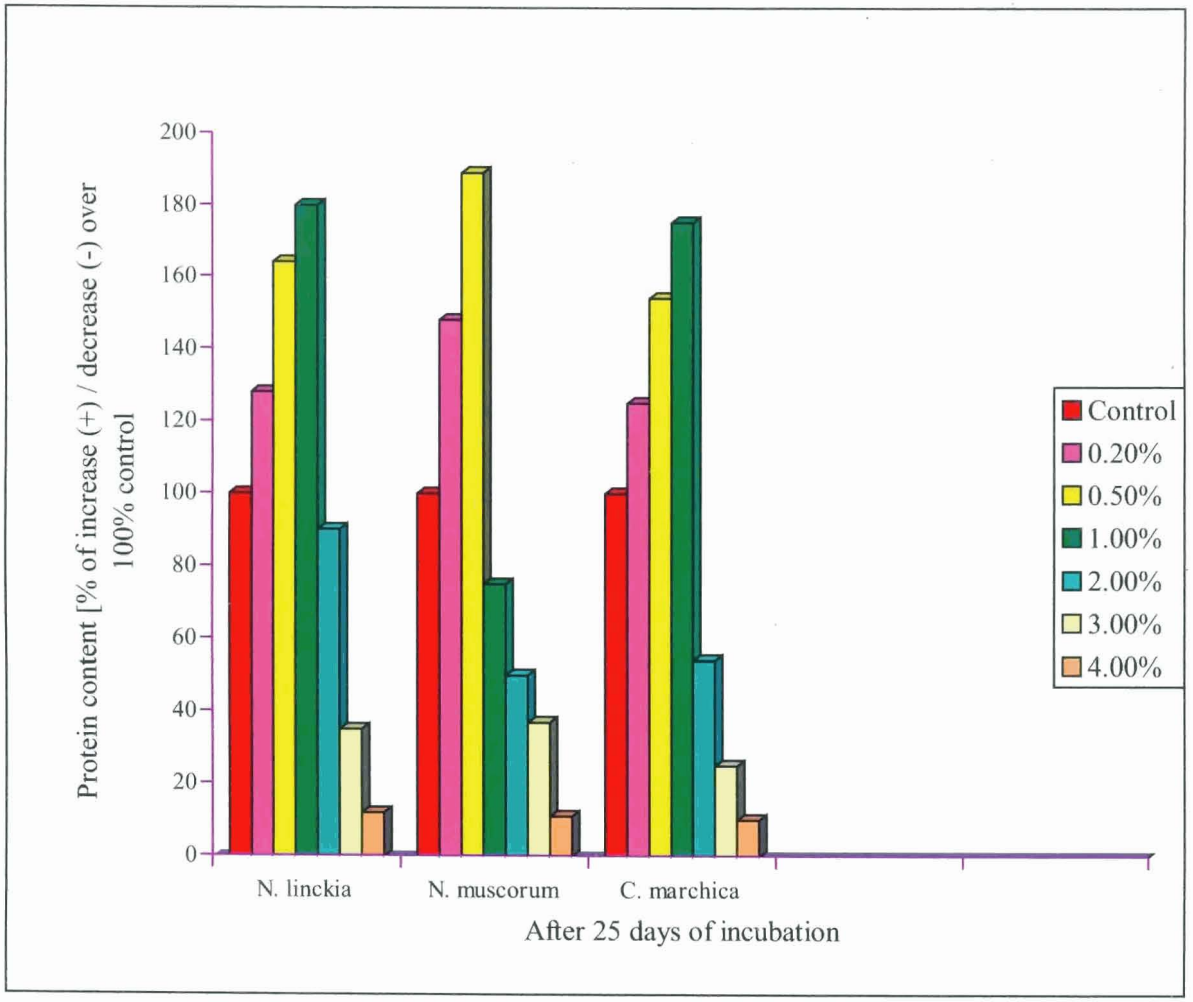


Fig. 52. Neemcake response
Growth in terms of Protein content



Discussion:

The results showed varying response of the three cyanobacteria to neemcake. All the species grew well in lower concentrations of neem cake, i.e. up to 0.5%. Two species *viz., Nostoc linckia* and *Calothrix marchica* var. *intermedia* - exhibited maximum growth at 1%. This increase in growth, chlorophyll *a* and protein content indirectly suggests the nitrogen fixing potential of the species, which in turn is exploited in the biofertilizer producton.

From the above results, it is seen that neem cake acted as a good activator and energizer for cyanobacterial growth. Reports of Aziz *et al.* (1981), Kannaiyan and Sundaravarathan (1998) also support the above findings. Neem cake application increased the chlorophyll *a* content of all the three cyanobacterial strains, which indirectly increased their nitrogenase activity. In cyanobacteria, photosynthesis is the ultimate source of all the ATP and reductant for nitrogenase activity and there is a close relationship between these two processes (Peters, 1976). The strong interaction between photosynthesis and nitrogen fixation was also demonstrated by the active spectrum for nitrogenase–catalyzed C_2H_2 reductase (Peters, 1976). Later works also support this view (Mishra and Adhikary, 1997; Kannaiyan and Sundaravarathan, 1998). The biomass increase of these cyanobacteria in neem cake extracts over control may be due to its high nutrient content. Neem cake has been reported to be a good manure (Devkumar and Goswami, 1992). It reduces the loss of N_2-fertilizer by preventing the population of nitrifiers (Reddy and Prasad, 1975; Chhonker and Mishra, 1978; Subbaiah and Kothandaraman, 1980; Devkumar and Mukherjee, 1985). It also increases the population of cyanobacteria in flooded soil (Aziz *et al.*, 1981; IRRI, 1982). Recommended field application dose of neem cake is 120 kg / ha which will be equal to 0.02% (200ppm) in the waterlogged soil. Neem cake blended with urea increase the uptake of 'N', 'P' and 'K' (Harishankar and Rathi, 1976).

In the present study neem cake extract did not show any adverse effect even up to 0.5% - 1% (w/v), rather increased the biomass of the cyanobacteria, *Nostoc muscorum*, *Calothrix marchica* var. *intermedia* and *Nostoc linckia*

respectively, suggesting that using neem cake for crop purpose will not adversely affect the growth and establishment of the free living cyanobacteria of rice fields, instead, augment nitrogen utilization efficiency by reducing denitrifiers and help pest management by controlling pests. Similar reports already available (Mishra and Adhikary, 1997) support the findings in the present study. Neem cake at 500 kg ha^{-1} significantly reduced pest(s) problem in *Azolla pinnata* (Nandabalan and Kannaiyan, 1984).

Neem application repeatedly favours cyanobacterial growth in wetland soils by serving as a good pesticide (Grant *et al.*, 1985). Plant pesticides such as neem seeds can control invertebrate grazers, which in turn favour, establishment of nitrogen fixing population. Dominic (1997) observed that in neem treated soils of Sandy Alluvium and Kayal fields, *Anabaena* group became dominated by 7^{th} day itself and the group continued the dominance for the entire period of experiment due to inhibition of grazers. The success of neem cake to perform its role as a stimulating agent is due to the dual role performed by the neemcake – as a manure and as a pesticide (Kannaiyan *et al.*, 1983; Kannaiyan and Sundaravarathan, 1998). The present study also asserts the role of neemcake as a good tonic and pesticide for cyanobacterial growth. To sum up, application of neem and neem products in rice cultivation supplements the biomass of both rice and cyanobacteria. This will boost the nitrogen utility and fertility and will provide sound pest management at a very low cost.

Fig. 53. Carbofuran response
Growth in terms of Absorbance at 760nm

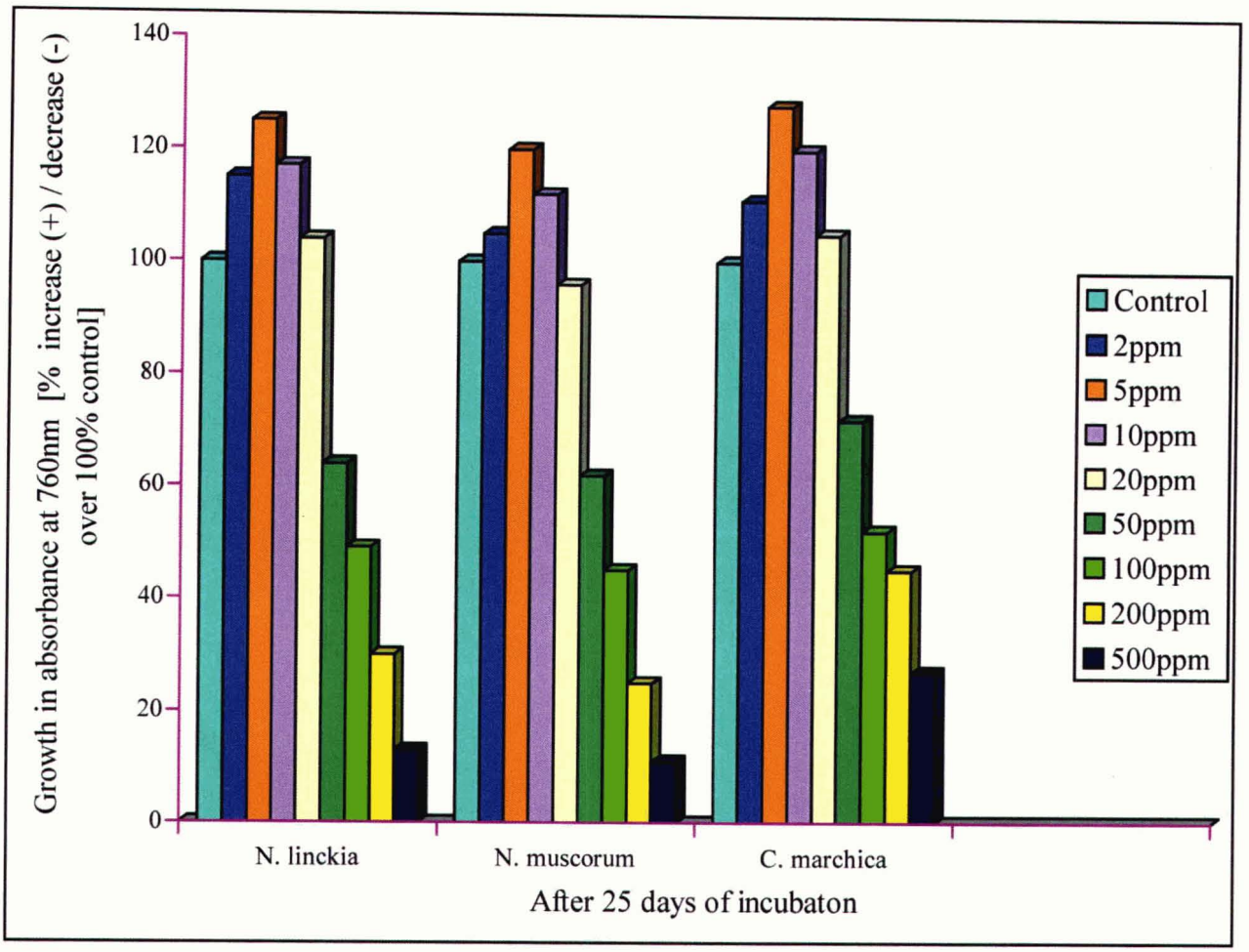


Fig. 54. Carbofuran response
Growth in terms of Chlorophyll *a* content

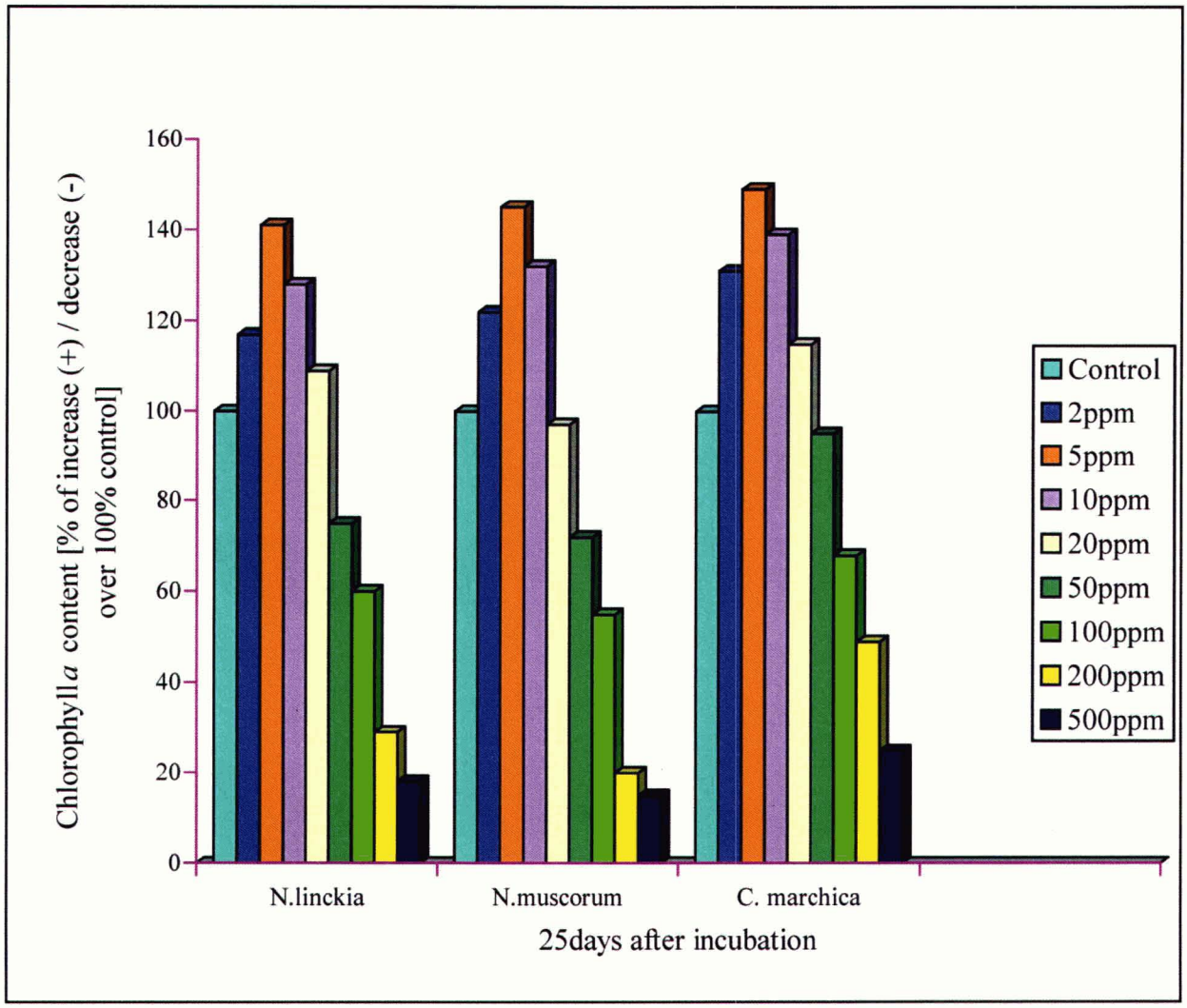
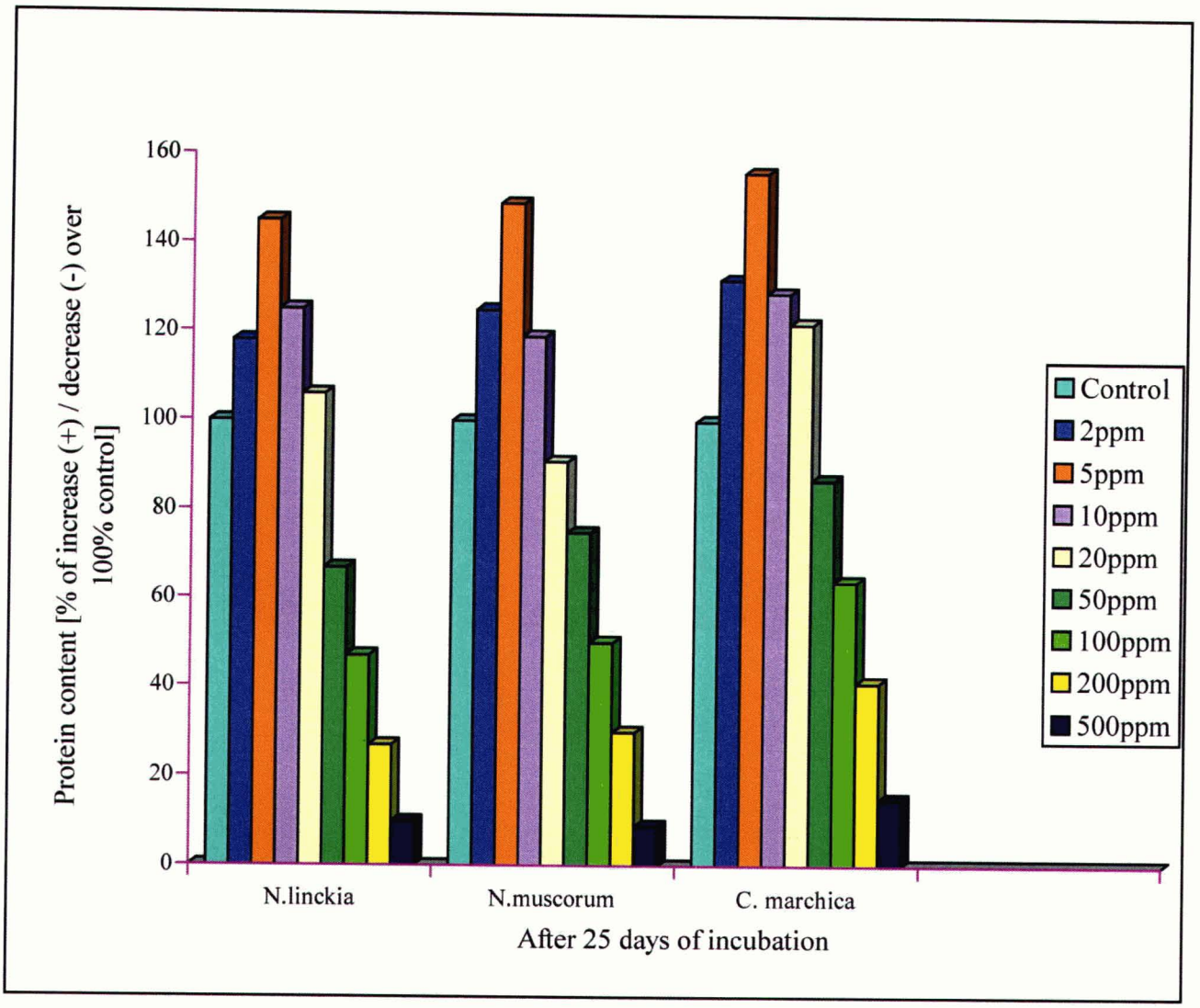


Fig. 55. Carbofuran response
Growth in terms of Protein Content



Carbofuran (Furadan) response:

Carbofuran (furadan) is a carbamate insecticide, which offers a broad range of toxicity to insect-pests. The three cyanobacterial species, *viz.*, *Nostoc linckia, Nostoc muscorum* and *Calothrix marchica* var. *intermedia* were tested for their response to the pesticide, Carbofuran (furadan). For this, the three cyanobacterial species were grown in BG-11 N_2 free medium amended with desired concentration of furadan ranging from 2 ppm - 500 ppm (2, 5, 10, 20, 50, 100, 200 and 500 ppm) for a period of 25 days under culture conditions and their growth rate were compared with that of control in terms of growth in absorbance at 760 nm, chlorophyll *a* content and protein content.

Growth in absorbance of *Nostoc linckia* increased with increase in the concentration of furadan level up to 20 ppm followed by a tolerance up to 100 ppm. The cyanobacterium showed higher growth rate at furadan concentration levels 2-20 ppm than control, with maximum growth rate at 5 ppm. It exhibited tolerance to furadan after 20 ppm and its growth was comparatively less than the control. After 100 ppm growth was completely arrested. *Nostoc muscorum* and *Calothrix marchica* var. *intermedia* also showed a stimulatory growth effect up to a furadan concentration of 10 ppm and 20 ppm respectively than control with high growth record at 5 ppm. While *Nostoc muscorum* tolerated the furadan range up to 100 ppm, *Calothrix marchica* var. *intermedia* tolerated up to 200 ppm (Fig. 53).

The chlorophyll *a* content was found increasing up to 20 ppm furadan level in *Nostoc linckia* and *Calothrix marchica* var. *intermedia* whereas in *Nostoc muscorum* the increase was noted up to 10 ppm only. In all the three species maximum chlorophyll *a* content was recorded at 5 ppm furadan amended BG -11 nitrogen free medium. The protein estimation also revealed a maximum content at 5 ppm level of furadan in all the three species (Fig 54). But an increase in protein content was noticed in furadan-amended media than in control up to 10 ppm in *Nostoc muscorum* and up to 20 ppm in *Nostoc linckia* and *Calothrix marchica* var. *intermedia* (Fig. 55).

Discussion:

Analysis of the above results reveals that furadan application is stimulatory in all the three experimental cyanobacterial species up to 10-20 ppm ranges with a maximum growth results at 5 ppm. The application of furadan and other pesticides affects cyanobacteria under laboratory conditions differently (Gangawane and Saler, 1979; Anand and Veerappan, 1980; Kannaiyan, 1985; Gangawane, 1990; Rath and Adhikary, 1994 and Padhy *et al.*, 2001). Accordingly the pesticides are classified as stimulatory, inhibitory and ineffective (Metting, 1985). These pesticides show their differential effect in various metabolic processes of cyanobacteria (Das and Singh, 1977; Zargar and Dar, 1990). Mostly they are stimulatory at lower concentrations (Anand and Subramanian, 1997). Sometimes, at the higher rate, carbofuran significantly affected the development of the pests and acted as a growth promoter for *A. pinnata* (Satapathy and Singh, 1987).

In laboratory and field conditions the addition of 10 µg carbofuran per gram dried soil had no inhibitory effect on the mineralization of native soil nitrogen. The addition of up to 15 ppm carbofuran had no effect on the acetylene-reduction activity of *Gloeotrichia sp.*, but 20 ppm caused a significant lowering of that activity (Tirol *et al.*, 1981). A significant enhancement in cyanobacterial growth, water-soluble pigments and carbohydrates was, however, observed by them at 50 ppm concentration of the pesticide. Higher doses (500 -1000 ppm) reduced the chlorophyll a content and a progressive decrease in cyanobacterial growth (Ahluwalia *et al.*, 1998). In the present investigation a stimulatory effect was noticed in all the three cyanobacteria on applying furadan up to 10 – 20 ppm ranges with a maximum growth at 5 ppm. The growth declined at higher doses of furadan (200 -500 ppm) and the chlorophyll *a* content and protein content reduced sharply.

Variation in the tolerance level to a specific pesticide existed among species of the same genus and also between genera. When compared for their response, in terms of growth and metabolic activities, to the application of the insecticide carbofuran, the micro alga *Chlorella vulgaris* and the

cyanobacterium *Nostoc muscorum* were found more sensitive than *Nostoc linckia* (Megharaj *et al.*, 1993). It is also reported that recommended field rates of the insecticide did not generally affect growth and nitrogen fixation of the cyanobacteria (Pandher *et al.,* 1995). Transmission electron microscopy of the cultures *Chlorella vulgaris* and the diazotrophic cyanobacteria *Nostoc linckia* and *Nostoc muscorum*, all isolated from a rice soil, exposed to 50 µg carbofuran/ml showed certain cellular abnormalities, indicating interference of the insecticide with membrane properties. The significant toxicity of the insecticide (at 20 and 50 µg/l) to nitrogenase activity in *Nostoc linckia* was reversed by the addition of ATP at 10 µM (Megharaj *et al.*, 1993). There was limited evidence that the application of pesticides promoted the development of cyanobacterial populations in the absence of readily available N_2 (Pipe, 1992). *Lyngbya major, Gloeocapsa atrata, Calothrix parietina* and *Scytonema pascheri,* all of which have well-defined sheaths, survived in the soil in the presence of 1000, 500 and 250 ppm of the carbamate, organophosphate and organochlorine pesticides, respectively, where other cyanobacterial forms could not (Jayanti *et al.*, 1992). Though some of the species belonging to the genera *Calothrix* and *Scytonema*, which possess a sheath layer around their trichome, were comparatively tolerant to the pesticides, the presence of additional cell envelope layers was not always the only factor responsible for their tolerance to higher levels of the pesticides (Das and Adhikary, 1996). In the present investigation also, it was found that *Calothrix marchica* var. *intermedia* performed better than the two *Nostoc* species, may be due to possession of a firm sheath around their trichome. Yet, its performance was not that much excellent, pointing out at some factors in addition to sheath that may have a role for the cyanobacterial tolerance to high doses of pesticides.

Carbofuran has been used extensively in India to fight *Nilaparvata lugens* causing harm to paddy cultivation. Under flooded conditions, 2 kg/ha significantly increased cyanobacterial populations for 20 days. In general, filamentous cyanobacteria were favoured by carbofuran application. Unicellular forms appeared especially in flooded soil samples when carbofuran was applied only at 0.5 kg/ha. Concentrations up to 20 and 50 µg/ml of carbaryl and

carbofuran, respectively, initially increased chlorophyll *a* in *Nostoc linckia* significantly, which again led to a subsequent inhibition (Megharaj *et al.*, 1988,1989).

Fertilizer response

The response of the three cyanobacterial species *Nostoc linckia, Nostoc muscorum* and *Calothrix marchica* var. *intermedia* to various fertilizers like urea, potash and super phosphate was analysed in the study. For this 1 ml of the homogenized suspension of either strain was grown in 10 ml of BG-11 N_2 free medium and medium amended with either type of fertilizers in desirable concentrations, *viz.*, (0.01, 0.05, 0.1, 0.5, 1.0, 2.0, 3.0 mg/ml) in a temperature (25±1°C) and light (L/D cycle 14/10 hours, light intensity 2000 lux) controlled culture room for a period of 15 days. Growth measurements were taken in terms of chlorophyll *a* content.

Of the three fertilizers tested, urea exhibited the least stimulatory effect on cyanobacterial growth. All the three cyanobacterial strains recorded growth higher than control in urea at a concentration of 0.01 mg/ml only, and further urea concentrations after 0.01 mg/ml were inhibitory. All the three strains tolerated the urea range up to 0.1 mg/ml. After that growth was completely arrested (Table 11).

The sensitivity to potash was found varying. All the species grew well in potash up to a concentration of 0.1 mg/ml and their growth were higher than that in control. The growth was inhibited from 0.5 mg/ml to the end (3 mg/ml). Yet the cyanobacterial species tolerated a wide range of potash level (Table 12). The *Nostoc* spp. were more resistant to potash fertilizer than *Calothrix marchica* var. *intermedia*.

The growth of the cyanobacterial species was slightly higher or equal to that of control at 0.01 mg/ml of super phosphate. After that the growth of the species was lower than that of the control and a concentration after 2 mg/ml arrested the growth totally (Table 13).

Microscopic observations were made for all the three species to observe the effects of fertilizers on the morphological features of different strains. The

various morphological parameters observed were the various cellular changes like size, shape and lysis of the cells, heterocyst differentiation (Table 14) and sporulation induction, *etc.* By observing the strains thoroughly on these basis the suitable concentration for survival and the toxic concentration of each cyanobacterium was determined.

Table 11: Effect of different levels of Urea on the growth of three different cyanobacterial species (Growth was measured in terms of chlorophyll *a* content).

Cyanobacterial species	Initial chloro-phyll *a* content	Chlorophyll *a* content (µg/ ml) after 15 days of incubation						
		Control	Urea mg/ ml					
			0.01	0.05	0.1	0.5	1.0	2.0
Nostoc linckia	0.069	4.45 ± 0.26	4.99 ± 0.33	2.34 ± 0.13	1.05 ± 0.01	0	0	0
Nostoc muscorum	0.068	4.30 ± 0.23	4.43 ± 0.28	2.05 ± 0.12	0.870 ± 0.01	0	0	0
Calothrix marchica var. *intermedia*	0.067	4.93 ± 0.25	5.10 ± 0.41	2.95 ± 0.11	0.450 ± 0.04	0	0	0

Table 12: Effect of different levels of Potash on the growth of three different cyanobacterial species (Growth was measured in terms of chlorophyll *a* content).

Cyanobacterial species	Initial chloro-phyll *a* content	Chlorophyll *a* content (µg/ ml) after 15 days of incubation							
		Control	Potash mg/ ml						
			0.01	0.05	0.1	0.5	1.0	2.0	3.0
Nostoc linckia	0.069	4.45 ± 0.26	4.79± 0.29	4.96± 0.35	5.2± 0.30	3.95± 0.2	2.75 ± 0	2.01 ± 0.12	1.35 ± 0.08
Nostoc muscorum	0.068	4.30 ± 0.23	4.65± 0.32	4.85± 0.34	5.02± 0.29	3.56± 0.11	2.48 ± 0.1	1.75 ± 0	0.52 ± 0.05
Calothrix marchica var. *intermedia*	0.067	4.93 ± 0.25	5.2± 0.31	5.55± 0.39	5.75± 0.41	4.01± 0.18	2.0 ± 0	0.80 ± 0.08	0.15 ± 0.02

Table 13: Effect of different levels of Super Phosphate on the growth of three different cyanobacterial species. (Growth was measured in terms of chlorophyll *a* content).

Cyanobacterial species	Initial chlorophyll *a* content	Chlorophyll *a* content (µg/ml) after 15 days of incubation						
		Control	Super phosphate mg/ ml					
			0.01	0.05	0.1	0.5	1.0	2.0	3.0
Nostock linckia	0.069	4.45 ± 0.26	4.61 ± 0.29	3.72 ± 0.21	2.52 ± 0.11	1.5 ± 0.08	0.75 ± 0.05	0.30 ± 0.02	0.08 ± 0.01
Nostoc muscorum	0.068	4.30 ± 0.23	4.40 ± 0.28	3.53 ± 0.18	2.19 ± 0.09	0.75 ± 0.05	0.55 ± 0.04	0.1 ± 0.02	-
Calothrix marchica var. intermedia	0.067	4.93 ± 0.25	5.05 ± 0.41	1.57 ± 0.08	0.55 ± 0.04	0.05	-	-	-

Table 14: Heterocysts differentiations in two *Nostoc* species under various fertilizer applications.

Cyanobacteria	*Control*	Urea (mg/ ml)	Potash (mg/ml)				Super phosphate (mg/ml)			
		0.01	0.01	0.1	.5	1.0	0.01	0. 1	0.5	1
Nostoc linkia	5.66 ± 0.33	6.0 ± 0.55	6.1 ± 0.50	6.6 ± 0.57	6.4 ± 0.51	6.0 ± 0.48	5.9 ± 0.48	7.1 ± 0.68	6.8 ± 0.61	6.0 ± 0.51
Nostoc muscorum	5.81 ± 0.31	6.2 ± 0.51	6.3 ± 0.48	6.7 5± 0.51	6.45 ± 0.52	6.2 ± 0.51	6.1 ± 0.46	7.3 ± 0.61	7.1 ± 0.58	6.1 ± 0.5

The cells were healthy and comparatively larger in size in all the three species especially in *Nostoc muscorum* in concentrations 0.01 mg/ml, 0.1 mg/ml and 0.01 mg/ml in fertilizers, in the order urea, potash and super phosphate. The thalli were bright blue green in *Nostoc* spp. and intensely brownish green in *Calothrix marchica* var. *intermedia.* The filaments were longer and false branches were observed in *Calothrix marchica* var. *intermedia.* Heterocyst differentiation was higher than control in *Nostoc* spp. Akinete formation was noticed in *Nostoc* spp. at 0.05 mg/ml urea and 0.1 mg/ml super

phosphate and 0.5 - 2 mg/ml in potash. In higher concentrations of these fertilizers the cells were abnormally larger in size and the filaments were highly coiled or shorter. Hormogones were seen in *Calothrix marchica* var. *intermedia* on increased application of fertilizers.

Discussion:

The present investigation showed that all the three cyanobacteria were found to prefer lower concentrations of the chemical fertilizers for their proper growth and functioning to higher concentrations. Various reports reveal the toxic effect of fertilizers, if used at higher doses (Anand and Karuppusamy, 1987). Reduced doses of application of fertilizers induce overall growth and enhance the efficiency of nitrogen fixation by cyanobacteria (Venkataraman, 1981d).

Fertilizer response reports reveal that phosphorus is an important nutrient affecting the growth, establishment and biomass production of cyanobacteria in general (Sinclair and Whitton, 1977) and nitrogenase activity in particular (Oh *et al.*, 1991). Phosphorus is the only nutrient used to increase the growth of native cyanobacterial strains as biofertilizer agents (Roger and Kulasooriya, 1980). In the present investigation enhanced growth was observed in all the three cyanobacterial species by the application of 0.01 mg/ml of super phosphate. This phosphate application is in addition to the phosphate component in the BG-11 medium, *i.e.*, K_2HPO_4 (0.04 mg/ml).

Studies showed that application of urea has an inhibitory effect on N_2 fixing cyanobacterial flora in low land rice fields (Roger and Watanabe, 1984). A very feeble stimulatory effect is noticed on application of 0.01mg/ml urea in laboratory conditions.

Cyanobacteria are known to exhibit a wide range of morphological variations especially in increased concentration of fertilizer application (Anand and Gunaseeli, 1978). Singh (1975) reported that application of potash and super phosphate did not suppress heterocyst differentiation and thereby favoured nitrogenase activity. While the non-nitrogenous fertilizers, superphospate and potash stimulated the growth and nitrogenase activity of the cyanobacteria; the nitrogenous fertilizer suppressed the growth and nitrogenase

activity (Anand, 1990a; Anand and Parameswaran, 1992). Application of potassium in the form of muriate of potash even at high dozes of 100 Kg/ha did not show any stimulatory or inhibitory effect on the growth and N_2 yield of cyanobacteria under field conditions (Singh and Bisoyi, 1989). The nitrogenase activity was significantly inhibited by the application of inorganic nitrogen fertilizers like ammonium sulphate (Anand and Radha, 1984), prilled urea (Sarma and Khattar, 1994) and farmyard manure (Singh and Dash, 1994). The results of the present investigations are in agreement with the early findings and support the cyanobacterial efficiency at lower doses of fertilizer application.

SUMMARY AND CONCLUSIONS

Umamaheswari N. A. "Isolation and Characterisation of Nostocales (Cyanobacteria) of the Paddy fields of Kerala " Thesis. Department of Botany , University of Calicut, 2005

Summary & Conclusions

SUMMARY AND CONCLUSIONS

The present work is on cyanobacteria belonging to the order Nostocales (*sensu* Desikachary, 1959) of Kerala. The Nostocales are characterized by filamentous plant body, presence of heterocysts, akinetes, hormogones, absence of true branching and presence of false branching.

The field study was conducted in all the eight types of paddy field soils of Kerala known as 1. Karappadom (pH 4.35), 2. Kari (pH 3.61), 3. Kayal (pH 4.18), 4. Kole (pH 4.56), 5. Laterite (pH 5.17), 6. Pokkali (pH 3.51), 7. Poonthalpadom (pH 7.74) and 8. Sandy alluvium (pH 5.04). Except for Poonthalpadom all the fields are acidic in nature. Poonthalpadom field is alkaline with a pH of 7.74.

Visible masses of cyanobacteria were collected from all the eight types of paddy fields of Kerala by conducting field trips. The nature of habitat and habit of the thallus (colony) were noted. The fresh samples were divided into two parts. One part was preserved in 4% formalin and used for identification of species and the remaining part was used for isolation and subsequent culturing of unicyanobacterial isolates. The cyanobacteria are identified with the help of literature and experts. Keys for the identification of families, genera and species are provided.

Culturing of heterocystous and non-heterocystous cyanobacterial species were done using BG11 N_2 ± medium. After selecting the suitable growth medium (BG11 N_2 free medium) for maintenance under laboratory conditions the following experiments were carried out.

1. Elucidation of growth and thallus characteristics of some selected nitrogen-fixing strains of cyanobacteria in solid and liquid media.
2. Analysis of response of three selected nitrogen fixing strains (*Nostoc linckia, N. muscorum* and *Calothrix marchica* var. *intermedia*) to various parameters like **a**. pH response, **b**. Acid tolerance, **c**. Salinity tolerance, **d**. Response to exogenous sugars, **e**. Autotrophic, mixotrophic and hetertrophic nutrition in presence of glucose and sucrose, **f**. Response to inorganic phosphate, **g**. Response to inorganic phosphate and combined

nitrogen, **h**. Neemcake response, **i**. Carbofuran response, **j**. Response to various fertilizers like urea, super phosphate and potash.

Results: Floristic analysis

Based on the taxonomic study the richness of cyanobacteria in the waterlogged paddy fields has been brought out.

- 111 species of cyanobacteria belonging to the order Nostocales distributed in different paddy fields of Kerala (belonging to eight soil types), under 17 genera, *viz.*, *Spirulina, Oscillatoria, Geitlerinema, Phormidium, Lyngbya, Microcoleus, Nostoc, Anabaena, Cylindrospermum, Aulosira, Plectonema, Scytonematopsis, Scytonema, Tolypothrix, Calothrix, Gloeotrichia and Rivularia* and four families (Oscillatoriaceae, Nostocaceae, Scytonemataceae and Rivulariaceae), were identified and treated systematically.
- *Oscillatoria* of family Oscillatoriaceae and *Nostoc* of family Nostocaceae were found to be the dominant genera of the two classes of Nostocales, non-heterocystous and heterocystous respectively.
- The study showed that 13 species are seen in all soil types /paddy fields of Kerala.
- Heterocystous and non-heterocystous members were almost equal in occurrence, but non-heterocystous were at slightly higher level to heterocystous.
- Of all the eight soil types/fields considered in this study, the Laterite fields showed maximum cyanobacterial diversity followed by Poonthalpadom fields, the only soil type where alkalinity is observed.
- Karappadom, Kari and Kayal fields showed poor cyanobacterial diversity.

Findings under laboratory conditions

- Majority of cyanobacteria preferred BG-11 N_2 free medium. However, *Cylindrospermum muscicola* preferred Fogg's medium. *Calothrix marchica* var. *intermedia* grows well in both media.
- *Nostoc linckia* was found to survive a wide range of pH (5-9); *Nostoc muscorum and Calothrix marchica* were observed to prefer narrow alkaline and acidic to neutral media respectively for their optimum growth.
- *Nostoc linckia and Calothrix marchica* can be used as biofertilizer in acid sulphate soils of Kerala since they can tolerate low pH.
- With regard to salinity, the order of tolerance to NaCl was *Nostoc muscorum* (0.3M) > *Nostoc linckia* (0.2M) > *Calothrix marchica* (Zero tolerance).
- All the three species are primarily autotrophic but capable of adapting to mixo/ heterotrophic mode of nutrition by utilizing the carbon source from external medium, if available.
- The growth of cyanobacteria was found to be optimum under P-rich condition and was poor in P-stress condition. However, supplement of combined nitrogen in the form of nitrates enhances further growth.
- Application of low quantity of neemcake (O.5-1.0%), carbofuran (5ppm) and fertilizers [urea and super phosphate (0.01mg/ml) and potash (0.1mg/ml)] stimulates growth and does not cause any harm to these cyanobacteria. However, in higher concentrations these are inhibitory.

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GROWTH PATTERN AND MORPHOLOGICAL ADAPTABILITY OF CERTAIN CYANOBACTERIA (NOSTCALES) TO VARIOUS pH LEVELS.

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ABSTRACT

pH response of three species of cyanobacteria belonging to *Nostocales* was investigated based on growth, chlorophyll-a content and protein content. *Nostoc linckia*, tolerated a wide range of pH, *Nostoc muscorum* preferred an alkaline pH to acidic while *Calothrix marchica* responded to an acidic pH. *Nostoc* species circumvent pH stress by synthesizing more mucilage content.

Table : 00 Figures : 03 References : 25

KEY WORDS : Cyanobacteria, growth and pH.

Introduction

Cyanobacterial abundance and growth, which influence the fertility of rice field soils, are controlled by several environment variables either singly or synergistically. The seasonal variations in the edaphic factors and the growth stages of paddy, which affect the attenuation of light, etc, have remarkable impact on the growth and multiplication of cyanobacteria [14,18,22].

The cyanobacteria are the major diazotrophic microbes in the rice field ecosystem and their biomass, rate of photosynthesis and nitrogen fixation vary widely due to variations in the physico-chemical and climatic conditions [12,14,24]. Further, salinity, mineralization and SRP concentrations correlate positively with the occurrence of heterocystous cyanobacteria in the rice fields[9].

Amongst the various factors affecting the distribution of blue-green algae, hydrogen ion concentration (pH) is very important in determining their distribution, growth, multiplication, and establishment in the soil[18]. The pH of the rice field soil or water varies in different parts of India. In general, the cyanobacteria augment in density pH of the near neutral as well as of the pH above 9.0[5]. However, the existence of cyanobacteria was reported in soil of acidic pH from various parts of India, especially from Kerala [1,2,3]. Wide pH is noticed in the rice fields, and this can be associated with the photon flux density and the photosynthetic removal of CO_2 changing the CO to HCO_3 buffering system [25,10]. The pH values reaching above 9.0, usually in the afternoon results in the reduction of N_2 content of the soil due to NH_3 volatilization[23]. Thus, it is clear that the rice field pH influences the growth and abundance of the cyanobacterial biomass, subsequently, their N_2 fixing capacity. In the present work, the growth response and morphological and physiological changes of three species of cyanobacteria isolated from paddy fields of Kerala, subjected to the ranges of pH, are studied.

Materials and Methods

Nostoc linckia (Roth) ex. Born. *et*. Flah (*CALI* 81305) *Nostoc muscorum* Ag ex. Born. *et*. Flah (*CALI* 81341) and *Calothrix marchica* Lemm.(*CALI* 81327) were isolated from paddy fields of Kerala using the standard streak plate and dilution methods. They were cultured in aseptic conditions in BG11 (N_2-free) medium [13] at 2000 Lux fluorescent light for 12 hours per day and incubated for 10 days at 25 ± 2° C. These Cyanobacteria were homogenized and grown in pH ranging from 4.0 -9.0 (pH 4.0, 5.0, 6.0, 7.0, 7.4 (Control),8.0 and 9.0) of the medium for a period of 25 days. Growth response (absorbance of the culture at 760nm) , chlorophyll-a and protein content of the cultures were studied. The readings were taken at intervals of 5 days. Chlorophyll-a content[7] and protein estimation were done[6]. The experiments were conducted in triplicates.

Results and Discussion

Of the three different species, *N. linckia* showed high growth rate, chlorophyll-a and protein content in the alkaline pH (8.0). However, it tolerated a wider pH range, from 5.0 to 9.0. Two peaks were noticed in the graph in this species, one in acidic pH (pH 6.0) and the other in the alkaline (pH 8.0). *N. muscorum* preferred an alkaline range to acidic. The growth in terms of chlorophyll-a and protein content were steadily increasing from acidic to alkaline range and the cyanobacterium recorded its maximum growth at pH 9.0. *C. marchica* on the other hand, exhibited an increase in chlorophyll-a and protein content from acidic to neutral pH (from 6.0 to 7.0). After 7.5, the chlorophyll-a protein content were found to be declining. (Figs. 1,2 & 3).

Analysis of the above data showed adaptation of *N. linckia* towards tolerance to wider pH range without any appreciable decrease in their growth activity. Thus, the above three cyanobacteria have been categorized into three classes, based on their response to pH. While *N. linckia* falls under the wide range pH-tolerating category, (acidic, neutral and basic), *N. muscorum* is restricted to alkalinity. It is found that acidic pH supports the growth of *C. marchica* more than an alkaline one. Yet, it tolerates slightly acidic-neutral-slightly alkaline pH.

The studies have shown that among the edaphic

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Fig. : 1-3

Figure 1- Growth in Absorbance.

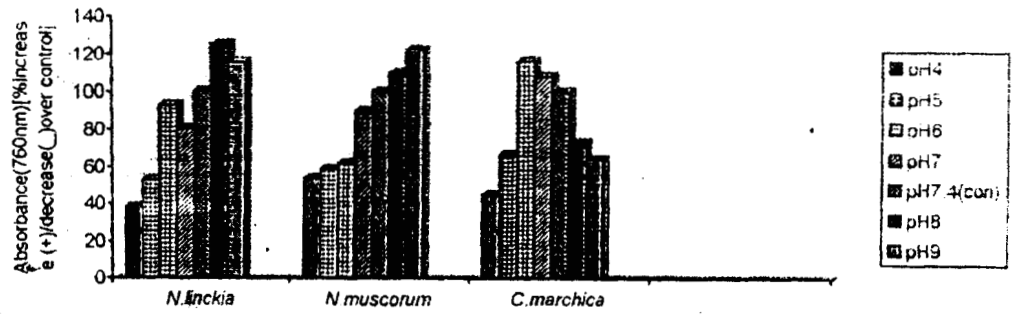


Figure2-Chlorophyll-a content

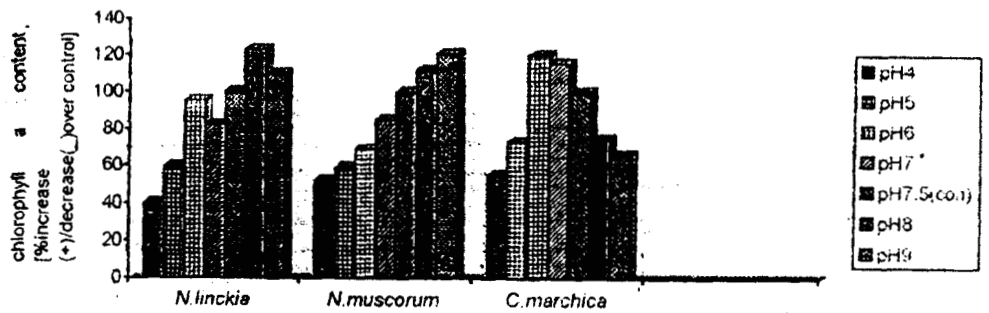
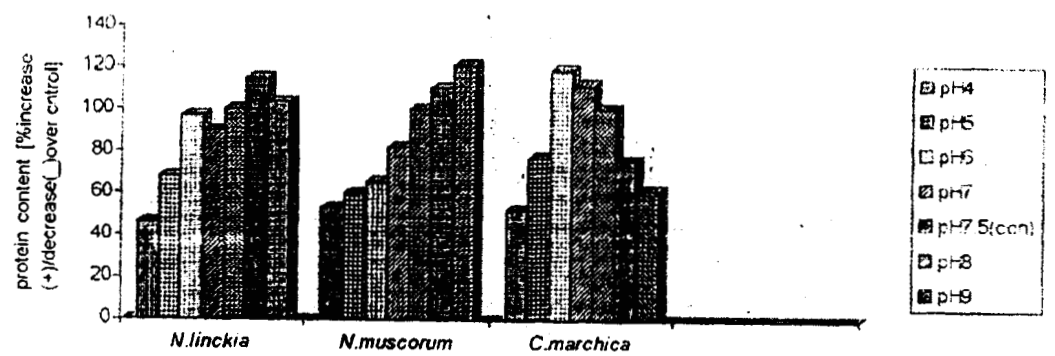


Figure3-Protein content



25 days incubation at different pH

factors, pH plays a vital and important role in determining the cyanobacterial floral composition of a given ecosystem. The positive correlation between the pH and cyanobacterial occurrence and establishment was explained by several experts[11,15]. In the culture media, the optimal range of pH for cyanobacteria growth varied from 7.5 to 10.0 in the upper limit and from 6.0 to 7.0 in the lower limit[4]. Reports have shown that cyanobacteria prefer a neutral to slighty alkaline soil for better growth. In addition, cyanobacteria thrive well in pH of 5.0-6.0[6],< 5.0[1,18,2],as well as in alkaline pH media.

The morphological changes, consequent to pH stress observed were production of high mucilage content, breakage of trichome at heterocyst region, release of various cellular contents and production of larger number of akinetes at low pH levels in both the species of *Nostoc*. Morphological variations were also seen in the cells; some were enlarged while some were disorganized. In *C. marchica*, akinete production was not observed. At low pH (pH 4.0 and pH 5.0) and at high pH (9.0) disruption of the thallus and production of many hormogones were observed. Favourable pH (7.0-7.4) induced false branch formation.

Previous studies have shown that some alkalotrophic cyanobacteria adapted to pH increase by synthesizing more polysaccharides [19,21]. Similarly, production of high mucilage, breakage of trichome, release of akinetes, heterocysts, cellular contents, etc in *Nostoc species* in the present analysis may be adaptations to circumvent the pH stress. The branched cyanobacteria *Mastigocladus laminosus* exhibited maximum branching at 9.5 and growth at 8.5[17]. Similar high rated false branch production is seen in *C. marchica* at pH from 7.0 to 7.4 and growth at 6.0. Neutral to slightly acidic range of the medium induced akinete differentiation in *Anabaena* sp.[20], for survival., Similar akinete differentiation is also noticed in *N. linckia* and *N. muscorum* at low pH values *i.e.*; pH 4.0 & 5.0 due to pH stress.

In this study, *N. linckia* is found to perfer a wide pH range and *C. marchica* a pH range from 6.0 to 7.4 for maximum growth. Therefore, they can be utilized as biofertilizers in the acidic fields of Kerala. *N. muscorum* seems to be a pH specific form in the present study. Such pH specific forms have been reported earlier[22]. In India, rice is cultivated in a variety of habitats with pH ranging from 3.5 to 8.5. Hence, it is essential to examine the response of these cyanobacteria to various pH levels is terms of their growth and biomass production, which in turn influence the efficiency of nitrogen fixation. For successful biofertilizer application technology in acidic and other pH environment, it is necessary to locate suitable cyanobacteria that can tolerate or exhibit maximum growth and nitrogen fixation in such environment. The strain isolated in the present study can be utilized as biofertilizers in their respective pH tolerating ranges in the rice fields of Kerala.

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