AQUATIC CYANOBACTERIA ISOLATED FROM COCHIN: GROWTH CHARACTERISTICS AND BIOACTIVE IMPACT ON SELECTED MICROFLORA

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Dedicated to my Parents,
Husband and Son.

Certificate

This is to certify that this thesis entitled "Aquatic Cyanobacteria Isolated from Cochin: Growth characteristics and bioactive impact on selected microflora." is an authentic record of research work carried out by Newby Joseph (Reg. No. 1755) under my guidance and supervision in the Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, in partial fulfilment of the requirements for the Ph.D degree in Microbiology of the Cochin University of Science and Technology and no part of this has previously formed the basis for the award of any other degree in any University.

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Declaration

I hereby declare that the thesis entitiled "Aquatic Cyanobacteria Isolated from Cochin: Growth characteristics and bioactive impact on selected microflora." is an authentic record of research work carried out by me under the guidance and supervision of Dr. A.V Saramma, Reader, Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, in partial fulfilment of the requirements for the Ph.D degree in Microbiology of the Cochin University of Science and Technology and no part there of has been presented for the award of any other degree in any University.

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CONTENTS

1
CHAPTER

INTRODUCTION

1.1 Significance of cyanobacteria.

Cyanobacteria belong to a diverse and widely distributed group of photosynthetic oxygen evolving prokaryotes of unique characteristics. They are found in both terrestrial and aquatic natural habitats. Extensive research on their fundamental and applied aspects has been carried out innovating their manifold significance,as a source of health food supplements, natural colourants, biofuel, fine chemicals, bioactive substances, lipids, enzymes, polysaccharides and glycerol. Besides their conventional use as food, feed and fertilizer, they are now extensively used in genetic engineering, agriculture, pollution control and pharmaceutical industry.

Cyanobacteria produce a large number of biologically active allelochemical substances, with a diverse range of biological activities. Such metabolites produced in large number and quantity may be directed against oxygenic photosynthetic processes regulating natural population and are potentially useful as biochemical tools and as herbicidal or biocontrol agents. Thus due to their applied biotechnological potential, amicability for gene manipulation and structural simplicity, they are explored in all fields of biological research.

These photosynthetic prokaryotes as assessed from the nutritive value serve as the best natural feed and supplements for fish as well as cattle. Several species of marine bacteria are potential source of large scale production of vitamins of commercial significance such as vitamins of the B-complex group and the vitamin E. A variety of pigments, the carotenoids and the biliproteins are of high

commercial value. Among the various types of carotenoids, β carotene is known to be the precursor of vitamin A and hence is of immense nutritional value. The cyanobacterium *Spirulina* is reported to be rich in this provitamin A. Phycobiliproteins are also found to be valuable in the food, drug and cosmetic industries as natural colourants. Isotopically labelled cyanobacterial metabolites such as aminoacids, lipids and sugars are commercially available.The marine cyanobacterium *Aphanothece halophytica* is rich in commercially important aminoacids like glutamate , aspartate, methionine and phenyl alanine. In addition marine cyanobacteria can be rich sources of several polyols, polysaccharides, lipids, homogenated compounds with varied properties employable as flocculants and surfactants.

Several cyanobacteria are capable of nitrogen fixation and thus contribute much to global nitrogen budget. Nitrogen fixation occurs in heterocyst which are structurally and biochemically different from vegetative cells. The photosynthetic vegetative cells fix CO_2 through the reductive pentose phosphate pathway and provide fixed carbon in the form of carbohydrate. They are the important primary producers of the aquatic ecosystems. The photosynthetic apparatus of cyanobacteria is very similar to plants producing oxygen through photosystem II.

The presence of structurally undefined and functionally specialized cells, heterocysts and akinetes is the most important characteristic feature of cyanobacteria which distinguish them from other prokaryotes. Such degree of

differentiation and development is peculiar to certain genera of filamentous species of cyanobacteria. Hormogonia formation and development of hair cells are other important cellular differentiations among the cyanobacteria.

Several species of cyanobacteria exhibit movement which may be either gliding, phototactic or chemotactic. There are different nutritional types in cyanobacteria - facultative chemoheterotrophs, obligate phototrophs and photoheterotrophs. Asexual reproduction occurs by the formation of hormogonia or endospores or by fragmentation of colonies.

Several species of cyanobacteria exhibit symbiotic associations which may be either extracellular or intracellular. The most common type of extracellular association is found in lichen where the algal partner is a cyanobacterium. Symbiotic association of cyanobacterium, *Anabaena azollae* with the water fern *Azolla* is significant as it increases the nitrogen budget in paddy fields. Symbiotic association of cyanobacteria is also found in marine sponges. Another instance of symbiotic relationship of cyanobacteria is seen in the coralloid roots of cycas.

In aquatic ecosystem, cyanobacteria are found distributed in marine, freshwater and estuarine ecosystem. In marine environment , they are found in all possible habitats – pelagic, benthic, littoral and oceanic. In the open ocean, most of the total photosynthetic capacity may be attributed to picophytoplankton. They are tiny, coccoid cyanobacteria with an average diameter of 2μ. Cyanobacteria larger

than picoplankton also form a significant part of the oceanic phytoplankton, the bloom of which often form discoloration of the water.

Freshwater cyanobacteria are distributed in lakes, rivers, canals, ponds and practically in all possible habitats. In many cases, the same species may grow in freshwater and in seawater. Freshwater blooms of these organisms are very common in ponds and reservoirs and lakes. Most freshwater blooms consist of *Microcystis, Anabaena, Aphanizomenon, Gloeotrichia, Oscillatoria and Lyngbya.*

Several species of cyanobacteria produce toxins which may be either neurotoxic or hepatotoxic. The neurotoxins produced by cyanobacteria are anatoxin and saxitoxin. The former is synthesized by the species of *Anabaena, Aphanizomenon, Oscillatoria and Trichodesmium.* The hepatotoxins include microcystins and nodularins. It is also known that bluegreens (cyanobacteria) produce extracellular substances in their various phases of growth which may either inhibit or enhance the growth of other microflora. Certain species of cyanobacteria have the capacity to reduce heavy metal load in the aquatic environment.

Investigation on these prokaryotic microbes has opened up vast opportunities where these tiny organisms could be used for the benefit of mankind and for alleviating human sufferings. Thus cyanobacteria can be considered as nature's unique gift to mankind.

Synechococcus elongatus bloom in a hatchery tank

1.2 Relevance of the work

Aquatic ecosystem in the south west coast of India is noted for its diversity of habitats. Very often these environments turn bluegreen when the bloom of bluegreen algae (cyanobacteria) appear consequent to eutrophication. This phenomenon occurs in these habitats one after the other or simultaneously. This conspicuousness make one curious enough to know more about these nature's gift bestowed upon mankind. While persuing the literature on the magnificent flora, it is understood that it may provide food fertilizer, chemicals and bioactive substances. These bioactive substances are likely to be involved in regulating natural populations and are potentially useful as biochemical tools and as herbicidal or biocontrol agents. The role of cyanobacteria in the aquatic food chain and contribution in abatement of heavy metals from the natural environment are well documented.

Considering the manifold utilization of the flora and their significance in the food chain, the present investigation has been undertaken. The objectives of which are given below:

1. Isolation and identification of cyanobacteria from different aquatic environments
2. To study the growth characteristics of selected species of cyanobacteria

3. To study the bacteria found associated with different species of cyanobacteria

4. To study the bioactive impact of cyanobacteria on selected microflora

5. To study the contribution of selected cyanobacteria in the abatement of heavy metals.

The thesis is presented in six chapters.The first chapter gives the significance of cyanobacteria, relevance of the present work and review of literature. In the second chapter the methods of collection and isolation followed and the species identified from different aquatic environments in and around Cochin are included. The third chapter is an account of bacteria associated with various species of cyanobacteria. The fourth chapter projects the growth characteristics of cyanobacteria, the effect of varying concentrations of salinity on the doubling time, growth characteristics of cyanobacteria in enriched and unenriched media and the production of cellular organic substance by ^{14}C technique and Winkler method. The fifth chapter discusses the work done on bioactive property of cyanobacteria. The extracellular product of selected species, which may contain bioactive substances, were estimated using ^{14}C technique. The effect of filtrate of *Synechocystis salina, Synechococcus elongatus* and *Gloeocapsa crepidinum* on cynaobacteria and other microfora were studied. The magnitude of effect varied with the source of the filtrate and test organisms. The algicidal property of tested

cyanobacteria can be exploited for the removal of undesired or harmful species from the ecosystem which would eventually enhance the bioproductivity of the aquatic environment. The role of cyanobacteria in metal detoxification is also presented in this chapter. The major findings of the study are summarized in chapter 6. This chapter is followed by the litrature referred.

1.3 Review of literature

Cyanobacteria have been generally recognized as one of the most diverse and the largest group among prokaryotes judged by the numerical preponderance of species. They have been traditionally classified as algae. This assemblage of diversified morphological forms characterised by the structural simplicity have been classified and identified by morphological, cytological or chemical characteristics. The autotrophic nature and macroscopic appearance for the assemblage of certain filamentous forms prompted the earlier workers to think that they were exclusively algae. However, there were reasons to connect the blue greens with bacteria, but the evidence was not overwhelming. It had been realized that blue greens lacked the nucleus and chloroplast, characteristic of other classes of algae. But the morphological characteristics of the blue greens and their ecological niches were identical with those of other microalgae. In cell size and morphological complexity the blue greens more closely resemble algae than bacteria. Their dual photosystems were almost identical with that of eukaryotic algae and higher green plants. The identical morphological characteristics of

blue green algae and other taxonomic classes of microalgae and similarity in their ecological niches made Fritsch(1945) to put them together. Cohn (1872,1875) however concluded that the blue green algae (Schizophyceae) and bacteria (Schizomycetes) be grouped in one division, Schizophyta.

In 1932, Geitler produced a comprehensive treatise which recognized 145 genera and 1300 species. Another comprehensive treatise was published by Elenkin (1936, 1938, 1949). Among other works on blue-greens on regional basis, that of Desikachary (1959) is a significant contribution as far as the bluegreens in India are concerned. He followed a classification similar to that of Geitler (1932). Drouet(1968, 1973, 1978, 1981) revised and consolidated bluegreens resulting in the drastic reduction of 2000 species in over 140 genera to 62 species in 24 genera. His classification was accepted by the biochemists and physiologists for its simplicity and left unaccepted by the taxonomists for its extreme simplicity resulted in the grouping of morphologically dissimilar forms. Drouet's system had become ineffective. In 1971 Stanier *et al.* introduced another system of classification based on the use of axenic cloned cultures and some of their morphological, cytological, genetic, chemical and physiological characteristics. Among the controversies in the classification of blue greens the one followed by Desikachary (1959) to describe the bluegreens has been adopted in the present investigation.

Inspite of the prevailing dispute in classification and the affinity of bluegreens, it is a fact that most of the blue greens occur as primary producers in aerobic environments, interspersed with the eukaryotic planktonic algae, tychoplankton and periphyton. Jupp *et al.* (1994) described the method of detection, identification and mapping of cyanobacteria using remote sensing to measure the optical quality of turbid inland waters.

The term " bacteria" is presently defined by bacteriologists as synonym with prokaryotes. From this it follows that the term 'cyanobacteria' is the taxonomically correct name for the bluegreens. Both "cyanobacteria" and "blue green algae" (cyanophyceae) should be considered usable and compatible names. (James.T.Stanley and Noel.R. Krieg, 1984). Here an attempt is made to study the distribution of cyanobacteria in the marine, estuarine and freshwater environments.

Although the class cyanobacteria includes 150 genera and about 2000 species (Fott, 1971) they represent the largest group of morphologically diverse cosmopolitan, photosynthetic microbes lacking motile stages and sexual reproduction. These organisms are frequently encountered in shallow, nearshore tropical seas, but appear in low densities in nearly all regions. Occasionally they build blooms in brackish or nearshore habitats. The size ranges from less than 1μm for single – celled forms to more than100μm for filamentous types. They

contain chlophyll a, phycobiliproteins, glycogen as a storage product and cell consisting of aminosugars and aminoacids.

Cyanobacteria can form not only chlorophyll 'a', but the phycobilin protein pigment complexes based upon phycocyanin, phycoerythrin and allophycocyanin which contribute to the characteristic colour. The majority of the species are characterized by the presence of thylakoids, considered to be the sites for both photosynthesis and respiration. The complementary chromatic adaptation, the presence of gas vacuoles, the formation of heterocyst and nitrogen fixation potential are other significant attributes of several species of cyanobacteria.

A wide spectrum of morphological complexities evolved among the cyanobacteria, ranging from small, bacteria- sized sphere and rods to truly multicellular macroscopic forms. Different cyanobacteria exhibit distinctive vegetative and reproductive cell division. They show cell differentiation in form and function, as well as intercellular communication and distinctive behavioural responses. A wide range of specialized functions evolved within the group, providing the basis for different competitive strategies. There are stenotopic as well as eurytopic cyanobacteria. Those which live in stable environments are more sensitive to environmental changes than those occupying fluctuating and extreme environments. The range of habitats and conditions, occupied by cyanobacteria as a group, however, is wider than that of most eukaryotic phototrophs. Cyanobacteria proved successful in occupying freshwater, brackish

and marine environments. Marine forms employ both halophily and halotolerance as survival strategies(Golubic, 1980). It is probable, that the cyanobacteria, the first oxygenic photosynthesizers, occupied the available marine niches early and over long geological time, evolved a broader spectrum of specializations and tolerances than their later evolving competitors. Consequently, cyanobacterial dominance remained unchallenged in most extreme environments, whereas in optimal ecological ranges they became tightly integrated into systems of higher ecological complexity.

About twenty percent of the identified cyanobacteria occurs in saline situations majority of which are truly marine. In India,85 species are known from saline situations including fifty marine species. A majority of these forms occurs in the intertidal and supratidal regions while a few are planktonic. (Desikachary, 1959).

Picophytoplanktonic cyanobacteria such as *Synechocystis* and *Synechococcus* contribute significantly to the primary productivity of lakes, oceans and lagoon waters. (Stockner, 1988; Charpy and Blanchot, 1996).

Bloom- forming nitrogen fixing filamentous cyanobacteria like *Kathagnymene* and *Trichodesmium* are common in tropical oceans. The ability to fix atmospheric nitrogen gives an obvious competitive advantage in large areas of the open ocean where nitrogen is a limiting nutrient. However, nitrogen activity is inhibited by the presence of oxygen. This problem has been solved in other

cyanobacteria most elegantly by the evolution of the heterocyst, a complex, structurally, biochemically and functionally differentiated, nitrogen-fixing cell within a multicellular trichome (Rippka *et al.*, 1979).

Benthic marine cyanobacteria are well adapted in exploiting low light conditions. Many of them are able to regulate pigment composition as well as phycobilisome ultrastructure, in response to low light intensity and spectral shift (Ohki and Fujitha, 1992) as part of the process of chromatic adaptation (Tandeau de Marsac, 1977).

Marine species of *Phormidium* with narrow trichomes are common epiphytes on other cyanobacteria and algae. Other marine epiphytes include small filamentous cyanobacteria *Plectonema golenkinianum* and *P battersii*. These organisms are externally attached to other cyanobacteria. *Spirulina subtilissima* and *S. tenerrima* tend to crawl inside sheaths of other cyanobacteria. Epizoic and endozoic habitats include sponges and didemnid ascidians which harbour coccoid *Synechocystis* and *Prochloron* (Lewin, 1989). Prochloralean prokaryotes apparently evolved from several separate branches of the cyanobacterial stock.(Chrisholm *et al.*, 1992)

Small coccoid epiphytic cyanobacteria (< 0.8 μm diameter) attached to sheaths of large *Lyngbya majuscula* (> 80 μm) illustrate the enormous cell size range represented among marine cyanobacteria (Hua et al., 1989). Coastal marine

environments are ecologically and biologically the most diversified ones. They may be exposed to wave energies or located in protected bays. Numerous attached cyanobacteria grows in the intertidal ranges on exposed rocky coasts. Some promote precipitation of species-specifically shaped carbonate minerals within their thalli.(Golubic and Campbell, 1981). Exposure to wave energy on exposed rocky shores requires structural firmness and most epilithic cyanobacteria adhere firmly to substrate. All surfaces overgrown by cyanobacteria are invariably darkly pigmented by extracellular protective pigments.

Microbial mats are most diversified along protected tropical coasts, where they are zonally arranged. Cyanobacteria constitute the main component of these microbial mats ecosystems. Under more arid climatic conditions, coastal ponds turn hypersaline and these ponds are dominated by specialized cyanobacteria with particular metabolic flexibility (Cohen *et al.*, 1975; Campbell and Golubic, 1985 ; Jorgensen et al., 1986)

Intertidal and supratidal ranges of carbonate coasts are sites of the most intensive bioerosion which effectively destroys rocky shores and contributes to fine grain sediment production at a geologically significant scale (Schneider, 1976). Epilithic and endolithic cyanobacteria are the principal primary producers in these ranges and the ultimate cause of coastal bioerosion. The significance of cyanobacterial colonization in these intertidal ranges is in providing the very base of a complex and diversified pyramid.

Freshwater cyanobacteria are abundant in eutrophic lakes and paddy fields. Many of these photosynthetic organisms are capable of fixing free dinitrogen. Singh(1961) pointed out the ways in which cyanobacterial growth can be encouraged and thus improve the soil fertility. Venkataraman (1961) introduced the term algalization for the practice of adding cyanobacteria to soils which is now widely used. According to Metting (1988) algalization is done in India using mixtures of *Anabaena, Aulosira, Nostoc, Scytonema and Tolypothrix.* This practice may be useful where fields undergo marked environmental changes during the year. They have gained special importance in tropical rice cultivation(Venkataraman, 1972). They are also important in soil erosion and increasing the organic content of the soil and probably in producing cer substances which enhancesthe growth of higher plants (Venkataraman et al.,1974). Cyanobacteria are widely distributed in the freshwater plankton and occur in lakes at almost every latitude. Certain genera are widely distributed, such as *Microcystis* and *Anabaena*. *Oscillatoria* species seem more characteristic of temperate zones, whilst *Anabaenopsis* and *Spirulina* occur more frequently at lower latitudes.

Planktonic cyanobacteria have become abundant in ponds which have been fertilized to increase fish production. The increase in planktonic cyanobacterial populations has sometimes been dramatic and has brought with it considerable practical problems. Dense populations cause great difficulties to the water-supply

industry, interfering with treatment processes and imparting taste and odour to the water. The production of toxins or the removal of oxygen by respiration or decomposition of dead cells can have serious consequences, causing death amongst fish, birds and occasionally cattle. Bathing in waters densely populated with *Aphanizomenon* can cause an unpleasant condition of the skin known as swimmer's itch (Schwimmer and Schwimmer, 1964).

There is a wide range of form in the plankton from minute simple cells *Synechococcus* sp.(Bailey-Watts et al., 1968) to simple filaments such as *Oscillatoria* sp. to relatively large rafts (*Aphanizomenon*), coils (*Anabaena*), spherical aggregates (*Coelosphaerium*, *Gomphosphaeria)* and indefinite masses (*Microcystis*). Eventhough planktonic cyanobacteria are more commonly encountered in eutrophic lakes, they are by no means confined to them. Significant populations may occur in nutrient-poor lakes in both high latitudes and the subtropics. The highly alkaline lakes produce massive crops of planktonic cyanobacteria(Talling ,1973) mainly *Spirulina platensis and Microcystis.* Cyanobacteria may also occur in association with other planktonic organisms. *Phormidium mucicola* is found in the mucilage of many colonial planktonic cyanobacteria and rotifers.

Research on toxigenic species of cyanophyceans is still in its infancy (Carmichael, 1981). The occurrences of toxic cyanobacteria have been reported in many countries of the world, with the species mainly restricted to *Microcystis*

aeruginosa, Aphnizomenon flos-aquae and Anabaena flos aquae. The toxigenic species which commonly occur are reported from aquatic environments. Blooms formed by toxin-producing cyanobacteria commonly occur in fresh and brackish water environments (Codd and Poon, 1988;Carmichael 1989; Sivonen *et al.*1990;Carmichael 1992). The toxins produced by them involve secondary metabolites like peptides, alkaloids and phenols(Campbell,1984). Cyanobacteria produce two main types of toxins, cyclic peptide hepatotoxins and alkaloid neurotoxins (Codd and Poon 1988; Carmichael 1989).Toxic cyanobacterial blooms have caused mortality among wild and domestic animals and they constitute hazards to human health (Falconer 1989;Carmichael 1992). Toxin production is common among members of the genera *Anabaena, Aphanizomenon, Microcystis, Nodularia, Nostoc and Oscillatoria* (Carmichael 1989; Sivonen *et al.* 1989 1990, Harada *et al.*, 1991). It is not possible to determine the toxicity of cyanobacterial blooms by their appearance or species composition because not all strains of the same species produce toxins.

Toxin production by cyanobacteria leading to the death of cattle and birds is a world-wide phenomenon that is reviewed by Gorham(1964). In the case of the toxin from *Microcystis aeruginosa*, Bishop *et al.* (1959) and Ramamurthy & Capindale (1970) have shown that it consists of a polypeptide containing D-serine, L-ornithine and some protein L-aminoacids. Several species of cyanobacteria produce hepatotoxic peptides called microcystins. Toxic

cyanobacteria may affect sensitive organisms and populations and also fundamental ecological processes such as microbial production and microbial activity. Rai and Prakasham (1994) isolated toxic cyanobacterium *Microcystis aeruginosa* and determined its toxicity to mice which exhibited clinical signs of toxicity.

Baker and Humpage (1994) studied the toxicity associated with commonly occurring cyanobacteria in surface waters. Marine and freshwater phytoplankton may produce phycotoxins under certain environmental conditions. In the marine environment, dinoflagellates produce fatty polyethers which accumulate in shellfish and can cause diarrhetic shellfish poisoning when ingested. They found that in freshwater, the toxins are microcystins and nodularin which have caused massive poisoning of wild animals or domestic livestock and now are a threat for human beings. Pushparaj *et al* (1999) found that the active substance in *Nodularia harveyana* has allelopathic activity against other cyanobacteria, Gram positive bacteria and pathogenic fungi.

Much work has been carried out on the growth characteristics of cyanobacteria. Fogg (1949) studied the growth constant, k′ and doubling time , tg for *Anabaena cylindrica*. Their values being 0.68 and 25 hours respectively. Kratz and Myers(1955) recorded the growth constant ,k′ as 3.55 and doubling time tg as 2 hrs. Joseph and Nair (1975) recorded a highest growth constant of 0.048 hr^{-1} with a corresponding generation time of 14.6 hrs in an estuarine *Synechocystis*

salina. Ikemoto and Mitsui (1994) recorded the growth attributes of an anaerobic nitrogen fixing *Synechococcus* strain Miami BG 043511.

Physiologically, cyanobacteria are well adapted to tie over physicochemical stresses such as hypersalinity, desiccation, excessive irradiance and extreme temperature fluctuations. Numerous taxa are encapsulated in mucilaginous sheaths and slimes that exhibit antidesiccation, strong irradiance absorbing and selective gas diffusion characteristics. Fogg *et al.*,1973; Castebholz 1998). Cyanobacteria are well adapted to environmental excesses. Consequent to eutrophication, proliferation of harmful blooms appear in estuarine and coastal ecosystems (Horstmann, 1975; Fogg, 1982; Paerl, 1988 Larson *et al.*,1990; Kahru *et al.*,1994; Sellner, 1997). Blooms pose serious threat to water quality, fisheries resource, aquaculture and human health problems, including disruption of food webs (Porter and Orcutt, 1980; Fulton and Paerl, 1987, 1988).

There are several physical and chemical constraints to cyanobacterial growth and expansion. Elevated salinity(Thomas *et al.*, 1988), phosphorus deficiency (Doremus, 1985), relatively low supply rates and supply of organic matter(Fogg, 1969), and specific trace metals such as Fe and Mo deficiencies in brackish and full salinity systems(Howrath and Cole , 1985) have been identified as potential physico-chemical barriers for growth and multiplication of cyanobacteria in various aquatic ecosystems.

In marine environment, salinity has been recognized as potential barrier for growth and proliferation of cyanobacteria(Thomas *et al.*,1988). Fixation of nitrogen seems particularly susceptible to osmotic stress and organisms unable to adjust by the production of compensatory factors show inhibition of activity at increasing salt concentrations (DuBois and Kapustka 1981; LeRudilier *et al.*, 1984). Cyanobacteria introduced into an estuarine environment from terrestrial or freshwater origins may not be able to compensate for increasing salinities and osmotic stress (Paerl et al., 1983). Indigenous populations are often able to adjust salinities by producing compatible osmolytes (Reed and Stuwart, 1985).

Certain freshwater species such as *Microcystis aeruginosa* and *Aphanizomenon* sp. can be highly sensitive to a few ppt salinity when discharged into estuarine waters (Paerl *et al.*, 1983). Moore (1995) analysed the influence of light and temperature on growth, pigments and absorptive properties of *Synechococcus* and *Prochlorococcus*. It was found that the temperature optima for growth varies with the species. Lee and Rhee (1999) studied the kinetics of growth and death in *Anabaena flos-aquae* under light limitations and supersaturations. At lower light intensities the growth rate was found to be proportional and at higher light intensity it was inhibited.

Several bacteria are found attached to various microflora including cyanobacteria. Cyanobacteria are noted for their interactions with bacteria which may be pathogenic, saprophytic or symbiontic. Ganapati and Amin (1972) studied algal-

bacterial symbiosis in which they found that cyanobacterium (*Oscillatoria chalybea*) seems to inhibit bacteria. Investigations carried out by Paerl (1976, 1978) and Cladwell(1977) showed that cyanobacteria are frequently the sites of extensive bacteria and fungal colonization. The bacterial attachment sites known as microzones, where chemical and physical properties are determined by microbial exchange process (Cladwell, 1978)

There are several reports on the association between cyanobacteria and other prokaryotes. Cyanobacteria may be the extra or intracellular symbionts and may exchange with the other organism the dissolved organic carbon. Wherever the cyanobacteria is capable of nitrogen fixation, then this nitrogen is apparently passed to the other organism. Considering the significance of this exchange phenomenon, the bacteria associated with various aquatic cyanobacteria were studied.

Comprehensive studies for bioactive substances in unicellular and multicellular algae made earlier revealed that several species showed growth inhibitory and promoting effect on bacteria and algae. Several substances were found to be toxic to other fauna including man. Smith and Ng (1999) observed cyanobacterial metabolites with bioactivity against photosynthesis in cyanobacteria. Srivastava et al (1999) found that broad spectrum inhibitory metabolites were produced by a benthic cyanobacterium *Fischerella muscicola* which inhibited the growth of eukaryotic

algae, cyanobacteria and eubacteria. In the present investigation the impact of the filtrate and the organisms on the growth of other species were analysed.

Cyanobacteria are great performers of various metabolic feats. A vast range of pharmacologically important secondary metabolites have been extracted from these microorganisms and characterized. More than 600 secondary metabolites have been isolated from marine algae. About 60%of these are terpenes and 20% of these are fatty acids with nitrogenous compounds and compounds of mixed biosynthesis each making up only about 10%. Secondary metabolites are compounds which are not used by the organism for the cell division and primary metabolism and act as hormones, antibiotics, allelochemicals, carcinogens and 'oxins. The members of this group of organisms are also being utilized as biofertilizers in agriculture, as sources of protein and vitamin in food, in bioremediation, for heavy metal removal and reagents for biochemical research. Many of these compounds are bioactive and have been extensively studied using laboratory and pharmacological assays. However, their natural functions under ecologically realistic conditions have been investigated only recently. Many active compounds remain unidentified either because they degrade during collection, storage and extracellular and because they are not tested against appropriate target organisms. A clear understanding of the structure, functions, effects and mechanisms of action of secondary metabolites would provide useful

information for the development of economically important natural products from the sea.

During recent years,there seems a shift in the need for the production of natural pigments for use as food colours because of the proven carcinogenecity of hitherto used coal tar dye based colours. Chlorophyll a extract of the cyanobacterium, *Spirulina* having iron oxide and higher alcohols (stearyl and catyl) is patented as strong deodorant (Hasting, 1968). Glycosidic xanthophylls occur only in cyanobacteria. β carotene is of great commercial interest, in view of its anticancer property (Pero et al., 1981), its involvement in physiology and reproduction. Xanthophylls are usually present at 0.5% of total algal biomass. Cantaxanthin of *Spirulina* is used for intensifying the colour of fancy gold fish (Matsuno*et al.*, 1979). The only known source of phycobilins are algae belonging to Rhodophyceae, Cryptophyceae and Cyanophyceae or cyanobacteria. But it is a highly expensive fine chemical and used as fluorescent dye (phycoflour probe) in immunoassay and is an important component of diagonostic kits, natural colouring pigment in food, drug and cosmetic industries. Phycobilins are chromoproteins and occurs as C-phycocyanin, C- phycoerythrin and allophycocyanin in cyanobacteria.

Many cyanobacteria are nitrogen fixers. Since nitrogen is a common limiting nutrient in many marine and some freshwater environments, their contribution to the nitrogen budget can be extremely important. They have a long history of

usage in agriculture as a biofertilizer. *Aulosira, Plectonema, Tolypothrix, Nostoc* and *Anabaena* are examples. Addition of cyanobacteria to paddy fields has reported to increase aggregate stability and improve rice yield by 10-15%. *Nostoc* and *Spirulina* balls are consumed as a staple or as delicacies. Cyanobacterial single cell protein is used as a supplement or replacement for conventional protein sources in livestock feed. Trials with poultry, pigs and ruminants conclude that concentrations of *Spirulina* upto 10% are satisfactory replacements for conventional protein sources, while higher concentrations reduce growth. Morse *et al.* (1984) found molecules from cyanobacteria that induced larval selltlement and metamorphosis in the mollusc *Haliotis rufescens.*

The production of a great variety of extracellular substances by algae which play an important role in algal growth and physiology as well as in aquatic food chain is now well established. Microorganisms form a rich source of activity of bioactive products in aquatic ecosystem. Marine environment is a potent source of new antibiotics and other biologically active substances. The studies on antagonistic microorganisms of the marine environment, eventhough limited, clearly indicate that the sea would be a vast reservoir of potential drugs (Lakshmanaperumalsamy,1978, Padmakumar and Ayyakkannu 1994, Padmakumar, 1994). Cyanobacteria are known to produce a wide range of secondary metabolites, including antifungal, antiviral and antialgal substances.

During the various stages of growth, a considerable quantity of the synthesized organic substances is liberated from the cells as carbohydrates, enzymes, aminoacids and peptides, vitamins and growth substances, autoinhibitors and antibiotics, toxins, enzymes etc.(Fogg, 1962). Considering the significance of extracellular product studied the total quantity of extracellular product released by a few species during their growth. Practically very little studies have been undertaken in India using ^{14}C isotope.

Extracellular metabolites of five species of cyanobacteria were screened against a range of bacteria, fungi and yeast in addition to anticancer screen. Among them, the particular interest focused on *Nostoc muscorum*, which was shown to produce a broad spectrum antibiotic active against Gram negative, Gram positive and eukaryotic organisms (Bloor, 1990).

Patterson and Smitt, (1991) screened laboratory cultures of blue green algae as a source of anti neoplastic, antimycotic, antiviral or pharmacologically active agents. Approximatly 1000 cyanophytic strains from diverse habitat culture to provide extracts for testing were to have many metabolites particularly those of polypeptide and polyketide origin bioactive compounds. Mule *et.al.* (1991) found bioactive compounds present in methanol extracts and extracellular products from *Nostoc muscorum* evoked a significant inhibition in the growth of the phytopathogen *Sclerotinia sclerotiorum* and no significant difference was found between either treatment.

Lipophilic extracts of five species of cynobacteria isolated from mangrove showed inhibitory activity against all seven strains of bacteria tested. Among them *Nostoc paludosum* and *Schizothrix sp.* showed maximum activity against *Bacillus subtilis* (Rao, 1994). A number of associated bacteria and cyanobacteria in sponges were found to be source of antibiotics and other bioactive compounds in marine environment. The culture of a bacterial isolate from sponge produced an aminophenol which inhibited the growth of *Staphylococcus aurius* and *Bacillus subtilis* (Oclarit *et al* ., 1994).

A large number of antibiotics and pharmaceutically active compounds with novel structures have been isolated and characterized. Similarly many cyanobacteria have been shown to produce antiviral and antineoplastic compounds. Several of the bioactive compound may find application in human or veterinary medicine or agriculture (Borowitzaka, 1994).

In1994, Fish and Codd reported extracelluar antimicrobial material produced by a thermotolerant species of *Phormidium* (cyanobacterium) is effective against many Gram positive and Gram negative heterotrophic bacteria, *Candia albicans* (Fungi) and *Cladosporium resinae.*

Cyanobateria are rich sources of biologically active cyclic peptides like majusculamide C, scytonemin A, a major active metabolite in the culture cyanobacteria *Schizothrix sp.* and it was found that a protein residue attached to

amino group of a 2 hydroxy 3-amino long chain acid residue is common among all the above compounds for the biological activity.

Moore(1996) reported that an elaborate array of structurally ,novel and biologically active cyclic peptides and desipeptides present in cyanobacteria. These include antibiotics, algicides, toxins, pharmaceutically active compounds and plant growth regulators. The production of secondary metabolites varies with the environmental conditions and these cyanobacterial extracts were found to be active against many plant pathogens(Metting and Pyne, 1986). Cyanobacteria are found to be remarkably active against AIDS virus (HIV-1)(Gustafson *et al.*, 1989).

Various survey programmes aimed primarly at discovery of biopharmaceuticals for treatment of such catastrophic diseases as cancer and AIDS have identified the cyanobacteria as one of the most promising groups of microorganisms for finding new bioactive natural products. Cyanobacteria can be used for the generation of biomass. It is possible to use cyanobacteria for the direct production of energy-rich fuels, especially hydrogen.

Secondary metabolites of cyanobacteria are now known to specifically disrupt the structure and function of microtubules, microfilaments and intermediate filaments of eukaryotic cells. Microcystins and Nodularins produced by *Microcystis* and *Nodularia* are examples. Extracellular substances produced by cyanobacteria can

be broadly divided into two categories- physiologically active compounds which inhibit or stimulate growth at low concentrations and nutritive substances. An example of the former are the siderochromes. Keating (1978) made a systematic study of cell-free filtrates from cultures of the dominant cyanobacteria from Linsley Pond, U.S.A., showed that they were inhibitory to diatoms isolated from the same lake. Swierczynski & Czerniawska (1992) examined the dynamics of mortality of selected species of hydrofauna according to changes of blue-green (*Microcystis* sp.) algal concentrations. Oufdou, *et al*(1998) studied the effect of bioactive compounds produced by a cyanobacterium *Synechocystis* sp. on bacteria It was found that the extracellular substances released during algal culture in stationary phase reduced the growth of *E.coli* and *Salmonella* sp. by 85% and 90% respectively. Smith (1999) revealed that cyanobacterial metabolites which affects the metabolic process within the cell. Such chemicals are likely to be involved in regulating natural populations and are potentially useful as biochemical tools and as herbicidal or biocontrol agents.

Certain species of algae have the capacity to reduce the heavy metal load in aquatic environment. Screening of various species of cyanobacteria for its capability of heavy metal uptake and further studies on this line is significant for water pollution abatement.

Three species were selected in the present investigation to assess their potentiality for heavy metal removal in aquatic environment.

Recent observations show that naturally occurring bacterial-cyanobacterial assemblages (including *Phormidium* and *Microcoleus*) are capable of utilization of n-alkanes, leading to the breakdown of crude oil spills. Metals can be concentrated using cyanobacteria. Specific metals shown to be absorbed or accumulated by cyanobacteria include Al, Cd, Cu, Pb, Hg, Ni and Zn. A recent review on the potential cyanobacteria as biological control agents concludes that cyanobacteria, because they produce antibacterial and antifungal materials, do not pose a threat to the environment and are suitable for exploitation as biocontrol agents for plant pathogenic bacteria and fungi.

Pandey *et al.*(1992)studied the copper uptake in the diazotrophic cyanobacterium *Nostoc calcicola.* It was noted that the uptake of copper was accompanied by inhibitions in photosynthesis. The cyanobacterial cells while saturated for copper uptake within one hour at 40 mμ M Cu showed more than 50% of inhibition of PS II. Corder and Reeves (1994) studied the abatement of Ni by cyanobacteria. They were evaluated as biosorbents for removing Ni at concentration of <20ppm. Among four filamentous cyanobacterial species studied *Anabaena torulosa* was found to be the most sensitive species which could be used as a bioindicator for chromium ions in industrial effluents. *Anabaena variabilis* was found to have a chromium ion tolerance upto 10 ppm and would be suitable as a bioscavenger of chromium ions from industrial waste water. Bilgrami *et al.* (1996) studied the river biota as indicators and scavengers of heavy metal pollution. Studying the bioaccumulation

of different groups of organisms such as algae, macrophytes and animals, they found that all these organisms including algae such as *Anabaena* and *Cladophora* can be used as scavengers as well as indicators of high levels of metal pollution in aquatic ecosystems. Nagase *et al* (1997) succeeded in the selective cadmium removal from the hard water by *Tolypothrix tenuis*. Twenty four strains of 191 marine microalgal strains were found to exhibit cadmium resistance. They were tested for their Cd removal ability by Matsunaga *et al.* (1999) and found that six strains out of 19 green algae and one out of five cyanobacteria removed more than 10 % of Cd from the medium.

2
CHAPTER

OCCURRENCE OF AQUATIC CYANOBACTERIA IN COCHIN

Tropical aquatic ecosystems are characterized by the diversity and magnitude of photosynthetic microflora of which the role of cyanobacteria is very significant in initiating and supporting the food chain. These bluegreens of nature occur in all the habitats, freshwater and marine and even withstand the stress caused by the fluctuation of environmental parameters such as light, temperature, pH and salinity. In the present study the cyanobacteria distributed in the marine, freshwater and estuarine habitats of Cochin in the southwest coast of India is discussed (Fig.1). As photosynthetic organisms, they are important contributers to benthic and pelagic primary production but their main role in the tropical waters appears to be as nitrogen fixers (Hoffmann, 1999). Though several studies have been carried out in India on various aspects of cyanobacteria such as that of Venkataraman (1979, 1981), Raju and Meka (1989), Misra and Kaushik (1989), Singh and Singh (1990) and Subramanian,(1998) very little has been done on their occurrence and distribution of marine, freshwater and estuarine cyanobacteria in the southwest coast of India. In the present study, the occurrence and distribution of cyanobacteria in the marine, freshwater and estuarine environments of Cochin, their isolation and maintenance are discussed.

The temporal and spatial variation of cyanobacteria is controlled mainly by oxygen and light. Within the photic zone, oxygen levels can be at constant high, constant low or alternate between aerobic and anaerobic conditions. Cyanobacteria can adapt to any of these conditions as they possess photosystem I

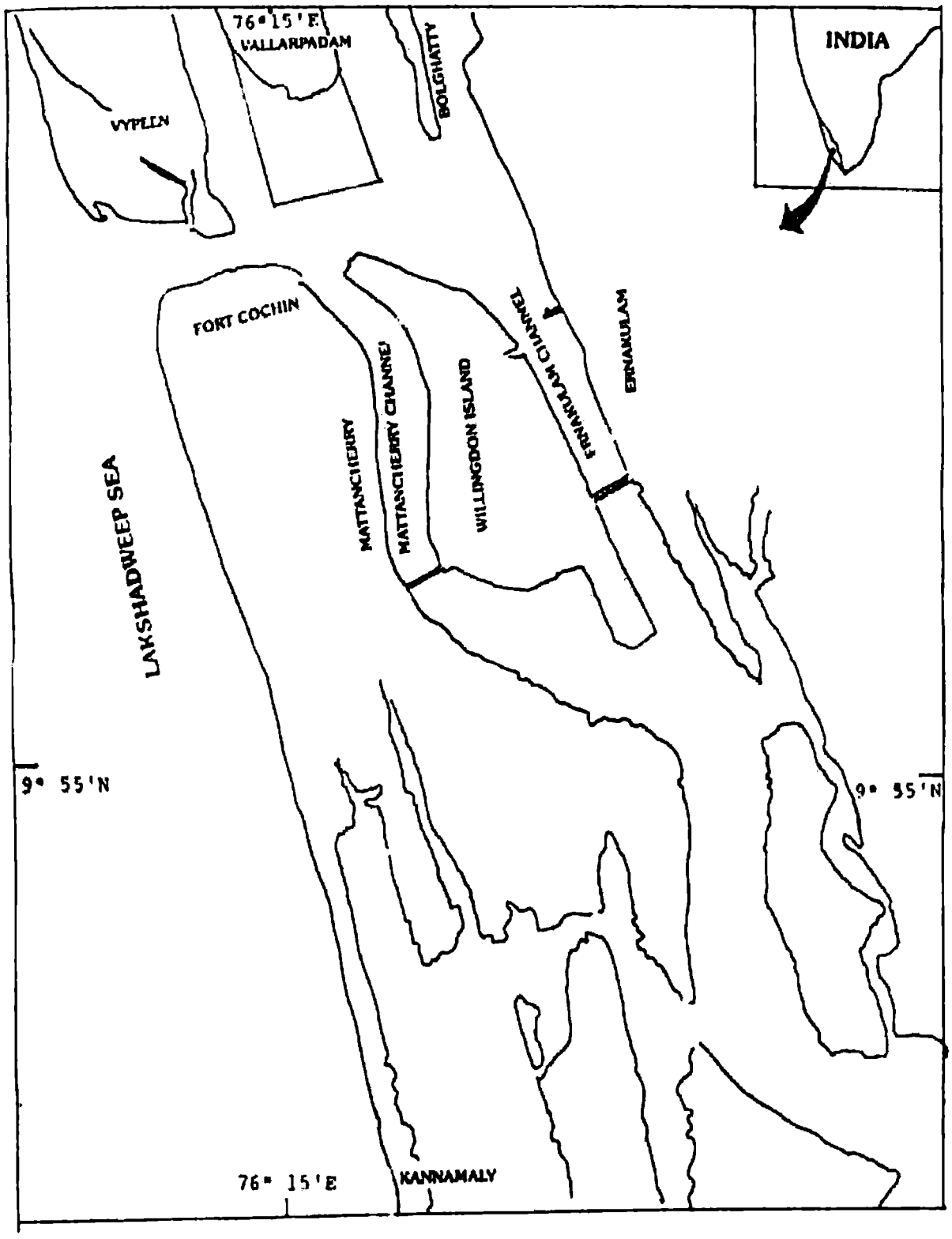


Figure 1 - Study Area

and II and that under microaerophilic conditions photosystem I can utilize H_2 S as the electron donor. Hence the two extremes with regard to the oxygen availability can be exploited. In the photic zone, the light passing through the water column varies in its intensity and spectral composition. These changes induce the development of layers composed of different species within the water column.

Cyanobacteria are world-wide in distribution. In the range of habitats which they occupy, the cyanobacteria are rivalled only by the bacteria (Fogg *et al.*, 1973). They grow in a variety of habitats such as freshwater systems, soils, hotsprings, brines etc. They are also abundant in eutrophic lakes and paddy fields. In 1849 , Montagne described the first blue-green alga from India. Many workers have studied the cyanobacterial flora of ricefields in India (Aiyer, 1965; Kolte and Goyal, 1985; Shaji and Pannikar, 1994).

Methodology

2.1 Collection and isolation of cyanobacteria

Water samples from pelagic and benthic environment of various aquatic ecosystems such as freshwater, estuary and sea were collected using a water sampler and transferred aseptically to sterile bottles of 100ml volume and transported to laboratory in thermocol box. Besides, cyanobacteria attached to various organic and inorganic substrata were also collected by using scalpel or

Primary enrichment of natural water samples

Laboratory culture of cyanobacteria

forceps. The collected samples were either transferred to 100ml clean glass bottle or in polythene bags.

Composition and mode of preparation of media

Composition of media used are given in the Table 2.1

After adding all the ingredients as per table in either marine, estuarine, pond water as stipulated, the media were autoclaved at 15 lb pressure for 15 minutes.

Isolation of cyanobacteria was done by the following methods:

1. Pipette method.

Filamentous forms and colonial types were isolated using a micropipette under microscope and transferred to culture tubes containing suitable medium.

2.Centrifuging or washing method.

Samples were centrifuged repeatedly at different revolutions and by inoculating the deposits obtained at different revolutions, different species were obtained.

3. By exploiting the phototactic movements.

Some species of cyanobacteria like *Oscillatoria* are capable of gliding motion and shows a tendency to move towards light. Gliding takes place within a mucilage sheath, with the sheath sticking to the substrate and being left behind the advancing trichome. Phototactic movement may be either towards or away from a light source. The genus *Phormidium* showed positive phototactic movement. In

Table 2.1
Composition of growth media used

Sl No.	Ingredients /liter	BG-11 Stainer et.al (1971)	ASN-111 Waterbury(1976)
1	Distilled water(ml)	1000	1000
2	Aged sea water(ml)	0	0
3	NaCl(g)	30	30
4	$MgSO_4.7H_2O$(g)	0.095	3.5
5	$MgCl_2.6H_2O$(g)	0	2
6	KCl(g)	0	0.5
7	$CaCl_2.2H_2O$(g)	0.36	0.5
8	$NaNO_3$(g)	1.5	0.75
9	$K_2HPO_4.3H_2O$(g)	0.04	0.02
10	Na_2CO_3	0.02	0.02
11	$Na_2SiO_3.9H_2O$(g)	0	0
12	EDTA,(Na,Mg Salt)(g)	0.001	0.0005
13	$C_6H_8O_7.H_2O$(g)	0.006	0.003
14	$FeNH_4C_6H_4O_7$(g)	0.006	0.003
15	Trace metal solution(A-5)ml	1ml	1ml

Composition of trace metal solution

Sl No.	Ingredients	Amount
1	Distilled water	1000ml
2	H_3BO_3	2.86g
3	$MnCl_2.$	1.81g
4	$ZnSO_4.7H_2O$	0.222g
5	$Na_2MoO_4.2H_2O$	0.039g
6	$CuSO_4.5H_2O$	0.079g
7	$Ca(NO_3)_2.6\ H_2O$	0.0494g

Composition of Allen and Nelson medium(1910)

	Ingredients	Quantity
Solution-A	KNO_3	2.2g
	Distilled water	100ml
Solution-B	$NaHPO_4$	4g
	$CaCl_2$	4g
	$FeCl_3$	2g
	Conc.HCl	2g
	Distilled water	80ml

Allen and Nelson's medium

For the preparation of Allen and Nelson's media,solution A and solution B were prepared separately as per the composition given in Table To each 1000ml aged seawater added 2ml solution A and 1ml solution B and sterilized by heating to 70^0 C for 30 minutes.

Composition of Walne's medium
(Stock solution -A)

Sl. No.	**Ingredients**	**Quantity**
1	Ferrous chloride	1.3g
2	Manganese chloride	0.36g
3	Boric acid	33.6g
4	EDTA disodium salt	45g
5	Sodium orthophosphate	20g
6	Sodium nitrate	100g
7	Trace metal solution	1ml
8	Distilled water	1000ml

(Stock solution-B)

Sl.No	Ingredients	Amount
1	Vitamin B_{12} (Cyanocobalamine)	10mg
2	Vitamin B_1(Thiamine)	200mg
3	Distilled water(sterile)	100mg

Walne's medium

Three stock solutions A,B and C were prepared. Stock solutions A and C were sterilized by autoclaving and stock solution B which contained vitamins like cyanocobalamine (B12) and Thiamine (B1) was sterilized by filtering through Millipore filter. 1 ml of each stock solutions was added to one litre of aged seawater.

Anabaena sp., the tip of the filament turns in the direction of light source. At high light intensities the organism moved away from light. In such cases this method was effective.

4. By agar plating method.

Agar medium was prepared by adding 1.5 g of agar to 1 litre of the culture medium. This agar solution was sterilized in an autoclave for 15 minutes under 120 lb pressure and 100°C temperature. Now this medium was poured in petri-dishes and kept for 24 hrs.

Samples containing required cyanobacterial species were streaked on to agar plates. These plates were incubated in an incubation chamber for a week providing light of 1000 lux and constant temperature of 25^0 C. After a week, the incubated plates were observed for the cyanobacterial colonies, which were transferred to culture tubes.

5. Serial dilution technique.

In this method, mainly five dilution steps the inocula corresponding to 1, 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} were involved for the isolation of the required cyanobacterial species. The samples containing the species to be isolated were inoculated in five series of culture tubes in various concentrations. These were kept under sufficient light (1000 lux) with constant temperature. After one week, some discolouration was observed in the culture tubes due to the growth of cyanobacteria If

Culture of cyanobacteria and other microflora.

monospecific culture was not obtained, the process was repeated. On attainment of monospecific culture, this was transferred to the enriched medium and by repeated and frequent subculturing it was made pure as far as possible. This was asceptically transferred to sterile 250ml flasks containing sterilized medium. On development of cyanobacterial culture, the same was transferred to sterilized flasks containing sterilized medium.

2.2 Identification

Cyanobacteria collected from marine environment had been identified and maintained in culture as per the methods of Burlew, J.S(ed) 1976, Kinne, 1976 , Fogg, 1975, Desikachary, 1959,Staley *et al.,* 1989 and Golubic *et.al* (1996) and Bergy's Manual of Systematic Bacteriology, (1989). They were classified based on observations of natural populations recognizable by their distinctive morphological characteristics and their distribution along environmental gradients. In fact, there is no specific single reference exclusively for the taxonomical determination of aquatic cyanobacteria.

Key used for identifying the common genera of toxigenic species of cyanobacteria.

(Skulberg et al,1984)

I. Unicellular or colonial, reproduction by binary fission

A. Cell shape coccoid or ellipsoid , forming aggregates

1. Cells elongate, dividing lengthwise----------*Coelosphaerium*
2. Cells egg shaped or heart shaped, division in three planes- *Gomphosphaeria*

3. Cells coccoid, division in two or three planes----------*Microcystis*
4. Cells elongate, division in one plane only----------*Synechococcus*
5. Cells coccoid, division in one plane only----------*Synechocystis*

B. Cells rod shaped or elongate, in short chains

1. Cells short rods with rounded or squarish ends ----------*Pseudanabaena*

II Multicellular, forming filaments

A. Trichomes with non- differentiated cells,
reproduction by fragmentation (hormogonia)

(a) Filaments single or in loose masses, sheath
usually not present

1. Trichomes more or less straight, end cell distinctly marked--*Oscillatoria*
2. Trichomes in bundles (marine)----------*Trichodesmium*

(b) Filaments single or in loose masses, sheath present

1. Trichomes many in a sheath----------*Schizothrix*
2. Trichomes single in a firm sheath----------*Lyngbya*
3. Trichomes single in a mucilaginous sheath----------*Phormidium*

C. Trichomes with heterocysts, reproduction by fragmentation (hormogonia) and akinetes

1. Heterocysts generally terminal on the trichomes,
A single akinete adjoining----------*Cylindrospermum*
2. Heterocysts generally intercalary, cells and heterocysts cylindrical,
end cells elongated, filaments in flake-like colonies—*Aphanizomenon*
3. Heterocysts generally intercalary, vegetative
cells homogenous, filaments flexuous and
contorted, developing in gelatinous colonies----------*Nostoc*
Heterocysts generally intercalary, cells spherical or longer than wide,
filaments separate or in tangled masses---------- *Anabaena*
4. Heterocysts intercalary, trichomes ,

5. more than one in a sheath-- ----------*Hormothamnion*
6. Heterocysts intercalary, cells and heterocysts compressed (discoid) --*Nodularia*
7. Heterocysts basely, akinetes next to the heterocyst, Colonies spherical or hemispherical----------*Gloeotrichia*

The aim of this investigation was to study the occurrence of different species of aquatic cyanobacteria in and around Cochin(Table2.2). Collections were made from various aquatic environments in Cochin. Emphasis was given to the qualitative collections rather than their quantitative assessment. The various aquatic environments had been screened for the presence of cyanobacteria during various seasons viz., summer, winter and rainy seasons. The occurrence and distribution of these cyanobacteria collected for a period of one year (1999- 2000) is given in Table 2.2.

The distribution pattern is shown below:

Cyanobacteria collected from freshwater environment included the following genera

Synechocystis, Gloeocapsa, Chroococcus, Gloeothece, Synechococcus, Microcystis, Aphanocapsa, Aphanothece, Merismopedia, Eucapsis, Coelosphaerium, Chlorogloea, Chaemosiphon, Crinalium, Microcoleus, Hydrocoelom, Schizothrix, Lyngbya, Symploca, Trichodesmium, Oscillatoria, Spirulina, Phormidium, Microchaete, Fortiea, Anabaenopsis, Cylindrospermum,

Table 2.2

	Freshwater			Marine			Estuarine		
	S	R	W	S	R	W	S	R	W
Order : Chroococcales									
Family : Chroococcaceae.									
Synechocystis aquatilis Sauv.	x	x	x	x		x	x	x	
S. salina Wislouch.		x	x	x	x	x		x	x
G rupestris Kutz.		x	x						
G. crepidinum Thuret.	x		x	x		x		x	x
G. punctata Nag.		x	x	x	x				
G. decorticans (A.Br.) Richt. Forma		x	x						
Chroococcus turgidus (Kutz) Nag.	x	x						x	x
C. minutus (Kutz) Nag.		x	x						
C. limneticus Lemm.				x	x	x	x	x	
C. cohaerens (Breb.) Nag.	x			x		x	x		
C. montanus Hansgirg		x							
G. membranaceae(Rabenh.) Bornet	x		x	x					
G. rupestris (Lyngb.) Bornet.		x							
Dactylococcopsis raphidioides Hansg.				x					
Synechococcus aerug(Hansg) Forti		x							
S elongatus Nag.		x	x	x		x	x		
Microcystis litoralis (Hansg.) Forti				x	x				
M.robusta (Clark) Nygaard.	x								
M. aeruginosa Kutz.	x			x		x			
M. flos-aquae (Wittr.) Kirchner	x		x						
M.elabens(Breb.) Kutz.	x	x							
Aphanocapsa littoralis Hansg.	x								
A. biformis A. Br.				x	x				
A. montana Cramer				x		x			
A. muscicola (Menegh.) Wille	x								
A.pallida (Kutz) Rabenh.	x		x						
Aphanothece saxicola Nag.	x		x						
A. nidular(Kutz) Rabenh.	x		x						
A. microscopica Nag.		x							
Merismopedia miniata Beck				x	x				
M.punctata Meyen.				x		x			
M. tenuissima Lemm.		x							
Eucapsis minuta Fritsch		x						x	x
Coelosphaerium dubium Grunow		x							
C.kuetzingianum Nag.		x							
Gomphosphaeria lac Mitra				x					
Family : Entophysalidaceae									
Johannesbaptistia pellucida (Dickie) Tayler et Drouet				x					x
Chlorogloea fritschii		x							
Order: Chaemosiphonales									
Family : Chaemosiphonaceae									
Chaemosiphon fuscus (Rostaf.) Hansgirg		x							
Family : Dermocarpaceae									
Dermocarpa clavata (Setchell et Gardner) Geitler				x					
Dermocarpa olivacea var. gigantea Rao, C.B				x					
Order: Pleurocapsales.									
Family:Pleurocapsaceae									
Myxosarcina burmensis Skuja				x					
Family : Hyellaceae									
Hyella caespitosa Born. Et Flah.				x				x	
Order : Nostocales									
Family: Oscillatoriaceae									
Crinalium magnum Fritsch et John		x							
Microcoleus acutissimus Gardner		x							
M.chthonoplastes Thuret ex Gomont				x					
M.subtorulosus (Breb.) Gomont		x							

M. lacustris (Rabenh.) Farlow			x							
Polychlamydum variurn Ghose					x					
Hydrocoleum lyngbyaceum Kutz(after gomont)									x	
H. heterotrichum Kutz em Gomont			x							
Schizothrix lacustris A. Br.ex Gomont			x							
Lyngbya mesotrichia Skuja			x		x					
L. gardeneri (Setchell et Gardner) Geitler			x							
L. rivularianum Gom					x					
L. mucicola Lemmermann			x							
L. borgerti Lemmermann			x							
L. truncicola Ghose Klebahn					x					
L. martensiana Menegh. Ex Gomont			x							
Symploca hydnoids Kutzing ex Gomont					x					
Trichodesmium lacustre Klebahn			x							
T. thiebautii Gomont					x					
Oscillatoria nigroviridis Thwaites ex Gomont					x					
O. miniata(Zanard) Hauck ex Gomont			x							
O. anguina (Bory) Gomont					x					
O. princeps Vaucher Ex Gomont			x							
O. proboscidea Gomont			x							
O. amphibia Ag. Ex. Gomont			x		x					
O. brevis (Kutz) Gomont			x							
O. formosa Bory ex Gomont					x					
O. limnetica Lemm.			x							
O. laetevirens (Craun) Gomont			x		x					
O. rubescens D.C. ex. Van Goor			x							
O. sancta (Kutz) Gomont			x							
O. pernornata, Skuja			x							
O. acuminata Gom.					x					
O.limosa Ag.			x							
Spirulina platensis (Nordst)			x							
Spirulina meneghiniana Zanard. Ex Gomont			x							
Phormidium ceylanicum Wille					x					
P. molle (Kutz.) Gomont			x		x					
P. foveolarum (Mont) Gomont			x		x					
P. orientale West, G.S.			x							
P. valderianum (Delp.) Gomont					x					
P. hansgirgi (Schmiddle)			x							
Family: Microchaetaceae										
Microchaete elongata (Fremy) comb. Nov			x							
Fortiea incerta Skuja			x							
Family : Nostocaceae										
Richelia intracellularis Johs. Schmidt.					x					
Anabaenopsis arnoldii var. indica Ramanathan				x	x				x	
Cylidrospermum alatosporum Fritsch			x							
Anabaena constricta Szafer					x					
Anabaena fertilissima Rao C.B			x							
Nostoc hatei Dixit			x		x					
Nostoc calcicola Brebisson ex Born and Flah			x							
N. commune Vaucher ex.Born and Flah			x							
N.muscorum Ag. Ex. Born.and Flash			x							
Pseudanabaena schmidlei Jaag. O.			x		x					
Nodularia spumigena Mertens ex Born et Flah			x		x		x			
Aulosira pseudoramosa Bharadwaja			x							
Family: Scytonemataceae										
Plectonema terebrans Bornet ex Gomont					x					
P. thomasinianum(Kutz) Born		x	x							
P. indica Dixit			x							
P.wollei Farlow ex Gomont					x					
Camptylonemopsis lahorensis (Ghose)Desikachary			x							
Scytonema bohneri Schmidle					x					
S. subtile Moeb			x							
S. rivulare Borzi			x							
S.fremyii nom.nov					x					
Calothrix brevissima West, G.S.					x					
Dichothrix gypsophila (Kutz) Born et. Flah			x							
Rivularia hansgirgi Schmidle			x							
Gloeotrichia echinulata (J. E) Smith P.Richter			x							
Order: Stigonematales										
Family: Mastigocladaceae										
Mastigocladus laminosus Cohn					x					
Hapalosiphon welwitschii West, W and G.S.			x		x					
Family: Stigonemataceae										
Stigonema dendroideum Fremy (after Fremy)			x							
Westiellopsis prolifica Janet			x				x			

Anabaena, Nostoc, Pseudanabaena, Nodularia Aulosira, Plectonema, Camptylonemopsis, Scytonema, Dichothrix, Rivularia, Gloeotrichia, Stigonema, Westiellopsis.

In marine environment ,following genera were found.

Synechocystis, Gloeocapsa, Chroococcus, Gloeothece, Synechococcus, Microcystis, Aphanocapsa, Aphanothece, Merismopedia, Gomphosphaera, Johannesbaptistia, Dermocarpa, Myxosarcina, Hyella, Microcoleus, Polychlamydum, Lyngbya, Symploca, Trichodesmium, Oscillatoria, Phormidium, Richelia, Anabaenopsis, Anabaena, Nostoc, Pseudanabaena, Nodularia, Plectonema, Scytonema, Calothrix, Mastigocladus.

Estuarine genera included *Synechocystis,Synechococcus, Gloeocapsa, Chroococcus, Eucapsis, Johannesbaptistia, Hyella caespitosa, Hydrocoleum, Anabaenopsis.*

It is significant that the cyanobacteria collected includes the following toxigenic genera.

Coelosphaerium, Gomphosphaeria, Microcystis, Synechococcus, Synechocystis, Anabaena, Cylindrospermum, Gloeotrichia, Hapalosiphon, Lyngbya, Nostoc,Oscillatoria, Pseudanabaena, Trichodesmium.

Cyanobacterial genera found only in freshwater are *Eucapsis, Coelosphaerium, Chlorogloea, Chaemosiphon, Crinalium, Hydrocoleum, Schizothrix,* Spirulina,*Michrochaete, Fortiea, Cylindrospermum, Aulosira, Camptylonemopsis, Rivularia, Gloeetrichia, Stigonema, Westiellopsis.*

Genera found only in marine or estuarine habitats.
Johannesbaptistia, Gomphosphaeria, Dermocarpa, Myxosarcina, Hyella, Microcoleum, Calothrix and Mastigocladus.

Genera found in both freshwater and marine habitats.
Synechocystis Gloeocapsa, Chroococcus, Gloeothece, Synechococcus, Microcystis, Aphanocapsa , Aphanothece, Merismopedia, Microcoleus, Lyngbya, Symploca, Trichodesmium, Oscillatoria, Phormidium, Cylindrospermum, Anabaena, Nostoc, Pseudanabaena, Nodularia, Plectonema, Scytonema.

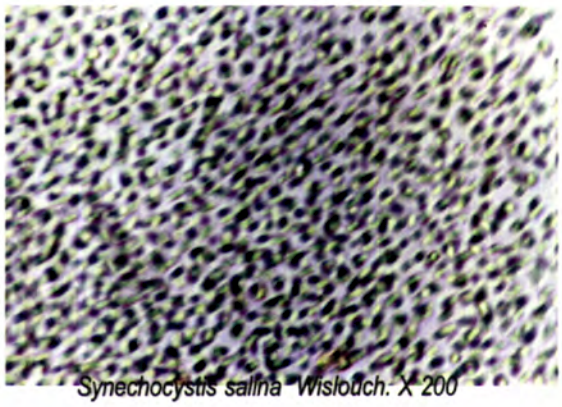
In the present investigation, 116 species of cyanobacteria have been recorded of which 36 belonged to family Chroococcaceae, 2 to Entophysalidaceae, 1 to Chaemosiphonaceae, 2 to Dermocarpaceae, 1 to Pleurocapsaceae, 1 to Hyellaceae, 42 to Oscillatoriaceae, 2 to Microchaetaceae, 12 to Nostocaceae, 13 to Scytonemataceae, 2 to Mastigocladaceae and 2 to Stigonemataceae. All these species were distributed among different aquatic environments viz., freshwater (79sp.), marine (54spp.) and estuarine(10spp.). Several species were found to occur in both freshwater and marine environments. Among these, 17 genera were

found exclusively distributed in freshwater and 8 genera were found in marine and 22 genera were found distributed in both marine and freshwater environment. It is inferred from the distribution pattern of cyanobacteria that several species have wide salinity tolerance as in the case of *Synechocystis* sp. and *Synechococcus*.sp.

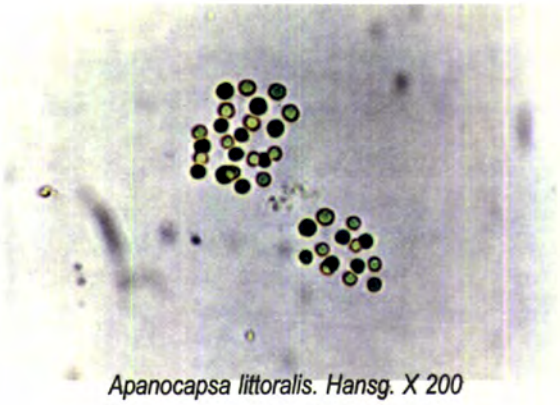
On analysis of seasonal variation it was found that maximum number of cyanobacteria was invariably found during rainy season in freshwater,marine and estuarine environment.

Studies on the occurrence, distribution and contribution of picoplanktonic cyanobacteria are sparce in India. However, Odate et.al. ,(1989) studied the distribution of cyanobacteria and other picophytoplankton in the western northpacific ocean. They found that cyanobacteria were the most abundant forms in the surface of subtropical water. The standing crop of picophytoplankton was however high in the northern part and low in the subtropical water. Their studies revealed that algae other than cyanobacteria may significantly contribute to the picophytoplankton community under the low water temperature conditions of open waters.

Laloraya and Mitra(1973.) studied the cyanobacteria in the paddy fields of India and identified 122 forms belonging to different families. Prasad (1998) recorded cyanobacteria of ricefields and soil in Nepal belonging to different genera Thajuddin and Subramanian(1992) studied the cyanobacterial flora of the east

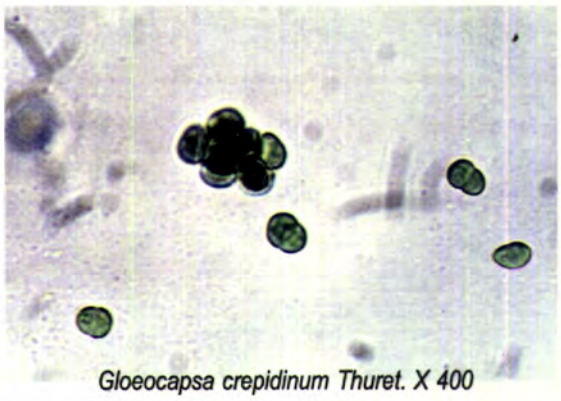


Synechocystis salina Wisloudh. X 200



Apanocapsa littoralis. Hansg. X 200

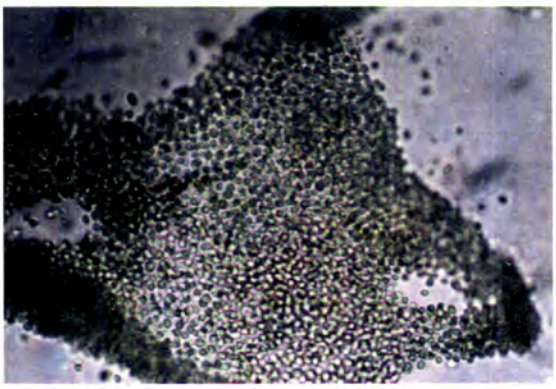
Gloeothece rupestris Kutz. X 400



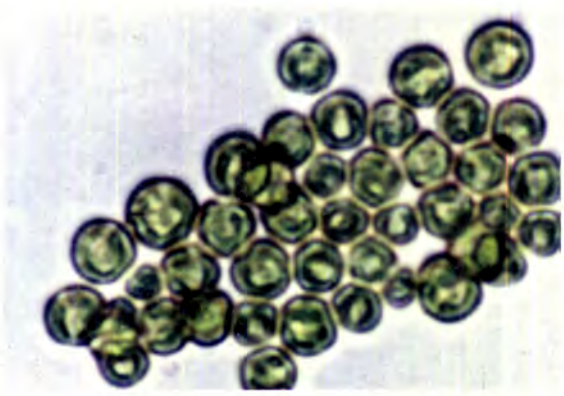
Gloeocapsa crepidinum Thuret. X 400

Gloeocapsa rupestris Kutz. X 400

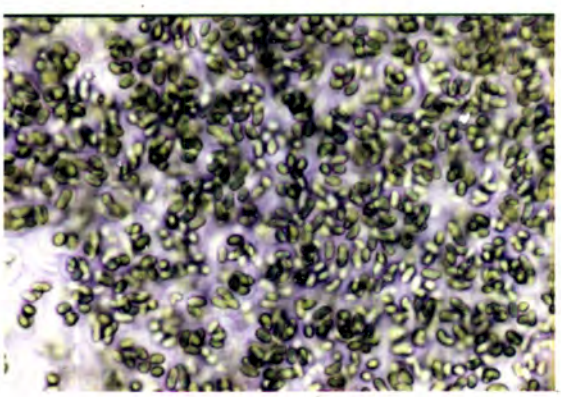
Chroococcus turgidus Kutz. X 400



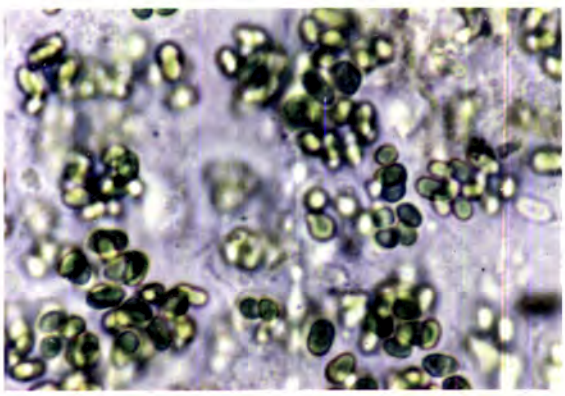
Microcystis aeruginosa Kutz X 100



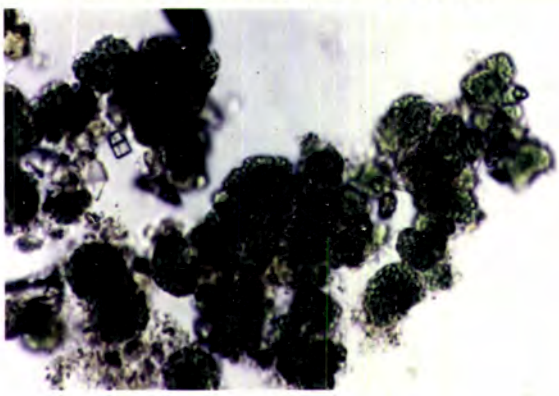
M.aeruginosa X 400



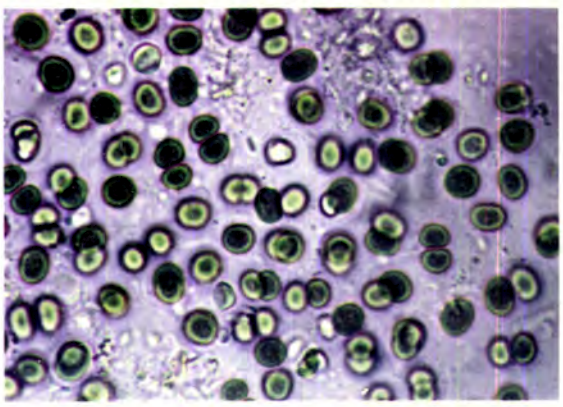
Aphanothece pallida (Kutz) Rabenh X 200



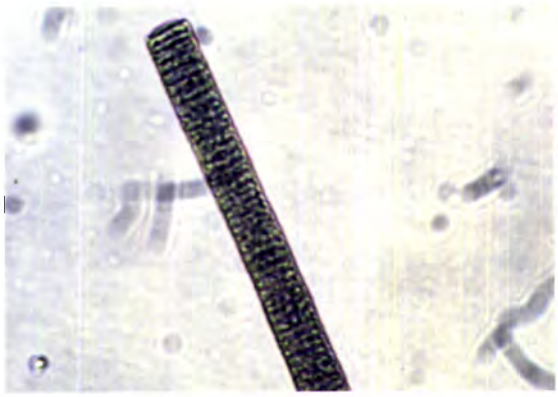
Gloeothece membranaceae (Rabenh.) Born. X 400



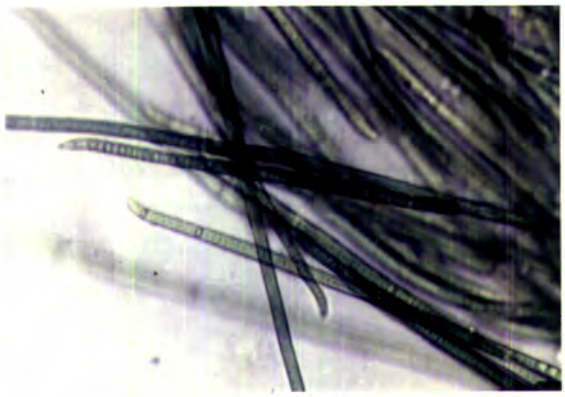
Myxosarcina burmensis Skuja. X 200



Gloeocapsa decorticans (A. Br) Richter X 400



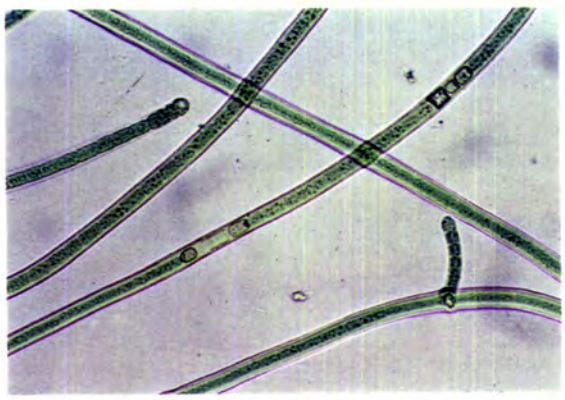
Oscillatoria limosa. Ag. X 400



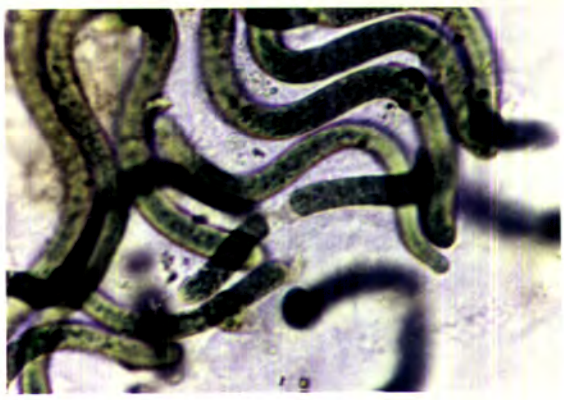
Oscillatoria acuminata.Gom. X 200

Stigonema dendroideum Fremy. X 200

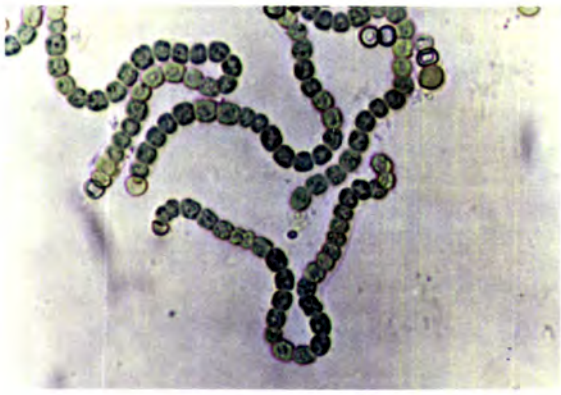
Fischerella muscicola (Thuret) X 200



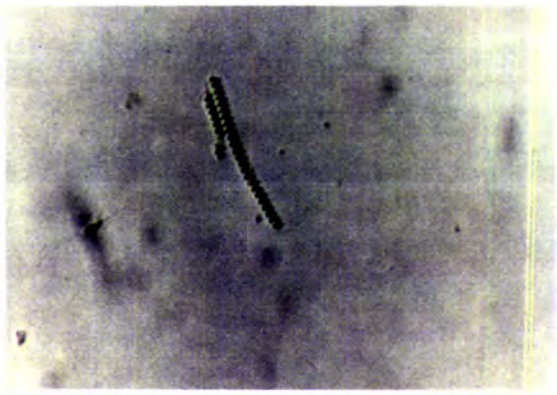
Hapalosiphon stuhlmannii Hieron. X 200



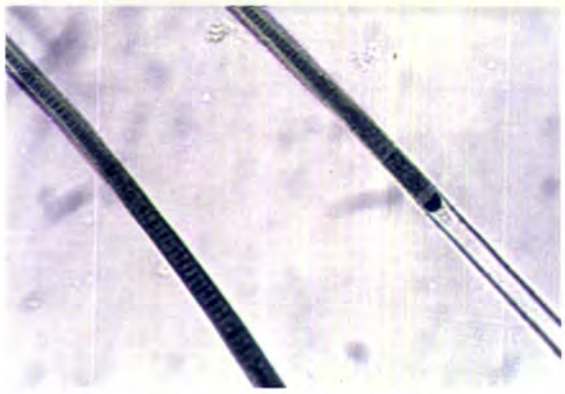
Spirulina platensis(Nordst.) Gom. X 400



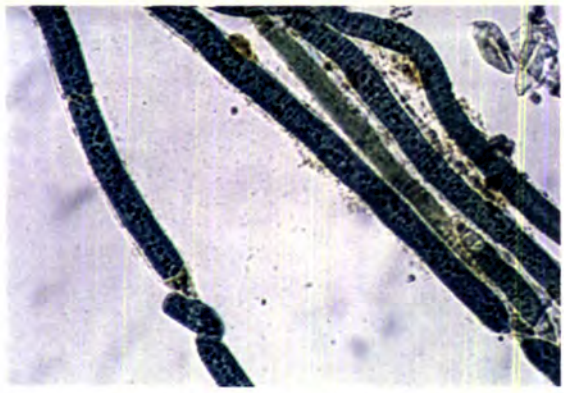
Nostoc linckia (Roth) X 200



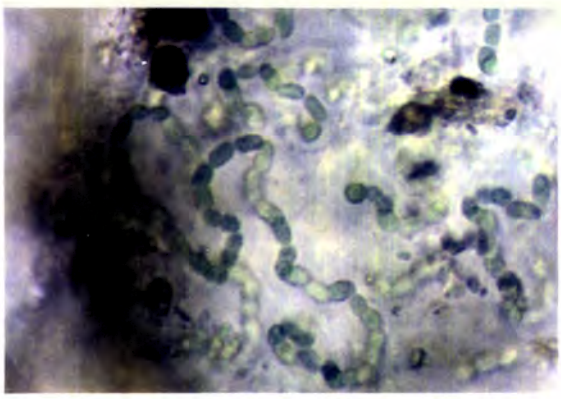
Spirulina meneghiniana Zanard X 400



Lyngbya rivularianum Gom. X 200

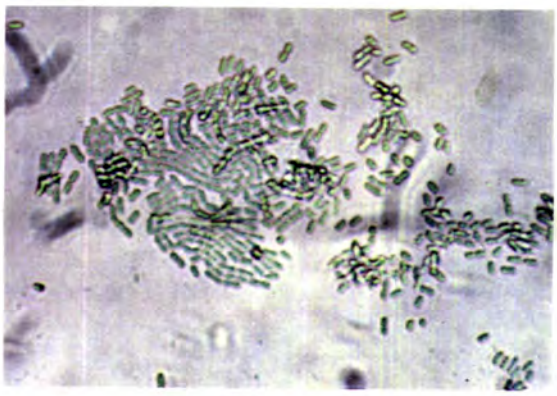


Plectonema thomasinianum (Kutz) Born X 200

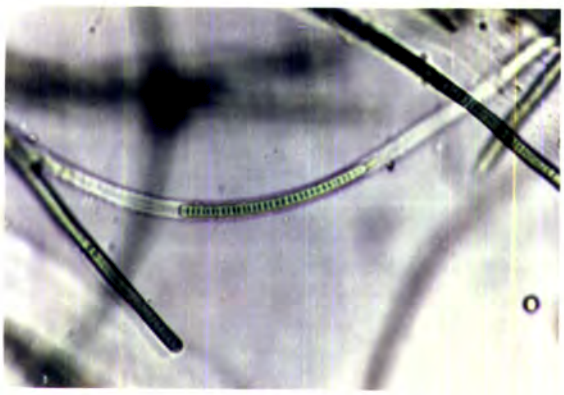


Nostoc hatei Dixit. X 200

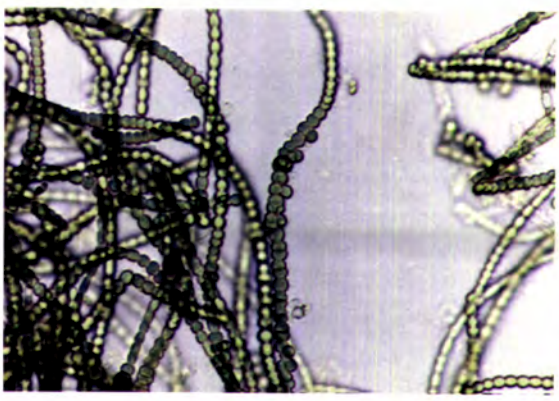
Phormidium orientale West G.S X 400



Synechcoccus elongatus Nag. X 400



Lyngbya martensiana Menegh. X 200



Westiellopsis prolifica Janet X 200

W. prolifica – lateral branches arising lfrom one side X 200

coast of India and found that the habitat exhibited such diversity of cyanobacteria. It is very difficult to distinguish marine and freshwater cyanobacteria from literature surveys. Further, many cyanobacteria appear to have an innate flexibility to adapt themselves to either habitats (Subramanian, 1998). Though several aspects of cyanobacteria such as nitrogen metabolism , fatty acid metabolism , toxicity and bioremediation have been investigated, the distribution of aquatic flora of a region with diverse aquatic habitats in and around Cochin is first of its kind.

3
CHAPTER

BACTERIA ASSOCIATED WITH CYANOBACTERIA

In aquatic environment autotrophic and heterophic planktonic organisms compete for nutrient sources. Competition studies in chemostat cultures inoculated with natural phytoplankton assemblages, have clearly shown that under continuous nutrient supply only as many species coexist as there are limiting factors (Sommer, 1983; 1985). A pulsed nutrient regime can help to maintain the coexistence of more species and the frequency of nutrient addition affects the resulting species richness(Robinson and Sandgren,1983; Sommer, 1985), competitive outcome and community structure (Sakshaug and Olsen,1986) as well as cell size structure (Turpin and Harrison, 1980; Suttle et al., 1987)

Bacteria are normal component of both internal and external surfaces of higher organisms. They act as decomposers and transformers of organic matter to vitamins and aminoacids, besides being served as direct food sources for higher organisms. Thus they play a ubiquitous role in energy transfer and nutrient flux at all trophic levels. Several bacteria are able to degrade pollutants and some are indicators of faecal contamination. Many human pathogens such as *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Aeronomonas* are components of aquatic microbial ecosystems. The presence of *Vibrio* in aquatic environments is a serious problem in aquaculture industry. (Singh *et al.*, 1985, Singh, 1990; Hipolito,*et al* 1996)

The heterotropic planktonic bacteria are the most important heterotrophs in marine and freshwater pelagic ecosystems, possible utilizing the bulk of primary production (Cole et al.,1988;) In Lake Balaton,it was estimated that about a half of the planktonic primary production is channeled through bacterioplankton on yearly basis (Voros et al.,1996).Although planktonic bacteria play a key role in the degradation of organic carbon substrates, recent studies suggest that bacteria function as sinks rather than as sources of phosporus. Gude et al. (1992) failed to observe a net phosporus transfer from bacteria to algae in natural water samples.

Several work such as Currie and Kalff, 1984, suggested strong competion between algae and bacteria for phosphorus when the concentration of phosphorus is low. Fractionated uptake experiments with natural plankton comunites showed that the concentration rather than the quality of the phosphorus source may be decisive for the outcome of competion for phosphorus between algae and bacteria (Gude et al., 1992).

Several bacteria are found attached to various photosynthetic microflora including cyanobacteria. Many workers have studied the inhibition properties of photosynthetic microflora on various species of *Vibrios* (Gauthier *et al.*, 1978, Austin and Day, 1990, Viso *et al.*,1987, Kogure *et al.*,1979). In the present work,

associated bacteria with marine, freshwater and estuarine species of cyanobacteria are discussed.

Cyanobacteria are noted for their interactions with bacteria which may be pathogenic, saprophytic or symbiontic. Paerl and Kellat (1978) and Cladwell(1977) showed that cyanobacteria are frequently the sites of extensive bacteria and fungal colonization. The bacterial attachment sites are called microzones, where chemical and physical properties are determined by microbial exchange process (Cladwell and Cladwell, 1978).

Bacillus rods are frequently visible on staining the sheaths of bluegreen algae. *B.polymyxa*, a plant pathogen, produced an extracellular pectinase. This pectinase might presumably be expected to attack the mucilages of blue- green algae.

Bacteria associated with cyanobacteria utilizes the extracellular products of the latter. Many bacteria, with their ability to lyse the cyanobacteria and to grow in the lysate, have several survival strategies for their maintenance in nature.

According to Cladwell(1979) ensheathed bacteria pose another situation both in purification techniques and the relationship between the cyanobacteria and the bacterium. However, working with non-axenic cultures reflects a more 'realistic'

approach to the study of organisms in relation to their natural environment than axenic cultures.

Atleast in certain cases it is found that several species of cyanobacteria shows slow growth rate in the absence of associated bacteria. Bacteria may be pathogenic utilizes extracellular products or enhance the growth of cyanobacteria .

In the present investigation, special attention was taken to study the bacteria associated with cyanobacteria. Aquatic cyanobacteria collected and isolated from various habitats of freshwater, estuarine and marine environments were analysed for associated bacteria. For analysis of freshwater samples, nutrient agar medium was used while Zobell's agar was used in the case of marine samples Isolated bacteria were further subjected to morphological and biochemical tests and identified using Bergy's Manual of Systematic Bacteriology(Buchnan and Gibbons, 1974)

Media used for isolating the associated bacteria:

Nutrient Agar Medium

Peptone	5 g
Beef extract	3 g
Sodium chloride	5 g in 1000ml freshwater
Agar	20g

The pH was adjusted to 7.2 before autoclaving.

Zobell's Agar Medium

Peptone	5 g
Yeast extract	1 g
Ferric phosphate	0.02g
Seawater	1000 ml
Agar	20 g

The pH was adjusted to 7.2 before autoclaving.

Tests used for identification of bacteria:

Gram staining

Bacterial cultures 12-18 hrs old were taken and smears were prepared on clean glass slides for staining. The primary stain ammonium oxalate-crystal violet was added to the fixed smear and allowed to stand for one minute. Then the slides were rinsed in a gentle running water and allowed to dry. The sildes were flooded with mordant, Lugol's iodine solution and allowed to stand for one minute. The slides were then rinsed gently in running water. Flooded the slides with the decolouriser (acetone- alcohol) and allowed to stand for 30 sec. Rinsed in gentle running water and kept for drying. The counter stain Safranine O was added to the smear and allowed to stand for one minute. Then the slides were washed, air-dried and observed under an oil immersion microscope. Gram +ve bacteria appear in violet colour and Gram -ve in pink colour.

Spore staining

Smears were prepared using 60-72 hrs. old bacterial culture. The slides were flooded with malachite green and allowed to react at room temperature for one

minute. Then the slides were heated for steaming by using a Bunsen's burner periodically until the rise of the steam from the slide. The slide was then waved over the flame as the steaming ceases without allowing it to boil or dry. The slides were thus steamed for about three minutes replacing the malachite green as it evaporates from the slide.

The slides were then allowed to cool for five minutes and rinsed with water. The slides were then flooded with safranine counter stain. The stain was allowed to react for one minute. The slides were washed with water and allowed to air dry and slides were observed under oil immersion microscope. Bacterial endospores appeared as green oval shaped structures within pink vegetative cells.

Oxidase test

Small pieces of filter paper were soaked in 1% aqueous tetramethyl para phenylene diamine dihydrochloride and the papers were dried. A small portion of the culture was placed on the test paper with a clean platinum loop and the colour change (purple colour) within 15 seconds was observed.

Catalase test.

On a clean glass slide a smear of the bacterial culture was prepared A drop of hydrogen peroxide solution was added on the smear and the reaction was carefully observed. Effervescence or bubbling was noticed in the case of cultures producing catalase enzyme.

Marine Oxidation Fermentation Test.(MOF)

Hugh- Leifson's medium was prepared with specified composition, melted and distributed in culture tubes having aliquots of 5ml each and plugged with cotton, autoclaved and slants were prepared The test culture was inoculated in the hard agar and by stabbing and streaking on the slope taking care not to allow air bubbles in the agar medium, incubated for 48hrs. and observations were made.

Oxidative reaction

Change of pink to yellow colour in the slope area.

Fermentative reaction

Change of pink to yellow from the slope to the bottom of the butt i.e; the entire tube.

Alkaline reaction

Change of pink to deep pink at the slope..

MOF medium

22 g dehydrated medium in 1000ml water.

1% dextrose: 2% Agar
The pH was adjusted to 8

Mannitol- motility test

The medium was prepared and about 3-4 ml was distributed in test tubes for about 3-4ml. The tubes were sterilized in an autoclave and left for setting in a vertical position. After setting, clear test tubes were selected and inoculum from the culture was stabbed straight to the bottom. The tubes were then incubated at room temperature for 48-72 hrs.

Yellow colour in the medium shows the utilization of the mannitol. No colour change is negative. Motile bacteria diffuse from the culture and the medium become dense. Non- motile forms grow at stabbing line only with shaft margin.

Mannitol motility agar

Mannitol	10g
Beef extract	3g
Peptone	5g
NaCl	15g
Phenol	0.1g
Tap water	1000ml
Agar	4g
pH	7.2

Arginine hydrolysis

Incubate cultures in Arginine broth for 24- 28 hrs and add a few drops of Nessler's reagent. A brown colour indicates hydrolysis.

Arginine broth.

Tryptone	5g
Yeast extract	5g
Di potassium hydrogen Phosphate	2g
L-arginine monohydrochloride	3g
Dextrose	0.5g
Water	1000 ml

Results and discussion:

On examining the cyanobacteria collected from various environments, it was observed that different bacteria were attached to cyanobacterium. They were isolated and identified using standard methods according to Bergy's Manual of Determinative Bacteriology

The bacteria found associated to these selected freshwater and the marine cyanobacterial species examined are given in the tables 3.1 a &b. It was observed that while some species of cyanobacteria exhibited specificity in bacterial association while certain species welcomed several variety of bacteria for association. Bacteria belonging to nine genera were found attached to the various cyanobacteria.

Marine samples Table 3.1 a

	Bacillus	Coryneform	Nessena	Aerobacter	Acinelobacter	Alkaligenes	Micrococcus	Staphylococcus	Vibrio	Pseudomonas	Enterobacteriaceae
S. salina Wislouch.	x										
Gloeocapsa punctata Nag.		x	x								
G. crepidinum Thuret.	x			x							
G. punctata Nag.		x						x			
Chroococcus turgidus (Kutz) Nag.	x	x						x	x		
C. limneticus Lemm.		x			x			x			
C. cohaerens (Breb.) Nag.	x					x					
Gloeothece fusco-lutea Nag.		x					x				
G. rupestris (Lyngb.) Bornet.		x									
S.elongatus Nag.	x										
Microcystis litoralis					x						
M.robusta (Clark) Nygaard.	x								x		
M. aeruginosa Kutz.		x									
M. flos-aquae (Wittr.) Kirchner		x									
M.elabens(Breb.) Kutz.										x	
Aphanocapsa littoralis Hansg.									x		
A. biformis A. Br. (Hansg) Forti		x							x		
A. montana Cramer						x					
A. crassa Ghose	x										
Aphanothece saxicola Nag.											x
A. nidulans Richter, P.		x									x
Merismopedia miniata Beck	x										
Eucapsis minuta Fritsch.	x										
Gomphosphaena lacustris Chodat	x										
Johannesbaptistia pellucida (Dickie) Taylo	x										
Chlorogloea fritschii							x				
Dermocarpa clavata (Setchell et Gardner) Geitler									x		
Dermocarpa olivacea var. gigantea Rao, C B						x			x		
Myxosarcina burmensis Skuja		x					x				
Hyella caespitosa Born. Et Flah.	x										
Crinalium magnum Fritsch et John	x										
Microcoleus acutissim Mitra	x										
M.chthonoplastes Thuret ex Gomont	x										
M.subtorulosus (Breb.) Gomont	x					x					
M. lacustris (Rabenh.) Farlow			x			x					
Polychlamydum varium Ghose						x					
Hydrocoleum lyngbyaceum Kutz(after gomont)		x									
Lyngbya mesotrichia Skuja		x									
L. rivulananum Gom.	x										
L. truncicola Ghose	x										
Symploca hydnoids Kutzing ex Gomont	x										
Oscillatoria nigroviridis Thwaites ex Gomont	x										
O. anguina (Bory) Gomont	x										
O. amphibia Ag. Ex. Gomont							x				
O. formosa Bory ex Gomont						x					
O. laetevirens (Craun) Gomont											
Phormidium ceylanicum Wille									x		
P. molle (Kutz.) Gomont						x		x			
P. foveolarum (Mont) Gomont											
P. valderianum (Delp.) Gomont	x										
Anabaenopsis arnoldi var. indica Ramanatha	x										
Anabaena constricta Szafer	x										
Nostoc hatei Dixit	x										

Freshwater samples Table 3.1 b

	Bacillus	Coryneform	Neisseria	Aerobacter	Acinetobacter	Alkaligenes	Micrococcus	Staphylococcus	vibrio	Pseudomonas	Enterobacteriaceae	Pasteurella
G.crepidinum Nag.	x									x		
G.rupestris Kutz.											x	
G. punctata Nag.					x							
G. decorticans (A.Br.) Richt. Forma			x									
Chroococcus turgidus (Kutz) Nag.	x						x					
C. minutus (Kutz) Nag.	x	x										
Gloethece membranacea Nag				x								
G. rupestris (Lyngb.) Bornet.	x				x							
Synechococcus aeruginosus Nag.	x											
S.elongatus Nag.			x									
M.robusta (Clark) Nygaard.				x								
M. flos-aquae (Wittr.) Kirchner	x											
Aphanocapsa littoralis Hansg	x											
A. biformis A. Br.	x											
A. montana Cramer			x									x
A. muscicola (Menegh.) Wille												
A. microscopica Nag.							x					
M. tenuissima Lemm.		x										
Eucapsis minuta Fritsch Mehra	x											
Chlorogloea fritschii				x								
Chaemosiphon fuscus (Rostaf.) Hansgirg	x									x		
Microcoleus acutissimus Gardner	x											
Lyngbya mesotricha Skuja	x											
L. gardeneri (Setchell et Gardner) Geitler				x		x						
L. martensiana Menegh. Ex Gomont												
O. miniata(Zanard) Hauck ex Gomont		x										
O. princeps Vaucher Ex Gomont	x											
O. proboscidea Gomont	x											
O. amphibia Ag. Ex. Gomont												
O. brevis (Kutz) Gomont		x										
O. limnetica Lemm.				x								
P. molle (Kutz.) Gomont		x										
P. foveolarum (Mont) Gomont		x										
P. orientale West. G.S.							x					
P. hansgirgi (Schmiddle)		x										
Microchaete elongata (Fremy) comb. Nov												
Nostoc hatei Dixit				x								
Nostoc calcicola Brebisson ex Born and Flah	x					x						
N. commune Vaucher ex Born and Flah	x											
N.muscorum Ag. Ex. Born.and Flash				x					x			
Pseudanabaena schmidtei Jaag. O.	x											
Nodularia spumigena Mertens ex Born et Flah								x				
Aulosira pseudoramosa Klebahn	x											
Scytonema bohneri Schmidle	x									x		
Tolypothrix fremyii. nom.nov		x										
Westiellopsis prolifica Janet												

Genera isolated from freshwater samples :

Bacillus, Coryneform, Neisseria, Aerobacter, Acinetobacter, Alcaligenes, Micrococcus, Staphylococcus, Vibrio, Pseudomonas, Enterobacteriaceae, Pasteurella.

Genera isolated from marine samples:

Bacillus, Coryneform, Neisseria, Aerobacter, Acinetobacter, Alcaligenes, Micrococcus, Staphylococcus, Vibrio, Pseudonomas, Enterobacteriaceae.

Bacillus was the most common and dominant bacteria seen associated with cyanobacteria from both habitats. The other common genera include *Coryneform, Neisseria, Aerobacter and Acinetobacter.*

The **percentage composition** of various bacteria found attached to different species of cyanobacteria are shown below:

	Marine cyano' acteria	**Freshwater cyanobacteria**
Bacillus	35.82	38.46
Coryneform	19.04	15.38
Neisseria	2.99	5.76
Aerobacter	1.493	13.46
Acinetobacter	2.99	3.84
Alcaligenes	11.94	3.84
Micrococcus	5.97	5.77
Staphylococcus	4.48	1.92
Vibrio	10.45	1.92
Pseudomonas	1.49	5.77
Enterobacteriaceae	2.985	1.92
Pasteurella		1.92

Turley and Machie (1994) studied the biogeochemical significance of attached and free-living bacteria.. They observed in amorphous aggregates higher concentrations of bacteria, cyanobacteria and flagellates. In Cochin backwaters, several indicator bacteria namely coliforms , *E.coli* and *Streptococcus faecalis* are present in sediment and water samples. Shome *et al.* (1995) studied the bacterial flora associated with mangrove macroflora of Andaman. They found the following bacterial species. *Aeromonas, Vibrio, Escherichia, Enterobacter, Corynebacterium, Staphylococcus and Micrococcus* and reported that most species were Gram +ve. It is known that certain cyanobacteria can produce and release a wide variety of biologically active organic substances. In addition to toxins, cyanobacterial exudates showed antibiotic effects (Bloor, 1990). A broad spectrum antimicrobial substance was found produced by *Nostoc muscorum* during the post exponential phase of growth. The antibiotic inhibited the growth of bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The antibiotic property of such organism is nature's device to protect the organisms from harmful bacteria. However, many works have shown that bacteria influence the growth of other organisms.

Some bacterial species start to grow in sea water prior to an algal blooming. Bacterial community structure before and after some algal blooms and observed that high densities of the *Pseudomonas* sp. developed just before the alga *Asterionella glacialis*. The axenic culture of *Asterionella* fails to develop but when

bacteria were added the diatom grew well. Algal growth was stimulated most by adding *Pseudomonas* sp. They also found that the addition of a *Vibro* showed no growth at all. This shows that bacterial association beneficial to the organism. Cyanobacterial-bacterial consortial associations are taxonomically complex, metabolically interactive, self-sustaining prokaryotic communities representing pioneer and often the only biota inhabiting extreme aquatic and terrestrial environments.

In the present work, the bacteria attached to the various species of cyanobacteria were studied Many cyanobacteria exhibit tolerance of salinity. There are some species which are found distributed either only in marine or freshwater habitat. The distributional pattern of cyanobacteria in marine and freshwater environment was found to be similar. In the present study, several species belonging to twelve genera were found distributed. All the genera were found associated with one or more species of cyanobacteria. The genera of bacteria associated varied from one as in *Anabaenopsis* and *Anabaena* , to four as in *Chroococcus.* In Anabaena and Anabaenopsis, the attached bacteria was Bacillus. In *Chroococcus*, the bacteria found attached were *Bacillus*, *Coryneform*, *Staphylococcus* and *Vibrio.* The role of bacteria in the metabolism of cyanobacteria is still a matter of dispute. In *Dermocarpa* sp. and *Gloeocapsa* sp.,number of genera was limited to two. From the study, it was found that twenty nine cyanobacterial species showed single dominant attached bacterium.

In freshwater species of cyanobacteria most of the species found attached with single genus of bacterium the *Bacillus*. The percentage of the genera of associated bacteria varied from 1.92 % for *Vibrio* to 38.46% for *Bacillus* in freshwater. In the marine environment, the associated bacterial flora varied from 1.49 % for *Pseudomonas* and *Aerobactor,* to 35.82% for *Bacillus*.

4
CHAPTER

GROWTH CHARACTERISTICS OF CYANOBACTERIA

Growth of a living organism is defined as an increase in mass or size accompanied by synthesis of macromolecules, leading to the production of a new organized structure. In unicellular cyanobacteria, the increase in the number of cells is a measure of growth. In filamentous species, growth may include differentiation to produce cells for particular function. Any cell of such a filament is potentially capable of binary fission and hence growth in length of the chain is mainly the sum of the growth of the individual cells.

Cyanobacteria were isolated and cultured in the laboratory under varying physiochemical parameters in a closed system. A closed system has limited volume and limited life. It is often restricted with optimum physiochemical conditions. The growth cycle in the system passes through a series of phases from the inoculation through lag, log, stationary leading to the sessation of growth. Growth constant, generation time and productivity during various phases of growth were studied.

Growth of unicellular cyanobacteria under the typical regime of homogenous culture of limited volume is similar to that of any other unicellular algae. Increase in cell numbers in such a batch culture follows a characteristic pattern in which the following phases may be identified.

(i) Adaptation or lag phase in which no increase in cell numbers occurs

(ii) An exponential phase, in which the cell multiplication takes place in geometric progression (logarithmic phase)

(iii) A phase of declining relative growth

(iv) A phase in which cell numbers remain more or less stationary (stationary phase)

(v) A death phase.

Becker (1994) recognizes additional two intermediate phases i.e.,(a) accelerating growth phase placed in between lag phase and log phase (b) accelerated death phase between stationary phase and log death phase. Very often one or more of these phases may not be recognizable. The presence and duration of various phases of growth are indicative of the ecophysiological response of the organism to the physiochemical parameters to which the culture is exposed.

Such work has been carried out on the growth characteristics of cyanobacteria. Fogg,(1949) studied the growth constant, k′ and doubling time , tg for *Anabaena cylindrica.* Their values being 0.68 and 25 hours respectively. Kratz and Myers(1955) recorded the growth constant ,k′ as 3.55 and doubling time tg as 2 hrs. Joseph and Nair (1975) recorded a highest growth constant of 0.048 hr^{-1} with a corresponding generation time of 14.6 hrs in an estuarine *Synechocystis salina.* Ikemoto and Mitsui (1994) recorded the growth attributes of an anaerobic nitrogen fixing *Synechococcus* strain Miami BG 043511. In anaerobic conditions, the generation time varied from 19- 23 hrs and in anaerobic conditions the generation time varied from 19-15hrs at 30^{0} C respectively.

There are several species of cyanobacterium which are found distributed in marine as well as freshwater environment. Besides, the same species were found growing in estuaries when the salt contents are fluctuating with seasons. Hence in the present study, salinity tolerance of three species viz., *Synechocystis salina, Synechococcus elongatus* and *Gloeocapsa crepidinum* was studied. Attempt was also made to study the impact of enrichment of nutrients on the growth and productivity of cyanobacterium.

Procaryotes are one among the dominant primary producers in aquatic environments of both the marine and freshwater ecosystems. The productivity of various species of cyanobacteria under various environmental conditions has also been carried out. The productivity has been measured using oxygen method and ^{14}C method.

Methodology:

Nitrite (NO_2-N)

The determination of nitrite in sea water was made by Bendschneider and Robinson (1952) method.

Nitrate

This method is based on the reduction of nitrate to nitrite and its subsequent photometric estimation (Morris and Riley, 1963; Grasshoff et al, 1983)

Reactive phosphate (PO_4-P)

Reactive phosphate in water was measured photometrically according to the method of Murphy and Riley (1962)

Estimation of chlorophyll a.

Strickland and Parsons (1972) method was employed for the determination of pigments. Algal pigments present in an aliquot of 10-50 ml of the cyanobacterial culture were separated by filtering through a Whatman GF/C glass fibre filter. This filter then was extracted with 10ml of 90% acetone and kept in a refrigerator for 24 hours.

The absorbance of the clear acetone as blank at different wavelengths (750, 665,645, 630 and 450 nm). Concentration of various pigments were then calculated using Strickland and Parsons equations(1972).

Primary productivity:

Productivity of cyanobacterial cultures was estimated by ^{14}C technique introduced by Steemann Nielsen, (1950-52) and Light and dark bottle oxygen technique (Gaarder and Gran, 1927).

The difference in oxygen concentration between the light and dark bottles was converted into its carbon equivalents using a PQ of 1.25 for obtaining gross production values. The difference between the initial and dark bottle was taken

as the respiration of the cyanobacteria and that between the light bottle and the initial was taken as the net production (Steemann Nielsen and Hansen, 1959).

Primary productivity which is a measure of the uptake of carbondioxide by algal plankters was measured by ^{14}C technique introduced by Steemann Nielsen (1952) and as described by Strickland and Parsons (1972). A clean stoppered 19BOD bottle (50ml reagent bottle)was filled with the culture sample and then inoculated with 1ml of sodium bicarbonate solution in which the Carbon is labelled with ^{14}C

(5 μ cu). These samples were incubated under artificial constant light (4 k lux) for 1-2 hours in experiments with laboratory cultures of cyanobacteria and dark uptake also was determined simultaneously (corrections were applied for the efficiency of the counting system and for the slower uptake of $C^{14}O_2$ when compared with $C^{12} O_2$).

The samples were filtered through millipore membrane (0.45 μm) filter using a manifold filtering unit under suction. The filters were dried over silica gel and exposed to HCl fumes before counting. The activity of the filters was determined using Liquid Scintillation Counting Method (Jitts and Scott,1961)

Production rate per unit volume was calculated by the corrected counts of the filtered samples as fraction of the added activity and multiplying with the total CO_2 content

Primary production calculation

Primary production($mgC/m^3/hr$)= $\frac{(RS-RB)WX1.05}{RX\,N}$

Where,R-Total activity(dpm) of the bicarbonate added(for exactly 5μC I, this equals $1.11x10^7$ dpm)

N-Number of hours of incubation

RS-Sample count(dpm) corrected for quenching

RB-Dark bottle (or blank)count (dpm) corrected for quenching

W-Weight of total carbon dioxide present in mgC/m3

1.05- Correction factor

(w=12,000Xtc, Where TC is the total carbon dioxide as determined in the following method ie,

If the salinity,$S^0/00$, is known,then:

Total alkalinity= $S^0/00x0.067$ meq/l

Carbonate alkalinity= total alkalinity-0.05

Total carbon dioxide= 0.96x carbonate alkalinity

Salinity tolerance in different species of aquatic cyanobacteria

4.1Salinity tolerance of *Synechocystis salina* isolated from two different aquatic habitats

(a) marine

(b) freshwater

Salinity tolerance of *S.salina* isolated from freshwater environment was carried out for ten days. The growth of the species under varying conditions of salinity from 0-50 ppt was measured. Growth was found to occur at all levels of salinity. On the first day though the species showed a tendency to opt to favour higher salinity of 40 and 50ppt from third day onwards at all salinity the growth was

Table 4.1(a)

Salinity tolerance of *Synechocystis salina*. (Marine)

Age of culture (Days)	$S^0/_{00}$.0			$S^0/_{00}$.10			$S^0/_{00}$.20			$S^0/_{00}$.30			$S^0/_{00}$.40			$S^0/_{00}$.50		
	ln	k	tg	ln	k	tg	ln	k	tg	ln	k	tg	ln	k	tg	ln	k	tg
0	15.3	0	0	15.3	0	0	15.3	0	0	15.3	0	0	15.3	0	0	15.3	0	0
1	15.4	0.004	175	15.3	0	0	15	-0.01	-53.85	15	-0.013	-53.85	14.9	-0.02	-41.18	14.9	-0.02	-41.18
3	15.8	0.007	100	16	0.01	70	16.1	0.011	63.64	16	0.01	70	16	0.01	70	15.8	0.007	100
4	16.1	0.008	87.5	16.7	0.015	46.67	16.6	0.014	50	16.4	0.011	63.64	16	0.007	100	16.4	0.011	63.64
7	16.7	0.008	87.5	17.4	0.013	53.85	17.5	0.013	53.85	17.6	0.014	50	17.2	0.011	63.64	16.7	0.008	87.5
10	17.2	0.008	87.5	17.8	0.01	70	18.3	0.013	53.85	18.3	0.013	53.85	18.4	0.013	53.85	17.9	0.011	63.64

Table 4.1(a')

Salinity variation in *Synechocystis salina(Marine).*

ANOVA

rce of varia	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	17.272	5	3.4544	15.9236	4.5E-07	2.60299
Columns	2.92368	5	0.58474	2.69543	0.04419	2.60299
Error	5.4234	25	0.21694			
Total	25.6191	35				

Table 4.1(b')

Salinity variation in *Synechocystis salina* (Freshwater).

ANOVA						
rce of varia	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	425.591	6	70.9319	1.41715	0.24072	2.42052
Columns	250.919	5	50.1838	1.00262	0.43317	2.53355
Error	1501.57	30	50.0525			
Total	2178.09	41				

Table 4.1(b)

Salinity tolerance of *Synechocystis salina* .(Freshwater)

Age of culture (Days)	$S^0/_{00}$.50			$S^0/_{00}$.40			$S^0/_{00}$.30			$S^0/_{00}$.20			$S^0/_{00}$.10			$S^0/_{00}$.0		
	ln	k	tg	ln	k	tg	ln	k	tg	ln	k	tg	ln	k	tg	ln	k	tg
0	15.2	0	0	15.2	0	0	15.2	0	0	15.2	0	0	15.2	0	0	15.2	0	0
1	15.2	0	0	15.3	0.004	175	15.2	0	0	15.1	-0.004	-175	15.2	0	0	15.3	0.004	175
3	15.8	0.008	87.5	15.7	0.007	100	15.7	0.007	100	15.4	0.003	233.33	15.3	0.001	700	15.2	0	0
4	15.6	0.004	175	15.8	0.006	116.67	15.6	0.004	175	15.6	0.004	175	15.4	0.002	350	15.1	-0	-700
7	16.8	0.01	70	16.7	0.009	77.78	16.9	0.01	70	16.7	0.009	77.78	16.8	0.01	70	15.2	0	0
10	15.8	0.003	233.33	17.9	0.011	63.64	18	0.012	58.33	17.5	0.01	70	17.4	0.009	77.78	15.6	0.002	350

Fig 4.1 (b)

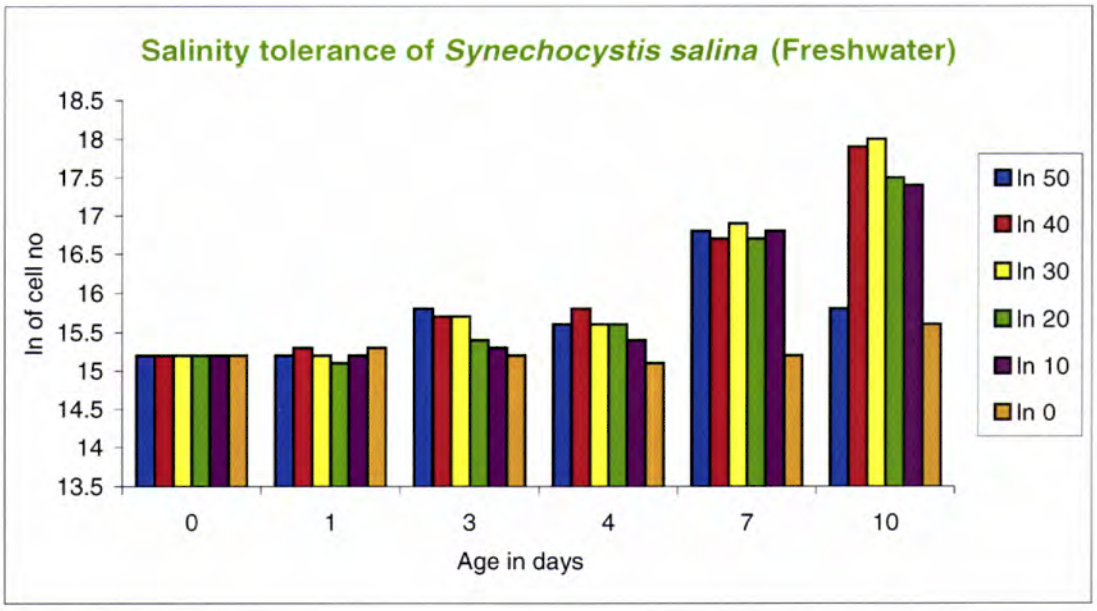
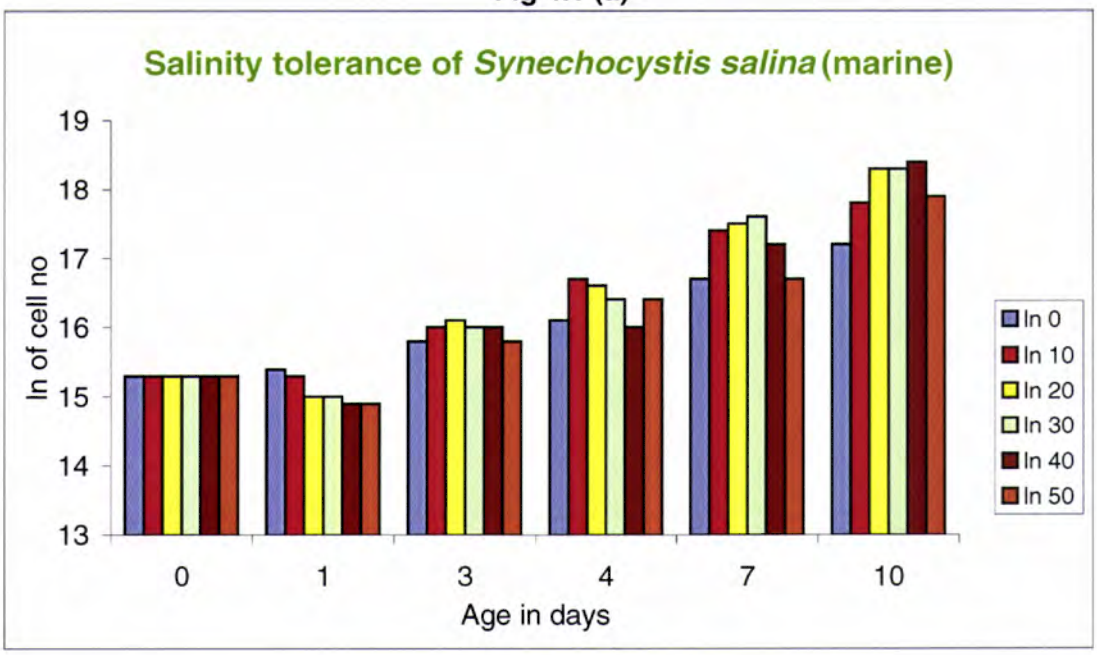


Fig 4.1 (a)



more or less equal indicating that the freshwater species of *S.salina* can tolerate wide fluctuations of salinity. Table 4.2 (a)

Synechocystis salina isolated from marine environment was tested for its salinity tolerance by growing in varying levels of salinities from 0-50 ppt. Initially the growth pattern was almost similar. But on the seventh day, the species exhibited considerable growth at all salinity levels above salinity ten. On the tenth day the cyanobacterium showed higher growth at salinity 30 and 40ppt. Table 4.2 (b).

Table 4.1(a′) gives ANOVA (analysis of variance) of salinity on the growth of *Synchocystis salina* isolated from marine environment. There is significant difference between salinities.($p < 0.05$). The least significant difference at 5% level is 0.5534. 30 ppt shows significantly higher values compared to 50, 40 and zero ppt salinities. There is no significant difference in cell count in zero and fifty. In zero salinity, the cell count is significantly lower than in all others.

Table 4.1 (b′) gives ANOVA of the growth of *Synechocystis salina* isolated from freshwater environment in different salinities and days. There is no significant difference in cellcounts between salinities at 5% level.

c) Salinity tolerance of *Synechococcus elongatus*.

The effect of varying salinities 0,10, 20,30 and 40 ppt on *S.elongatus* was studied for 14 days. The species exhibited growth at all salinities initially. However, the species showed higher growth at higher salinities in the present investigation. Table 4.1 (c).Fig 4.1c

At zero salinity, practically no growth was observed beyond ninth day of the experiment. The generation time varied from 87.5 to 233.3 indicating very slow growth. At salinity10 ppm, the generation time varied from 11.9hrs on the first day to 117hrs on the fourteenth day. At salinity 20 and 30 ppm, the generation time varied from 11.86 hrs on the first day to 70 hrs on the fourteenth day. At salinity 30ppm, variation of generation was also ranged from 11.86hrs to 70 hrs. But at salinity 40ppm, the lowest generation time was 9.59hrs and the maximum was 70hrs. On analysis, it was found that comparatively lower generation time was recorded at salinity 30ppm and 40ppm. From this, it may be inferred that the optimum salinity for this species is between 30 and 40 salinity. The effect of salinity on growth in unicellular algae was observed by Mc. Lachlan (1961). Brand (1984) had studied the salinity tolerance of marine phytoplankton isolates. Diaz Ramos and Reyez Vazquez (1992) incubated *Synechococcus* sp. at different NaCl concentrations and found that the concentration of NaCl had a significant influence on the population density of the cyanobacterium.

Table 4.1.(c)

Salinity tolerance of *Synochococcus elongatus.*

Age in days	$S^0/_{00}$. 0 cells/mm³	ln	k^{hr-1}	tg	$S^0/_{00}$.10 cells/mm³	ln	k^{hr-1}	tg	$S^0/_{00}$. 20 cells/mm³	ln	k^{hr-1}	tg	$S^0/_{00}$.30 cells/mm³	ln	k^{hr-1}	tg	$S^0/_{00}$.40 cells/mm³	ln	k^{hr-1}	tg
0	600	6.40	0	0	600	6.40	0	0	600	6.40	0	0	600	6.40	0	0.00	600	6.40	0	0
1	650	6.48	0.003	233.33	2500	7.82	0.059	11.9	2500	7.82	0.06	11.86	2500	7.82	0.06	11.86	3500	8.16	0.073	9.59
2	1000	6.91	0.011	63.636	3000	8.01	0.034	20.6	3750	8.23	0.04	18.42	5000	8.52	0.04	15.91	5000	8.52	0.044	15.9
3	1250	7.13	0.01	70	6000	8.70	0.032	21.9	6000	8.70	0.03	21.88	8000	8.99	0.04	19.44	9000	9.10	0.038	18.4
4	1500	7.31	0.009	77.778	6000	8.70	0.024	29.2	8750	9.08	0.03	25	9000	9.10	0.03	25.00	10500	9.26	0.03	23.3
5	1500	7.31	0.008	87.5	6000	8.70	0.019	36.8	11250	9.33	0.02	29.17	10000	9.21	0.02	30.44	12000	9.39	0.025	28
6	1500	7.31	0.006	116.67	5000	8.52	0.015	46.7	12000	9.39	0.02	33.33	12500	9.43	0.02	33.33	20000	9.90	0.024	29.2
7	1500	7.31	0.005	140	4000	8.29	0.011	63.6	12250	9.41	0.02	38.89	13000	9.47	0.02	38.89	22000	10.00	0.021	33.3
8	1500	7.31	0.005	140	2500	7.82	0.007	100	12500	9.43	0.02	43.75	15000	9.62	0.02	41.18	24000	10.09	0.019	36.8
9	1100	7.00	0.003	233.33	2500	7.82	0.007	100	15000	9.62	0.02	46.67	16000	9.68	0.02	46.67	24000	10.09	0.017	41.2
10	500	6.21	-0.001	-700	2500	7.82	0.006	117	16000	9.68	0.01	50	20000	9.90	0.02	46.67	24000	10.09	0.015	46.7
11	250	5.52	-0.003	-233.3	4000	8.29	0.007	100	16000	9.68	0.01	58.33	20000	9.90	0.01	53.85	24000	10.09	0.014	50
12	200	5.30	-0.004	-175	5000	8.52	0.007	100	20000	9.90	0.01	58.33	24000	10.09	0.01	53.85	28000	10.24	0.013	53.8
13	100	4.61	-0.006	-116.7	5000	8.52	0.007	100	20000	9.90	0.01	63.64	20000	9.90	0.01	63.64	28000	10.24	0.012	58.3
14	50	3.91	-0.007	-100	5000	8.52	0.006	117	16000	9.68	0.01	70	20000	9.90	0.01	70.00	20000	9.90	0.01	70

(d) Salinity tolerance of *Gloeocapsa crepidinum*

Salinity tolerance of the species *G.crepidinum* was carried out for 15 days at varying salinities of 0, 10, 20, 30 and 40ppm. Increase in cell numbers was found to occur at all salinities initially. But later, the growth was found to be more at salinity30. At zero salinity, the generation time varied from 12.96 hours on the first day to 140 hrs on the ninth day(Table and Fig.4.1d) During the period of fifteen days of observation, no growth was recorded beyond the ninth day. At salinity 10ppm, the generation time varied from 16.6 hrs on the second day to 350 hrs on the tenth day and no further growth was recorded beyond tenth day. At salinity 20 the lowest generation time was 12.96 hrs which was on the first day. The maximum was 700hrs which was on ninth day indicating the physiological stress exhibited by the organisms by the survival. At salinity 30ppm, the generation time varied from 24.14 hrs to 350 hrs and in the case of 40ppm salinity, it varied from 24.14 hrs to 700hrs. The comparatively lower generation time exhibited at 30 salinity showed the preference of *G.crepidinum* to this salinity. Table 4.1(d) Juergensen &Davey(1968) and Sharma &Kumar

(1975)observed that pH is one of the major factors which affect the distribution and the activity of cyanobacteria in soils.

Lara and Romero (1986) investigated on the interaction between nitrate and CO_2 fixation in cyanobacteria. Romero and Lara (1987) studied the photosynthetic assimilation of NO_3 by the intact cells of the cyanobacterium *Anacystis midulans*

Table 4.1(d).

Salinity tolerance of *Gloeocapsa crepidinum*.

Age in days	$S^0/_{00}$. 0 cells/mm^3	ln	k^{hr-1}	tg	$S^0/_{00}$.10 cells/mm^3	ln	k^{hr-1}	tg	$S^0/_{00}$. 20 cells/mm^3	ln	k^{hr-1}	tg	$S^0/_{00}$.30 cells/mm^3	ln	k^{hr-1}	tg	$S^0/_{00}$.40 cells/mm^3	ln	k^{hr-1}	tg
0	110	4.7	0	0	110	4.7	0	0	110	4.7	0	0	110	4.7	0	0	110	4.7	0	0
1	400	5.99	0.054	13	300	5.7	0.042	16.67	400	5.99	0.054	12.96	220	5.39	0.029	24.1	220	5.39	0.029	24.14
2	450	6.11	0.029	24.1	300	5.7	0.021	33.33	400	5.99	0.027	25.93	400	5.99	0.027	25.9	320	5.77	0.022	31.82
3	700	6.55	0.026	26.9	500	6.21	0.021	33.33	400	5.99	0.018	38.89	450	6.11	0.02	35	400	5.99	0.018	38.89
4	550	6.31	0.017	41.2	630	6.45	0.018	38.89	400	5.99	0.013	53.85	400	5.99	0.013	53.8	400	5.99	0.013	53.85
5	400	5.99	0.011	63.6	300	5.7	0.008	87.5	400	5.99	0.011	63.64	300	5.7	0.008	87.5	480	6.17	0.012	58.33
6	400	5.99	0.009	77.8	400	5.99	0.009	77.78	380	5.94	0.009	77.78	300	5.7	0.007	100	400	5.99	0.009	77.78
7	340	5.83	0.007	100	400	5.99	0.008	87.5	370	5.91	0.007	100	400	5.99	0.008	87.5	400	5.99	0.008	87.5
8	340	5.83	0.006	117	300	5.7	0.005	140	230	5.44	0.004	175	300	5.7	0.005	140	370	5.91	0.006	116.7
9	350	5.86	0.005	140	280	5.63	0.004	175	150	5.01	0.001	700	400	5.99	0.006	117	350	5.86	0.005	140
10	30	3.4	-0.005	-140	180	5.19	0.002	350	100	4.61	0	_	300	5.7	0.004	175	350	5.86	0.005	140
11	30	3.4	-0.005	-140	50	3.91	-0.003	-233	100	4.61	0	_	350	5.86	0.004	175	150	5.01	0.001	700
12	30	3.4	-0.005	-140	100	4.61	0	_	100	4.61	0	_	250	5.52	0.003	233	150	5.01	0.001	700
13	20	3	-0.005	-140	80	4.38	-0.001	-700	100	4.61	0	_	250	5.52	0.003	233	120	4.79	0	_
14	20	3	-0.005	-140	60	4.09	-0.002	-350	100	4.61	0	_	250	5.52	0.002	350	140	4.94	0.001	700
15	0	_		_	60	4.09	-0.002	-350	100	4.61	0	_	300	5.7	0.003	233	140	4.94	0.001	700

Fig.4.1 (c)

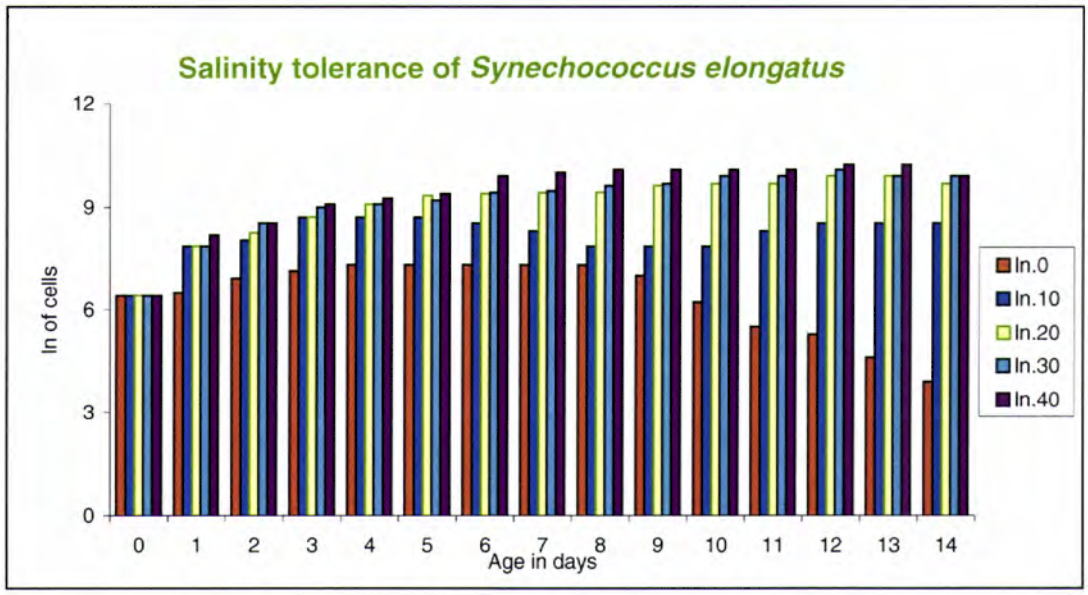
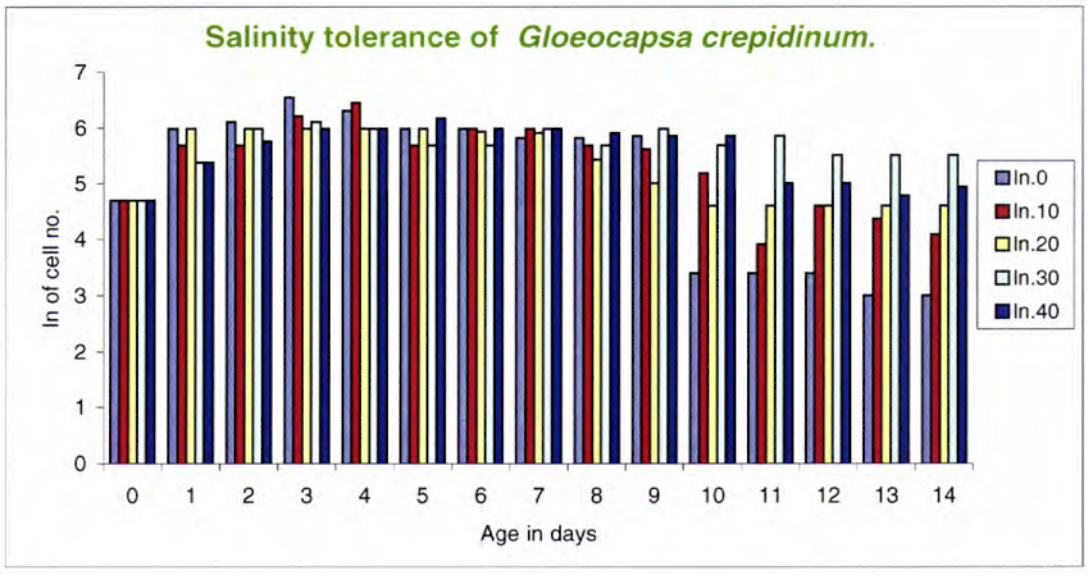


Fig.4.1 (d)



.They found that illuminated suspension of the cyanobacterium, supplied with saturating concentration of CO_2 evolved O_2 at a greater rate when nitrate was simultaneously present.

Palaniselvan et al, 1998 studied the salinity and age induced changes in pigments and biomass production in the marine cyanobacterium *Phormidium tenue*. It was observed that the salinity of 40 x 10^{-3} induced higher mass production which was lower by 15.8 %and 25% at salinities 18 and 100 x 10^{-3} respectively.

Cyanobacteria have the remarkable tolerance potential to adjust to several environmental stresses such as heat, salinity and osmotic changes which are frequently encountered in nature (Kaushik and Sharma, 1997). Several species grow well at varying salinities and the doubling time was found to be unaffected considerably. However, very high salinity was found to affect the protein content. Kaushik and Sharma (1997) studied the effect of salinity stress on the halotolerant form *Nostoc linckia, Westiellopsis prolifica and Tolypothrix ceylonica* and found that there was a 3-5% reduction in total protein content when these forms were grown in 100 mM NaCl. Many cyanobacteria appear to have an innate flexibility to adapt themselves to either habitats (Subramanian, 1998).

Gotto et al.(1979) studied the effect of varying concentration of NaCl on the various strain of the genus Anbaena sp. Growth of the organisms was characterized with respect to NaCl concentration and one of the marine strain did

not show requirement for NaCl. In fact several other strains also showed low generation time indicating high rate of growth than at higher salinities.

Growth of isolates in ASP-2 medium (values given are generation time) Gotto et al.(1979)

Organism	**optimum**	**NaCl (g/l)**					
		0	5	10	15	30	40
1F	42	n.g	5.8	5.7	5.4	6.8	7.8
2A	42	n.g	6.8	5.8	5.5	6.3	7.8
2C	42	n.g	5.1	6.0	6.2	11.0	16.8
21A	35	n.g	5.9	5.1	5.7	6.4	7.4
22B	39	n.g	5.9	6.1	6.3	10.3	19.5

Atrie, V (1998) studied the response of a freshwater cyanobacterium *Lyngbya birgei* Smith to Sodium chloride salinity. It was found that the growth of these organisms in the presence of NaCl depends on the availability of nutrients and the rate and mode of carbon fixation. Growth of cyanobacteria in response to salt stress showed contrary results(Atrie,V ,1998). In *Lyngbya*, low salt concentrations stimulated growth and photosynthesis but decreased at high comcentrations which was directly proportional to the chlorophyll content and the rate of photosynthesis. The freshwater form *Nostoc muscorum* grows well at 200 mM NaCl, where the biomass, photosynthetic activity and sugar accumulation was stimulated (Blumwodd, 1982) but at 400 mM it fails to grow. Anand and

Hopper (1982) have shown that salt concentration above 10% brought major changes in photosynthetic pigments and also photosynthesis(Atrie, V, 1998)

4.2 Growth and productivity of *Synechocystis salina* in enriched and non-enriched media:

Cyanobacteria is one of the major primary producers in any aquatic environment. Their major role has been ignored due to their extremely small size.

Cyanobacteria are distributed in the whole of pelagic ecosystem serving as living food for a wide variety of fishes ,crustaceans and molluscs. For a realistic interpretation of the biomass at different trophic levels and assessment of productivity of various species their growth rate and productivity must be understood. The present work is planned to study the growth characteristics and primary productivity at different concentrations of nutrients.

Methodology

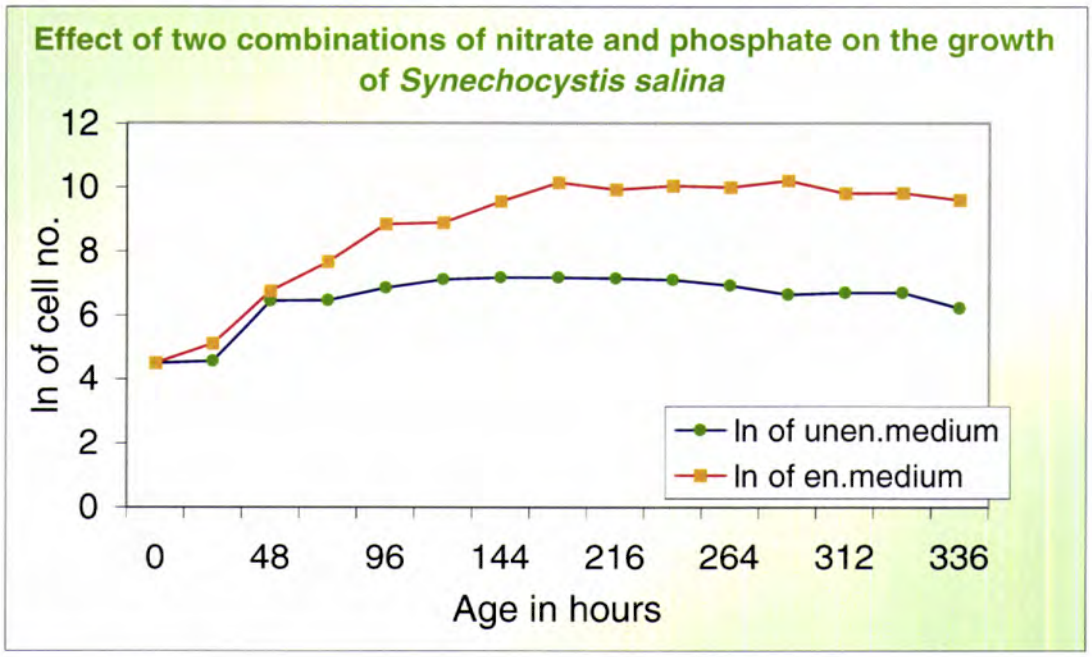
Two culture media (salinity 35.3 ppt) one without enrichment and the other enriched with phosphate and nitrite were used for the experiment (duplicate). The first set of medium without enrichment had phosphate and nitrite concentration 1.6 μg PO_4 P and 0.5 μg NO_2 N/ litre respectively and the medium enriched had phosphate and nitrite concentration of 44.8 μg PO_4 P and 2.35 μg NO_2 N/litre respectively. Each of the 4 two litre culture flask was inoculated with a known volume of *Synechocystis salina* so that the initial concentration was 90 cells/ml.

Table 4.2

Growth characteristics of *Synechocystis salina* in unenriched and enriched media.

Age in hours	Cell concentration							
	Unenriched medium				Unenriched medium			
	Cell no/mm^3	ln of unen.medium	khr^{-1}	tg	Cell no/mm^3	ln of en.medium	khr^{-1}	tg
0	90	4.5	0	0	90	4.5	0	0.0
24	95	4.55	0.002	350	165	5.11	0.025	28.0
48	620	6.43	0.04	17.5	840	6.73	0.046	15.2
72	630	6.45	0.027	25.9	2100	7.65	0.044	15.9
96	930	6.84	0.024	29.2	6750	8.82	0.045	15.6
120	1220	7.11	0.022	31.8	7080	8.87	0.036	19.4
144	1300	7.17	0.019	36.8	13800	9.53	0.035	20.0
168	1300	7.17	0.016	43.8	25000	10.13	0.034	20.6
216	1250	7.13	0.012	58.3	20100	9.91	0.025	28.0
240	1200	7.09	0.011	63.6	22750	10.03	0.023	30.4
264	1000	6.91	0.009	77.8	21500	9.98	0.021	33.3
288	750	6.62	0.007	100	27000	10.2	0.02	35.0
312	800	6.68	0.007	100	17900	9.79	0.017	41.2
360	800	6.68	0.006	116.7	18000	9.8	0.015	46.7
336	500	6.21	0.005	140	14500	9.58	0.015	46.7

Fig.4.2


Effect of two combinations of nitrate and phosphate on the growth of Synechocystis salina
ln of cell no.
12
10
8
6
4
2
0
0
48
96
144
216
264
312
336
Age in hours
ln of unen.medium
ln of en.medium

The initial gross and net productivity as estimated by oxygen method was 220µg C/l/hr. and 80.4µg C/l/hr.

Increase in cell numbers was taken every day and primary productivity was estimated every alternate days using light and dark bottle oxygen method. Both gross and net productivity were calculated.

Results and discussion

In the unenriched medium after 24 hrs of incubation,the generation time recorded was 350 hrs as against 165 hrs in the enriched medium, the corresponding growth constants , k^1 being 0.002 and 0.025 respectively. On the second day i.e., after 48 hrs in the unenriched medium, the doubling time was 17.5 hrs recording a growth constant of 0.027 k^1 The high generation time on the first day of inoculation of cyanobacteria was due to a short lag phase. This was not due to the depletion of nutrients but due to the time required for acclimatization. Table 4.2(a)

The available nutrients on the second day might have been optimum or near optimum so as to demand the shortest doubling time as far as the unenriched medium is concerned.

The initial gross primary production was 220mgC/l/hr. the corresponding net production being 80.4 mgC/l/hr. On the second day ,the gross production was increased to 455.6 mgC/l/hr which was double the value of initial production and the net production was increased to 96.5 mgC/ l/hr. With the minimum available

nutrients of nitrite and phosphate the maximum yield in terms of cell numbers supported by the maximum gross production. Table 4.2 (b).

On the third day, the doubling time of cells was 25.9 hours as against 17.5 hours on the second day. From the third day onwards,the doubling time was found to be increased and on the seventh day it was 43.8 hrs the constant being 0.16 k^1

Gross and net primary productivity showed a gradual decrease from 455.6 and 96.5 mgC/l/hr on the second day to 69.6 mgC/l/hr and 37.5 mgC/l/hr on the fourth day and 75.0 mgC/l/hr on the sixth day.

In the enriched medium where the concentration of phosphate and nitrite were 44.8 mg PO_4 and 2.35 NO_2 N/l the values of concentration and productivity, both gross and net were of higher magnitude. Higher growth constants and lower generation time were the characteristics of enriched cyanobacterial culture. The shortest generation time was 15.2 hrs with a high growth constant of 0.46 K^1 on the second day. Thereafter too the generation time was less indicating a sustained growth for the remaining period till the seventh day.

The relative abundance of nitrogen and phosphorus present in the waterbodies has been found to have both qualitative and quantitative effect on cyanobacterial population. Presence of the dissolved nutrient is a critical determinant of population climax. Nitrates produced as a result of high rate of nitrification under

water logged conditions form a preferred non-toxic source of combined nitrogen for most cyanobacteria. Microcystis is an efficient bioaccumulator of nitrogen (Gerloff and Skoog, 1954) which has been held responsible for its dominance

Phosphorus is also essential for the growth of cyanobacteria in aquatic habitats. But its concentration required for the luxuriant growth of the organism is low compared to nitrogen. Lara et al (1986) investigated the interaction between nitrate and $C0_2$ fixation in cyanobacteria. Romero and Lara (1986) studied the photosynthetic assimialation of NO^{3-} by intact cells of the cyanobacterium *Anacystis nidulans*. They found that illuminated suspension of the cyanobacterium supplied with saturating concentration of CO_2 evolved O_2 at a greater rate when nitrate was simultaneously present.

4.3 Growth characteristics of (a)*Synechococcus elongatus*

(b) Synechocystis salina.

10ml of *Synechocystis aquatilis* from exponentially growing culture was inoculated into one litre of Allen and Nelson medium (salinity 30 ppt ; 25^0 C ; pH 7.4) and incubated under 5 K.lux. The increase in the cells was studied for 23 days.

There was apparently no lag phase and the cells started dividing from the first day onwards. The shortest generation time was 11.9 hours which was on the first day.

The cells continued to grow almost at the same rate for three days indicating prevalance of optimum conditions especially with regard to nutrients and light. The growth rate found reduced afterwards as indicated by the high generation time and low values of growth constants. A gradual decrease in the growth rate was observed with time.[Table 4.3 (b)].

As the cell numbers increased the effective illumination available to the cells would be less compared to the initial days. With the increase in cell numbers the nutrients also would be depleted retarding the growth rate. *Synechocystis aquatilis* during the period of growth for 23 days did not show any conspicuous death phase. Though the doubling time was increased with time, *S. aquatilis* showed sustained growth in this culture system of limited volume. Such a sustained growth and consequent postponement of death phase may be viewed from the mixotrophic characteristic of cyanobacteria.

The growth pattern of *S. salina* (Table 4.3) in Allen & Nelson medium of salinity 30 ppt salinity was found to be peculiar and different from that of *Synechococcus aquatilis*. The increase in cell numbers was recorded for 23 days and growth constant and generation time were calculated. The highest growth constant k′ of 0.058 and lowest generation time of 12.1 hrs were obtained on the second day. The highest generation time of 175 hrs was recorded on 22nd and 23 rd day. But another intermediate depression in the growth and subsequent regeneration phase was noted. This phenomenon may be explained by the mixotrophic characteristics

Table 4.3(a)&(b)

Growth characteristics of *Synechocystis aquatilis* and *Synechocystis salina* in Allen and Nelson medium.

Age of culture in days	*Synechocystis aquatiis*				*Synechocystis salina*			
	Cells /ml	In. of cell no.	k	tg	Cells /ml	In. of cell no.	k	tg
0	61000	11.02	0.0	0.0	219000	12.3	0.0	0.0
1	250000	12.43	0.059	11.9	700000	13.46	0.048	14.6
2	900000	13.71	0.056	12.5	3550000	15.08	0.058	12.1
3	3800000	15.15	0.057	12.3	2250000	14.63	0.032	21.9
4	5250000	15.47	0.046	15.2	1950000	14.48	0.023	30.4
5	5250000	15.47	0.037	18.9	1550000	14.25	0.016	43.8
6	8500000	15.95	0.034	20.6	1550000	14.25	0.014	50.0
7	8500000	15.95	0.029	24.1	1000000	13.82	0.009	77.8
8	16500000	16.62	0.029	24.1	1100000	13.91	0.008	87.5
9	13250000	16.4	0.025	28.0	1100000	13.91	0.007	100.0
10	15250000	16.54	0.023	30.4	700000	14.25	0.008	87.5
11	14500000	16.43	0.02	35.0	5500000	15.52	0.012	58.3
12	16250000	16.6	0.019	36.8	5750000	16.56	0.015	46.7
13	19500000	16.79	0.018	38.9	5750000	16.56	0.014	50.0
14	25750000	17	0.018	38.9	5750000	16.56	0.013	53.8
15	32000000	17.28	0.017	41.2	5750000	16.56	0.012	58.3
16	25750000	17.06	0.016	43.8	26750000	17.1	0.013	53.8
17	21750000	16.9	0.014	50.0	26750000	17.1	0.012	58.3
18	27750000	17.14	0.014	50.0	26750000	17.1	0.011	63.6
19	27750000	17.14	0.013	53.8	21000000	16.86	0.01	70.0
20	27750000	17.14	0.013	53.8	21000000	16.86	0.01	70.0
21	19500000	16.79	0.011	63.6	13250000	16.39	0.008	87.5
22	28500000	17.17	0.012	58.3	1900000	14.46	0.004	175.0
23	44500000	17.61	0.012	58.3	1550000	14.25	0.004	175.0

Fig 4.3.(a)

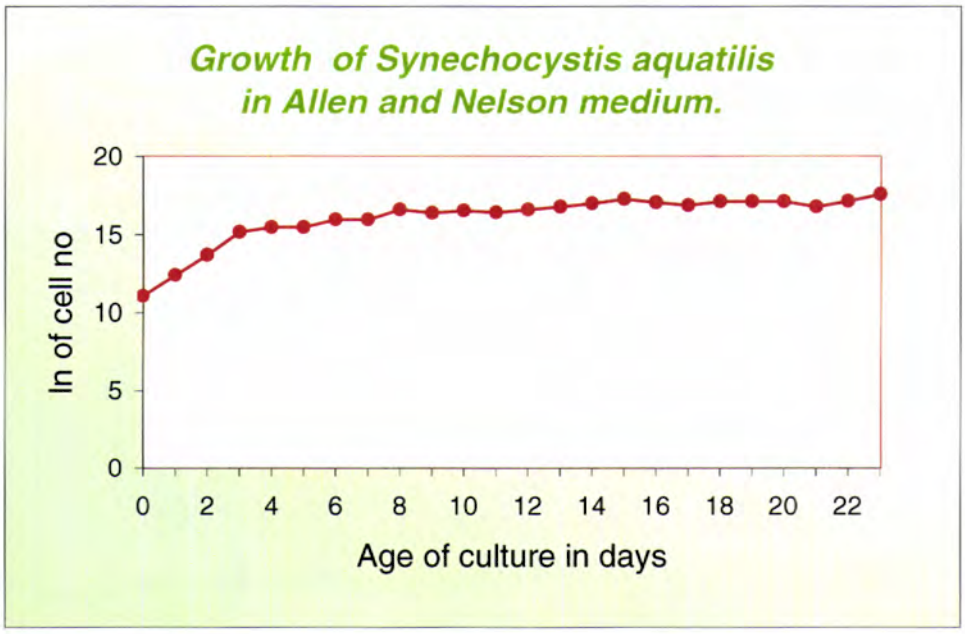
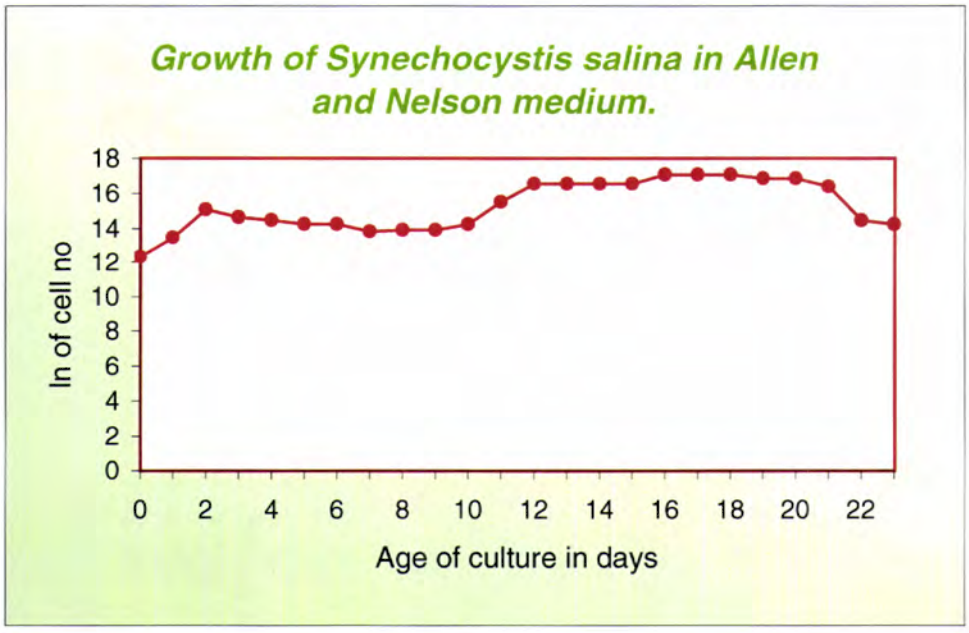


Fig 4.3.(b)



where dissolved organic substances are utilized by the cyanobacterial species and consequent regreening effect whereby the cells are rejuvenated and start growing.

Estimation of production of cellular organic substances(productivity) by aquatic cyanobacteria (^{14}C method)

The cellular productivity of *Synechocystis salina*, *Synechococcus elongatus* and *Gloeocapsa crepidinum* , grown in Allen and Nelson medium of salinity 30 ppt , temperature 25^{0}C and pH 7.2 were studied.

4.4 (a) Synechococcus elongatus

For an initial concentration 1.5 x 10 ^{6} cells, the productivity was found to be 0.249 µg C/l/hr and the extracellular product estimated was 0.058 ie., 23.49 % of the productivity. On the third day for 3.0 x 10 ^{6} cells, the productivity and the extracellular release of organic substance was recorded to be 0.931µg C/l/hr and 0.251 µg C/l/hr respectively. The extracellular product was estimated to be 26.96 % of the productivity. On the sixth day for a cell concentration of 18.0 x 10 ^{6}cells/l the productivity was 2.74 µgC/l/hr, the extracellular product being 0.92µgC/l /hr (33.58 %). On the twelvth day, the productivity recorded was 9.167 µgC/l/hr and the extracellular product was 3.56µgC/l/hr. The extracellular production was 38.83 % of the cellular productivity. On the fifteenth day for a concentration of 4.0 x 10 ^{6} cells the productivity and the rate of extracellular

release were 18.655 µgC/l/hr and 0.17 µgC/l/hr respectively. The extracellular product was estimated to be 0.91% of the productivity. Table 5.1 (c)& 4.4 (a)

4.4 (b)Synechocystis salina

The initial concentration of the cell was 0.5 x 10^6 cells/l. The initial productivity was 0.27 µg C/l/hr and the extracellular products was found to be 0.092 µg C/l/hr as measured by C^{14} technique as described in material and methods. Hence the total synthesized organic substance was 0.362 µgC/l/hr and the extracellular products itself was34.04 % of the productivity. The extracellular product was found to have both growth promoting and growth inhibiting substances rather it was found to have bioactive substance that would affect the growth of other flora and fauna. (Saunders,1957; Gleason and Baxa, 1986;Gleason and Paulson, 1984)

On the third day, the cell concentration was increased to 3.6 x 10^6 cells/l, the productivity being 2.53 µgC /l/hr and the extracellular product 0.59 µgC/l/hr which was 23.3 % of the productivity. With the increase of cells, the extracellular product also found to have increased. On the sixth day, for a concentration of 9.5 x 10^6 cells/ l, the rate of production of cellular and extracellular substances were 3.321 µgC/l/hr and 0.916 µgC/l/hr respectively. The extracellular product was estimated to be 27.58 % of the observed productivity. On the twelvth day, the cell concentration was increased to 28.5 x 10^6 cells/l and the productivity recorded a considerable hike in the value (15.59µgC/l/hr). The extracellular product was

found to be only 6.22% of the productivity. Thus in the exponential phase of *Synechocystis salina*, the extracellular release of organic substance was found to be considerably low. On the 15th day, for a cell concentration of 40 x 10^6 cells/l, the productivity was 26.356µg C/l/ hr. The extracellular product was estimated to be 0.75µgC/l/hr ie. 2.85% of the intracellular organic substance synthesized per hour.

The intracellular and extracellular organic substance (µg C/hr) per 1 x 10^6 cells /l for the various days of the experiment are given in table 5.1 (a) & 4.4(b)

4.4 (c) Gloeocapsa crepidinum

For an initial concentration of 0.155 x 10^6 cells, the productivity was 0.222 µgC/l/hr. The extracellular product was 0.136 µgC/l/hr (61.3 % of the productivity). On the third day, for a cell concentration of 0.4 x 10^6 cells /l, the productivity was 0.235 µgC/l/hr. The extracellular product was 0.145 µgC/l/hr which was calculated to be 61.7 % of the productivity. On the sixth day, for a cell concentration of 0.5 x 10^6 cells, the productivity recorded was 1.388 µg C/l/hr. The extracellular productivity was 0.308 µgC/l/hr ie. 22.24 % of the productivity. In the case of *Gloeocapsa*, the highest release of extracellular product was in the initial phase and there was a conspicuous reduction in the extracellular release on the sixth day. Table 5.1 (b) & 4.4(c).

Table 4.4(a)

Production of cellular organic substances by *Synechcoccus elongatus* (^{14}C method)

Age in days	Cell no/ml	Chlorophyll *a* µg/l	Production of cellular organic substance				µgC/l/hr/10^6cells
			LB(dpm)	DB(dpm)	LB - DB(dpm)	µgC/l/hr	
0	$1.5x10^6$	110.67	30518	1264	29254	0.249	0.166
3	$3x10^6$	226.52	110010	657	109353	0.939	0.313
6	$18x10^6$	497.34	965878	2601	963278	2.74	0.152
12	$40x10^6$	546.71	1103706	26968	1076738	9.167	0.229
15	$40x10^6$	762.3	2196557	5342	2191215	18.65	0.466

Table 4.4(b)

Production of celiuiar organic substance by *Synechocystis salina* (^{14}C method)

Age in days	Cell no/ml	Chlorophyll *a* µg/l	Production of cellular organic substance				µgC/l/hr/10^6cells
			LB(dpm)	DB(dpm)	LB - DB(dpm)	µgC/l/hr	
0	$0.5x10^6$	116.12	33627	1867	31760	0.27	0.54
3	$3.6x10^6$	168.11	298353	1337	297016	2.53	0.702
6	$9.5x10^6$	188.57	393102	1491	391611	3.33	0.35
12	$18.5x10^6$	190.62	1843190	11941	1831249	15.59	0.843
15	$40x10^6$	370.07	3147904	52123	3095781	26.35	0.659

Table 4.4(c)

Production of cellular organic substance by *Gloeocapsa crepidinum* (^{14}C method)

Age in days	Cell no/ml	Chlorophyll *a* µg/l	Production of cellular organic substance				µgC/l/hr/10^6cells
			LB(dpm)	DB(dpm)	LB - DB(dpm)	µgC/l/hr	
0	$0.155x10^6$	95.39	27150	1055	26095	0.22	1.42
3	$0.4x10^6$	108.97	27981	359	27622	0.235	0.588
6	$0.5x10^6$	163.03	168087	5027	163060	1.388	2.776

As cyanobacteria are the dominant primary producers in the less nutrient and less illuminated zone of the aquatic environment. ^{14} C technique introduced by Steemann Neilson (1952) and recommended for oligotrophic waters has been widely used for the estimation of primary productivity of various microflora including cyanobacteria. Productivity values estimated by ^{14}C technique are comparatively low due to the extracellular release of organic substance. In the present work the amount of extracellular product released has also been estimated also estimated.

4.5 Estimation of production of organic substances by Winkler method:

In *Synechocystis salina* the production of organic substances per litre per hour was estimated for sixteen days at regular intervals. The initial production was 0.32 mg C/l/hr. The maximum production of 1.12 mg C/l/hr was recorded on the eighth day and the production was decreased to 0.41 mgC/l/hr on the sixth day. The pattern of growth in *Synechococcus elongatus* was found to be similar to that of *Synechocystis salina*.(Table 4.5 a&b) From an initial concentration of 0.32 mg C/l/hr it was enhanced to 1.31 mgC/l/hr on the eighth day. On the sixteenth day the production was found to be decreased to 0.48 mgC/l.hr. Both the organisms were grown in Allen and Nelson medium. On analysis of productivity of these two organisms it was found that rate of production was found to be more or less same.

Table 4.5(a)&(b)

Estimation of production of organic substances by Winkler method

Synechocystis salina			
Age of culture	**pH**	**O.D**	**Rate of production mgC/l/hr**
0	7.03	0.034	0.32
4	8.24	0.081	0.75
8	9.17	0.142	1.12
12	9.72	0.336	0.31
16	8.98	0.473	0.41

Synechococcus elongatus			
Age of culture	**pH**	**O.D**	**Rate of production mgC/l/hr**
0	7.1	0.023	0.32
4	1.01	0.106	0.74
8	8.96	0.196	1.31
12	9.35	0.314	0.51
16	7.82	0.397	0.48

On a critical analysis of the methods of estimation of primary organic production by oxygen and ^{14}C method, it may be concluded that the former method is suitable for the estimation of primary productivity in less productive waters. The growth constant and generation time recorded for Synechocystis salina is comparable to that of Joseph and Nair (1975) as various species of cyanobacteria tested for its salinity tolerance were found to grow at all salinities. They can be used in aquaculture as an excellent feed for fishes. Glover *et al.*, (1988) studied the variability and comparative distribution of Synechocystis species at Sargasso sea in 1986. Highest concentration of Synechocystis was found in the surface in the isothermal layer where they accounted for 79.5% of ultraplankton cells.

5
CHAPTER

BIOACTIVE IMPACT OF CYANOBACTERIA ON SELECTED MICROFLORA

Extracellular products are soluble substances liberated from various microflora during the different phases of their growth. Substances set free by injured cells or by autolysis or decomposition of dead cells are not considered as extracellular products.

Various species of cyanobacteria show qualitative and quantitative variation of extracellular product and the rate of release varies with the species. Fogg (1952) found that 5 to 60 % of the nitrogen fixed by *Anabaena cylindrica* may appear extracellularly in combined form. Substances liberated from algal cells may be used directly as carbon souces by other organisms. Although the actual concentrations of dissolved organic materials in the natural waters may be low, their rate of turn-over is probably high and ecologically significant. Steeman Neilsen (1952) demonstrated by ^{14}C technique that the membrane filtrate from samples of lake water contained extracellular organic substances liberated from phytoplankton. Fogg (1958) observed that in lake Erken, Sweden when *Gloeotrichia* and *Aphanizomenon* predominated in plankton, the extracellular products was 1.5% of the total carbon fixed. The extracellular products liberated by various species of microalgae include aminoacids and peptides, carbohydrates, vitamins and growth substances, autoinhibitors and antibiotics, enzymes, and various toxins. Aminoacid secreted from the cyanobacterium *Anacystis nidulans* and the diatom *Aulosira fertilissima* were found different according to the stages

of growth. Bentley (1960) found substances similar to auxins in their biological activity in culture filtrates of *Anabaena cylindrica* and *Oscillatoria* sp. About 11% of the species belonging to genera *Scytonema, Oscillatoria, Fischerella, Calothrix and Nostoc* showed antialgal properties.

In tropical and subtropical marine environments, cyanobacteria produce many unique metabolically active substances and these cyanobacteria are very good sources of new medicines and chemicals. The natural product cyanobacterin, shown to be toxic to most cyanobacteria, also inhibits the growth of most eukaryotic algae. *Scytonema hofmanni* was reported to inhibit the growth of other cyanobacteria and some eukaryotic algae when the organisms were cultured together(Mason et. al, 1982). Bentley (1960) found substances similar to auxins in their biological activity in culture filtrate of *Anabaena cylindrica* and *Oscillatoria* sp. Concentrated culture filtrates of *Cylindrospermum* sp. gave positive tests for gibberellin- like substances. Inhibitor –like substances were found at maximum amount in 20 to 26 days of algal growth. The cell-free filtrates from cultures of dominant cyanobacteria from water bodies were found inhibitory to other algal forms isolated from the same habitat.

Earlier investigations showed that extracellular products of algae belonging to various taxonomic classes are of considerable magnitude and ecologically very significant. In the present investigation, the release of extracellular products was

measured by isotopic method (^{14}C method). The biochemical components of the filtrate such as carbohydrate, protein and lipids were estimated. The impact of cyanobacterial filtrate in their own growth as well as other flora was studied.

The use of cyanobacteria to reduce the pollution load in different types of waste water has also been studied. The use of sewage effluent containing the heavy metals such as Fe, Zn, Mn, Pb, Cd, Ni, Co, Cr etc. favour the growth of cyanobacteria. In the present study, several species of cyanobacteria have been tested for their ability to abate lead and cadmium.

5.1 Estimation of extracellular products using ^{14}C technique.

Synthesis of organic material in photosynthetic flora is its characteristic feature and it can be used as an index of rate of growth. In aquatic microalgae, as in all other autotrophs, the rate of synthesis of organic substances varies with the species and phases of its growth and ultimately influenced by the solar radiation. While synthesizing organic material, a portion would be released into the medium in which they grow. This fraction of photosynthetic product occuring extracellularly is as important as the production retained in the cell. Both the fractions are significant as they are initiating the entire trophic food chain in the ecosystem through different pathways. The extracellular product, apart from having direct involvement in the food chain, serve as the food of bacterioplankton as it controls the growth and multiplication of other organisms.

They either inhibit or enhance the survival and survival and growth of other organism and sometimes act as the regulating factor for the physiological process of other organisms. Thus such extracellular products, whatever be their composition affect the ecophysiology of other flora and fauna and control overall bioproductivity of the aquatic ecosystem.

Test organisms:

Synechocystis salina
Synechococcus elongatus
Gloeocapsa crepidinum.

The test organisms were grown in Allen and Nelson culture medium (salinity 30 ppt ; pH 7.2 temperature24^{0}C +1). Each of these cultures with known cell concentration was collected in two light bottles and two dark bottles (50 ml). To these samples (12 bottles ie.,two light and two dark bottles for each species) a known quantity of ^{14} C in the form of $Na_2 HC^{14}O_3$ (5μg)was added to each sample and the samples incubated at 5 Klux. After incubation, the samples are filtered through Millipore filters. The filters were dried and kept in a desiccator over concentrated hydrochloric acid fumes for a fortnight. An aliquot of the filtrate, of both light and dark bottles was directly collected over a Millipore filter during filtration. They were also dried and kept in a desiccator over hydrochloric acid fumes for a fortnight. The activity of all the filters were determined in dpm using

a scintillation counter. The dark filtrate counts were substracted from the light filtrate counts. From the activity of the light bottle samples (LB) those in the dark bottle samples (DB) were substracted (LB-DB) to get the net dpm. Similarly, the dark filtrate (DF) was substracted from that of the light filtrate to get a net activity (LF- DF) attributed to the extracellular product. The calculation given for the estimation of primary productivity was also used for the estimation of extracellular products as described in chapter 4.

Results and discussion:

The extracellular products of various species of cyanobacteria *Synechocystis salina*, *Synechococcus elongatus* and *Gloeocapsa crepidinum* grown in Allen and Nelson medium of salinity 30 ppt temperature 25^0C and pH 7.2,were determined by ^{14}C technique. It was found that all the species released extracellular substances at various stages of growth.

5.1 (a) *Synechocystis salina*

On the initial day, when the cells were in the phase of adaptation (lag phase), the extracellular product was estimated to be 0.92 µg/l/hr, which was 34.07 % of the cellular organic substances. The initial cell concentration was 0.5 x 10^6 cells/l. Inspite of comparatively lower cell concentration, the rate of release of extracellular product was higher. This was probably because of the physiological stress encountered during the process of adaptation. There was a progressive

Table 5.1(a)

Productivity and extracellular products (^{14}C method)

Synechocystis salina

Age in days	LB(dpm)	E. Product LB (dpm)	DB(dpm)	LB-DB (dpm)	Productivity mgC/l/hr	E.cellular productivity mgC/l/hr	E.Cellular Product(%)	E. Product DB (dpm)	E. Product (LB-DB)dpm
0	37830	25706	3734	34096	0.27	0.092	34.07	23233	2473
3	298353	46800	1337	297016	2.529	0.59	23.33	12325	34475
6	393102	68350	2980	390122	3.321	0.916	27.58	7283	61067
12	1843190	93025	11941	1831249	15.59	0.97	6.22	38500	54525
15	3147904	60050	52123	3095781	26.356	0.75	2.85	16267	43783

increase in the extracellular product upto the 12th day where it was the maximum of 0.97 µg/l. On the 15 th day there was a conspicuous decrease in the total quantity of extracellular substances and the percentage of release of extracellular product with respect to the total production was only 2.85µgC/l/hr. On analysis of the percentage of release of extracellular product, it was found that there was gradual decrease with the increase in cell concentration and time. This is probably due to the fact that the cells have already adapted themselves to the medium and no physiological stress was felt when compared to the adaptation phase.(Table and Fig.5.1 a)

5.1 (b) *Gloeocapsa crepidinum*

The initial concentration of extracellular products and that released on third and sixth day were measured.. The release of extracellular product varied from 22.24 µg C/l/hr on sixth day to an average of 61% on the early phase of growth. The extracellular product was comparatively high in *Gloeocapsa crepidinum* while there was no thick mucilage layers for the other two species studied namely *Synechcystis salina* and *Synechococcus elongatus*, more mucilaginous substances were released in the case of *Gloeocapsa*. High percentage of extracellular release recorded in *Gloeocapsa* may be due to its mucilaginous excretion.(Table5.1b)

Table 5.1(b)

Productivity and extracellular products(^{14}C method)

Gloeocapsa crepidinum

Age in days	LB(dpm)	E. Product (dpm)	DB(dpm)	LB-DB (dpm)	Productivity mgC/l/hr	E.cellular productivity mgC/l/hr	E.Cellular Product(%)	E. Product DB (dpm)	E. Product (LB-DB)dpm
0	27150	21440	1055	26095	0.222	0.136	61.3	13450	7990
3	27980	16525	360	27620	0.235	0.145	61.7	12275	4250
6	168085	31000	5027	163058	1.388	0.308	22.2	12933	18067

5.1(c) *Synechococcus elongatus*

^{14} C estimation of extracellular product in *Synechococcus elongatus* showed that on the initial day when the cell concentration was 1.5 x 10^6 cells,the extracellular release of organic substance was 0.058 µg C/l/hr recording 23.49 % of cellular productivity. On the second, sixth and twelfth day, a steady increase in the extracellular release was noted, their values being 0.251, 0.92 and 3.56. On the fifth day,there was a conspicuous decrease in the release of extracellular product, its concentration being 0.17 µg C/l. The percentage of release of extracellular product was the least on the fifteenth day the value being 0.91 %(Table &Fig 5.1c)

On quantitative analysis of the extracellular release of the three species studied, the highest concentration of 61.7 % was observed for *Gloeocapsa crepidinum* on the third day of inoculation. There was quatitative similarity in the release of extracellular product of *Synechocystis*.sp and *Synechococcus* sp. Comparatively higher values of extracellular release was recorded by *Gloeocapsa* sp.

As picocyanobacteria are the dominant primary producers in the nutrient poor and less illuminated zone ^{14} C technique recommended by Steemann Nielson for oligotriphic waters is used for the growth and productivity studies of cyanobacteria. Productivity values estimated by ^{14}C technique are usually low

Table 5.1(c)

Productivity and extracellular products (^{14}C method)

Synechococcus elongatus

Age in days	LB(dpm)	E. Product LB (dpm)	DB(dpm)	LB-DB (dpm)	Productivity mgC/l/hr	E.cellular productivity mgC/l/hr	E.Cellular Product(%)	E. Product DB (dpm)	E. Product (LB-DB) dpm
0	30518	30438	1264	29254	0.249	0.058	23.29	11500	18938
3	110010	49950	657	109353	0.931	0.251	26.96	20425	29525
6	965880	171100	2601	963279	2.74	0.92	33.58	9800	161300
12	1103700	200175	26968	1076732	9.167	3.56	38.83	22175	178000
15	2196550	39150	5342	2191208	18.655	0.17	0.91	9467	29683

Fig. 5.1(a)

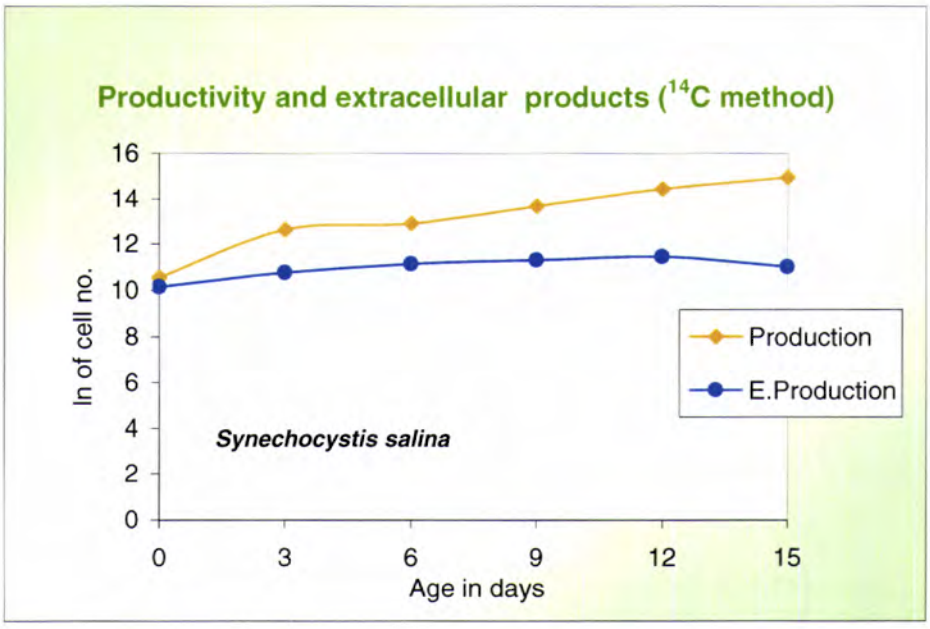
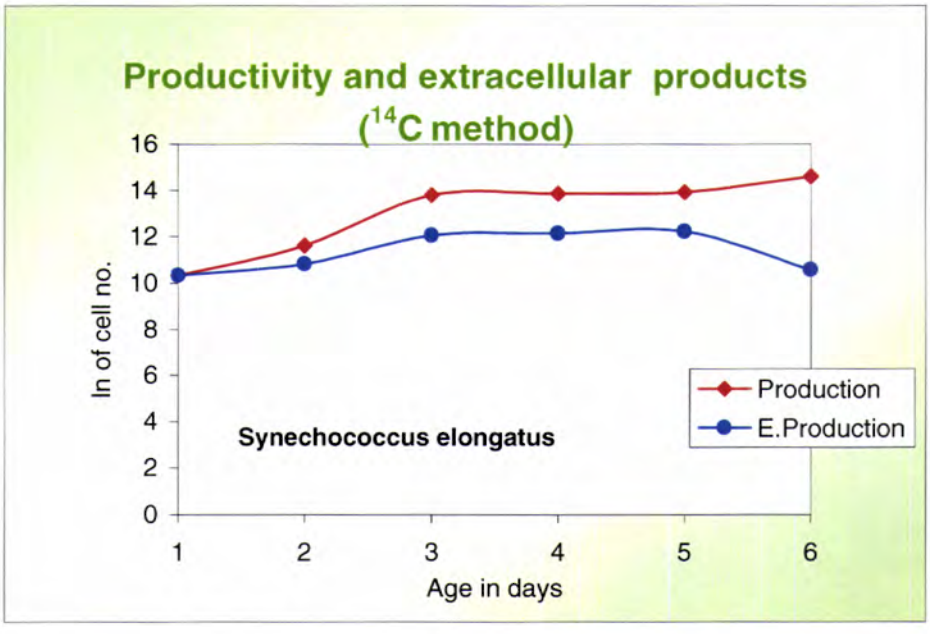


Fig. 5.1(c)



due to the extracellular release of organic substance. In this work the amount of extracellular products released were also estimated.

5.2 Estimation of extracellular protein, carbohydrate and lipid in cyanobacteria

During the growth of three species of cyanobacteria *Synechocystis salina*, *Synechococcus elongatus* and *Gloeocapsa crepidinum* in Allen and Nelson medium for fifteen days, the extracellular substances were released of which protein (Herbert et al, 1971),Carbohydrate(Dubois et al., 1966) and lipid (Bligh and Dyer, 1969) were quantitatively estimated. In *S. elongatus*, no detectable quantity of protein was recorded for the first fourteen days and on the fifteenth day protein obtained was 18.7 mg/l and in *Gloeocapsa* too the first fourteen days did not give protein and on the fifteenth day it was 64.1 mg/l.

In *Synechocystis salina* the carbohydrate content varied from 0.6 mg/l on the initial day to 509.4 mg/l on the sixteenth day. There was gradual increase in the release of carbohydrate. In *Synechococcus elongatus,* the carbohydrate released varied from 0.8 to 44.9 mg/l. In *G. crepidinum* , the carbohydrate content varied from 1.6 to 58.4 mg/l.

Release of lipid was comparatively less and occasional during the period of fifteen days. In *Synechocystis salina,* it was recorded only on the third day the concentration recorded was (0.6mg/l) in *Synechococcus elongatus* it was found

Table 5.2

Extracellular protein ,carbohydrate and lipid in cyanobacteria

Age of culture in days	*Synechocystis salina*					*Synechococcus elongatus*					*Gloeocapsa crepidinum*				
	Optical density	pH	Protein mg/l	Carbohydrate mg/l	Lipid mg/l	Optical density	pH	Protein mg/l	Carbohydrate mg/l	Lipid mg/l	Optical density	pH	Protein mg/l	Carbohydrate mg/l	Lipid mg/l
0	0.042	6		0.6		0.036	5.94				0.048	7.26		2.2	0.4
3	0.056	7.01		10.1	0.6	0.098	7.05		8.2		0.04	6.35		1.6	
6	0.15	7.66				0.298	6.5		44.9	14	0.089	7.07		58.4	
9	0.27	7.14		12.2		0.43	6.94		8.4		0.215	6.31			
12	0.525	6.47		9.8		0.507	7.58				0.396	6.87		10.5	
15	0.789	6.7		509.4		0.729	7.36	18.7	0.8		0.586	6.68	64.1	2.9	

Table 5.3

Cellular composition of carbohydrate, protein and lipid of selected species of cyanobacteria

Test organism	Carbohydrate(mg/100mg wet wt)	Protein (mg/100mg wet wt.)	Lipid (mg/100mg wet wt.)
Synechocystis salina	0.5855	0.0119	0.0164
Synechococcus elongatus	0.7906	0.0074	0.0014
Gloeocapsa crepidinum	7.777	0.0013	0.4587

only on the sixth day (14 mg/l) and in *G.crepidinum* ,it was recorded initially was 0.4mg/l). Table 5.2.

5.3 Estimation of cellular composition of carbohydrate, protein and lipid in cyanobacteria

Among the various biochemical components of three selected species three components viz., carbohydrate(Carrol, et al ,1956), protein(Lowry et al., 1951) and lipid (Frings and Dunn 1970) were estimated In *Synechocystis salina*, *Synechococcus elongatus* and *Gloeocapsa crepidinum*, the percentage composition of carbohydrate was found to be approximately 0.6%, 0.8% and 7.8% respectively. The percentage composition of protein in these species was approximately 0.01, 0.007 and 0.001 and that of lipid was 0.016, 0.001 and 0.5 respectively. Comparatively higher percentage of carbohydrate and lipids were found in *G.crepidinum*. The cellular composition of carbohydrate, protein and lipid of *Synechocystis salina, Synechococcus elongatus and Gloeocapsa crepidinum* is given in table 5.3.

5.4 Effect of cyanobacterial filtrate on various species of cyanobacteria.

Preparation of filtrate

Cultures of *Synechocystis salina, Synechococcus elongatus and Gloeocapsa crepidinum* in the stationary phase were selected. These cultures were filtered through Millipore filters (0.45μ pore size) and the cell free filtrate was collected.

Preparation of test cultures

Each of the test culture contained definite volume of the filtrate of the selected species of cyanobacteria and the culture containing rapidly dividing cells of cyanobacterial species on which the effect of the filtrate was to be studied.

All the test cultures were incubated under light (7500 lux) and temperature of 25^0 C for three weeks. The normal growth in the control and the effect of filtrate on the growth of the test was assessed at regular intervals of two days by recording their absorbance using a spectrophotometer.

5.4 (a) Effect of *Synechocystis salina* on its own growth.

When *Synechocystis salina* filtrate was added to *Synechocystis salina* culture,an initial decrease in growth was measured by optical density was observed upto fifteenth day of incubation. Later on an enhancing effect was noticed as evidenced by optical density. Here slight inhibition was observed only upto the first fifteen days. Consequently,the growth was found considerably enhanced and on the twenty fourth day the enhancement was about 25 %. Table&Fig 5.4 (a)

5.4 (b) Effect of *Synechocystis salina* on the growth of *Synechococcus elongatus*

When *S.salina* filtrate was added on *Synechococcus elongatus* culture a conspicuous inhibition of the growth was observed. The percentage of inhibition increased with the age of the culture and during the last five observations the

Table 5.4(a)

Effect of *Synechocystis salina* filtrate on its own growth

Age in days	Optical Density	
	Control	*S.salina* +filtrate
0	0.100	0.100
3	0.181	0.182
6	0.232	0.213
9	0.238	0.219
12	0.222	0.216
15	0.223	0.218
18	0.262	0.277
21	0.239	0.291
24	0.202	0.268
27	0.222	0.284
30	0.216	0.276
33	0.229	0.272

Table 5.4(b)

Effect of *Synechocystis salina* filtrate on the growth of *Synechococcus elongatus*

Age in days	Optical Density	
	S.elongatus Control	*S.elongatus +filtrate*
0	0.100	0.1
3	0.177	0.159
6	0.202	0.203
9	0.204	0.199
12	0.199	0.198
15	0.203	0.202
18	0.234	0.202
21	0.233	0.116
24	0.24	0.101
27	0.247	0.091
30	0.242	0.085
33	0.23	0.079

Fig.5.4(a)

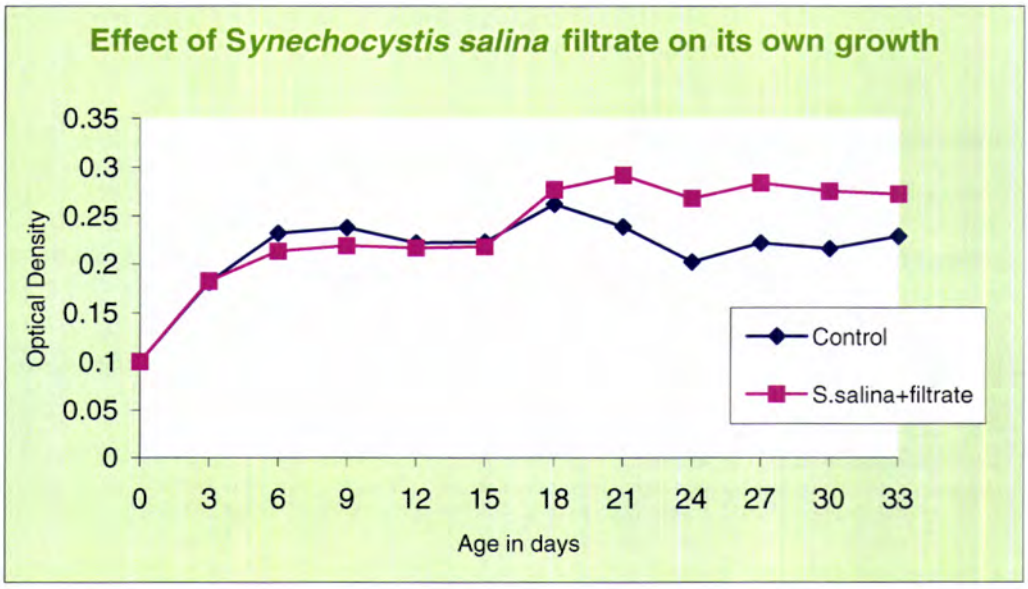
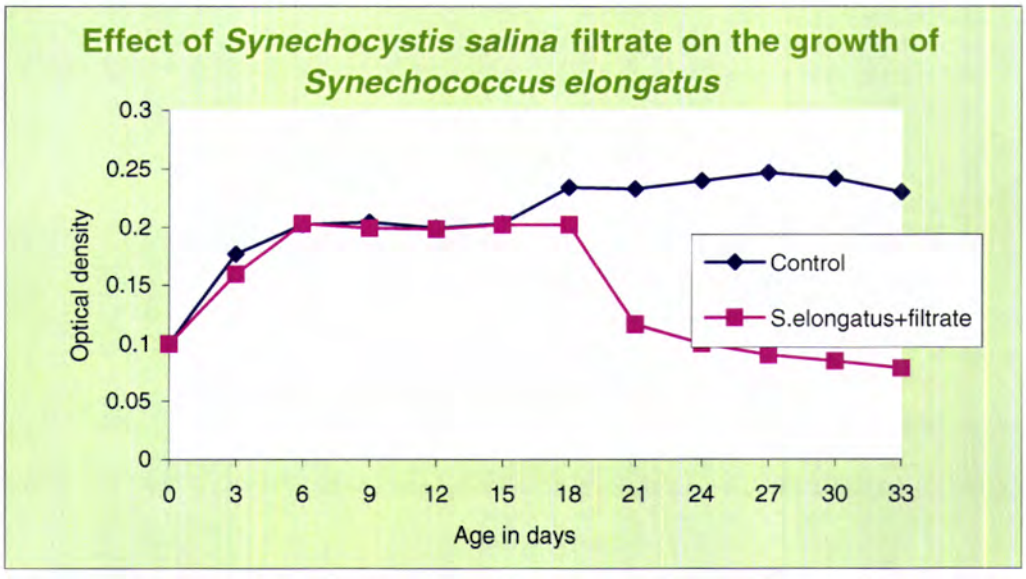


Fig.5.4(b)



percentage of inhibition were 50%, 58%, 53%, 65% and 67% respectively. It is evident that these two species cannot exist together in batch culture because of the inhibiting property of *Synechocystis salina.* Table &Fig 5.4 (b).

5.4 (c) Effect of *Synechocystis salina* filtrate on the growth of *Gloeocapsa crepidinum*

S. salina filtrate was found to inhibit the growth of *G. crepidinum.* The inhibition was found throughout the period of the experiment i.e., for all the thirty three days. The percentage of inhibition varied from 5% on the third day to 39% on the last day of the experiment. The extent of inhibition shows that *G. crepidinum* cannot thrive with *S.salina.* Table &Fig5.4 (c).

5.4 (e) Effect of *Synechococcus elongatus* filtrate on the growth of *Synechococcus elongatus.*

S. elongatus with its own filtrate showed general enhancement of growth upto eighteenth day. The maximum percentage of enhancement (16.5%) was recorded on the twelfth day. From the twenty first day onwards the growth was found inhibited and the percentage of inhibition varied from 32 on the twenty first day to 48% on the thirty third day. It was observed that the percentage of inhibition increased with the age of the culture. In the early growth phase of the culture the filtrate content of the culture was optimum and the growth was enhanced with the increase in the extracellular product liberated by the algae. (Table &Fig 5.4 e).

Table 5.4(c)

Effect of *Synechocystis salina* filtrate on the growth of *Gloeocapsa crepidinum*

Age in days	Optical Density	
	G. crepidinum Control	*G. crepidinum +filtrate*
0	0.100	0.100
3	0.183	0.174
6	0.235	0.186
9	0.237	0.212
12	0.239	0.2
15	0.262	0.207
18	0.305	0.215
21	0.284	0.221
24	0.288	0.231
27	0.291	0.211
30	0.283	0.211
33	0.318	0.232

Table 5.4(d)

Effect of *Synechococcus elongatus* filtrate on the growth of *Synechocystis salina*

Age in days	Optical Density	
	S.salina Control	*S salina +filtrate*
0	0.100	0.100
3	0.182	0.168
6	0.232	0.187
9	0.255	0.184
12	0.244	0.197
15	0.257	0.201
18	0.239	0.221
21	0.234	0.227
24	0.225	0.211
27	0.231	0.214
30	0.229	0.213
33	0.232	0.219

Fig.5.4(c)

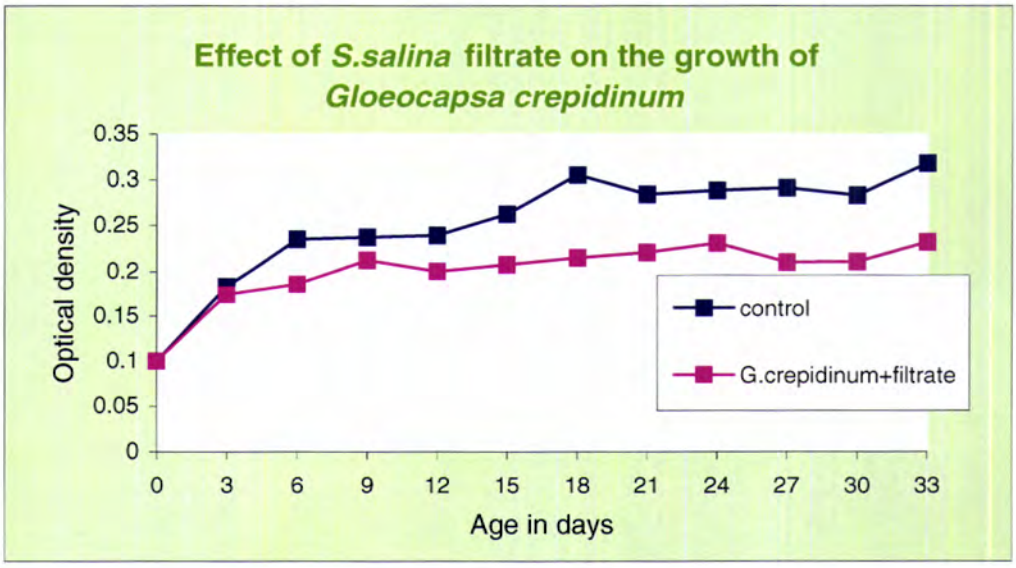
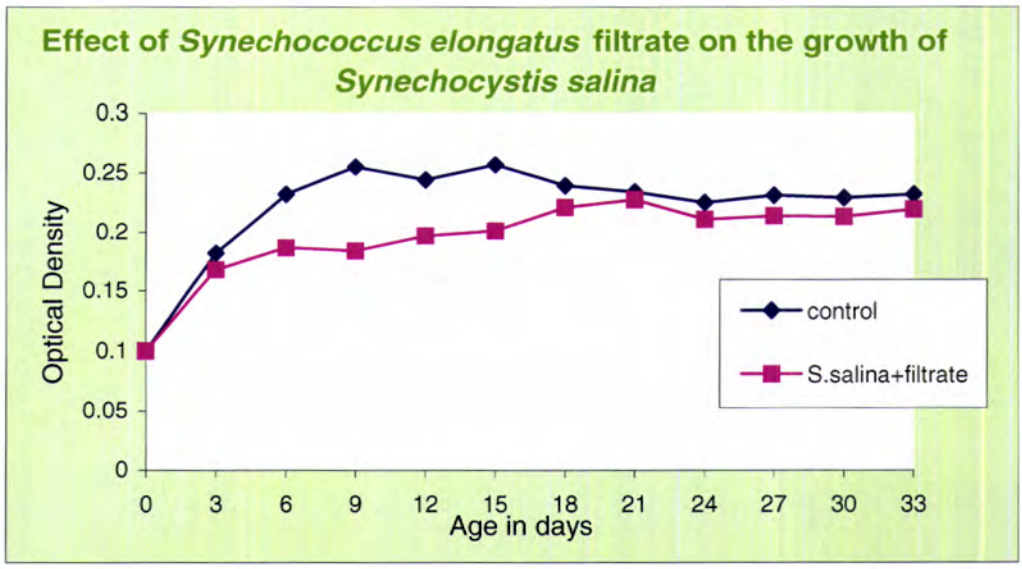


Fig.5.4(d)



5.4 (d) Effect of *Synechococcus elongatus* filtrate on the growth of *Synechocystis salina*

Synechococcus elongatus filtrate was found to inhibit the growth of *S.salina* also. But the rate of inhibition was low compared to that of *S.salina* filtrate on *S. elongatus*. The inhibition was found to be reduced with the time indicating a tendency to counteract the inhibition process or rather some degree of adaptation by *Synechocystis salina* to the media with *Synechococcus elongatus* fitrate.(Table & Fig 5.4 d.)

5.4 (f)Effect of *Synechococcus elongatus* filtrate on the growth of *Gloeocapsa crepidinum*.

With the *S. elongatus* filtrate *Gloeocapsa crepidinum* showed decrease in growth upto eighteenth day. The percentage of inhibition varied from 1.8% to 12.5%. From twenty first day onwards,the growth of *G. crepidinum* was found to be enhanced and the percentage of enhancement varied from 8.8% on twenty first to 145 % on the thirtieth day. (Table &Fig5.4 f)

5.4 (g) Effect of *Gloeocapsa crepidinum* filtrate on the growth of *Synechocystis salina.*

Gloeocapsa crepidinum filtrate was found to have a conspicuous enhancing effect from twelfth day onwards and the percentage of enhancement varied from 15.7% to 144%. The percentage of enhancement on 12^{th}, 15^{th}, 18^{th}, 21^{st}, 24^{th}, 27^{th}, 30^{th} and 33^{rd} day were 15.7%, 29.8%, 38.9% 100, 110.6, 97.2, 109.6 and 144

Table 5.4(e)

Effect of *Synechococcus elongatus* filtrate on the growth of *Synechococcus elongatus.*

Age in days	Optical Density	
	S.elongatus Control	*S.elongatus +filtrate*
0	0.100	0.100
3	0.17	0.174
6	0.189	0.221
9	0.218	0.221
12	0.206	0.24
15	0.214	0.209
18	0.203	0.211
21	0.205	0.141
24	0.177	0.129
27	0.176	0.099
30	0.166	0.093
33	0.129	0.067

Table 5.4(f)

Effect of *Synechococcus elongatus* filtrate on the growth of *Gloeocapsa crepidinum*

Age in days	Optical Density	
	G. crepidinum Control	*G. crepidinum +filtrate*
0	0.100	0.100
3	0.162	0.159
6	0.209	0.183
9	0.212	0.192
12	0.194	0.184
15	0.181	0.196
18	0.221	0.196
21	0.193	0.21
24	0.156	0.199
27	0.114	0.208
30	0.087	0.213
33	0.097	0.226

Fig.5.4(e)

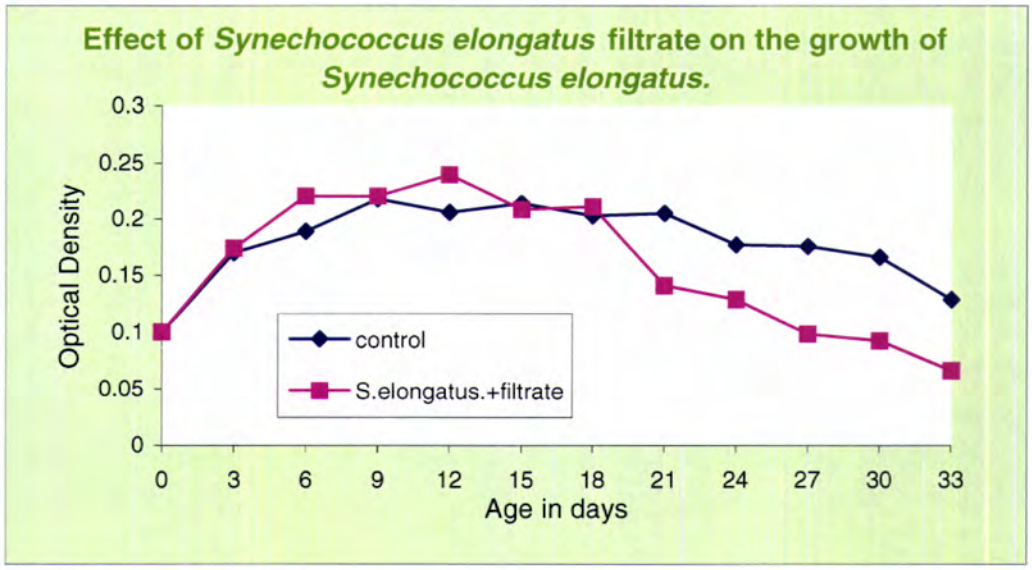


Fig.5.4(f)

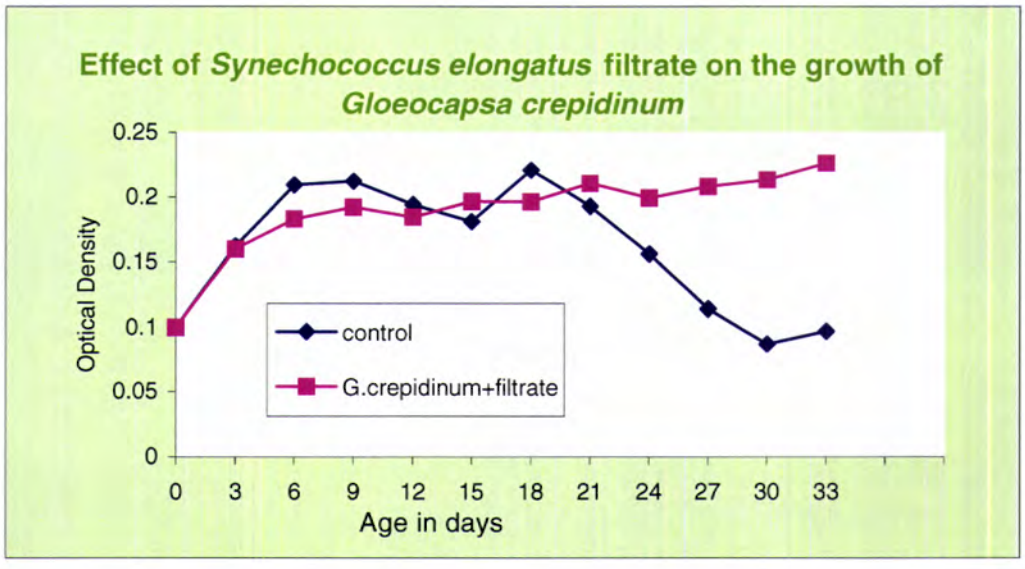


Table 5.4(g)

Effect of *Gloeocapsa crepidinum* filtrate on the growth of *Synechocysis salina*

Age in days	Optical Density	
	S.salina Control	*S.salina +filtrate*
0	0.100	0.100
3	0.164	0.182
6	0.23	0.204
9	0.225	0.216
12	0.198	0.229
15	0.188	0.244
18	0.203	0.282
21	0.184	0.369
24	0.18	0.379
27	0.214	0.422
30	0.21	0.439
33	0.188	0.459

Table 5.4(h)

Effect of *Gloeocapsa crepidinum* filtrate on the growth of *Synechococcus elongatus.*

Age in days	Optical Density	
	S. elongatus Control	*S.elongatus +filtrate*
0	0.100	0.100
3	0.166	0.197
6	0.206	0.219
9	0.218	0.218
12	0.187	0.204
15	0.182	0.205
18	0.167	0.194
21	0.232	0.165
24	0.191	0.147
27	0.207	0.118
30	0.162	0.101
33	0.149	0.076

Fig.5.4(g)

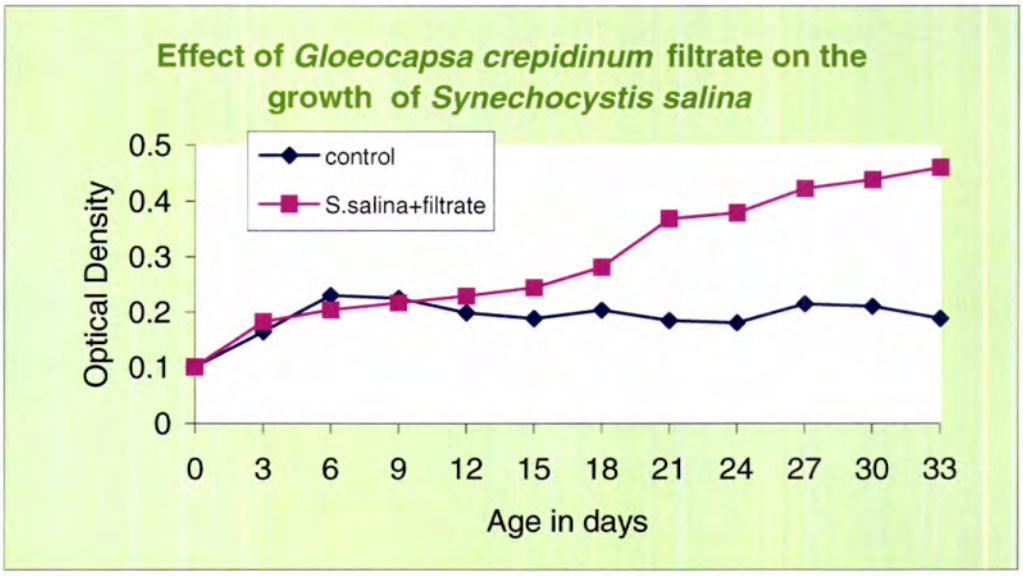


Fig.5.4(h)

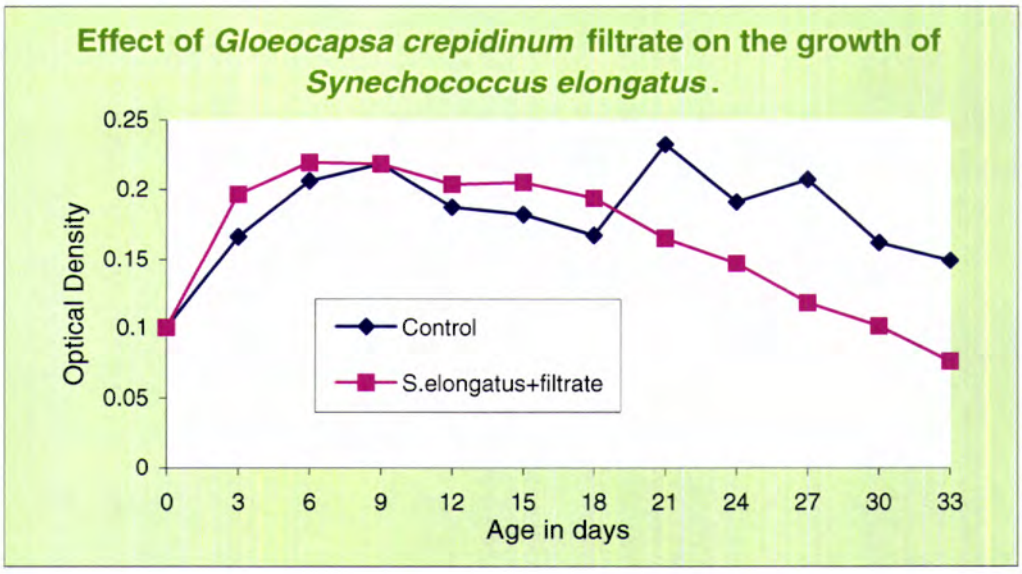
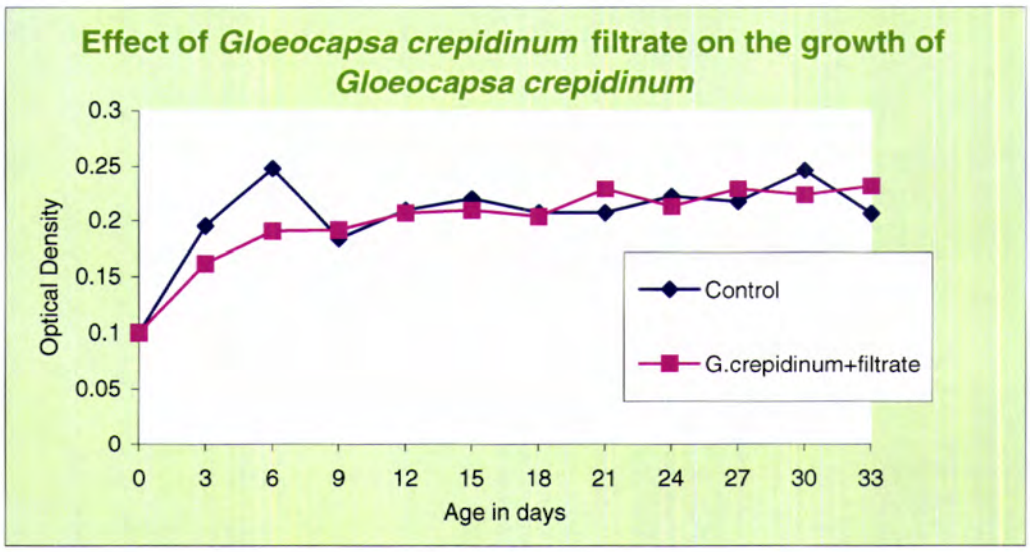


Table 5.4(i)

Effect of *Gloeocapsa crepidinum* filtrate on the growth of *Gloeocapsa crepidinum*

Age in days	Optical Density	
	G. crepidinum Control	*G. crepidinum +filtrate*
0	0.100	0.100
3	0.195	0.162
6	0.248	0.191
9	0.184	0.192
12	0.209	0.207
15	0.22	0.209
18	0.207	0.204
21	0.207	0.229
24	0.222	0.213
27	0.217	0.229
30	0.246	0.224
33	0.206	0.232

Fig.5.4(i)


Effect of Gloeocapsa crepidinum filtrate on the growth of Gloeocapsa crepidinum
Optical Density
0.3
0.25
0.2
0.15
0.1
0.05
0
0
3
6
9
12
15
18
21
24
27
30
33
Age in days
Control
G.crepidinum+filtrate

respectively. Thus a gradual increase in enhancement rate was observed. (Table & Fig5.4 g).

5.4 (h)Effect of *Gloeocapsa crepidinum* filtrate on the growth of *Synechococcus elongatus*.

The effect of *G. crepidinum* filtrate showed enhancement in the growth of *S.elongatus* upto eighteenth day and conspicuous inhibition was observed for the rest of the period of the experiment and on the thirtythird day the percentage of inhibition was 48.9%. (Table& Fig 5.4 h).

5.4 (i) Effect of *G. crepidinum* filtrate on the growth of *Gloeocapsa crepidinum*.

The effect of G. crepidinum filtrate on its own growth was found to be peculiar and complex in the sense that there was not a regular pattern of impact to be noticed. This would explain the heterotrophic uptake of carbon reducing the O.D value and the release of organic substance in large quantity increasing the value.(Table&Fig 5.4i).

5.5 . Effect of cyanobacterial filtrate on other microflora.

Preparation of the filtrate

Cyanobacterial cultures , viz., *Synechocystis salina* and *Synechococcus elongatus* in stationary phase were filtered through millipore filters to make it cell free.

Test organisms.

Chaetoceros affinis
Dunaliella marina

Isochrysis galbana
Tetraselmis gracilis

Each of the test culture contained a known concentration of test organism in Allen and Nelson medium of 30 ppt salinity along with the culture filtrate of cyanobacteria.

Control of the test organisms was also prepared. The experiment was carried out in duplicates. Growth and productivity were measured at definite intervals.

5.5(a) Impact of *Synechocystis salina* filtrate on *Dunaliella marina.*

Synechocystis salina filtrate was found to inhibit the productivity of *Dunaliella marina* and the high percentage of inhibition (76.0%) was found on the fourth day. Thus the inhibition was remarkable in the exponential phase. In the stationary phase no considerable impact was noticed and the pH was found to be varying much from 8.16 to 6.16. (Table & Fig 5.5a).

5.5 (b) Impact of *Synechocystis salina* filtrate on *Isochrysis galbana*:

In *Isochrysis galbana* the *S.salina* filtrate enhanced the productivity remarkably. On the initial day itself a remarkable increase in production, thrice that of control was observed.

The pH was also found to vary compared to that of the control and the lowest pH recorded was 5.83 which was on the twelfth day when there was 40% enhancement in production. Table &Fig 5.5 (b)

Table 5.5(a)&(b)

Impact of *Synechocystis salina* filtrate on other microflora

Age in days	*a. Dunaliella marina*						*b. Isochrysis galbana*					
	control			filtrate+			control			filtrate+		
	OD	pH	Production mgC/l/hr	OD	pH	Production mgC/l/hr	OD	pH	Production mgC/l/hr	OD	pH	Production mgC/l/hr
0	0.009	7.06	426.25	0.007	7.06	426.25	0.009	7.06	65	0.003	6.88	212.5
4	0.03	8.16	1383	0.024	6.16	319.13	0.031	7.46	106.5	0.029	7.84	319
8	0.043	7.99	306.13	0.042	7.99	204.13	0.065	8.4	408.13	0.061	8.56	306.13
12	0.04	7.9	102	0.041	7.9	102	0.098	8.43	510.25	0.119	5.83	714.25
16	0.031	7.3	80.5	0.033	7.3	100	0.124	7.95	520.5	0.176	8.1	754

Fig 5.5(a)

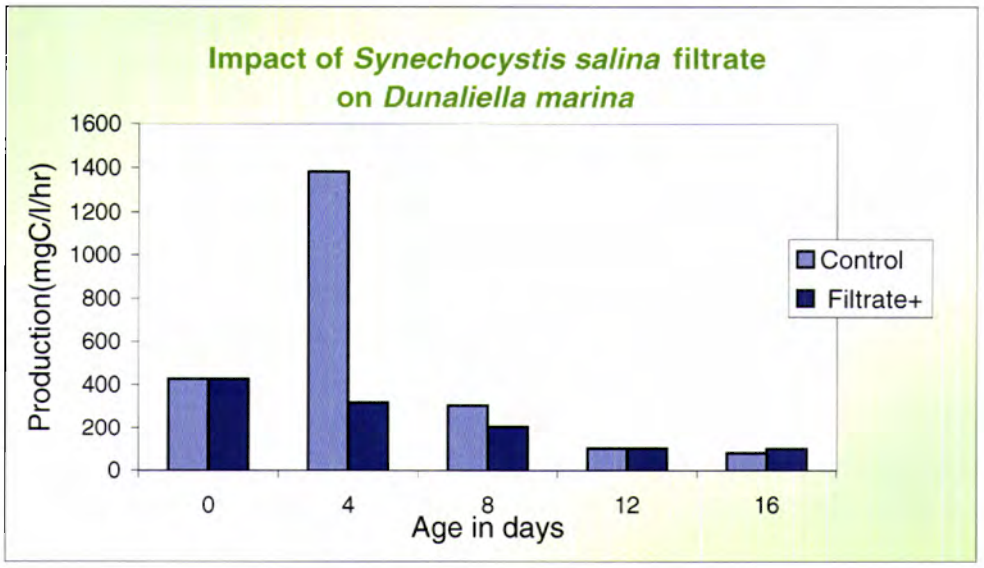
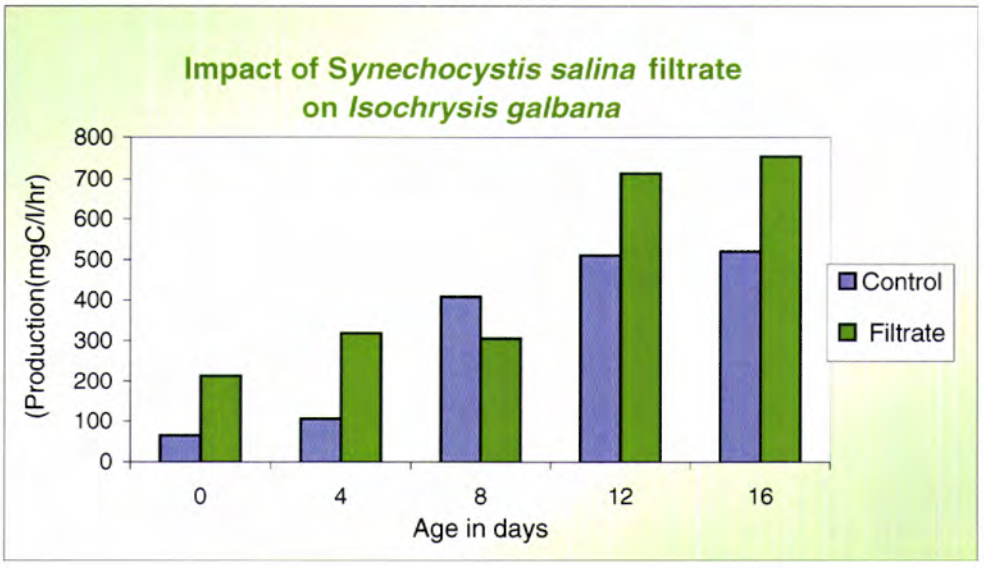


Fig 5.5(b)



5.5 (c) Impact of *Synechocystis salina* filtrate on *Tetraselmis gracilis* filtrate:

The filtrate of *S.salina* had conspicuous adverse effect on the productivity of *Tetraselmis gracilis* The initial productivity itself had decreased to one third of that in the control. On the fourth day, the production was reduced to half. The percentage of inhibition appeared to be decreasing with the age of the culture. The percentage of inhibition on the initial, 4^{th} , 8^{th}, 12^{th} and 16^{th} day were 66.4, 58.3., 20.0, 44.6, 36.6 respectively. Table &Fig 5.5 (c)

To study the significance of the difference between productivity in control and the filtrate for different days, the experimental data were subjected to statistical analysis using two way ANOVA. The mathematical model employed for this purpose is

$$X_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij}$$

Where X ij = the productivity for i^{th} day using j^{th} treatment , μ overall effect αi i^{th} day effect, βj j^{th} treatment effect and ε ij is the random error. The results are given in Tables 5.5.(a′) , 5.5 (b′) & 5.5 (c ′).

The first table 5.5(a) gives ANOVA(analysis of variance) on the effect of *Synechocystis salina* filtrate on productivity of *Dunaliella marina.* There is no significant difference in productivity between control and filtrate at 5% level.

Table 5.5(c)

Impact of *Synechocystis salina* filtrate on other microflora

Age in days	*3.Tetraselmis gracilis*					
	control			filtrate+		
	OD	pH	Production mgC/l/hr	OD	pH	Production mgC/l/hr
0	0.013	6.99	318.75	0.019	6.99	106.25
4	0.026	7.63	425.63	0.024	7.63	212.875
8	0.035	8.09	510.13	0.038	8.09	408.125
12	0.075	8.5	920.5	0.068	8.5	510.13
16	0.1	6.12	1120.3	0.094	8.12	710.4

Fig 5.5(c)

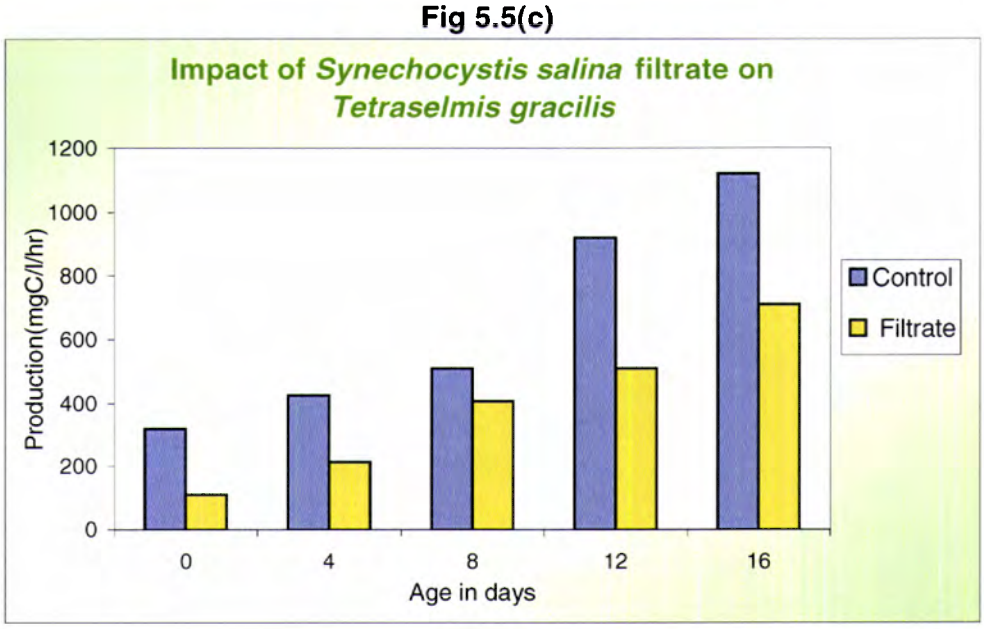

Impact of Synechocystis salina filtrate on Tetraselmis gracilis
Production(mgC/l/hr)
1200
1000
800
600
400
200
0
0
4
8
12
16
Age in days
Control
Filtrate

Table 5.5(a')

Synechocystis salina filtrate on *Dunaliella marina*

ANOVA

rce of varia	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	6.12174	4	1.530435	6.75288	0.45619	6.388234
Columns	0.274286	1	0.274286	1.21026	0.333038	7.70865
Error	0.906537	4	0.226634			
Total	7.302563	9				

Table 5.5(b')

Synechocystis salina filtrate on Isochrysis galbana

ANOVA

rce of varia	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	4.565513	4	1.141378	10.734	0.020551	6.388234
Columns	1.026397	1	1.026397	9.65265	0.035981	7.70865
Error	0.425333	4	0.106333			
Total	6.017243	9				

Table 5.5(c')

Synechocystis salina filtrate on Tetraselmis gracilis.

ANOVA

rce of varia	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	3.200527	4	0.800132	15.2574	0.010885	6.388234
Columns	0.936571	1	0.936571	17.8591	0.013413	7.70865
Error	0.209769	4	0.052442			
Total	4.346867	9				

The table 5.5 (b)′ gives ANOVA of impact of *S. salina* filtrate on *Isochrysis galbana*. There is significant difference between productivity in control and filtrate ($p < 0.05$). Filtrate gives significantly high productivity values than in control.

The ANOVA of the impact of *S.salina* filtrate on *Tetraselmis gracilis* is given in Table 5.5(c) ′ There is significant difference between control and filtrate ($p < 0.05$). Control gives significantly higher values than filtrate.

5.5 (d) Impact of *Synechococcus elongatus* filtrate on *Chaetoceros affinis.*

The filtrate of *S. elongatus* showed enhancing effect on the growth of *Chaetoceros affinis,* indicating the presence of growth promoting substance in the filtrate. The percentage of increase in productivity of the diatom was 101.2, 75.1, 90.0, 6.7 and 7.4 respectively. Table&Fig 5.5(d).

5.5 (e). Impact of Synechocystis elongatus filtrate on Dunaliella marina.

In *Dunaliella marina,* upto eight days the rate of production was inhibited and thereafter considerable enhancement was noted. Thus *Dunaliella* had a mixed response to *Synechococcus elongatus* filtrate exhibiting initial inhibition and subsequent enhancement. Table&Fig 5.5 (e).

Table 5.5(d)&(e)

Impact of *Synechococcus elongatus* filtrate on other microflora.

Age in days	*1.Chaetoceros affinis*						*2.Dunaliella marina*					
	control			filtrate+			control			filtrate+		
	OD	pH	Production mgC/l/hr	OD	pH	Production mgC/l/hr	OD	pH	Production mgC/l/hr	OD	pH	Production mgC/l/hr
0	0.006	7.09	105	0.031	7.03	211.25	0.009	7.06	426.25	0.009	6.62	106.25
4	0.061	8.39	425.5	0.059	8.89	744.88	0.03	8.16	1383	0.027	8.6	212.875
8	0.106	8.86	1020.38	0.109	8.92	1938.75	0.043	7.99	306.13	0.038	8.15	204.13
12	0.207	8.99	1530.63	0.205	9.1	1632.63	0.04	7.9	102	0.052	7.79	306.13
16	0.171	7.52	1425.25	0.283	8.2	1530.25	0.031	7.3	80.5	0.031	7.5	205.2

Fig 5.5(d)

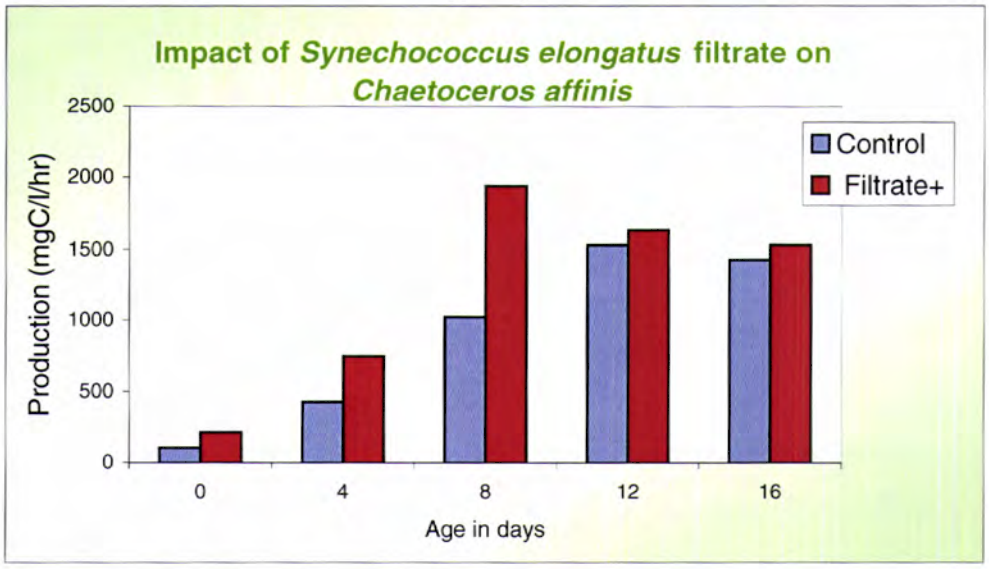
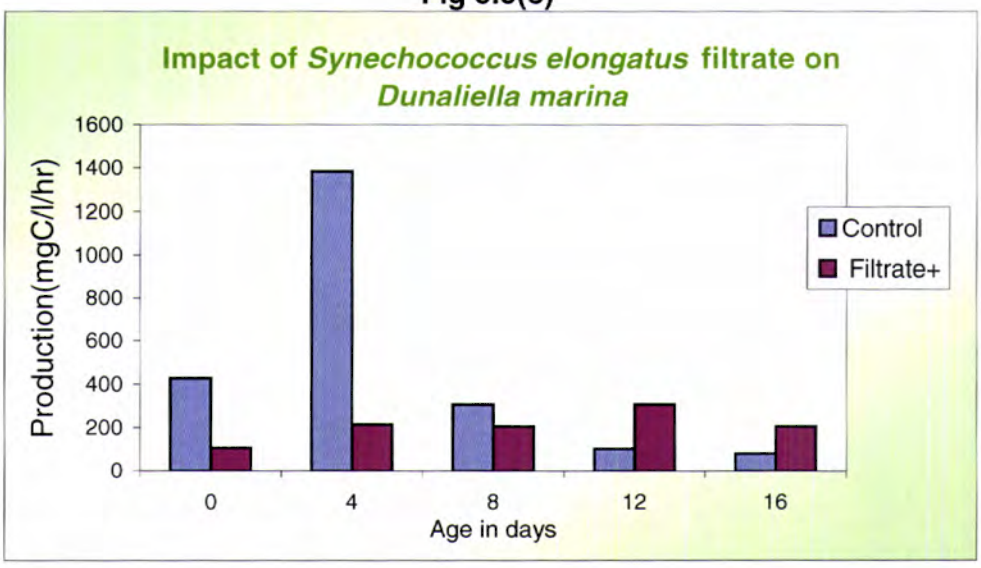


Fig 5.5(e)



5.5 (f) Impact of Synechococcus elongatus filtrate on Isochrysis galbana.

Isochrysis galbana with *S.elongatus* was as good as enriched with nutrients and there was a steady increase in production. The percentage of enhancement of production for initial, 4^{th} , 8^{th} , 12^{th} and 16^{th} day was 228.8, 200, 125, 60 and 130 respectively. Table &Fig 5.5(f).

5.5 (g) Impact of *Synechococcus filtrate* on *Tetraselmis gracilis.*

In *Tetraselmis gracilis*, the filtrate of *Synechococcus* had adverse effect on productivity except for the initial day. The percentage of inhibition on 8^{th} , 12^{th} and 16^{th} day was 20, 22.4 and 9.0 respectively. Table &Fig 5.5 (g).

The ANOVA of the impact of *Synechococcus elongatus* filtrate on *Chaetoceros affinis* is given in Table 5.5 (d)′ There is significant difference in productivity ($p< 0.05$). Addition of filtrate gives high productivity value compared to that of control.

Table 5.5 (e)′gives the ANOVA of impact on *Dunaliella marina*. There is no significant difference between productivity in control and test at 5% level.

The ANOVA of impact of *Synechococcus elongatus* filtrate on *Isochrysis galbana* is shown in Table 5.5 (f)′ There is significant difference between productivity in control and test ($p< 0.01$). The presence of filtrate gives significantly high productivity value.

Table 5.5(f)&(g)

Impact of *Synechococcus elongatus* filtrate on other microflora.

Age in days	*3.Isochrysis galbana*						*4.Tetraselmis gracilis*					
	control			filtrate+			control			filtrate+		
	OD	pH	Production mgC/l/hr	OD	pH	Production mgC/l/hr	OD	pH	Production mgC/l/hr	OD	pH	Production mgC/l/hr
0	0.009	7.06	65	0.009	6.86	213.75	0.013	6.99	318.75	0.015	7.01	532.5
4	0.031	7.46	106.5	0.02	7.67	319.25	0.026	7.63	425.63	0.031	7.69	425.63
8	0.065	8.4	408.13	0.059	8.35	918.38	0.035	8.09	510.13	0.026	8.04	408.13
12	0.098	8.43	510.25	0.125	8.94	816.38	0.075	8.5	920.5	0.072	8.69	714.25
16	0.124	7.95	520.5	0.183	8.4	1200	0.1	6.12	1120.3	0.11	7.98	1020.38

Fig 5.5(f)

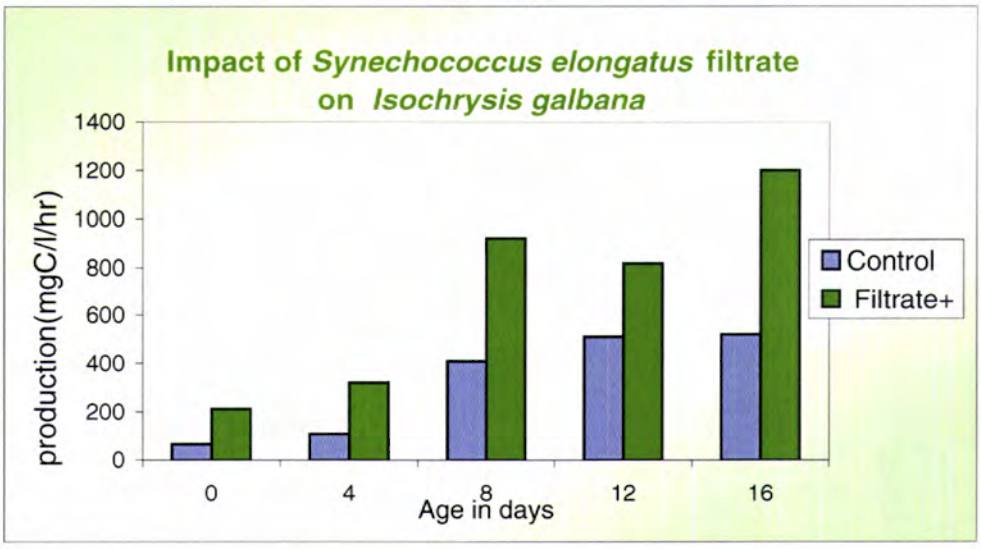


Fig 5.5(g)

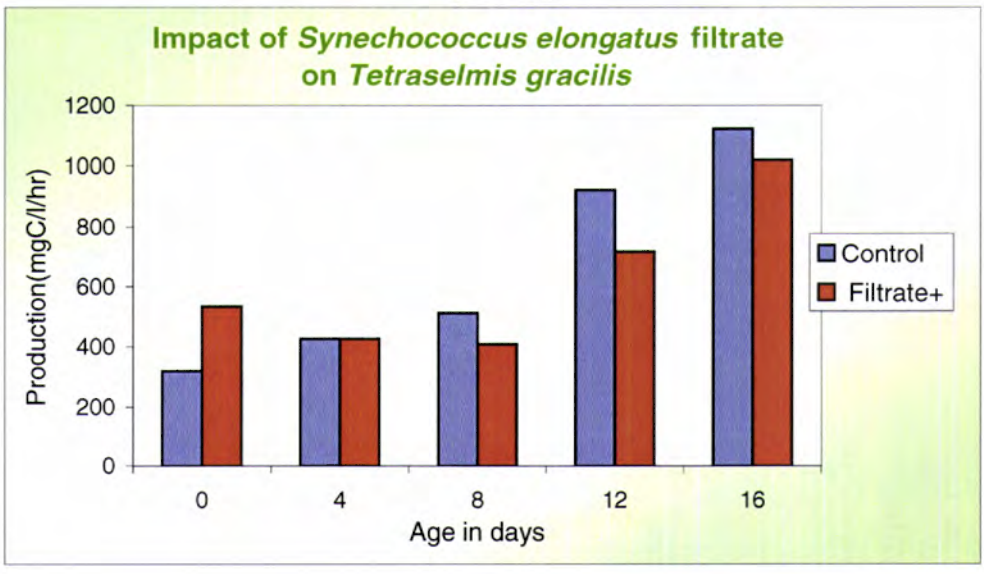


Table 5.5(d')

Synechococcus elongatus* filtrate on *Chaetoceros affinis.

ANOVA

rce of varia	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	8.21226	4	2.053065	41.539	0.001632	6.388234
Columns	0.42025	1	0.42025	8.502782	0.043419	7.70865
Error	0.1977	4	0.049425			
Total	8.83021	9				

Table 5.5(e')

Synechococcus* filtrate *on Dunaliella marina

ANOVA

rce of varia	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	3.24896	4	0.81224	3.076667	0.150994	6.388234
Columns	1.849	1	1.849	7.003788	0.057192	7.70865
Error	1.056	4	0.264			
Total	6.15396	9				

Table 5.5(f')

Synechococcus elongatus* filtrate on *Isochrysis galbana

ANOVA

rce of varia	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	5.865225	4	1.466306	36.80951	0.002062	6.388234
Columns	1.969998	1	1.939998	48.70086	0.002217	7.70865
Error	0.15934	4	0.039835			
Total	7.964563	9				

Table 5.5(g')

Synechococcus elongatus* filtrate *on Tetraselmis gracilis

ANOVA

rce of varia	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	1.51204	4	0.37801	7.843606	0.035467	6.388234
Columns	0.000325	1	0.000325	0.006744	0.938496	7.70865
Error	0.192774	4	0.048193			
Total	1.705138	9				

Table 5.5 (g)′ gives the ANOVA of the impact on *Tetraselmis gracilis*. There is no significant difference in productivity between control and test at 5% level.

The filtrate of *Synechocystis salina* had an inhibitory effect on the productivity of *Dunaliella* and *Tetraselmis* while the productivity of *Isochrysis* was enhanced upto the eighth day. The inhibitory effect was maximum in the case of *Dunaliella* which showed a productivity of 1383mgC/l/hr in control and 319µgC/l/hr in test culture with filtrate.

But in contrast to this, the filtrate of *Synechococcus elongatus* had a conspicuous enhancing effect on all tested microflora except *Dunaliella* Maximum enhancing effect was noticed on the sixteenth day in the case of *Isochrysis* where the filtrate of *S.elongatus* led to an increase in productivity upto 1200µgC/l/hr while that in control was only 520.5µgC/l/hr.The pH in this case was 8.94 instead of 8.43 in the control.

Casamata and Wickstrom. (2000) reported that cyanobacterium *Microcystis aeruginosa* releases a variety of bioactive compounds during growth. Robert andMorris(1962) examined products of actinomycetes and fungal isolates with respect to their antialgal distribution and specificity as well as their possible significance in eliminating selective algal populations. After a cyanobacterial bloom(*Oscillatoria* sp.) in a lagoon, the diversity of the subsequent diatom growth

was greatly reduced suggesting a residual inhibitory effect caused by the *Oscillatoria* sp. (Revelante and Gilmartin,1982).

The present study has shown that the filtrate of various species of cyanobacteria contains varying concentration of growth promoting and growth inhibiting substances suggesting the presence of a sort of antagonism between the two. The liberation of such substances in the medium has been also reported by Bentley(1960). Singh and Trehan (1973) reported a complex of substances with activity similar to gibberellins in culture filtrates of *Aulosira fertilissima.* The percentage of the inhibition and enhancement is indicative of the magnitude of growth promoters and inhibitors.

5.6 Heavy metal abatement by aquatic cyanobacteria

An attempt has been made to test the ability of cyanobacteria to abate metal pollution.

5.6(a) Abatement variation with different concentrations of heavy metals

Inspite of the immense heavymetal abatement potential comparatively only very few attempts have been made so far to use them for this purpose. In the present study the abatement of Cd and Pb of different concentrations by three cyanobacterial species – *Synechocystis salina Synechococcus elongatus and Gloeocapsa crepidinum* commonly seen in aquatic environments in and around of Cochin were studied. Further investigation was carried out to know the change of the percentage of abatement with time.

The quantity of removal of the above mentioned heavy metals was determined using the Atomic Absorption Spectrometer,Perkin Elmer AAS 3110.

In AAS, the sample was first converted into an atomic vapour and then the absorption of atomic vapour was measured at a selected wavelength which is characteristic of each individual element. Quantitative measurements in AAS are based on Beer's law and therefore, the measured absorbance is proportional to the concentration.

For the estimation of Lead the instrument was calibrated using a standard solution of 20 ppm lead and the concentrations of lead in the samples were then directly measured from AAS. The lamp used was hollow cathode lamp of Lead and the flame used was air- acetylene. The monochrometer wavelength selected was 283.3 nm.

For the estimation of Cadmium, hollow cathode lamp of Cadmium was used as the primary light source. The instrument was calibrated using the standard solution of Cadmium (1.5ppm). Samples dosed with higher ppm Cadmium were diluted ten times. The monochrometer wavelength selected was 228.8nm.

Test organisms:

Synechocystis salina
Synechococcus elongatus
Gloeocapsa crepidinum

Methodology:

The organisms were grown in Allen and Nelson medium of salinity 30 ppt temperature 25 ^{0}C and pH 7.2. Different concentrations of Cadmium and Lead for each organism were prepared along with their respective controls. To this, uniform concentration of each test organism was added. Test samples were taken in duplicates. After mixing the samples in a shaker at 200 rpm for 30 minutes under a constant temperature 25 ^{0}C, the samples were centrifuged and the supernatant was collected for the determination of heavy metal concentration by atomic absorption spectrophotometry (Perkin Elmer AAS 3110)

Results and discussion:

One among the significant factors influencing the abatement rate by cyanobacteria is the concentration of heavy metal. Two effective concentrations of lead and Cadmium as measured by AAS 8.88ppm and 0.14 ppm were used for the experiment. It was found that for all the three species viz., *S. salina* , *S. elongatus and G. crepidinum* ,the percentage of abatement of lead was higher at lower concentration For *S. salina* at higher concentration, the percentage of abatement was 34% where as at lower concentration , the abatement by the organism was 42.86%. This may be due to the fact that higher concentration of heavymetal lead tilts the physiological balance of the organism inhibiting its abatement potential.

In the case of *Synechococcus elongatus*, at high concentration the percentage of abatement was only 0.9% as against 64.29 % for lower concentration. In the case of *G.crepidinum* too,the same pattern of abatement of Pb was observed i.e., lower abatement for high concentration and high abatement for lower concentration, their values being 5.52% and 78.57% respectively.

It is evident that even though higher concentration was found to be generally harmful to the organisms the tolerance limit varied with the species which are shown below.

G. crepidinum > *Synechococcus elongatus* > *Synechococcus salina.*

In the abatement of lead also,the organisms maintained the same trend as shown in the table & Fig 5.6 (a).

Just as in the case of Lead, the percentage of abatement of Cadmium was found varying with concentration. In *Synechocystis salina*, the percentage of abatement for the higher concentration was 5.24 and for the lower concentration it was 25.24. It is evident that the higher concentration of heavy metal adversely affect the physiology of the organism and consequently reduce the abatement potential.

Synechococcus elongatus also exhibited the same trend but the percentage of abatement at higher concentration was comparatively low, recording the

percentage of abatement as (2.77). At lower concentration the percentage of abatement was 32.04.

In *G. crepidinum,* at higher concentration of Cadmium the percentage of abatement was 10.33 and at lower concentration it was 36.89. Thus the cyanobacterium *G. crepidinum* was found to abate more cadmium compared to other species selected. Hence among the species selected *G. crepidinum* was found to be more effective for the abatement of the heavy metal Cadmium.

The resistance to Cadmium toxicity and Cadmium abatement capability of various species can be represented as follows:

G. crepidinum > *Synechocystis salina* > *Synechococcus elongatus.*

5.6 (b) Abatement of Lead and Cadmium by aquatic cyanobacteria with time:

From the studies conducted on the rate of abatement of Pb and Cd by three species of cyanobacteria it was found that *G. crepidinum* was more efficient than the other unicellular species selected. It was also found that the rate of abatement was more at lower concentration. The rate of abatement of Pb and Cd by *G. crepidinum* with the varying time of 15 to 1440 minutes was studied. It was observed that in the first fifteen minutes it was found that the rate of abatement was 28.6 % Within the first 30 minutes, it was 39.3 % indicating that in the

Table 5.6(a)

Abatement of Lead and Cadmium with concentrations

Test organism	Concentration of Pb supplied (ppm)	Concentration of Pb in the filtrate (ppm)	Pb abated by the organism (ppm)	% of Pb abatement	Concentration of Cd supplied (ppm)	Concentration of Cd in the filtrate (ppm)	Cd abated by the organism (ppm)	% of Cd abatement
Synechocystis salina	8.88	8.85	0.03	0.34	6.87	6.51	0.36	5.24
	0.14	0.08	0.06	42.86	1.03	0.77	0.26	25.24
Synechococcus elongatus	8.88	8.8	0.08	0.9	6.87	6.68	0.19	2.77
	0.14	0.05	0.09	64.29	1.03	0.7	0.33	32.04
Gloeocapsa crepedinum	8.88	8.39	0.49	5.52	6.87	6.16	0.71	10.33
	0.14	0.03	0.11	78.57	1.03	0.65	0.38	36.89

Table 5.6(b)

Abatement of Lead and Cadmium by *Gloeocapsa crepidinum* with time

Time in minutes	Pb supplied (ppm)	Pb in the filtrate (ppm)	Pb abated (ppm)	% of Pb abatement	Cd supplied (ppm)	Cd in the filtrate (ppm)	Cd abated (ppm)	% of Cd abatement
15	0.14	0.1	0.04	28.6	0.14	0.03	0.11	78.6
30	0.14	0.085	0.055	39.3	0.14	0.024	0.116	82.9
60	0.14	0.06	0.08	57.1	0.14	0.017	0.123	87.9
120	0.14	0.04	0.1	71.4	0.14	0.011	0.13	92.9
1440	0.14	0.02	0.12	85.7	0.14	0.006	0.134	95.7

Fig. 5.6(a)

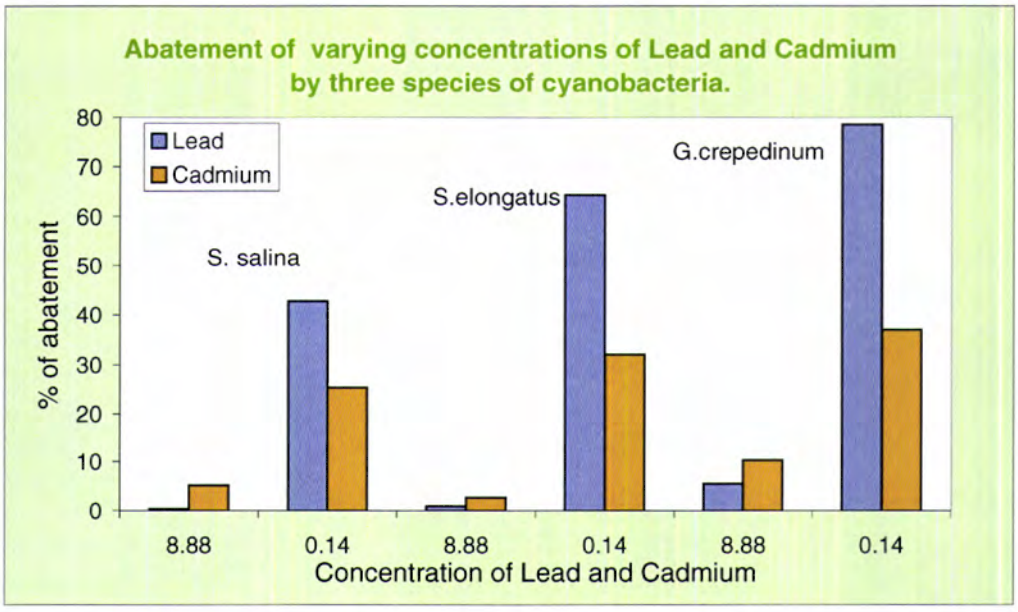
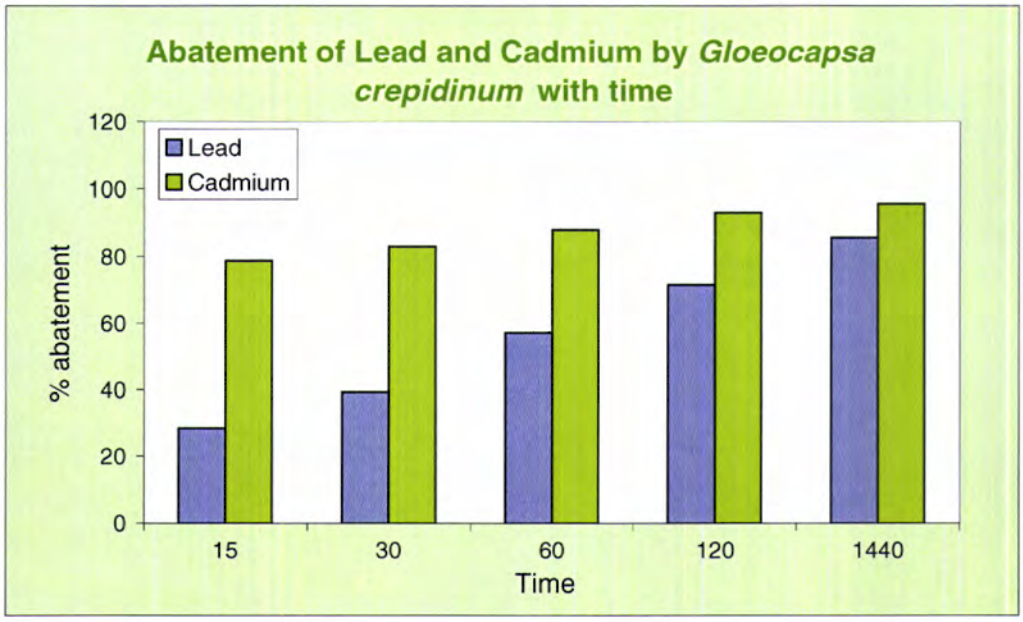


Fig. 5.6(b)



second fifteen minutes the percentage of abatement was only 10.7 %. Thus, it was found that the maximum abatement has taken place in the first fifteen minutes. The abatement rate was found to be reduced with time. 71% of the Pb was found abated by two hours. The rate of abatement even after 24 hours was found to be only 85.7 % ie., the lead abated for 22 hrs was only 14.3 %. (Table&Fig 5.6 b).

78.6 % Cadmium was found removed from *G. crepidinum* culture within the first fifteen minutes. As time advances, the rate of abatement was found reduced considerably. Thus after two hours the percentage of abatement was 92.9. After 24 hours, the cadmium abated by the species was 95.7 %, for 22 hrs the cadmium abated was as little as 2.8 %.

Inthorn *et al.* ,(2001) studied the rate of abatement of three heavy metals, Pb, Cd and Hg by two strains of filamentous cyanobacteria viz., *Calothrix, Tolypothrix tenius* and three strains of green algae. It was observed that the kinetics of three heavy metal removal were similar showing a fast removal in the first five minutes and reaching an equilibrium within 10 to 20 minutes. The results showed that most microalgae could toleratewell the toxicity of three metals and the relative toxicity observed was Hg < Cd < Pb. The removal of heavy metals from comparatively wider area by ion exchange, precipitation and reverse osmosis is complicated and expensive (Manahan, 1993). Biological methods by using

various microflora appear to offer a promising alternative. Their advantages include natural occurrence, cheap production availability to treat large volumes of waste water due to rapid kinetics and high selectivity in terms of removal and recovery of specific heavy metals. (Genter, 1996).

5.7 Toxin producing cyanobacteria

The frequency of nuisance blooms is increasing due to the progressive eutrophication. This may lead to the episodes of poisoning causing much damage to animals and human beings. Skulberg, et al. (1994) disclosed fifteen toxigenic species of cyanophytes in an extensive survey of lakes in the South of Norway. The present known toxigenic cyanobacteria constitute about 40 species(Skulberg et al, 1993). Research on toxic cyanobacteria is still in its infancy (Carmichael, 1981)

All the three species viz., *Synechocystis salina, Synechococcus elongatus and Gloeocapsa crepidinum* on which detailed studies of their release of extracellular products and their bioactive impact on cyanobacteria and other microflora have been tested for toxicity.The test algae were digested in HCl and estimated for PSP as per standard mouse bioassay (AOAC, 1990). The toxin was injected into the mice having a weight of 20grams intraperitonially. The clinical symptoms produced were paralysis and death. Of the three species tested, two species

namely *Synechocystis salina and Gloeocapsa crepidinum* were found to produce neurotoxin (saxitoxin) as evidenced by clinical signs of toxicity exhibited by the mice used in bioassay.

Toxin production by cyanobacteria leading to the death of cattle and birds is a world-wide phenomenon that is reviewed by Gorham(1964). Toxic cyanobacteria may affect sensitive organisms and populations and also fundamental ecological processes such as microbial production and microbial activity. Rai et al (1994) isolated toxic cyanobacterium *Microcystis aeruginosa* and determined its toxicity to mice which exhibited clinical signs of toxicity. A particular problem regarding toxicity relates to the clonal differences and toxicity in nature and varies considerably between different strains and populations of cyanobacteria.

Hepatotoxic and neurotoxic bloom samples as well as isolates of cyanobacteria were found toxic to *Artemia* brine shrimp larvae, but some laboratory grown strains also caused mortality, although they were non-toxic to mice. Hence,it may be inferred that the mouse bioassay, using intraperitoneal injection of cyanobacterial cells or extract is not sensitive to mild toxicity exhibited by several cyanobacterial species.In *Artemia salina* bioassay among the various strains of Anabaena used, three strains *Anabaena flosaquae*, *Anabaena circinalis* and *Anabaena circinalis* were found to have anatoxin –a, where *Synechococcus* sp. was found to be nontoxic. In the present study also , *Synechococcus* sp.was found

to be nontoxic. Similarly, reproduction in planktonic rotifers was also found to be inhibited by the presence of the toxic strain of *Anabaena flosaquae* (IC-1) producing the neurotoxic alkaloid antoxin- a (Gilbert, 1994).

6
CHAPTER

SUMMARY & CONCLUSION

Though several aspects of cyanobacteria such as nitrogen metabolism , fatty acid metabolism , toxicity and bioremediation have been investigated, the distribution of aquatic flora of a region with diverse aquatic habitats, their growth kinetics and bioactive impact have not been studied earlier in India.

The present work deals with the investigations on the occurrence of the cyanobacteria in Cochin ,situated in the south west coast of India. The aquatic habitat is unique with the proximity of the Arabian sea, Periyar river and Vembanad estuary. Cyanobacteria distributed in the varying aquatic habitats were collected, isolated and identified and maintained in enriched media. In all, 116 species of cyanobacteria were collected On analysis of seasonal variation it was found that maximum number of cyanobacteria was invariably found during the rainy season in freshwater,marine and estuarine environment.

In several instances the very survival of an organism was found to depend on its associated flora. The bacteria seen associated on various species of cyanobacteria were isolated and identified. No specificity of association was noticed and in marine and freshwater habitats all the genera were recorded. Generally, a single dominant bacterium was found attached to a cyanobacterial strain and the genus *Bacillus* was found to be associated with a majority of cyanobacterial species. This pattern of association was found both in the marine as well as freshwater forms.

Methods of collection varied with the habitat where the bacteria had been distributed. The planktonic forms were isolated by collecting water samples and the attached forms by setting them detached mechanically. Isolation of cyanobacteria was done by five methods- pipette method, centrifuging, exploiting the phototactic movements, agar plating method and serial dilution method. The isolated species were cultured in Allen and Nelson medium.

Same species of cyanobacteria were found in freshwater, marine and estuarine environment. *S. salina* isolated from the marine and freshwater environments were cultured in varying salinity from zero ppt to 50 ppt. Both the species were found to grow well at this salinity range.

Synechococcus elongatus was isolated from marine, freshwater and estuarine environment and that which is isolated from marine environment was studied at varying salinity 0, 10, 20, 30, 40 ppt. In all varying salinity except at zero ppt, the species exhibited comparitively good growth. At zero ppt, the initial generation time taken was 233.37 hrs. So also on the ninth day. Beyond this period no growth was recorded showing the limit of time within which this same species can survive in freshwater medium. At higher salinity the lowest generation time observed was 9.59 which was for 40 ppt. *Gloeocapsa crepidinum* isolated from the coastal waters of Cochin had exhibited initial tolerance at all the salinities studied. But the sustained growth beyond 10 th day was not obtained for lower

salinity expecially at zero ppt. On analysis of the salinity tolerance of various species of cyanobacteria studied it may be difficult to arrive at a conclusion deviating from that of Atre (1998) that growth of cyanobacteria in response to salt stress showed contrary results.

Growth characteristics of *Synechocystis salina* in enriched and uneriched media were studied. In the unenriched medium the growth rate and the generation time varied from 17.5 to 350 hrs whereas, in the enriched medium sustained growth was observed as evidenced from comparatively lower generation time and the generation time varied from 15.2 on the second day to 46.7 on the 14^{th} day.

Production of organic substances by three unicellular species of cyanobacteria- *Synechocystis salina, Synechococcus elongatus* and *Gloeocapsa crepidinum* was carried out using ^{14}C technique. The rate of production for *Synechocystis salina* recorded was found to be low due to the extracellular substance released The extracellular substances released was also estimated. In *S.salina,* the initial productivity recorded was 0.27 µgC/l/hr and a steady increase in productivity was recorded till the 15 th day of the experiment when the productivity was 26.356 µgC/l/hr.

Though the production of organic substances was found to increase with the age of culture and production per unit number of cells was found fluctuating

indicating the variation in the productivity of the cells. The production of organic substance in relation to the cell numbers and chlorophyll a for a period of fifteen days was observed in the case of *Synechocystis elongatus*. A steady increase in all the parameters was observed and the production of organic substance was found to vary from 0.249 on the initial day to 18.65 µgC/l/hr on the fifteenth day. The maximum production of 0.466 µgC /l/hr / 10^6 cells was obtained on the fifteenth day. In *S.elongatus* an increase in the production of organic substances was noticed with the age of the culture The rate of production/unit number of cells was found fluctuating. The rate was the minimum on the sixth day(0.35 µgC/l/r/10^6 cells) and it was the maximum on 12^{th} day (0.843µg C/l/hr/ 10^6 cells).

In *Gloeocapsa crepidinum* a steady increase in cell numbers followed by proportional increase in chlorophyll a concentration and production of organic substance was recorded. From the initial concentration of 0.22 µgC/l/hr it increased to 1.388 µgC/l/hr. The production ranged from 1.42 µg C/l/hr/10^6 cells in the initial day to 2.776 µgC/l/hr/ 10^6 cells on the sixth day. In *Gloeocapsa* , the rate of release was found to be higher compared to that of other species. From 61.3% on the initial day it was found to be reduced to 22.2 %.

In *S. elongatus* the percentage of liberation of extracellular product was estimated to vary from 0.9µgC/l/hr on fifteenth day to38.83µgC/l/hr on the 12^{th} day .The cellular composition of carbohydrate, protein and lipids were estimated for the

three species - *Synechocystis salina, Synechococcus elongatus* and *Gloeocapsa crepidinum*. It showed that comparatively higher percentage of carbohydrate and lipids were found in *G.crepidinum*.

Extracellular organic substance was analysed for the composition of carbohydrate, protein and lipids in the species *Synechocystis salina, Synechococcus elongatus and Gloeocapsa crepidinum*. It was found that carbohydrate was present in all the three species. Protein and lipids were recorded occasionally. The apparent absence of the components at regular or irregular interval may be attributed to the heterotrophic uptake of the organic substances. This can be a plausible explanation as several species of cyanobacteria are mixotrophic

Studying the impact of cyanobacteria on selected microflora it was shown that various cyanobacteria release a considerable percentage of organic substances synthesized in the cells was released in the medium. These extracellular product was proved to be bioactive as shown by their impact on growth and multiplication of other organisms. The quantity of extracellular product released was measured by ^{14}C technique. The percentage of extracellular product was found to vary with the species and age and phase of culture.

In *S.salina* culture, the extracellular product varied from 0.092 to 0.916μgC/l/hr.

There was a gradual decrease in the production of extracellular product from 34.07 % on the initial day to 2.85% on the 15th day.

Biological effect of the filtrate of several species of cyanobacteria such as that of *S.salina. Synechococcus elongatus, G.crepidinum* were studied on their own growth and on other species of cyanobacteria. The filtrate of *S.salina* showed 25% enhancement on its own growth inspite of an initial inhibition. The growth of *Synechococcus elongatus* was found to have increased with the age of the culture and the percentage of inhibition being 67% on the last day of the experiment.

The filtrate of *S.elongatus* is growth inhibiting as evident from its effect on the growth of *Synechocystis salina.* The bioactive impact of the filtrate of *Synechococcus elongatus* was examined on its own growth and it was observed that its growth was inhibited upto 48% on addition of filtrate. It may be noted that there was an initial enhancement in growth.

Synechococcus elongatus filtrate had an enhancing effect on the growth of *G. crepidinum* and the percentage of enhancement varied form 8.8% to 145%

Gloeocapsa crepidinum filtrate enhanced the growth of S.salina from 15.7% to 144% and the percentage of enhancement increased with the age of the culture.

Inspite of initial enhancement *Synechococcus elongatus* showed inhibition in the growth by 48.9% with the filtrate of *G. crepidinum*

The effect of *G. crepidinum* filtrate on its own growth was found to be complex as no regular pattern of impact was observed. This may be explained by the heterotrophic uptake of organic substance affecting the O.D values.

The bioactive impact of the filtrate *Synechocystis salina* and *Synechococcus elongatus* was studied on other photosynthetic microflora such as *Dunaliella marina, Isochrysis galbana, Tetraselmis gracilis, Chaetoceros affinis.*

Synechocystis salina filtrate was found to inhibit the productivity of *Dunaliella marina* upto 76.9% and enhanced the productivity of *Isochrysis galbana* upto 40%..

The filtrate of *Synechococcus elongatus* showed enhancement on the growth and productivity of the diatom *Chaetoceros affinis* indicating the presence of growth promoting substance. The percentage of enhancement in productivity varied from 6.7 to 101.2 %.In *Dunaliella marina* remarkable inhibition and subsequent enhancement was observed with the filtrate of *Synechococcus elongatus Isochrysis galbana* with the filtrate of *Synechococcus elongatus* was found to be enhance growth with considerable in the productivity on all days. The percentage of enhancement of production varied from 60 to 228 .8%. In *Tetraselmis gracilis*

the filtrate of *Synechococcus elongatus* had an adverse effect on productivity , the percentage of inhibition varying from 9 to 22.4.

Toxicity studies conducted by mouse bioassay have shown that *Synechocystis salina* and *G.crepidinum* are toxin producing strain.

Microalgae are known to respond physiologically on exposure to sublethal concentration of toxic metals. Functional responses may be due to the result of the mode of the action of the metal, or due to adaphic responses by the organism. The abatement of Lead and Cadmium by *S.salina* was found to be influenced by the concentration of the heavy metal in the medium. Among the two concentrations used for the studies, at the higher concentration the percentage of abatement was 0.34 % as against 42.86 % in the lower concentration. The percentage of abatement by *S.elongatus* and *G.crepidinum* was higher at lower concentration and vice versa. The percentage of abatement of Cadmium by the same species also exhibited the same trend of abatement i.e., higher the concentration lower the abatement. This may be due to the physiological response of the cyanobacteria to the sublethal concentration of the toxic metal. In the present investigation, the abatement of heavy metals by the species of cyanobacteria suggests that they can be effectively used for the bioremediation of metals as they are efficient bioaccumulators of heavy metals.

From the present study,it is evident that cyanobacteria are distributed widely in the aquatic habitat-freshwater, marine and estuarine. They play a very significant role in bioproductivity , growth and survival of other flora and fauna and also in abating metal pollution.

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