TERRESTRIAL CYANOBACTERIA OF SELECTED HABITATS OF KODUNGALLUR, THRISSUR, KERALA

Dissertation submitted to the

UNIVERSITY OF CALICUT

In partial fulfilment of the requirements for the award of degree of

MASTER OF SCIENCE IN BOTANY

Submitted by

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POST GRADUATE AND RESEARCH DEPARTMENT OF BOTANY

M.E. S ASMABI COLLEGE, P. VEMBALLUR

2024

POST GRADUATE AND RESEARCH DEPARTMENT OF BOTANY

M.E.S ASMABI COLLEGE, P.VEMBALLUR

CERTIFICATE

This is to certify that the dissertation entitled **"Terrestrial Cyanobacteria of Selected Habitats of Kodungallur, Thrissur, Kerala"** submitted by **AYISHA HENNA P P** in partial fulfilment for the degree of Master of Science in Botany of M.E.S Asmabi College, P. Vemballur, Thrissur affiliated to University of Calicut is a bonafide work carried out by her during the fourth semester of the course.

SHAHEEDHA T.M — Supervising Teacher

Dr. GIRIJA T.P — Head of the Department

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I, **Ayisha Henna P P** hereby declare that this dissertation entitled **"Terrestrial Cyanobacteria of Selected Habitats of Kodungallur, Thrissur, Kerala"** is submitted by me under the supervision and guidance of **Mrs. Shaheedha T.M,** Assistant Professor, M.E.S Asmabi college, P. Vemballur for partial fulfilment of the requirements for the degree of Master of Science in Botany, in Calicut University and that no part of this dissertation has been presented earlier for any degree or diploma in any of the Universities.

AYISHA HENNA P P

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AYISHA HENNA P P

CONTENTS

ABSTRACT

Terrestrial cyanobacteria of selected habitats of Kodungallur, Thrissur, Kerala were analysed in the present investigation. The fresh samples from different habitat including tree trunk, soil surface, pots, cemented walls, building walls were collected by scrapping them in sterile bottle. Small portion of the samples were stored in freshwater and the remaining samples were cultured in BG 11 medium for isolating single species. Cyanobacteria were identified using Monograph and documented with digital photograph.

A total of 35 species of cyanobacteria coming under 14 genera were recorded. The species belongs to 7 families under 4 orders. Nostocales is the prominent order with 3 families. Most number of genera belong to Oscillatoriaceae family. The most established genera are *Oscillatoria* and *Lyngbya* with 7 species each. Among 35 species of identified cyanobacteria, 10 species have soil habitat, 5 species epiphytic, remaining 20 species epilithic. The dominant order is Nostocales with 26 species, (19 species Oscillatoriaceae and 6 species Nostocaceae, 1 scytonemataceae) followed by Chroococcales with 7 species. Third dominated order is Chamaeosiphonales and Stigonematales with 1 species each.

KEY WORDS: Cyanobacteria, Terrestrial habitat, Diversity

1. INTRODUCTION

Cyanobacteria or blue green algae are one of the earliest living forms in the planet. They represent a group of prokaryotes. They are phylogenetically the primitive group of gram-negative oxygen evolving photosynthetic prokaryotes. They have been on the earth almost from the beginning of life. The origin of cyanobacteria is estimated to be 2.7 billion years ago. They have chloroplasts within a typical bacterial cell, and they shared an evolutionary position with higher plant. Cyanobacteria possess light harvesting pigment such as chlorophyll a and carotenoids. About 20–30% of a global primary photosynthetic productivity originates only from cyanobacteria. Taxonomically they are classified as Cyanophyta but recently as cyanobacteria, and also introduced one more term `Cyanoprokaryota `for this group. There are an estimated 8000 species of cyanobacteria of which described species are about 5000, with about 3000 undescribed species.

Cyanobacteria are morphologically, physiologically and developmentally most diverse group of prokaryotes with low level of cellular differentiation, it constitutes one of the major phylum eubacteria. This diversity includes morphological features like -unicellular, filamentous, heterocystous, habitats and mode of nutrition. They are characterized by a low state of cell organization. The cell lacks the well-defined nucleus. Cell division is by division of protoplast by ingrowth of septum. These organisms are characterised generally by a blue green colouration of the cell, the chief pigments being Chlorophyll a, Carotenes, Xanthophylls, c-phycocyanin and c-phycoerythrin. It lacks flagellated reproductive bodies and there is a total lack of sexual reproduction.

They are unique in the living world because of their ability to carry out two key phenomena; oxygenic photosynthesis and nitrogen fixation within the same cell. The exposure to various environment stresses like high/low temperature and light intensity and ultra violet radiation forced gradual evolution in cyanobacteria to maintain the cell viability by the synthesis of many photon absorbing pigments. They can survive at temperature range 45-70^{0}C and pH range of 4-10. Cyanobacteria show great variability in occurrence, distribution, ecology and periodicity because they show specific growth pattern in a specific environment.

Cyanobacteria are ubiquitous in their distribution and it grow in different habitats such as fresh and marine water, on soil surface, in the paddy field, on animals and plants as epiphytes etc. Cyanobacteria are most diverse group found in all habitats from hot springs to the site having the permanent ice cover of the Antarctic. Cyanobacteria also form the major population in the extreme environments of sub-aerial habitats like external wall of building facades, desert crusts, and barks of trees as well as exposed rock surfaces of temples and monuments in different parts of the world.

It is also found on the bricks and cemented wall that form characteristics mats. The blackish look of monuments, temple stone and building facades is due to phototrophic biofilms of cyanobacteria. They could survive in biofilms even during summer season when the temperature exceeds 60^0C coupled with high light intensity and extreme dryness and they sprout receiving the moisturizer and grow utilizing the minerals leading to the deterioration of building facades. Cyanobacteria usually cause damage to the exposed surface of walls, make them discoloured and cause deterioration of the substratum texture forming blackish brown, greenish -black, greyish brown and black patches.

Terrestrial ecosystem is well balanced with diverse group of microorganisms. In terrestrial environment cyanobacteria, along with other phototrophic and heterotrophic microorganism have significant role in subaerial biofilm formation (SAB). SAB is the process of establishing the complex microbial communities on solid surface. These biofilm population can hold in extreme or harsh environmental condition. Subaerial biofilm formation depends on the specific biology of microorganisms and also on the physical and chemical property of substrata.

An ecological community is an organised system of interaction of individuals of different species and also with the environment with respect to environmental changes. Habitat quality and habitat composition are primary factors affecting the spatial distribution of organisms. The community always remains dynamic. The diversity of organisms will make a reflection on the community. Their wide occurrence in all possible biotopes reflects a broad spectrum of their physiological properties and tolerance to environmental stress. The four important survival strategies of cyanobacteria include formation of resting structures, presence of aerotopes, exopolysaccharides, production of UV absorbing factors.

Cyanobacteria and other microorganisms can be found forming a biological soil crust (BSC) in the sun-exposed upper few millimetres of the surface. Apart from the capability of carbon fixation, some BSC organisms are also able to fix N2 and support the accumulation of N-compounds in the soil. They perform ecosystem functions such as help stabilize soil or mobile sand surfaces by binding dust and mineral particles, hinder wind erosion, and influence hydrological cycles. Sand stabilization is promoted by the excretion of extra cellular polysaccharides (EPS) during cyanobacterial growth.

High fertility of the paddy field soil contributed to the occurrence of nitrogen fixing blue green algae which increases the nitrogen content of the soil and supplies nitrates in the symbiotic relationship and increase the nutritive quality of the host plant. Some act as biofertilizer which improve plant nutrition in rice and wheat production. These qualities have been employed extensively in Asia to enhance agricultural production.

Irrespective of their colour, all algae have chlorophyll a, and by their photosynthetic action they take up carbon dioxide and aerate their habitat. The blue green algae thus participate actively in aeration of fish ponds. *Nostoc* is known to secrete an antibiotic known as bacteriocin that can kill related strains of alga. *Scytonema hofmanni* Agardh is known to secrete cyanobacterin, a chlorine containing gamma lactone. These antibiotics play a role in inhibiting the growth of competing organisms.

Blue green algae are useful in land reclamation as the first colonizers of marshlands. They hold soil and dust particles as they dry up. Thus, they are important in ecological succession. They also help in the reclamation of saline-alkali soils.

The ability of Cyanobacteria *Scytonema, Oscillatoria* to bind sand and soil particles, helps to prevent erosion. Their growth in soil seems to influence the physical and chemical properties of the soil. It increases the water-stable aggregates; and the soil aggregation and arrangement influence infiltration rate, aeration and soil temperature. Cyanobacteria *Anabaena* is used as a biological control agent against mosquitoes.

Cyanobacteria are source of single cell protein. Some species are delicacies in human diet. *Spirulina* is used as a food supplements. It contains 60 – 70% protein, 20 % carbohydrate, 5 % lipids, 7 % minerals and 6 % moisture. Moreover, it is a rich source of beta-carotene, thiamine and riboflavin and is one of the richest sources of vitamin B12.

Present study analyses the cyanobacterial flora and their diversity. Very few investigations have been carried out on cyanobacterial diversity in the terrestrial environment. This study will be a contribution to our knowledge of cyanobacterial biodiversity in terrestrial ecosystem to generate data on the future ecological studies.

OBJECTIVES

- Identification and documentation of cyanobacteria from various terrestrial habitats to understand the diversity of the cyanobacteria.
- Taxonomic analysis of cyanobacteria up to species level.
- To provide microphotographs and description of cyanobacteria in terrestrial habitat.

SIGNIFICANE OF THE STUDY

- Isolation and culturing of cyanobacteria helps to identify the species present in various habitat.
- By this study the distribution of specific cyanobacteria in each habitat can be identified.
- Investigation of these prokaryotes provide vast opportunities in several fields.

2. REVIEW OF LITERATURE

CYANOBACTERIA IN TERRESTRIAL ECOSYSTEM

Ortega-Calvo *et al.* (1993) considered terrestrial algae form biofilms on the exposed surface of the solid substrata on almost all climatic zone. Helene *et al.* (2006) made a study on the Diversity of algae and cyanobacteria growing on building façades in France. They suggest that algae and cyanobacteria in biofilms cause discolouration of the building surfaces they colonise. Ciferri (1999) made a study on the microbial degradation of paintings and suggested that paint surface contain organic and inorganic contents that facilitate the growth of cyanobacterium which led to the degradation of art works. Kala and Pandey (2021) reported that the bio colonization and growth of cyanobacteria as lithobionts are influenced by various properties of monuments, buildings or building stones as well as by environmental factors. As photoautotrophs and primary colonizers of lithic substrates, they facilitate and promote the growth and development of heterotrophic microbes, such as bacteria and fungi. The production of extracellular polymeric substances (EPS) leads to the formation of cyanobacteria-dominated phototrophic biofilms or sub-aerial biofilms on exposed surfaces of monuments, buildings and rocks or stones.

Sethi *et al.* (2012) identified twenty-four species of cyanobacteria and six species of micro-algae were recorded in biological crusts from different sub-aerial habitats e.g. Cemented building facades, tree trunks, soil surface of barren land and rice fields in certain locations of eastern region of India. These belong to seventeen genera. The brownish coloured crusts on soil contained sheathed cyanobacteria species whereas the greenish-brown crusts on rice field soils harboured green algae and cyanobacteria. The cemented building facades and tree trunks were colonized by filamentous cyanobacteria species. The green algal forms occurred in the crust only during the rainy season by their study cyanobacteria and micro-algae in biological crusts on soil and sub-aerial habitats of eastern and north eastern region of India.

Riahi *et al.* (2022) conducted study on the Diversity and distribution of heterocystus cyanobacteria across solar radiation gradient in terrestrial habitats of Iran. The study was conducted from the soil of 21 sites and the result suggest that ecological factors such as solar radiation, relative humidity, and soil salinity can affect the diversity and distribution of cyanobacteria in terrestrial ecosystems. Also, taxa like *Nostoc* were dominated in sites with high solar radiations. Samad and Adhikary (2008) made a study on the Diversity of Micro-

algae and Cyanobacteria on Building Facades and Monuments in India and reported that cyanobacteria dominate from warm temperate to tropical region.

DIVERSITY OF CYANOBACTERIA IN KERALA

Anand and Hopper, (1987) made a study on the blue green algae from the rice field of Kerala. Geethu and Shamina (2021) identified 18 cyanobacterial taxa from western ghats of north Kerala. Highest diversity of cyanobacteria was found on rocks surface. Keshri and Datta (2014) investigated soil and subaerial cyanobacteria and encountered 22 taxa of blue green algae. Fourteen members are non heterocystous and remaining eight are heterocystous forms. Suresh *et al*. (2012) conducted a systemic study on the microalgal flora of western ghats and other parts of eastern ghats, 91 species of microalgae were identified of which 41 species belonging to Cyanophyceae.

Thomas (2013) identified and listed seventy-seven species of algae belonging to the three major classes -Chlorophyceae, Cyanophyceae and Bacillariophyceae from the soils of Pathanamthitta. Arun (2022) reported a total of 89 cyanobacterial species which include 5 orders,15 families and 28 genera by studying the ecology and diversity of mangrove associated cyanobacteria of Kerala. Out of these 44 records were new to India and 29 were new to Kerala.

Paul (2012) made a study on the algal flora of Kole lands in Trissur and recorded total of 591 taxa of phytoplankton belonging to 105 genera, of this information 42 are new to India, 235 are new to Kerala and 484 are new addition to the algal flora of Trissur district. Vijayan and Danya (2015) made an investigation on the topic Ecology of Soil Algae of Wetland Paddy fields of Kuttanadu and provide a valuable information on the taxonomy and ecology of soil algae of Kuttanadu paddy field.

Philip *et al.* (2016) conducted a study on the cyanobacterial flora of Nelliyampathy totally 15 species of cyanobacteria belonging to 4 families were identified. Ram and Shamina (2016) conducted research on the biodiversity of epilithic cyanobacteria of Kerala. Cyanobacterial species were collected from the rock surfaces. Yusuf and Parambil (2023) identified 25 epilithic *Nostoc* spp. From western ghats regions of Kerala. Ammini and Smitha (2015) made an investigation of the filamentous algae in the paddy fields of Kuttanad and kole lands of Kerala. Thirty-two species of blue green algae and eight species of green algae were identified from

Kuttanad. Twenty-eight species of blue green and six species of green algae were identified from kole lands.

DIVERSITY OF CYANOBACTERIA IN INDIA

Maya Shree *et al* (2010) conducted a study on the Significance of cyanobacterial diversity in different ecological conditions of Meghalaya, India. The present study deals with preliminary investigation of cyanobacterial diversity in Meghalaya. A total of 75 samples were collected from 10 different ecosystems and analysed. 65 strains of cyanobacteria isolated under 11 genera include *Nostoc, Anabaena, Calothrix, Cylindrospermum, Gleocapsa, Fischerella, Plectonema, Tolypothrix, Stigonema, Loriella and Westiellopsis*. *Nostoc* was most abundant. This study revealed the cyanobacterial strains, which can withstand acidic pH and prevail in the region.

Adhikary *et al.* (2015) identified A total of 17 species of cyanobacteria, belonging to the genera *Gloeocapsa, Gloeocapsopsis, Porphyrosiphon, Leptolyngbya, Lyngbya, Phormidium, Nostoc, Scytonema, Tolypothrix, Hassallia and Stigonema* were found in biofilms of stone temple of Bhubaneswar during the hot months of the tropical summers. Maurya *et al.* (2019) reported seventeen species of cyanobacteria representing 15 genera and belonging to 10 families and 4 orders from high altitude of Uttarakhand. Banerjee and Pal (2020) reported 7 new unicellular cyanobacteria from west Bengal.

Mahanandia and Singh (2023) reported the diversity of western Odisha total fifty subaerial cyanobacterial taxa belonging to 21 different genera of five orders were documented. Nayak *et al.* (2011) isolated 22 taxa of cyanobacteria, 5 are chroococcales and 7 from stignonematales were found to occur on lithic surface of monuments of western Odisha. Sharma and Srivastava (2023) made a study of epilithic cyanobacteria of Rajasthan.

Pradhan (2018) carried out an investigation on the systematic of both epilithic and endolithic cyanobacterial flora on temples and caves of western Odisha. This investigation is carried out in both summer and winter showed that a total of 54 cyanobacterial taxa were found on exposed rock or cement surfaces of temples and 29 species on cave substrata during both the seasons. Thiyagarajan (2005) reported 83 species of cyanobacteria from the surface of moist or dry surface stone, cemented walls, tree bark, soils respectively from different altitude of Kolli hills.

Devi and Indrama (2013) listed 112 cyanobacterial strains from the rice field of Manipur, they coming under 16 genera and 52 species. Kharkongor and Ramanujam (2014) made a study on the diversity and species composition of subaerial algal communities from tree barks of closed undisturbed sacred grove, mixed plantation, and open disturbed forest. A total of 85 taxa with 30 cyanobacteria and 55 algal species were recorded. Sarmah and Rout (2018) studied algal colonization on polythene carry bags in a domestic solid waste dumping site of Assam. They identified 36 algae from the surface of polythene bags in solid domestic sewage dumping site.

APPLICATION OF CYANOBACTERIA

Falch *et al*. (1995) investigated the cyanobacteria of both terrestrial and freshwater and suggest that it have biological activity like antimalarial, antifungal and antibacterial. Tyagi *et al.* (2019) made a study on the topic cyanobacterial application in biotechnology and concluded that cyanobacteria synthesize a novel array of secondary metabolites with antifungal, antibacterial, antiviral activities.

Chlipala and Orjala (2011) conducted a study on Chemo diversity in freshwater and terrestrial cyanobacteria - a source for drug discovery. It highlights the structural diversity of natural products from freshwater and terrestrial cyanobacteria. This is mainly focus on three areas: cytotoxic metabolites, protease inhibitors, and antimicrobial metabolites. Malathi *et al.* (2014) isolated three Cyanobacterial strains such as, *Tolypothrix tenuis, Anabaena variabilis and Cylindrospermum sp*. from the soil samples collected from paddy fields of Telangana State in sterilized nitrogen free BG-11 medium. In this study of Screening of cyanobacterial strains for antimicrobial activity antimicrobial activities of these three strains were studied.

Borah *et al.* (2016) by studying the Biochemical composition and chemotaxonomy of cyanobacteria isolated from Assam, North-East India isolate Six strains of two closely related genera – *Nostoc and Cylindrospermum* from the biodiversity hotspot zone of Assam. A detailed study was made on the biochemical composition and chemotaxonomy of the strains based on Fatty acid profile and Attenuated Total Reflectance - Fourier Transform Infrared Spectroscopy (ATR-FTIR). Sanz *et al.* (2015) reported that among the structurally diverse secondary metabolites produced by these organisms, peptides are by far the most frequently described structures.

Roy *et al. (*2017) made an investigation on Cyanobacterial flora from Sidpur geothermal spring, Jharkhand, India and justified its role in protecting cellular constituents from photooxidation within response to higher light and temperature. Raja *et al*. (2016) stated that cyanobacteria produce biologically active and chemically diverse compounds belonging to cyclic peptides, lipopeptides, fatty acid amides, alkaloids and saccharides. Their role as anti-viral, anti-tumor, antimicrobial, anti-HIV and a food additive have also been well established. Samson *et al.* (2022) reported cyanobacteria has emerged as one of the promising resources to combat the issues of global warming, disease outbreaks, nutrition insecurity, energy crises as well as persistent daily human population increases.

Malathi *et al. (*2018) conduct a study on Biochemical analysis of cyanobacterial species isolated from paddy field of Warangal district, Telangana state, India. The aim of the present work was to study the biochemical constituents of four species of Cyanobacteria isolated from paddy fields of Warangal district. The biochemical constituents were analysed in terms of chl-a, carotenoids, total proteins, total carbohydrates and phycobiliproteins content of four Cyanobacterial species, *Anabaena circinalis, Nostoc punctiforme, Oscillatoria princeps and Phormidium mucosum* were analysed.

TAXONOMY OF CYANOBACTERIA

Schopf (1974) state that cyanobacteria are photosynthetic prokaryotic microbes actively involved in the nitrogen fixation evolved in the Precambrian period. Sachs (1874) named the prokaryotic microbe as Cyanophyceae. Smith (1938) and Desikachary (1959) bring a new nomenclature as Cyanophyta. (Bourrelly, 1970; Golubic, 1979; Anand, 1981) given the name blue green algae according to its morphology. Stainer *et al. (*1978) introduced the name cyanobacteria under the ICNP (International Code of Nomenclature Prokaryotes).

Geitlar (1925) made first attempt for the classification of cyanobacteria. He suggested 7 type cyanophytes namely Chroococcales, Entophysalidales, Pleurocapsales, Dermocarpales, Siphononematale Nostocales and Stigonematales. Geitlar (1932) identified 1300 species which fall under 145 genera, 20 families and 3 orders and also provided a manual for determination of the specimen. Geitlar also suggested two unicellular Chroococcales and Chamaesiphonales and one order of filamentous cyanobacteria, the Hormogonales. Desikachary (1959) brought a revised system for the classification of cyanobacteria and conducted a detailed study on Stigonematales.

Morphological, ecological and genetic observations are widely used in the recent taxonomic description and termed as polyphasic approach and it was suggested by several scientists in the following years (Castenholz, 1992; Castenholz&Norris, 2005; Komarek, 2003, Komarek *et al*.,2014). Hoffmann *et al*. (2005) proposed a new system of classification and brought two subclasses for each Chroococcales and Oscillatoriales. Palinska *et al*. (2006) made an attempt to do molecular research on the basis of historical and dried environmental sample of cyanobacteria. Palinska *et al.* (2014) suggested that the taxonomic distinction of cyanobacteria is based on phenotypic properties. Komarek and Anagnostidis (1998, 2005) published two volumes of monographs of cyanoprokaryota as part1 and part2.In 2013 Komarek published a manual of cyanoprokaryota as part3.

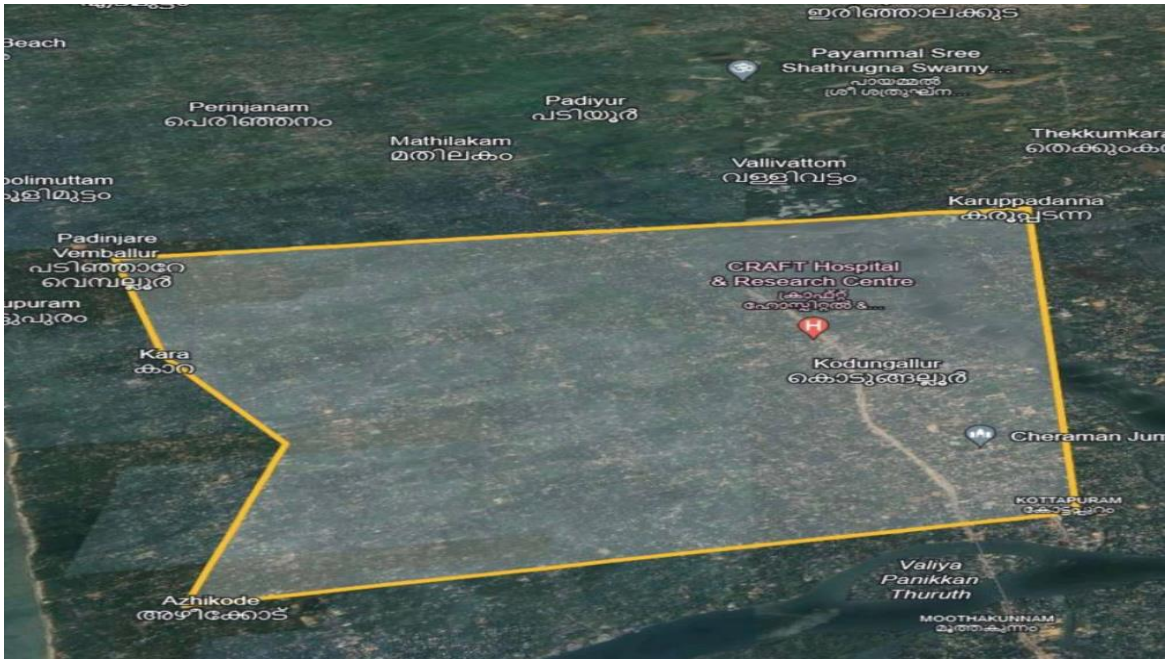
Komarek *et al*. (2014) introduced a new system of classification based on the polyphasic approach and they classified whole cyanobacterial genera in to 8 orders and 46 families. Walter *et al*. (2017) made a new attempt for the exploration of cyanobacteria by combining the taxonomy and eco genomics. Using taxonomic signatures, they first establish a phylogenomic frame work and using the metagenomic data set the relative abundance of genome is identified. Komarek (2020) opposed the new taxa proposed by Walter *et al*. (2017) by the opinion as new taxa are not obeying the nomenclature. Komarek (2020) suggested that in the taxonomic identification of all the cyanobacterial unit, polyphasic approach is defined as necessary and unavoidable.

In the modern world all researchers depend on the databases for the identification, documentation and classification of the cyanobacteria. In the present scenario several databases related to cyanobacteria such as CyanoDB2.0, CyanoType, AlgaeBase and CyanoOmicsDB are developed it provide updated information on the taxonomy, phylogeny and genomic data of cyanobacteria.

3. MATERIALS AND METHODS

1.STUDY AREA

This study evaluates the diversity of Cyanobacteria in the Terrestrial habitats of Kodungallur area, Thrissur district, Kerala, India. Kodungallur is a historically significant town situated on the banks of river Periyar on the Malabar coast in Trissur district of Kerala. It lies between 10° 14′ 1.54″ North latitudes and 76° 11′ 40.68″ East longitudes. It is a port city at the northern end of Kerala lagoons. The small country town of Kodungallur is situated 35 kilometres south of Thrissur. It is situated on the west coast. It is surrounded by backwater and sea. Its total area is 145km^2.



P VEMBALLUR

P Vemballur is a coastal village in Thrissur district in the state of Kerala, India. It is situated about 10 kilometers from the town of Kodungallur. The village is surrounded by lush green paddy fields and coconut groves. The Arabian Sea lies to the west of the village. It is situated between latitude10° 15′ 58″ N and longitude 76° 8′ 45.78″ E.

AZHIKODE

A coastal village situated between latitude 10° 11′ 0″ N and longitude 76° 9′ 0″ E. Azhikode was one of the major ports.

KARUPADANNA

It is a small village located in Kodungallur thaluk. It is lies between latitude 10°16'08.8"N and longitude 76°12'25.2"E.

EDAVILANGU

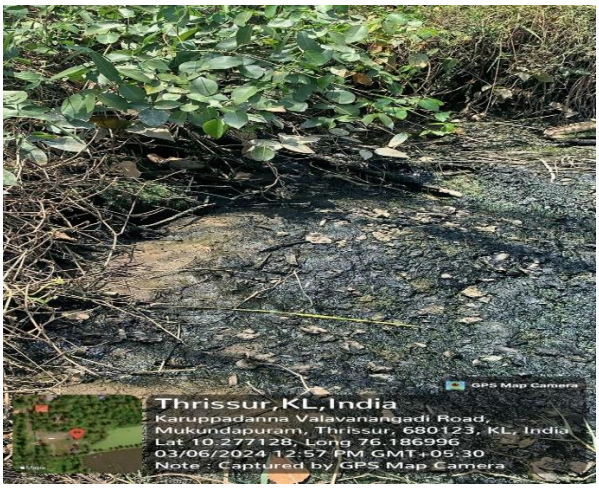
Edavilangu is a village in Kodungallur. The west side of Edavilangu is the Arabian sea. It is located between lattitude 10° 14′ 30″ N and longitude 76° 10′ 0″ E.

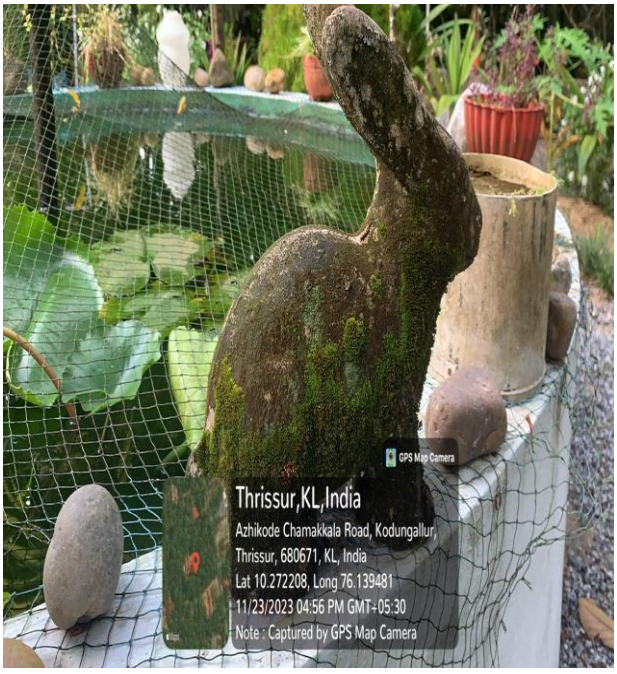
KOTTAPPURAM

Kottapuram is a village in Kodungallur. Kottapuram is the southern boundary of Kodungallur. It was surrounded by rivers on all three sides and had the landscape needed to become an early small port by sea. It is located between latitude 10° 40′ 0″ N and longitude 76° 11′ 0″ E.

The study was carried out in selected habitats like soil, epiphytic and epilithic etc. It plays an important role in terrestrial habitats. Soil cyanobacteria play important role in soil health and maintain nutrient balance. Epiphytic cyanobacteria were collected from bark of trees. The common substrata for epilithic cyanobacteria where calcareous rocks, shells etc. For this study around 50 samples were collected from habitats like bark, soil, monuments, concreated walls, PVC flex, flower pot etc.

SAMPLE COLLECTION



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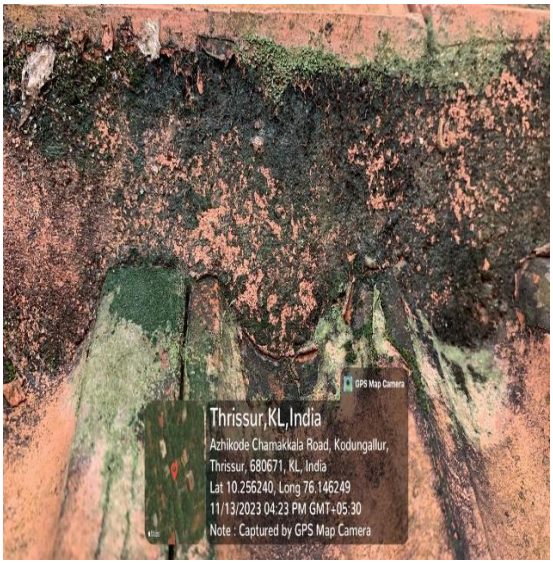
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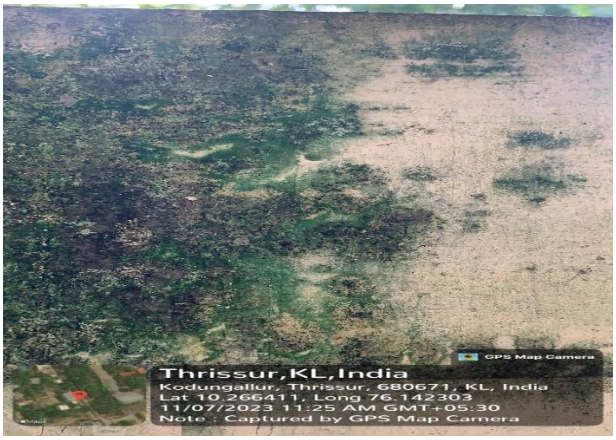
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Note : Captured by GPS Map Camera

2. COLLECTION AND PRESERVATION OF SAMPLE

For this study algal sample were collected between the period of August 2023 to December 2023. These were collected from different habitats like bark of tree, soil, monuments, concrete walls etc. Blackish brown crusts and mats of cyanobacteria were collected by scrapping them using sterile blade from the exposed stone, wall, bark, soil etc. The sample is kept in a beaker for the long storage of organism.

3. CULTURING OF SAMPLE

For the isolation of single species from a sample, culturing of the sample is carried out. Culturing is done in BG11solid medium. For the isolation streak plate method is carried out. It is maintained in the culture room 24 hours under a white fluorescent lamp. After 10-15 days subculture is done. After that it is maintained in liquid BG11 medium for pure culture.

CULTURE VESSELS

Petri plate is used for pouring solid culture medium. Agar is used for solidifying the culture medium. Conical flask of 250 ml is used in liquid medium culture. The volume of the liquid medium is kept 100ml and it is closed with cotton plug.

CULTURE MEDIUM

Varieties of culture media are available for culturing cyanobacteria. In this study BG11 medium without nitrogen is used as algal culture medium under laboratory condition. Four stock solution is prepared to make the medium. After mixing them in appropriate quantity medium is prepared. Solid media is prepared by adding 1% agar-agar.

Fig 3.1.A: Preservation of sample, B: Preparation of medium

BG-11 MEDIUM

SL.NO	INGREDIENTS	Wt.(g/L)
1	STOCK 1	
	Na_2MGEDTA	0.1g/L
	Ferric ammonium citrate	0.6 g/L
	Citric acid.H_2O	0.6 g/L
	Calcium chloride	3.6g/L
2	STOCK 2	
	Magnesium sulphate	7.5g/L
3	STOCK 3	
	KH_2PO_4	3.05g/L
	K_2HPO_4	4.0g/L
4	STOCK 5	
	Boric acid	2.86g/L
	Manganese chloride	1.81g/L
	Zinc sulphate	0.222g/L
	Copper sulphate	0.079g/L
	Cobalt chloride	0.050g/L
	Sodium molybdate	0.391g/L

Table 3.1. Showing the composition of BG11 medium

CULTURE IN BG-11 MEDIUM

Stock solution per litre of medium

Stock 1 10ml

Stock 2 10ml

Stock 3 10ml

Na_2NO_3 0.02g

Stock 5 1.0ml

$NaNO_3$ 1.5g

For preparing BG-11 medium, combine above stock make into 1000 ml and adjust pH to 7.5.

STERILIZATION

All the glass vessels like conical flask, beaker, measuring jars, Petri plate were sterilized in autoclave at 121^0C for 15 minutes after washing with liquid detergent and then keeping in hot air oven (100^0C) for 30 minutes. The culture medium used were sterilized separately, properly for 20 minutes in 15 psi pressure.

PROPAGATION AND MAINTAINCE OF CULTURE

In this study streak plate method is used for isolation.

STREAK PLATE METHOD

This method is employed for the separation of individual species. It is used for obtaining monoculture of algae. To solidify 5g of agar is boiled in 500 ml of culture medium. Then cool the medium to room temperature. The inoculation has been done in laminar air flow for avoiding contamination after turning UV light for 15 minutes. The plates were illuminated with 40-W cool-white fluorescent lamps.

- Prepare the Petri plate by pouring medium and kept it overnight for solid medium.
- Flame the inoculation loop and cool it, then streaked the samples collected back and forth, edge to edge and culture.
- Isolated mass were cultured in BG-11 medium

Fig 3.2. CULTURE IN BG-11 MEDIUM

Fig 3.3. PETRIPLATES WITH ALGAE STREAKED

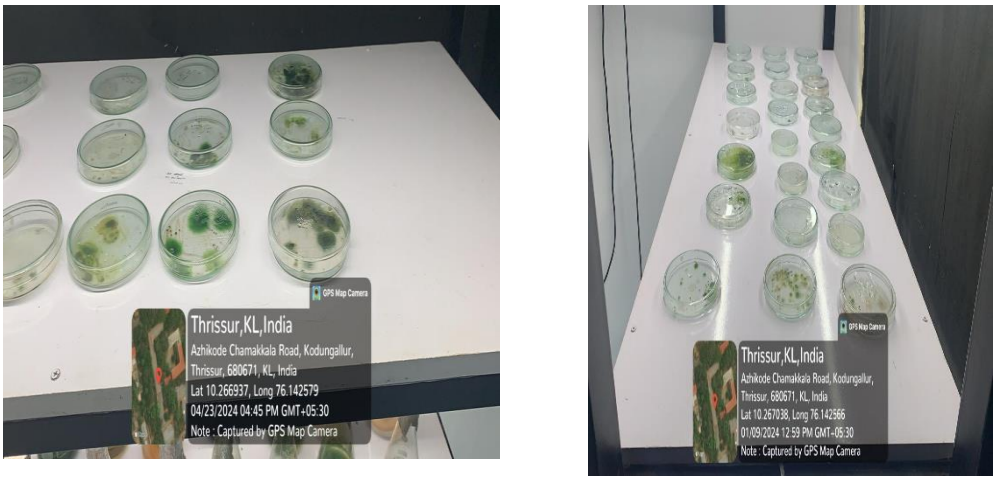


Fig 3.4. MAINTAINCE OF PURE CULTURE

CULTURE CONDITIONS

The experimental algal culture were incubated and grown in culture room under controlled condition of temperature of 25^0C, and illuminated with 2000 lux white fluorescent tube.

4.IDENTIFICATION OF SAMPLE

The identification of cyanobacteria is carried out under a microscope. The microphotograph is taken with the help of a camera attached to the microscope. Morphology, shape and size of cells were analysed. By using this photograph identification of cyanobacteria is done with the help of taxonomic literature Desikachary, T.V (1959).

4. RESULT AND DISCUSSION

The present investigation analysed and documented the diversity of cyanobacteria in the Terrestrial habitat of Kodungallur area Thrissur district, Kerala. This study recorded cyanobacteria in a particular time and space. It summarizes the information regarding the diversity of cyanobacteria. The objective of the investigation is to document the distribution of cyanobacterial diversity in the terrestrial habitat.

The cyanobacterial samples collected form soil, epilithic and epiphytic habitat were cultured, and identified up to species level using keys and description. Digital photographs were taken for the documentation.

The present study made an investigation on the diversity, distribution, and taxonomy of cyanobacteria in terrestrial habitat. In this study a total of 35 species of cyanobacteria were found during the study period. It belonging to 7 families under 4 orders. Nostocales is the prominent order with 3 families. Most number of genera belong to Oscillatoriaceae family. 14 genera were identified in this study. The most established genera are *Oscillatoria* and *Lyngbya* with 7 species each.

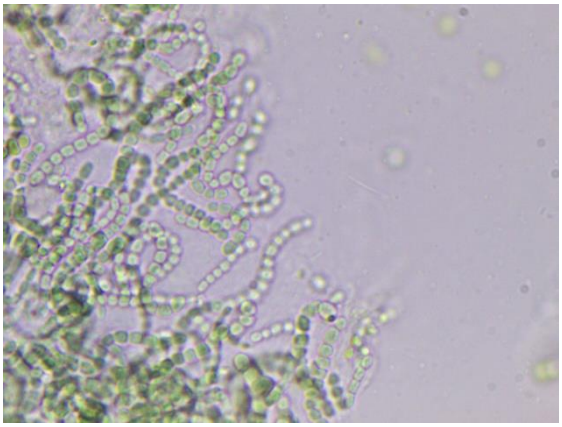
Systemic order of identified cyanobacteria up to species				
CLASS	ORDER	FAMILY	GENUS	SPECIES
Cyanophyta	Chroococcales	Chroococcaceae	*Gloeocapsa*	*Gloeocapsa atrata*
				Gloeocapsa magma
			Chroococcus	*Chroococus minutus*
				Chroococus tenax
			Aphanotheca	*Aphanotheca saxicola*
				Aphanotheca castagnei
		Entophysalidaceae	*Chlorogloea*	*Chlorogloea fritschii*
	Chamaesiphonales	Cyanidiaceae	*Johannesbaptistia*	*Johannesbaptistia pellucida*
	Nostocales	Oscillatoriaceae	*Oscillatoria*	*Oscillatoria princeps*
				Oscillatoria perornata
				Oscillatoria subbrevis
				Oscillatoria margaritifera
				Oscillatoria vizagapatensis
				Oscillatoria psuedogeminata
				Oscillatoria curviceps
			Phormidium	*Phormidium rotheanum*
				Phormidium pachydermaticum
				Phormidium calcicola

				Phormidium purpurascens
			Lyngbya	*Lyngbya limnetica*
				Lyngbya connectens
				Lyngbya polysiphoniae
				Lyngbya dendrobia
				Lyngbya sordida
				Lyngbya martensiana
				Lyngbya trunicola
			Microcoleus	*Microcoleus subtorulosus*
		Nostocaceae	*Cylindrospermum*	*Cylindrospermum doryphorum*
			Nostoc	*Nostoc muscorum*
				Nostoc punctiforme
			Anabaena	*Anabaena variabilis*
				Anabaena naviculoides
				Anabaena anomala
		Scytonemataceae	*Scytonema*	*Scytonema guyanense*
	Stigonematales	Mastigocladaceae	*Mastigocladus*	*Mastigocladus laminosus*

Table 4.1. showing the Systemic order of identified cyanobacteria up to species

IDENTIFIED SPECIES

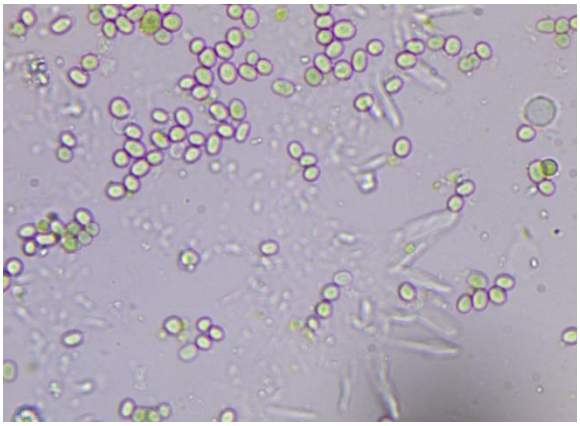
A-*Scytonema guyanense*



B-*Nostoc muscorum*

C-*Phormidium rotheanum*

D-*Oscillatoria princeps*

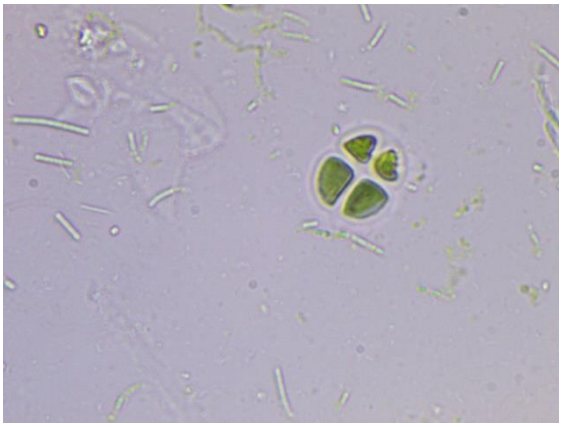


E-*Aphanotheca castagnei*

F-*Anabena variabilis*

G-*Phormidium purpurascens*

H-*Chroococcus minutes*



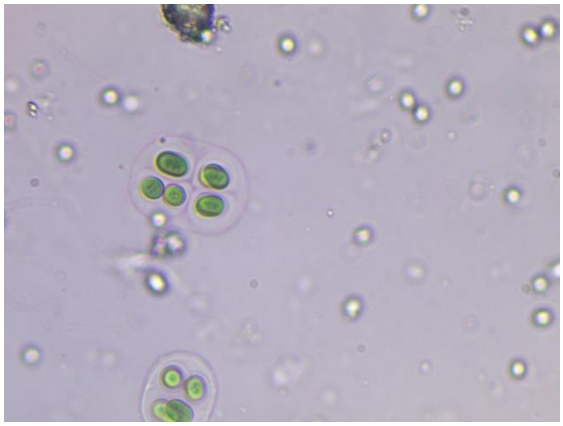
I-*Chroococcus tenax*

J-*Phormidium calcicola*

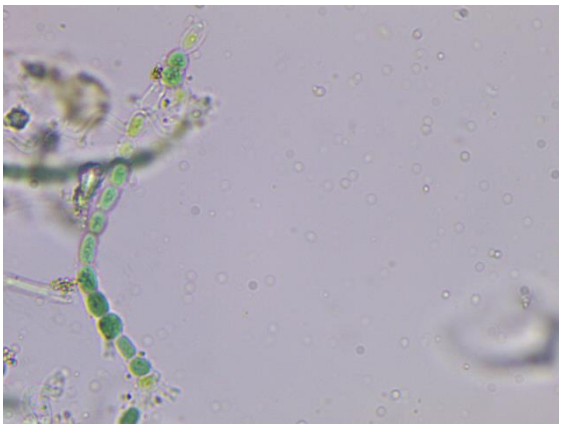
K-*Anabaena anomala*

L-*Lyngbya dendrobia*

M-*Microcoleus subtorulosus*



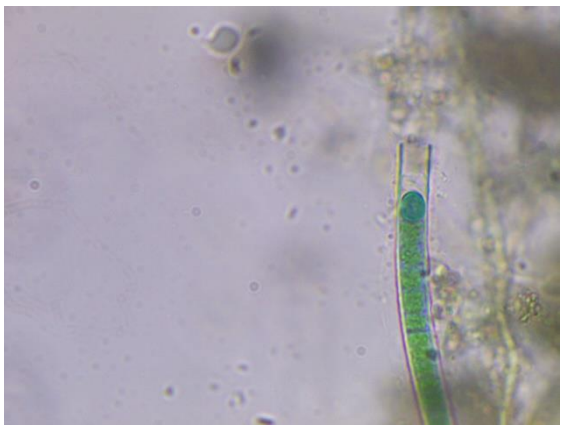
N-*Gloeocapsa atrata*



O-*Anabaena naviculoides*

P-*Lyngbya trunicola*

Q-*Oscillatoria curviceps*



R-*Lyngbya martensiana*

Among 35 species of identified cyanobacteria, 10 species have soil habitat, 5 species epiphytic, remaining 20 species epilithic. The epiphytic species include *Lyngbya* and *Phormidium* of which *Lyngbya* is prominent.10 species is recorded as soil cyanobacteria, it includes, *Anabaena, Chlorogloea, Nostoc, Cylindrospermum* and *Oscillatoria* spp. Remaining 20 epilithic cyanobacteria is collected from different sites like cemented walls, building facades, plant pot, banners, post, stones etc. The reported epilithic cyanobacterial species include *Aphanotheca, Gloeocapsa, Chroococcus, Phormidium, Lyngbya, Oscillatoria*.

The present study reported a total of 35 species from the different terrestrial habitat of Kodungallur area. The dominant order is Nostocales with 26 species, (19 species Oscillatoriaceae and 6 species Nostocaceae 1 scytonemataceae), followed by Chroococcales with 7 species. Third dominated order is Chamaeosiphonales and Stigonematales with 1 species each.

An illustration is plotted according to the above result:

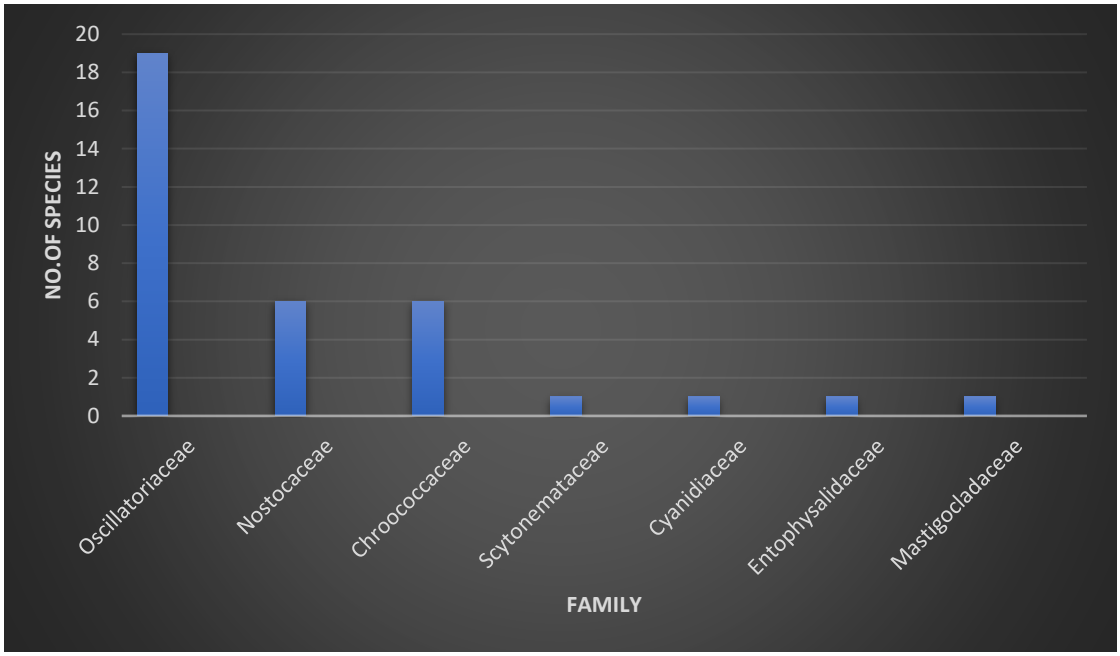


Fig 4.1. Family wise diversity of cyanobacteria

From this data, species from Oscillatoriaceae is dominant with 19 species. The second predominant family is Nostocaceae and Chroococcaceae with 6 species each. Single species is identified each from the family Scytonemataceae, Cyanidiaceae, Entophysalidaceae and Mastigocladaceae.

The nineteen species of Ocillatoriaceae belong to 4 genera, *Oscillatoria*, *Phormidium, Lyngbya* and *Microcoleus*. The second dominant famiy is Nostocaceae with 3 genera, *Cylindrospermum, Nostoc, Anabaena* and Chroococcaceae with 3 genera, *Gleocapsa, Chroococcus* and *Aphanotheca*.

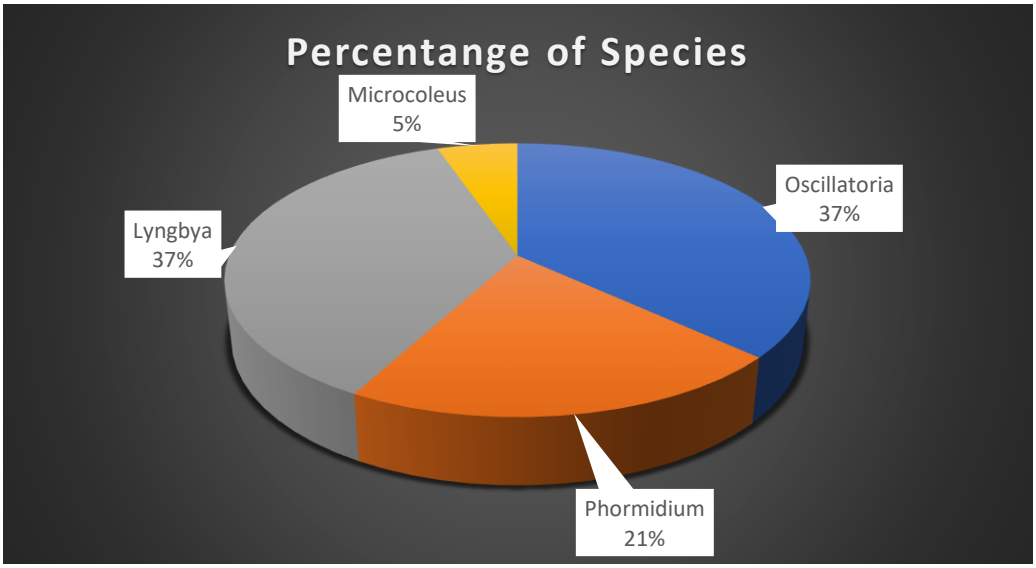


Fig 4.2. Species diversity of Oscillatoriaceae

From the obtained data *Oscillatoria* and *Lyngbya* is well established with 37%. Second predominant is *Phormidium* with 21% and the least established is *Microcoleus* with 5 %.

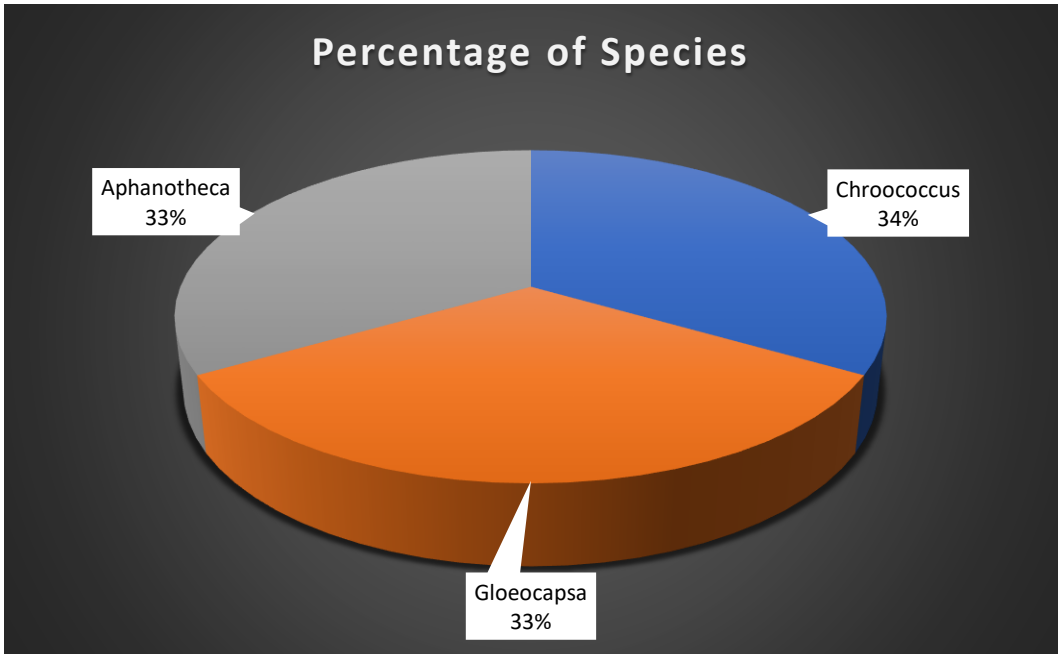


Fig 4.3. Species diversity of Chroococcaceae

From this data the diversity of Chroococcaceae species is nearly same. With 34%, *Chroococcus* is the dominant species. At 33%, *Gloeocapsa* and *Aphanotheca* come next.

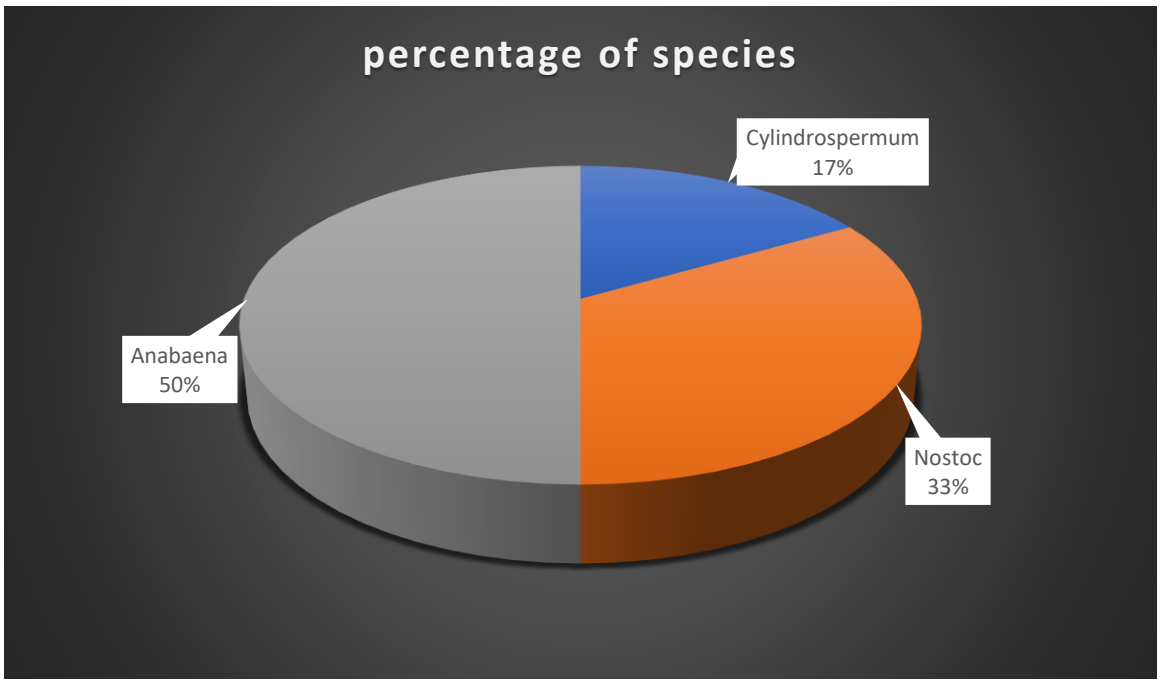


Fig.4.4. Species diversity of Nostocaceae

According to this findings, *Anabaena* is the most diverse species with 50%, followed by *Nostoc* (33%). *Cylindrospermum* has the lowest percentage (17%). Only one species is present in the other families.

The cyanobacterial diversity is more prevalent in natural habitat with water presence. Its highest species diversity is found in Vemballur and nearby area. Vemballur is a high diversity area with *Oscillatoria, Phormidium, Lyngbya* species. Kottappuram and Karupadanna have dominant species like *Chroococcus, Aphanotheca, Gleocapsa*. *Oscillatoria* and *Lyngbya* is found to be the dominated species in most of the area.

Nowadays terrestrial habitats are facing serious threats. The human interference and the development cause declining in the occupancy of cyanobacteria. It effects the diversity of cyanobacteria. The diversity of cyanobacteria is much reduced in urban area when compared to rural area. This is due to unfavourable environment conditions caused by human beings. By conserving the natural habitat we can protect the native species.

Cyanobacteria is a diverse organism that occur in a wide range of habitats from extremely cold deserts of the Arctic and Antarctic zones and the very extreme hot springs, thus representing their life form in diverse habitats of various aquatic and terrestrial environments (Dyer & Gafford, 1961, Amarouche-Yala et al., 2014; Vincent et al., 1993; Zaki et al., 2020). In terrestrial habitat it is found primarily on the surfaces of bare rocks, building materials, soil, and tree bark, all areas where little or no precipitation may occur. Stal (1995) reported cyanobacteria is the dominant species in microbial mat. They excrete massive amounts of polysaccharides as extracellular slime or sheaths. 35 cyanobacterial species were found in the results, of which 10 were soil, 20 were epilithic, and 5 were epiphytic.

Ortego-Calvo *et al.* (1991) reported the presence of Chlorophyta on stone monuments can lead to biodeterioration process. The most commonly reported cyanobacteria on stone monuments include *Gleocapsa, Phormidium and Chroococcus*. In this present study *Phormidium calcicola* is identified from monuments. Samad and Adhikary (2008) reported fifty-seven taxa of cyanobacteria and 15 taxa of Chlorophyta from the building facades and monuments in India. They reported *Chroococcus*, *Aphanotheca* and *Phormidium* species from wall surfaces similarly *Phormidium purpurascens, Aphanotheca castagnei* and *Chroococcus minutus* identified from wall surface of this study. *Oscillatoria princeps* is also reported from wall surface.

Philip *et al.* (2016) identified 15 species of cyanobacteria from 4 families Chroococcaceae, Nostocaceae, Scytonemataceae and Rivulariaceae. Of these *Chroococcus tenax and Gleocapsa atrata* were also reported in this study. The observation of *Lyngbya limnetica, Oscillatoria perornata, Oscillatoria vizagapatensis* agree with the report of Arun and Shamina (2016).

Thiyagarajan (2005) recorded that *Oscillatoria, Lyngbya, Scytonema* is the dominant species in the tree bark. In this study the identification of *Lyngbya connectans, Lyngbya dendrobia, Lyngbya martensia* showed that it is the dominant bark species. The observation of *Scytonema gayanase* also agree with the report of Thiyagarajan. In the present study *Phormidium rotheanum* is also reported from tree bark. The presence of *Phormidium* species is identified in the study of Sethi *et al* (2012).

Dash and Kumar (2022) identified *Anabaena* and *Nostoc* has the most diverse organism in the rice field. In this study *Anabaena naviculoides* and *Anabaena variabilis* were identified. Nostoc species were also identified. Umamaheshwari (2005) reported *Nostoc muscorum* were similarly recorded from this study also.

Rai *et al.* (2018) identified 22 cyanobacterial species from the soil crust along a rural-urban gradient out of which *Anabaena anomala, Anabaena variablis, Cylindrospermum sp, Nostoc muscorum* were identified from the present investigations. The presence of *Cylindrospermum doryphorum* agree with the study of Sethi *et al*. (2012)

5. SUMMARY AND CONCLUSION

The present investigation analysed and documented the diversity of cyanobacteria of selected terrestrial habitat of Kodungallur, Thrissur, Kerala. This study mainly focuses on the soil, epilithic and epiphytic cyanobacteria.

During the study, samples were collected from different habitats like concrete structure, tree barks, pots, soils, stones etc by scraping into sterile bottles. A portion of the collected sample cultured in solid BG-11 medium by streak plating method. Sample collected on seasonal aspects were identified and tabulated based on standard publications and digital photographs were taken for the documentation.

Total of 35 species coming under 14 genera are documented. The species are belonging to 7 families including 4 orders. Study of natural and artificial substrata including tree bark, soil, concrete surface promotes the diverse form of cyanobacteria. The most established family was Oscillatoraceae with 19 species. *Oscillatoria* and *Lyngbya* is the well-established species. Highest species composition is seen in P Vemballur. This study reported the presence of 54% of Oscillatoriaceae and it is followed by 17% Nostocaceae and 17% Chroococcaceae. The remaining family represent 3% each.

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