Quantitative and Qualitative Analysis of Photosynthetic Pigments and Lipids in Cyanobacteria

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DECLARATION

I hereby declare that the data presented in this Dissertation report entitled, "Quantitative and Qualitative Analysis of Photosynthetic Pigments and Lipids in Cyanobacteria" is based on the results of investigations carried out by me in Botany Discipline at School of Biological Sciences and Biotechnology, Goa University under the supervision of Dr. Rupali Bhandari and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will not be responsible for the correctness of observations experimental or other findings given in the dissertation.

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ABSTRACT

This study presents a comprehensive assessment of photosynthetic pigments and lipids in five different cyanobacteria: *Oscillatoria pseudogeminata*, *Oscillatoria trichoides*, *Oscillatoria proteus*, *Nostoc spongiaeforme, and Nostoc calcicola*. Thin Layer Chromatography and spectrophotometric techniques were used for quantitative and qualitative analysis. Quantitative analysis of photosynthetic pigments, including chlorophyll a, carotenoids, and phycobiliproteins, was conducted using spectrophotometric methods, providing precise measurements of pigment concentrations in cyanobacterial samples. These quantitative data were complemented by qualitative thin layer chromatographic analysis, which allowed for the separation and identification of individual pigment components, providing insights into pigment diversity and distribution within the cyanobacterial species.

1. INTRODUCTION

1.1 Cyanobacteria

Cyanobacteria, also known as blue-green algae, represent an ancient and diverse group of microorganisms having different sizes and shapes. They resemble the oldest fossils, going back more than 3.5 billion years. Because they contributed to the current oxygenic environment, they are also significant from the perspective of evolution (Chittora et al., 2020). They engage in oxygenic photosynthesis, a process identical to that observed in the chloroplasts of higher plants (Criscuolo & Gribaldo, 2011).

In terms of cellular organization, cyanobacteria resemble bacteria because they are devoid of the mitochondria, chloroplast, nucleus, and other membrane-bound organelles seen in true photosynthetic algae. Similar to higher plants, cyanobacteria produce oxygen during the photosynthetic process. They appear to act as a bridge between microbes and higher plants. They typically form vast colonies and are tiny, unicellular organisms. Cyanobacteria-driven development of the early Proterozoic led to the advent of an atmospheric oxygen content near current levels, shaping the circumstances necessary for creating aerobic microbes (Zavarzin, 2001).

Cyanobacteria, gram-negative bacteria found abundantly, have a lengthy evolutionary background and are unique among prokaryotes for their capacity for plant-like oxygenic photosynthesis. Cyanobacteria's extraordinary productivity and efficiency, attributed to their capacity for photosynthesis and nitrogen fixation, effective nutrient absorption mechanisms, and adaptation to low light levels, highlight their substantial ecological and economic importance. Ecologically, they make notable contributions to the primary productivity of various ecosystems, especially in freshwater and marine environments (Tomitani et al., 2006).

Waterbury et al., (1979) have documented their pivotal roles in carbon, oxygen, and nitrogen cycling, emphasizing the significance of cyanobacteria in maintaining ecosystem balance.

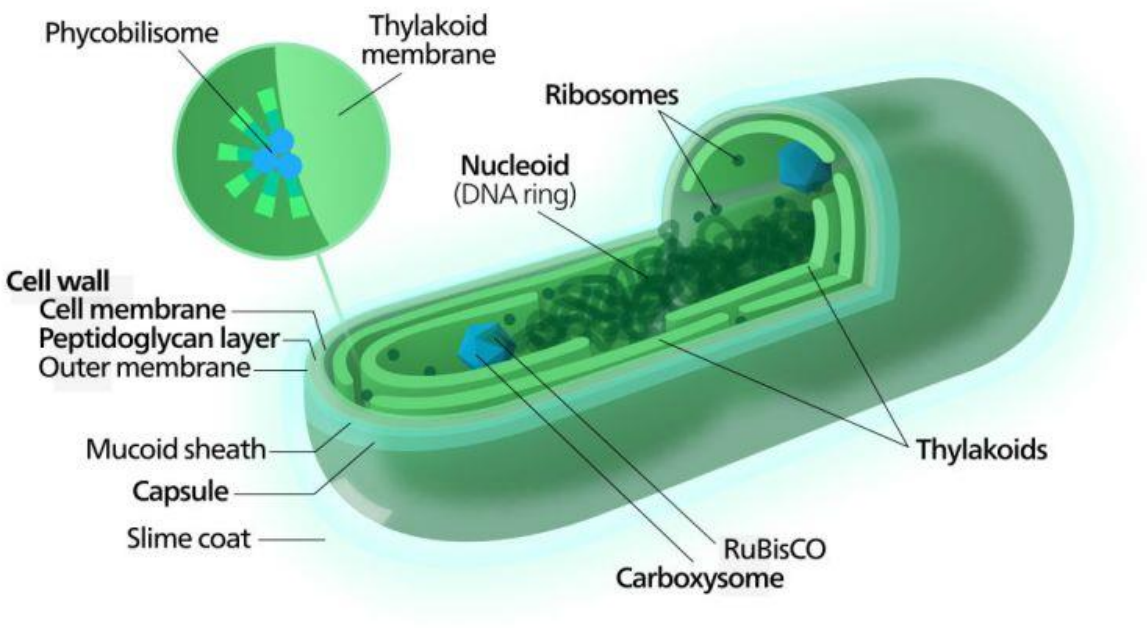


Fig. 1 Structure of Cyanobacteria

In recent years, cyanobacteria have been of considerable interest due to their promising applications in biotechnology. They have been recognized as a valuable reservoir of bioactive substances possessing antiviral, antibacterial, antifungal, and anticancer properties. Cyanobacteria also find use in aquaculture, wastewater treatment, food production, fertilizer development, and synthesizing secondary metabolites like exo polysaccharides, vitamins, toxins, enzymes, and pharmaceuticals (Abed et al., 2009). They offer numerous benefits as hosts for biotechnological purposes due to their straightforward growth needs, genetic manipulability, and potential for creating carbon-neutral processes. Using photosynthetic cyanobacteria to convert carbon dioxide into biofuels is an increasingly promising field. With

their capability to break down pollutants and eliminate heavy metals, cyanobacteria hold significant potential as tools for bioremediation and treating wastewater (Lau et al., 2015).

Large-scale cultivation of cyanobacteria and other microalgae is essential due to their beneficial properties in various areas. Economic sustainability is the primary determinant of the success of large-scale biomass production of economically significant products. Cyanobacterial growth is determined by five essential abiotic parameters: light, pH, temperature, water, carbon dioxide, and nutrient supplementation (C, N, P, S, K, Fe, etc.) (Pulz, 2001; Meena et al., 2017).

Microalgae and cyanobacteria are cultivated under controlled conditions or harvested from their natural environments for sale as food supplements globally. Cyanobacteria are known to produce a wide range of biologically active compounds, some of which hold promise for drug development. The potential anticancer, antimicrobial, antiviral, anti-inflammatory, and other effects of specific active components derived from cyanobacteria are exploited for medical purposes. This multipurpose characteristic of cyanobacterial pigments is leading to a rising increase in the production of these organisms, as well as the development of new industrial-level techniques of extraction and purification of phycobiliproteins, the leading group of pigments in cyanobacteria (Pandey et al., 2013).

Cyanobacteria such as *Anabaena, Nostoc,* and *Spirulina* have been used as food sources for centuries (Jaiswal et al., 2018). Natural pigments like phycobiliproteins have various economic applications beyond their traditional uses. For instance, they can serve as additives in food, cosmetics, and medical diagnostic tools. *Spirulina*, a type of cyanobacterium, is globally recognized as a beneficial dietary supplement and incorporated into animal feed. It

can synthesize valuable compounds like phycocyanin and carotenoids, which function as antioxidants. Additionally, it produces polyunsaturated fatty acids like linoleic acid and γ-linolenic acid (GLA), which play crucial roles in human metabolic processes (Chaiklahana et al., 2008).

Cyanobacteria are well known for producing diverse natural compounds, many recognized as toxins with potential pharmaceutical applications. While the order of Nostocales has often been overlooked in this context, it is increasingly acknowledged as a source of toxin-producing organisms. These include various genera such as *Anabaena, Nostoc, Hapalosiphon, Fischerella, Anabaenopsis, Aphanizomenon, Gloeotrichia, Cylindrospermopsis, Scytonema, Raphidiopsis, Cuspidothrix, Nodularia, Stigonema, Calothrix, Cylindrospermum, and Desmonostoc* species (Nowruzi & Porzani, 2020).

Filamentous genera within the Nostocales orders are frequently found among the microscopic populations in freshwater lakes and reservoirs globally. Furthermore, they are among the most favorable groups of organisms from which novel, biologically active, natural toxic products have been isolated. (Rezanka & Dembitsky, 2006). Using wastewater as an alternative nutrient source for cultivation presents a promising method to improve pigment production's economic viability and sustainability. Cyanobacteria cultivation is integrated with wastewater from which nutrients are assimilated to produce biomass while aiding bioremediation efforts. This approach allows for the efficient utilization of resources while mitigating environmental impacts (Thevarajah et al., 2023).

Photosynthetic microorganisms such as microalgae and cyanobacteria are increasingly recognized for their economic potential. Recent research has focused on metabolic

manipulation techniques to leverage these organisms in producing biofuels and various bio-based chemicals using carbon dioxide and sunlight as primary inputs. This innovative approach holds significant promise for sustainable energy and chemical production, utilizing renewable resources while reducing greenhouse gas emissions. (Patel et al., 2023).

As photosynthetic prokaryotes, cyanobacteria can shield themselves from UV radiation by producing UV-absorbing secondary metabolites like mycosporines and scytonemin (Sorrels et al., 2009). Their photosynthetic capacity has been hypothesized to contribute to the oxygenation of the atmosphere, facilitating the emergence of diverse life forms. (Williams et al., 2002).

As photoautotrophic prokaryotes, cyanobacteria contain a range of pigments that display diverse colors. These pigments include chlorophylls, carotenoids (such as β-carotene, lutein, lycopene, astaxanthin, and fucoxanthin), and phycobiliproteins (including phycoerythrin, phycocyanin, and allophycocyanin) (Mandal et al., 2020). In cyanobacteria, phycobiliproteins function as the antenna pigments (Glazer ,1977). In aquatic ecosystems, microalgae, particularly cyanobacteria, are the primary producers. They have chlorophyll a and b found in green plants, although specific carotenoids (such as peridinin or fucoxanthin) are significant in particular algae. Cyanobacteria can produce necessary chemicals like phycocyanins and carotenoids in addition to chlorophyll. Chemical substances called pigments mainly absorb light in the visible spectrum. The green color of cyanobacteria is caused by chlorophyll, but under certain circumstances, nearly all species can also produce the red pigment phycoerythrin and the blue pigment "phycocyanin" (Lall & John, 2017).

Pigments are chemical substances that absorb light within the visible wavelength range. Cyanobacteria, being photosynthetic organisms, can produce chlorophyll and various valuable compounds like carotenoids, which serve as antioxidants and accessory pigments to capture light energy. These accessory pigments expand the light absorption range for photosynthesis and, in certain instances, protect against UV and other light-related cellular harm. Chlorophylls are associated with membranous thylakoids, resembling those found in plants and other algae (Bartley & Scolnick, 1995).

The photosynthetic action spectra of various cyanobacteria exhibit a peak around 650 nm, closely linked to the amount of allophycocyanin present in the strains. Allophycocyanin is more effective at capturing light compared to phycocyanin and phycoerythrin. In cells with typical pigment compositions, the absorption of light by chlorophyll a has minimal detectable impact on photosynthetic activity. However, in cells where phycobiliproteins have been depleted due to physiological changes, chlorophyll a becomes the primary pigment for light absorption (Lemmason et al., 1973).

The light energy absorbed by photosynthetic organisms is utilized in two main steps. Initially, they absorb radiation, followed by converting this absorbed energy into chemical energy. Light is captured through arrays of pigment molecules with absorption spectra complementary to the incident radiation's energy distribution. These arrays, known as antennae, are positioned near "reaction centers," where chlorophyll molecules in specialized environments facilitate energy transduction. The antennae absorb energy, which is then transferred to the reaction centers through radiation with lower energy (Glazer, 1977).

As the most common pigments in the families chlorophyceae, ochrophyta, and rhodophyceae, respectively, algae's primary groups are chlorophylls, carotenoids, and phycobiliproteins. These traits help group algae into distinct phyla. Phycocyanin is the most prevalent pigment in cyanobacteria. All autotrophic algae have chlorophylls, greenish, non-polar pigments that enable light conversion into biological energy. Chlorophylls comprise porphyrin or hydroporphyrin rings centrally bonded to a magnesium atom (Osario et al., 2020).

Cyanobacterial mats are stratified benthic microbial communities dominated by cyanobacteria. They are found at sediment-water interfaces in a variety of shallow aquatic settings. They create laminated multilayers, or biofilms, deeply enmeshed in the abundant polysaccharides that the benthic microbial community excretes. (Krumbein et al., 1977). *Nostoc*, a commonly found genus of cyanobacteria, generates cyanobacterins LU-1 and LU-2, which possess algaecidal properties, and nostocyclamide, a cyclic peptide that disrupts electron transport during photosynthesis (Smith & Doan, 1999, Juttner et al., 2001). *Nostoc spongiaeforme* has been documented to synthesize Nostocine A, a heterocyclic pigment capable of inhibiting green algae (Hirata et al., 2003).

Oscillatoria is a type of filamentous cyanobacterium named for its oscillating motion. It reproduces through fragmentation and is studied for its ability to naturally produce butylated hydroxytoluene (BHT), an antioxidant, food additive, and industrial chemical. *Oscillatoria* species are also known for producing vitamins, minerals, and viridamines and exhibiting anti-protozoal activity (Wu, 2008).

1.2 PHOTOSYNTHETIC PIGMENTS:

1.2.1 CHLOROPHYLL

In cyanobacteria and microalgae, chlorophylls (Chl) are the predominant natural pigments facilitating oxygenic photosynthesis, whereby they derive energy for metabolism and reproduction. In microalgae (eukaryotes), Chls are located in the chloroplast, but in cyanobacteria (prokaryotes), in the photosynthetic lamellae. All cyanobacteria have Chl a universally distributed. Other chlorophylls are present as given below: Chl b in Euglenophyta, Chlorophyta, and Charophyta; Chl c in Bacillariophyceae, Chrysophyceae, Xanthophyceae, Raphidophyceae, Phaeophyceae, Haptophyta, Cryptophyta, Dinophyta; Chl d in Rhodophyta; Chl f in cyanobacteria (Silva et al., 2020). Chlorophyll a (Chl a) plays a dual function in oxygenic photosynthesis, both in light harvesting and converting the energy from absorbed photons into chemical energy.

1.2.2 CAROTENOID

All photosynthetic organisms contain carotenoids, which are tetraterpenoid molecules that improve photosynthetic capacity to absorb light and dissipate energy. The biosynthesis process of carotenoids in cyanobacteria is well-established. In addition to the more prevalent molecules (including βcarotene, zeaxanthin, and echinenone), specific carotenoids, such as myxoxanthophyll, are also found there. Additionally, orange carotenoid is a protein complex that cyanobacteria have as a photoprotective mechanism (Pagels et al., 2021).

Microalgae such as *Chlorella, Dunaliella, Nannochloropsis*, and *Scenedesmus* produce various light-harvesting carotenoids and can handle oxidative stress through their capacity to scavenge free radicals. This characteristic makes them appealing as a natural source of

antioxidants. Efforts are concentrated on identifying the most promising strains that produce valuable carotenoids and refining methods for extraction and purification. Among the carotenoids produced by marine microalgae, fucoxanthin is the most abundant, constituting over 10% of the total carotenoid content (Zittelli et al., 2023).

1.2.3 PHYCOBILISOME

Cyanobacteria and red algae possess a unique light-harvesting structure known as the Phycobilisome (PBS), distinguishing them from other photosynthetic antennas. Unlike other complexes, the PBS is anchored to the stromal side of thylakoid membranes, occupying much of the space between individual thylakoids. It comprises protein subunits that assemble themselves and contain conserved cysteine residues facilitating the covalent attachment of light-absorbing chromophores called linear tetra-pyrroles (Adir et al., 2020). Phycobilisomes are the primary pigment-protein antenna complexes responsible for photosynthetic light harvesting in cyanobacteria, rhodophyte, and glaucophyte algae. These structures can store a significant amount of cellular nitrogen, accounting for up to 50%. Consequently, when nitrogen levels decrease, phycobilisomes undergo rapid degradation through a complex genetic mechanism. (Krause et al., 2021).

A particular group of conjugated proteins, phycobiliproteins, is responsible for the primary antenna pigments in red algae chloroplasts and cyanobacteria. In significant quantities, these proteins give these organisms their specific colors, giving rise to the fictitious names of red and blue-green algae. Most photosynthetic accessory pigments are not water-soluble. Early identification and characterization led to the discovery of two quantitatively important types of phycobiliproteins: the red phycoerythrins and the blue phycocyanins. Phycoerythrin,

Allophycocyanin, and C-Phycocyanin form the complex of proteins known as phycobiliproteins (Elumalai et al., 2014).

The primary constituents of these pigments are the protein subunits α and β, which combine to create the "monomeric" unit (αβ). Multiple units autonomously assemble to form phycobiliproteins, which in vivo arrange themselves on the thylakoid membrane to construct more extensive protein complexes known as phycobilisomes. The composition of phycobilisomes, in terms of the number and types of phycobiliproteins, exhibits variability both within individual organisms of a species (attributed to the organism's environmental adaptations) and among different species (Zittelli et al., 2023).

Phycobilins represent a significant pigment category that enhances the light absorption efficiency in phytoplankton cells through complementary chromatic adaptation. They hold promise as biomarkers for cyanobacterial species. However, quantifying the concentrations of these water-soluble molecules in their extracted state poses challenges, unlike solvent-soluble pigments, which can be accurately measured using chromatographic methods. Additionally, there is a limited understanding of the quantitative spectroscopic analysis of extracted phycobilins (Sasim et al., 2014).

The protein composition of cyanobacterial phycobilisomes is notably intricate. Approximately 15% of the overall protein content in phycobilisomes consists of a limited set of colorless polypeptides, all of which have a higher molecular weight than the chromopolypeptide subunits found in the phycobiliproteins (Marsac & Bazire, 1977). Phycobilisomes have rising utilization as biofertilizers in agriculture because of their diazotrophic capabilities, capacity to improve soil's physical and chemical attributes,

adaptability to various soil environments, and competitiveness against indigenous flora and fauna. Additionally, they are prolific producers of numerous biologically active or biocidal compounds (Singh et al., 2016).

1.3 LIPIDS

Lipids in cyanobacteria encompass various types, such as free fatty acids, membrane lipids, triacylglycerols, and wax esters, all of which contribute significantly to lipid metabolism and cellular functions. The lipid metabolism of cyanobacteria involves biosynthesis, secretion, degradation, and storage (Eungrasamee et al., 2024). Cyanobacteria contain primary cellular lipids such asMonogalactosyldiacylglycerol (MGDG), Digalactosyldiacylglycerol (DGDG), Sulfoquinovosyldiacylglycerol (SQDG), Phosphatidylglycerol (PG), akin to chloroplasts. Still, they do not produce phosphatidylcholine, phosphatidylethanolamine, or phosphatidylinositol, present in other algal classes (Loura et al., 1987). The yield of lipid extraction relies on various factors, including the solvent type, size of lipid or oil particles, sample-to-solvent ratio, temperature, and extraction duration. Solvents commonly employed for lipid extraction encompass hexane, ethanol, methanol, acetone, petroleum ether, and combinations like chloroform and methanol ($CHCl_3$ -MeOH) (Chaiklahan et al., 2008).

1.4 CHEMICAL COMPOSITION

The evolution of DNA-based organisms on Earth, which traces back over 3.5 billion years ago, suggests that RNA was the primary genetic molecule during that era. Before the emergence of RNA-based organisms, it is theorized that peptide nucleic acids (PNAs) might have been employed to convey genetic information by the earliest life forms on Earth. It has been found that cyanobacteria produce N-(2-aminoethyl) glycine (AEG), which acts as a backbone for peptide nucleic acids (Banack et al., 2012).

A sophisticated RNA-based regulatory mechanism facilitates their adaptation to various environmental changes and stresses in cyanobacteria. Despite being less explored than other model microorganisms like yeast and Escherichia coli, an increasing number of non-coding regulatory sRNAs have been identified in cyanobacteria over the past decades. (Hu et al., 2018). RNase E in cyanobacteria is crucial in reshaping the transcriptome during the UV stress response. Despite the increased protein turn over under UV stress conditions, the required activity level of RNase E is maintained, highlighting its importance in cyanobacterial adaptation to environmental challenges (Watanabe et al., 2023). Most cyanobacteria strains that can undergo DNA-mediated transformation are physiologically or naturally competent to take up DNA. Although the mechanism underlying this process is yet understood, it has been widely employed to chromosomally or plasmidally alter cyanobacterial cells (Cohen & Gurevitz et al., 2006).

1.5. HYPOTHESES

1. **Qualitative separation of photosynthetic pigments by Thin Layer Chromatography:** The different photosynthetic pigments, such as chlorophyll a and carotenoids, will exhibit distinct migration patterns on the thin layer chromatography (TLC) plate due to differences in their polarity. This migratory pattern will allow their separation and identification based on the distance they traveled relative to the solvent front.
2. **Quantitative estimation of photosynthetic pigments by spectrophotometer:** The concentration of photosynthetic pigments in a given sample can be accurately determined using spectrophotometric analysis.

3. **Quantitative estimation of phycobilisome pigments by spectrophotometer:** Phycobilisome pigments, such as phycoerythrin and phycocyanin, can be quantitatively measured using a spectrophotometer at their respective absorption maxima. The absorbance values obtained will correlate linearly with the concentration of these pigments in the sample, allowing for accurate quantification.
4. **Qualitative separation of phosphoglycolipids and neutral lipids by Thin Layer Chromatography:** Phosphoglycolipids and neutral lipids will exhibit different migration patterns on the TLC plate due to their varying polarities. This difference in polarity will enable the separation of these lipid classes, allowing for their qualitative identification based on their relative positions on the chromatogram.

1.6 RESEARCH GAP

Despite the extensive research on photosynthetic pigments derived from cyanobacteria, there remains a critical gap in our understanding of the potential interactions and applications of the pigment extracts. These cyanobacterial species inhabit different ecological niches and likely possess distinct pigment compositions and biochemical functionalities. Photosynthetic pigments exhibit a wide range of biochemical functionalities beyond their roles in photosynthesis. For example, carotenoids possess antioxidant properties, chlorophyll derivatives have photoprotective effects, and phycobiliproteins serve as light-harvesting antennae. However, these have not been fully elucidated. We want to look at the specific biochemical functionality of pigment extracts from cyanobacteria, which can provide insights into these species' ecological adaptations and potential applications in biotechnology, environmental monitoring, and biomedical research.

Cyanobacteria synthesize various lipids, including glycolipids, phospholipids, and neutral lipids, each with unique chemical structures and biological activities. However, cyanobacteria's lipid profiles and how they differ have not been thoroughly characterized. Investigating the lipid compositions of these cyanobacterial species can provide insights into their ecological adaptations.

There is a notable absence of research that comprehensively studies how different the yield of photosynthetic pigments and lipids from cyanobacteria is due to the diverse environmental parameters in which they grow. What differences help in the adaptability of cyanobacterial species in environments and how they impact cyanobacterial physiology and biochemistry is a matter of interest. Investigating the difference in the pigment and lipid profiles of the two cyanobacteria will help understand how they can adapt and tolerate the environments in which they thrive.

1.7 AIMS AND OBJECTIVES OF PRESENT WORK

This study aims to extract photosynthetic pigments and lipids from different cyanobacteria and quantitatively and qualitatively analyse them using thin-film chromatography and spectrophotometry. The main objectives of the study are given as follows:

Objectives

1. Qualitative separation of photosynthetic pigments by Thin Layer Chromatography
2. Quantitative estimation of photosynthetic pigments by spectrophotometer
3. Quantitative estimation of phycobilisome pigments by spectrophotometer
4. Qualitative separation of phosphoglycolipids and neutral lipids by Thin Layer Chromatography.

2. REVIEW OF LITERATURE

Several studies have contributed significantly to understanding the bio-optical characteristics and vertical distribution of photosynthetic pigments in cyanobacterial mats. Kuhl & Fenchel (2000) observed intense photosynthetic activity in artificial cyanobacterial mats and noted pronounced minima in spectral radiance measurements corresponding to absorption maxima of chlorophyll a, carotenoids, and phycobiliproteins throughout the mat. Colyer et al., (2005) investigated chromatographic and electrophoretic methods for analyzing cyanobacterial pigments and proteins, focusing on bilins and phycobiliproteins. Simis et al., (2007) explored the influence of phytoplankton pigment composition on remote sensing of cyanobacterial biomass, proposing methods for extraction and in situ measurement of pigment fluorescence. Vermaas et al., (2008) employed hyperspectral confocal fluorescence imaging to determine pigment localization and distribution in cyanobacterial cells, providing unique insights into pigment organization.

Jodlowska et al., (2011) compared spectrophotometric and high-performance liquid chromatography methods for analyzing photosynthetic pigments, revealing strong correlations between the two techniques. Sasim et al., (2014) assessed spectrophotometric and spectrofluorometric methods for quantitatively analyzing extracted phycobilin pigments in cyanobacteria, highlighting spectral differences between standard solutions and monoculture extracts. Deshpande et al., (2014) investigated sedimentary pigments as indicators of cyanobacterial dynamics in a hypereutrophic lake, demonstrating the utility of pigment analysis for identifying anthropogenic impacts. Jaiswal et al., (2018) conducted pigment analysis of various cyanobacterial strains, revealing variations in chlorophyll, carotenoids, and phycobilins among different species. Raman spectral analysis study of microbial pigment compositions in cyanobacterial cells, detecting changes in pigment composition during cell differentiation, was

done (Ishihara and Takahashi, 2023). Khatulistiani et al., (2023) investigated the antioxidant and anti-tyrosinase activities of *Halymenia durvillei* water extract containing R-phycoerythrin before and after microencapsulation, suggesting microencapsulation as a preservation method for antioxidant activity. These studies collectively advance our understanding of cyanobacterial pigments and their diverse applications. Palinska et al., (2011) conducted a taxonomic survey of the Phormidium-group of cyanobacteria, employing multiple criteria, including morphology, pigments, RAPD molecular markers, and RFLP analysis of the 16S rRNA gene fragment. They found that strains within this group possess eight major carotenoids and three mycosporines, emphasizing the necessity of modern, combined criteria for resolving taxonomic issues in filamentous cyanobacteria with narrow trichomes.

Renaudin et al., (2021) focused on quantifying moss-associated cyanobacteria using phycocyanin pigment extraction, identifying it as an easy, rapid, and efficient method for estimating cyanobacteria numbers. The chemical diversity of cyanobacterial compounds was analysed through chemoinformatics, revealing significant molecular property differences between marine and freshwater environments (Medina and Franco, 2019). Kleigrewe et al., (2016) investigated cyanobacterial biosynthetic gene clusters, highlighting their potential to encode a vast array of novel enzymes for unique chemical reactions with potential applications in synthetic biology. Strienth et al., (2020) developed a new strategy for the combined extraction of cyanobacteria's exopolysaccharides (EPS) and pigments, achieving the highest EPS yield through a combination of heat and ultrasonication.

The investigation into the pigments of non-heterocystous filamentous cyanobacteria belonging to the Oscillatoriales group, found in biological crusts and soda lakes, highlighted the variability in pigment types and quantities among different cyanobacterial strains. Across

all studied strains, a common observation was the presence of a single peak at 339 nm in their pigment spectra when exposed to typical fluorescence lighting. This expected peak suggests that these strains can synthesize compounds that offer protection against light-induced damage (Tomer et al., 2018).

Chaiklahan et al., (2008) observed an increased lipid and total fatty acid yield from the cyanobacterium *Spirulina* when the extraction temperature was raised, providing insights into lipid and fatty acid extraction methods. Sheekh et al., (2020) investigated the detrimental effects of UV-B radiation on various cyanobacteria and freshwater chlorophytes, concluding that UV-B exposure significantly impacts growth, photosynthetic pigments, metabolites, and cellular ultrastructure, highlighting the vulnerability of these organisms to environmental stressors. These studies collectively contribute to our understanding of cyanobacterial taxonomy, quantification methods, chemical diversity, biosynthetic potential, extraction techniques, and responses to environmental stress.

Lara et al., (2022) conducted a study focused on the characterization of the halochromic pigment gloeocapsin, produced exclusively by cyanobacteria, with implications for paleobiology and astrobiology. They meticulously characterized the spectroscopic features of gloeocapsin. They investigated potential masses and chemical formulas associated with this unique pigment, offering valuable insights into its chemistry and potential as a biosignature. Meanwhile, Dwivedi & Ahmad (2023) evaluated the impact of UV-B radiation on the growth, photosynthetic pigments, and antioxidant enzymes of various cyanobacteria strains. Their findings underscored the prominent antioxidant and radical scavenging properties exhibited by cyanobacteria, crucial for defending against UV-B-induced cellular damage, thereby

elucidating the mechanisms underlying their resilience in challenging environmental conditions.

Sanmartin et al., (2010) observed the relationship between color and pigment production in two stone biofilm-forming cyanobacteria (*Nostoc* sp. PCC 9104 and *Nostoc* sp. PCC 9025). Confirming that CIELAB color parameters are correlated with pigment content implies that changes in pigment content result in observable variations in color according to the CIELAB color space. This correlation underscores the importance of CIELAB as a robust and widely used color model for describing and quantifying colors perceptually.

Mehnert et al., (2012) evaluated the effect of thermal acclimation and photoacclimation on lipophilic pigments in an invasive and native cyanobacterium of temperate regions. The conclusion drawn from this is when the temperature is below 20°C, photoinhibition (the reduction or cessation of photosynthesis due to excessive light) is prevented in the invasive species *Cylindrospermopsis raciborskii* compared to the native species *Anabaena gracile*. Khajepour et al., (2015) conducted a study of the effect of light intensity and photoperiod on the growth and biochemical composition of a local isolate of *Nostoc calcicola* and the capacity to influence the biochemical makeup of the local *N. calcicola* isolate by adjusting the light regime. Under low light intensity and shorter photoperiods, the organism tended to produce valuable phycobiliproteins or proteins. Conversely, it shifted its production towards carbohydrates and carotenoids under higher light intensities and longer photoperiods. This ability to manipulate biochemical composition based on light conditions suggests a promising avenue for controlled production of specific compounds in *N. calcicola*, with potential applications in various industries such as biotechnology and biomedicine.

Hotos et al., (2022) suggest that the growth of marine cyanobacteria *Phormidium* sp. and *Cyanothece* sp. can be optimized by initially cultivating them under white light to build up biomass; subsequently, exposing them to colored light can then enhance the production of phycobiliproteins. Singh et al., (2024) investigated *O. subbrevis* MTC-20702, which can produce significant amounts of algal biomass and serve as a biofuel feedstock. It can yield Chl-a, carotenoids, and c-Allophycocyanin, with a maximum production of these bioactive compounds occurring on the 20^{th} day of growth.

3. MATERIALS AND METHODS

3.1 MATERIALS: Cyanobacterial cultures used for the study were *Oscillatoria pseudogeminate, Oscillatoria trichoides, Oscillatoria protecus, Nostoc spongiaeforme*, and *Nostoc calcicola*, which were collected from Goa University campus.

3.2 CULTURAL CONDITIONS

Cultures were maintained in 100 ml conical flasks filled to 50% of their volume, plugged with cotton, covered with paper, tied with a rubber band, and sterilized by autoclaving at 15 lbs pressure for 20 min. After sterilization and cooling, 5 ml of cyanobacterial healthy stock culture was inoculated in culture media using a sterilized pipette and placed in a culture room at a day/night temperature of 25ºC respectively under cool white fluorescent light tubes at 80 µmol m^{-2} s^{-1} with 14h of photoperiod.

Table 1: Composition of standard mineral media for freshwater cyanobacterium *Oscillatoria pseudogeminata, Oscillatoria trichoides, Oscillatoria proteus, and Nostoc spongiaeforme* (BG11) and Marine cyanobacteria *Nostoc calcicola* (ASN III) according to Rippka et al., (1979).

Ingredient	ASN III (Amount in g/l)	BG 11 (Amount in g/l)
Sodium chloride	25g	-
Magnesium chloride	2.0g	-
Potassium chloride	0.5g	-
Sodium nitrate	0.7g	1.5g
Potassium hydrogen phosphate	0.02g	0.04g
Magnesium sulphate	3.5g	0.075g
Calcium chloride	0.5g	0.036g
Citric acid	0.003g	0.006g
Sodium carbonate	0.02g	0.02g
Ferric ammonium citrate	0.003g	0.006g
EDTA (Di sodium salt)	0.005g	0.001g
Trace metal mixture **(A_5**+Co)	1 ml/l	1ml/l
Distilled water	1000ml	1000ml
pH after autoclaving and cooling	7.5	7.5

Table 2: Composition of trace metal mixture (A5+Co):-

Ingredient	ASN III (g/100ml)	BG 11 (g/100ml)
Boric acid	2.86g	2.86g
Manganese chloride	1.81g	1.81g
Zinc sulphate	0.22g	0.222g
Sodium Molybdate	0.390g	0.390g
Copper sulphate	0.079g	0.079g
Cobalt nitrate	0.0494g	0.0494g

Oscillatoria pseudogeminata *Oscillatoria trichoides* *Oscillatoria proteus*

Fig. 2 *Oscillatoria* cultures were grown in the culture room.

Nostoc Calcicola

Nostoc spongiaeforme

Fig. 3 *Nostoc* cultures were grown in the culture room.

3.3 PHOTOSYNTHETIC PIGMENTS ANALYSIS:

Quantitative and qualitative photosynthetic pigment analysis was done using thin-layer chromatography (TLC) and a UV-visible spectrophotometer (Shimadzu**).**

3.3.1. EXTRACTION OF PHOTOSYNTHETIC PIGMENTS: Photosynthetic pigments were extracted according to the method described by Sharma and Hall (1996). Cells were collected after centrifugation of culture at 5000 x g for 10 min. The supernatant was discarded. 0.1 g of cyanobacterial cells were weighed and homogenized with 80% acetone, and the final volume was made to 5 ml at 4ºC under dim light, followed by centrifugation at 5000 rpm for 10 min at 4ºC for 10 min. The supernatant was used for spectrophotometric analysis at 663 nm, 645nm, and 443nm.

- Chlorophyll a (chl a)(mg/g fw)= 12.27×A663-2.69×A645
- Carotenoids (mg/g fw)=4.7×A443-0.27×(20.2×A645)+(8.02×A663)

3.3.2. TLC ANALYSIS OF PIGMENTS: Photosynthetic pigment was separated using TLC on silica gel plates, according to Sankhalkar (2000). 0.1 g of wet tissue was homogenized with 1 ml of acetone. Transfer the mixture in the Eppendorf tubes and centrifuged at 5000 rpm for 10 min at 4ºC. The supernatant was transferred to another Eppendorf tube. A syringe loaded pigment samples (50 µl) as discrete spots on the silica plates, 1.5-2 cm from the bottom. The plates were developed using the solvent system, n-hexane: ethyl acetate: triethanolamine (50:38:12). The colour spots were identified using their R_F values.

3.4. SPECTROPHOTOMETRIC ANALYSIS OF PHYCOBILISOMES: The resuspended culture (5 ml) was centrifuged at 5000 x g for 10 min at room temperature and dissolved in 5 ml of 20 mM sodium acetate buffer with pH 5.5, and cells were broken using sonicator at 50%

power with nine cycles for 1 min. Phycobilisomes of sonicated ruptured cells were precipitated with 1% (w/v) streptomycin sulphate for 15 min at 4ºC and collected by centrifugation at 5000 x g for 30 min at 4ºC. The absorbance of phycocyanin, allophycocyanin, and phycoerythrin was measured at 620 nm, 650 nm, and 565 nm using a UV-visible spectrophotometer (Shimadzu, UV-2450) and calculated according to Liotenberg et al., (1996).

$$\text{Phycocyanin (PC) mg/ml} = \frac{\text{OD 620} - 0.474 \times \text{O.D650}}{5.34}$$

$$\text{Allophycocyanin (APC) mg/ml} = \frac{\text{OD 650} - 0.208 \times \text{O.D 620}}{5.09}$$

$$\text{Phycoerythrin (PE) mg/ml} = \frac{\text{OD 565} - 2.41 \times \text{PC} - 0.849 \times \text{APC}}{9.62}$$

3.5 EXTRACTION OF TOTAL LIPIDS: Total lipids were extracted according to the method described by Turnham and Northcote (1984). Freshly harvested wet cell pellets (5 g) were homogenized in chloroform: methanol to make the final volume 10ml. Lipid extract was centrifuged for 5 min at 2000-3000×g to remove cell debris. To the supernatant, 0.8 ml of distilled water, 5 ml of chloroform, and 5 ml of 0.88% potassium chloride were added in a separating funnel. The mixture was shaken vigorously for 5 min, and the total lipids were kept for separation for 30 min. The lower phase of chloroform contains an appreciable amount of extracted lipids. Total lipids were taken into 10 ml screw-capped vials.

3.5.1 SEPARATION OF TOTAL LIPIDS BY TLC: Total lipids were separated by thin layer chromatography (TLC) on silica gel H, according to Liljenberg and Von Arnold (1987). A uniform slurry of 25 g of silica gel H was prepared in 50 ml of distilled water and stirred for 1-2 h on a magnetic stirrer. Glass plates were cleaned with acetone and arranged on a table.

The slurry was pulled at a steady rate across the plate on the table, from left to right, using a glass rod. The plates were placed in the drying rack, left at room temperature for an hour, and dried at 120ºC for 2 h. They were then stored in the storage cabinet but should be heated again at 120ºC for 1 h before use. After cooling the plates, samples (100 µl) were applied as discrete spots, 1.5-2 cm from the bottom of the plate, in chloroform using a syringe, and the plate was placed in a chromatographic chamber containing eluting solvents. In a chromatography chamber, filter papers were placed on both sides to saturate the atmosphere inside with solvent vapors. This speeds up the analysis, especially with polar solvents, and may occasionally improve the resolution. The plates were run in the chamber up to the top of the plate with a solvent system containing chloroform: methanol: glacial acetic acid: water (85:15:10:3.5) for phosphoglycolipids and diethyl ether: water (90:1) for neutral lipids, Plates were then air-dried and spots were visualized as bands with iodine vapors in iodine chamber, identified by their R_F values (Liljenberg and Kates, 1985**).**

4. RESULTS

4.1 Morphological study:

The morphological studies of cyanobacteria *Oscillatoria pseudogeminata*, *Oscillatoria trichoides*, *Oscillatoria proteus*, *Nostoc spongiaeforme* and *Nostoc calcicola* were done using compound microscope (Fig 4 and Fig 5).

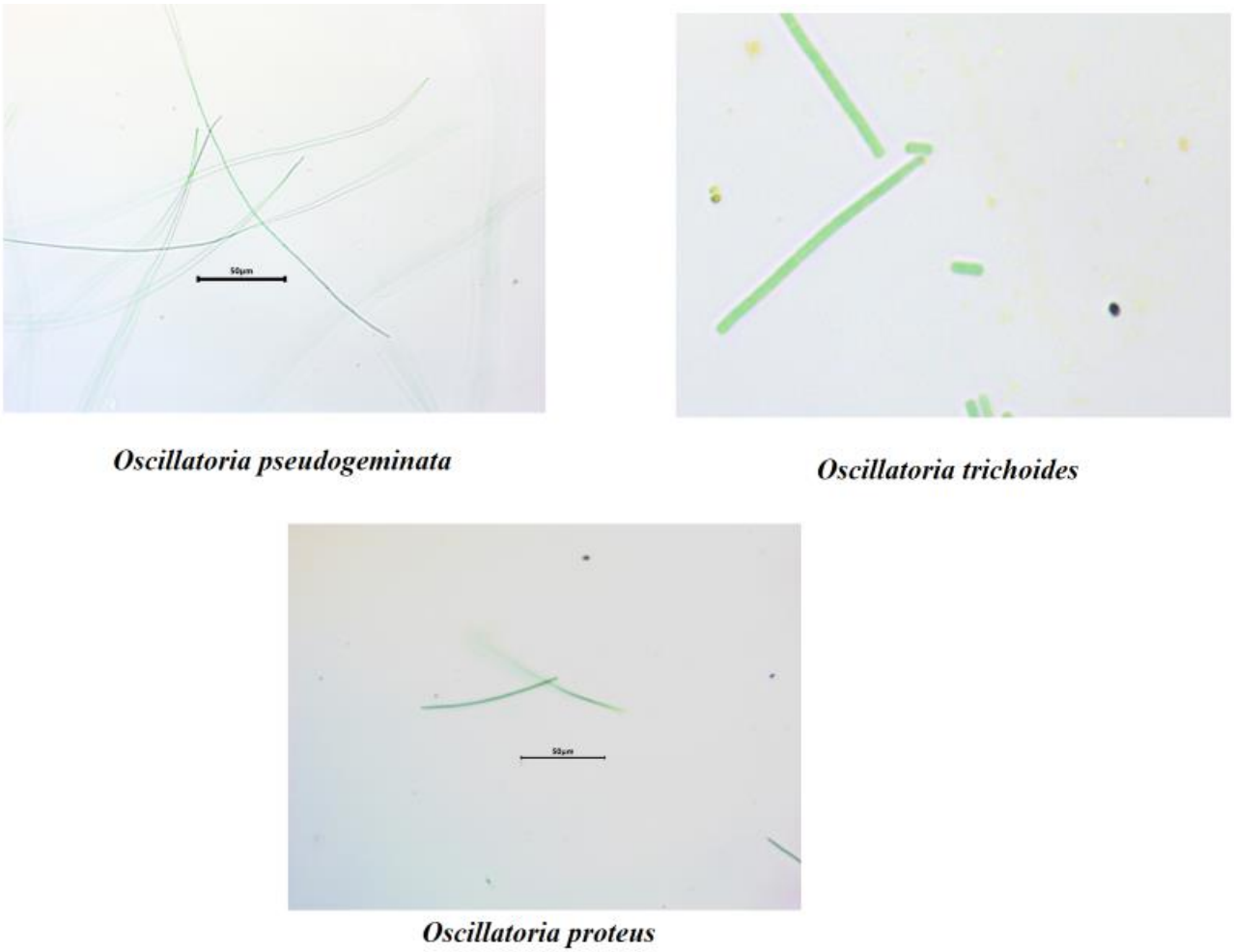
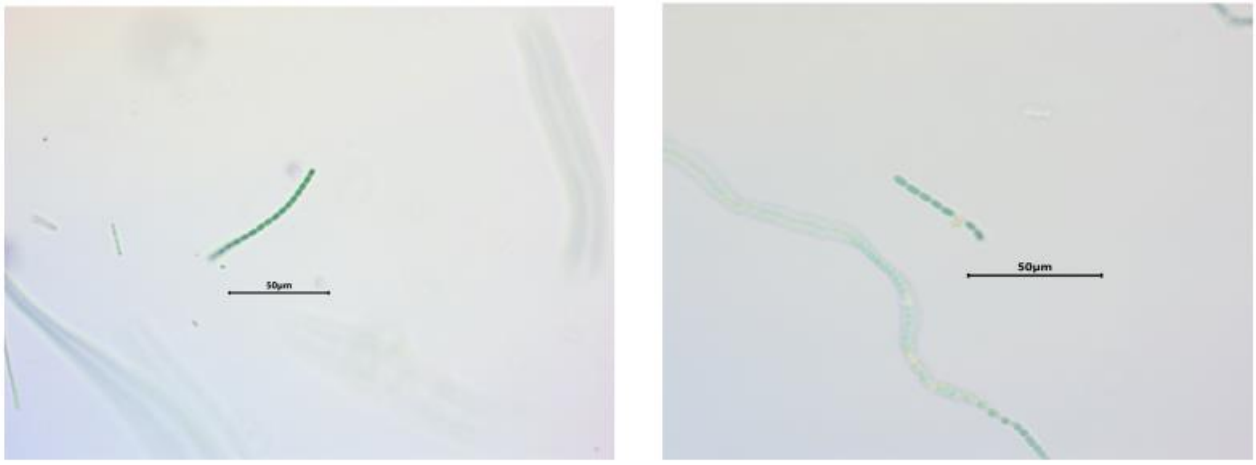


Fig. 4 Microscopic view (40X) of *Oscillatoria pseudogeminata, Oscillatoria trichoides* and *Oscillatoria proteus.*



Nostoc spongiaeforme *Nostoc calcicola*

Fig. 5 Microscopic (40X) view of *Nostoc spongiaeforme* and *Nostoc calcicola.*

4.2 Photosynthetic pigments

Photosynthetic pigments were studied using spectrophotometric methods and TLC (Fig. 6, 7 and 8). All the photosynthetic pigments, such as chlorophyll, carotenoids, and xanthophylls, were observed in five cyanobacteria studied.

4.2.1 Photosynthetic pigments (spectrophotometric analysis)

The spectrophotometric analysis of photosynthetic pigments in five cyanobacteria was conducted using a UV-visible spectrophotometer.

4.2.1a Chlorophyll a:

In *Oscillatoria pseudogeminata,* the concentration of chlorophyll a is 22.13 mg g^{-1}. In *Oscillatoria trichoides*, the concentration of chlorophyll a is 21.87 mg g^{-1} and in *Oscillatoria proteus*, it is 16.03 mg g^{-1}. *Nostoc spogiaeforme* has 5.321 mg g^{-1}, and *Nostoc calcicola* has 4.891 mg $g^{-1}$of chlorophyll a. The variation in chlorophyll a in different cyanobacteria is given in Table 3 and the corresponding bar graph is given figure 6.

Table 3: Chlorophyll-a concentration in different cyanobacteria.

Cyanobacteria	Concentration of Chl a (mg g^{-1})
Oscillatoria pseudogeminata	22.13
Oscillatoria trichoides	21.85
Oscillatoria proteus	16.03
Nostoc spongiaeforme	5.321
Nostoc calcicola	4.821

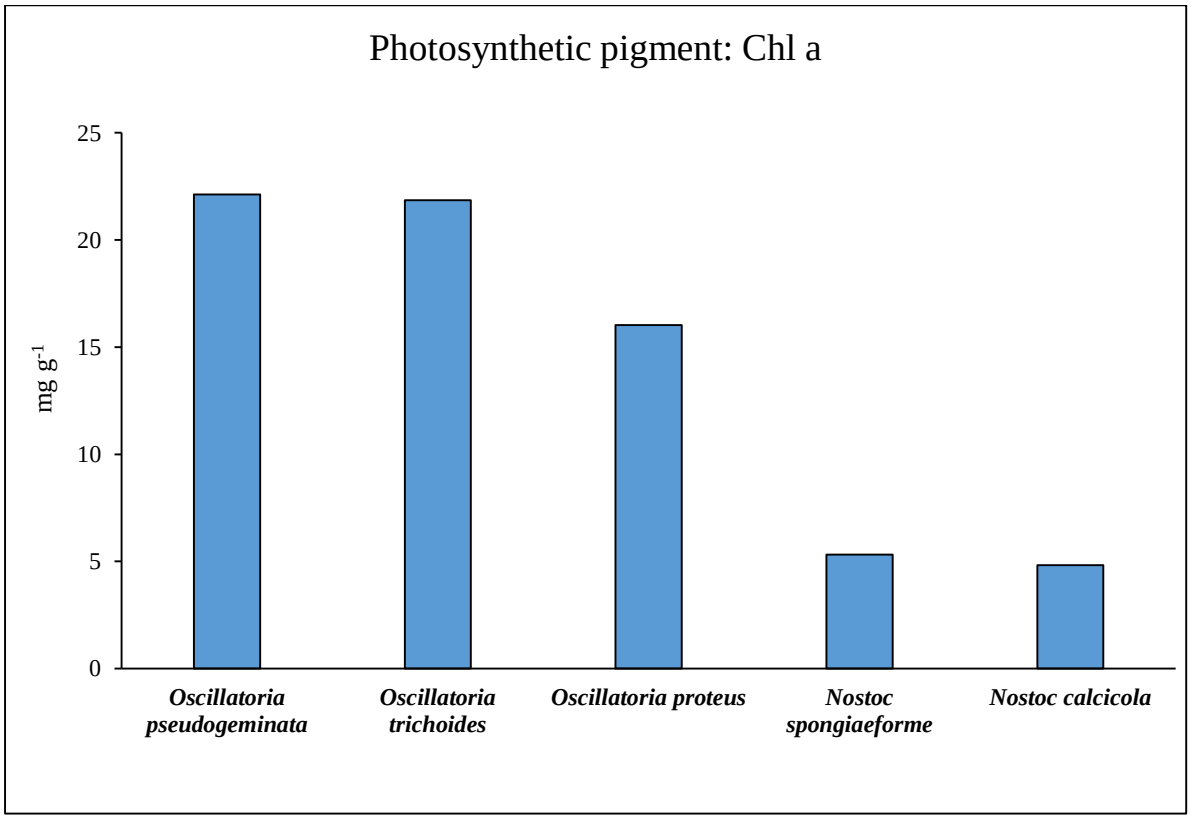


Fig.6: Variation in chlorophyll-a concentration in different cyanobacteria.

4.2.1 b Carotenoids:

The variation in concentration of the carotenoids in the five different algae is given in Table 4 and Figure 7 shows the bar graph of the concentration variation of carotenoids in different cyanobacteria. The concentration of carotenoids varies among different cyanobacteria. *Oscillatoria pseudogeminata* has a 3.653 mg g^{-1} of carotenoids, while in *Oscillatoria trichoides*, it is 1.54 mg g^{-1}, and *Oscillatoria proteus* contains 1.026 mg g^{-1} of carotenoids. *Nostoc spongiaeforme* and *Nostoc calcicola* contain less carotenoids (0.40 mg g^{-1} and 0.366 mg g^{-1}). The studies show that *Oscillatoria pseudogeminata* has the highest chlorophyll a and carotenoid concentration, whereas *Nostoc calcicola* has the lowest chlorophyll a and carotenoid concentration.

Table No. 4: Carotenoid concentration in different cyanobacteria.

Cyanobacteria	Concentration of carotenoids (mg g^{-1})
Oscillatoria pseudogeminata	3.60
Oscillatoria trichoides	1.54
Oscillatoria proteus	1.03
Nostoc spongiaeforme	0.40
Nostoc calcicola	0.36

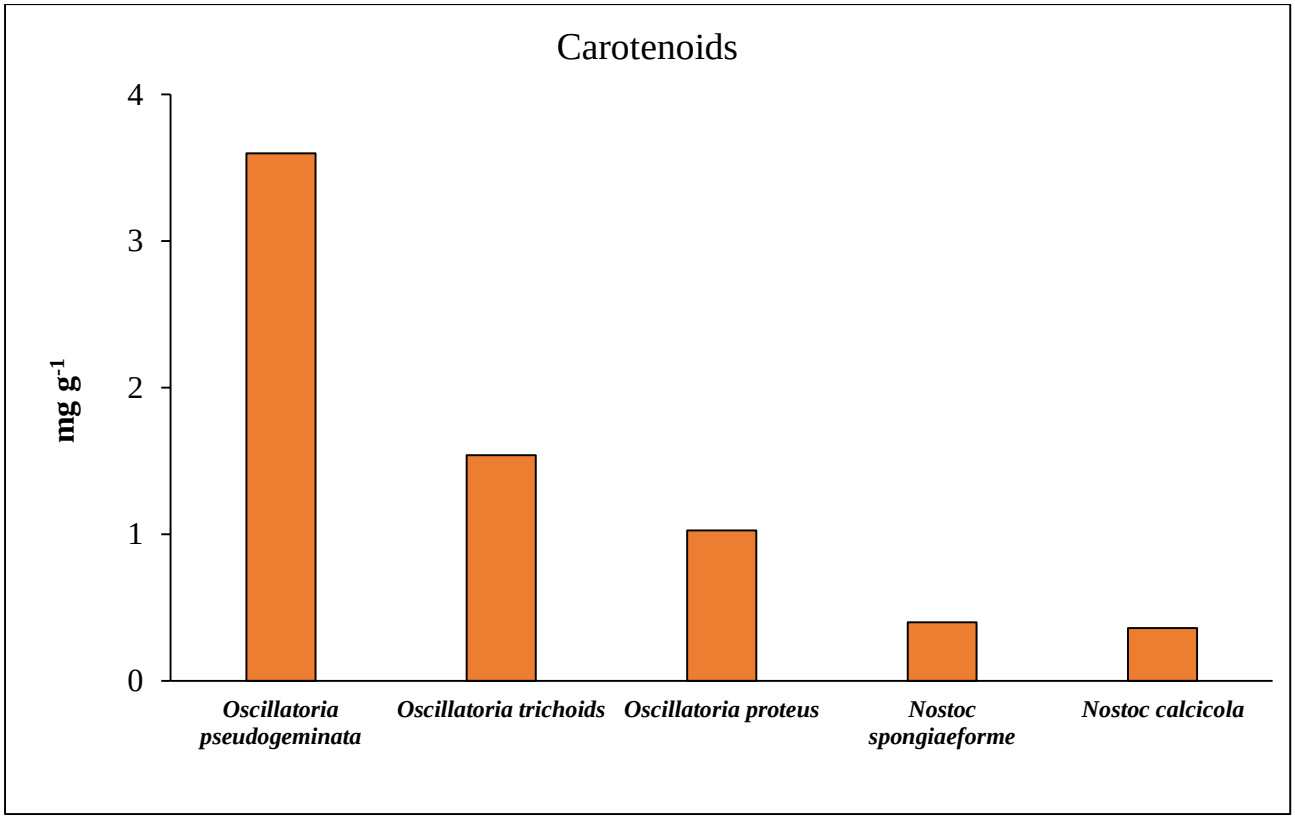


Fig 7: Variation in Carotenoid concentration in different cyanobacteria.

4.2.1 c. TLC analysis of photosynthetic pigments:

The photosynthetic pigments were studied using TLC in five different cyanobacteria (Table no. 5 and fig. .8) .

In *Oscillatoria pseudogeminata,* the three main photosynthetic pigments were identified as Chlorophyll a Xanthophyll 1, and Xanthophyll 2. Spot no.3, with an Rf value of 94, was green in colour and identified as chlorophyll a. Spot no.2, with an Rf value of 57 and yellow, was identified as xanthophyll 1, and Spot no 1, with an Rf value of 20, which was light yellow in colour as xanthophyll 2.

Oscillatoria trichoides has five main photosynthetic pigments: β-carotene, Lutein, Chlorophyll a, Xanthophyll 1, and Xanthophyll 2. Spot no.5, with an Rf value of 100, was orange and identified as β- carotene. Spot no 4, with an Rf value of 96 and grey, is recognized as lutein. Spot no 3 with Rf value 94, which was green, is identified as Chlorophyll a. Spot no.2 with Rf value 57 and yellow in colour is Xanthophyll 1. Spot no 1, with Rf value 20 and light yellow, is identified as Xanthophyll 2.

In *Oscillatoria proteus*, five main photosynthetic pigments were identified: β-carotene, Lutein, Chlorophyll a, Xanthophyll 1, and Xanthophyll 2. Spot no.5, with an Rf value of 100, was orange and identified as β- carotene. Spot no 4, with an Rf value of 96, grey is identified as lutein. Spot no 3, with an Rf value of 94, which was green in colour, was identified as Chlorophyll a. Spot no.2, with an Rf value of 57, yellow identified as Xanthophyll 1, and Spot no 1, with an Rf value of 20, which is light yellow colour is identified as Xanthophyll 2.

In *Nostoc spongiaeforme*, five main photosynthetic pigments were identified: β-carotene, Lutein, Chlorophyll a, Xanthophyll1, and Xanthophyll 2. Spot no.5, with an Rf value of 100,

was orange and identified as β- carotene. Spot no 4, with an Rf value of 96 and grey, was identified as lutein. Spot no 3, with an Rf value of 94, which was green, is chlorophyll a. Spot no.2, with an Rf value of 57 and yellow in colour, was identified as Xanthophyll 1. Spot no 1 with Rf value 20, which is light yellow, is recognized as Xanthophyll 2.

In *Nostoc calcicola*, only four main photosynthetics were identified: β-carotene, Lutein, Chlorophyll a, Xanthophyll 1, and Xanthophyll 2. Spot no.5, with an Rf value of 100, was orange and identified as β- carotene. Spot no 4, with an Rf value of 96 and grey, was identified as lutein, and Spot no 3, with an Rf value of 94, which was green in colour, is Chlorophyll a. Spot no 1, with an Rf value of 20 and light yellow in colour is identified as Xanthophyll 2.

Table 5: The photosynthetic pigments present in five different cyanobacteria obtained from TLC

Sr no.	Rf × 100	Colour of the compounds	*Oscillatoria pseudogeminata*	*Oscillatoria trichoides*	*Oscillatoria proteus*	*Nostoc spogiaeforme*	*Nostoc calcicola*
1	100	Orange	-	β-carotene	β-carotene	β-carotene	β-carotene
2	96	Grey	-	Lutein	Lutein	Lutein	Lutein
3	94	Green	Chlorophyll a	Chlorophyll a	Chlorophyll a	Chlorophyll a	Chlorophyll a
4	57	Yellow	Xanthophyll a	Xanthophyll a	Xanthophyll a	Xanthophyll a	-
5	20	Light yellow	Xanthophyll b	Xanthophyll b	Xanthophyll b	Xanthophyll b	Xanthophyll b

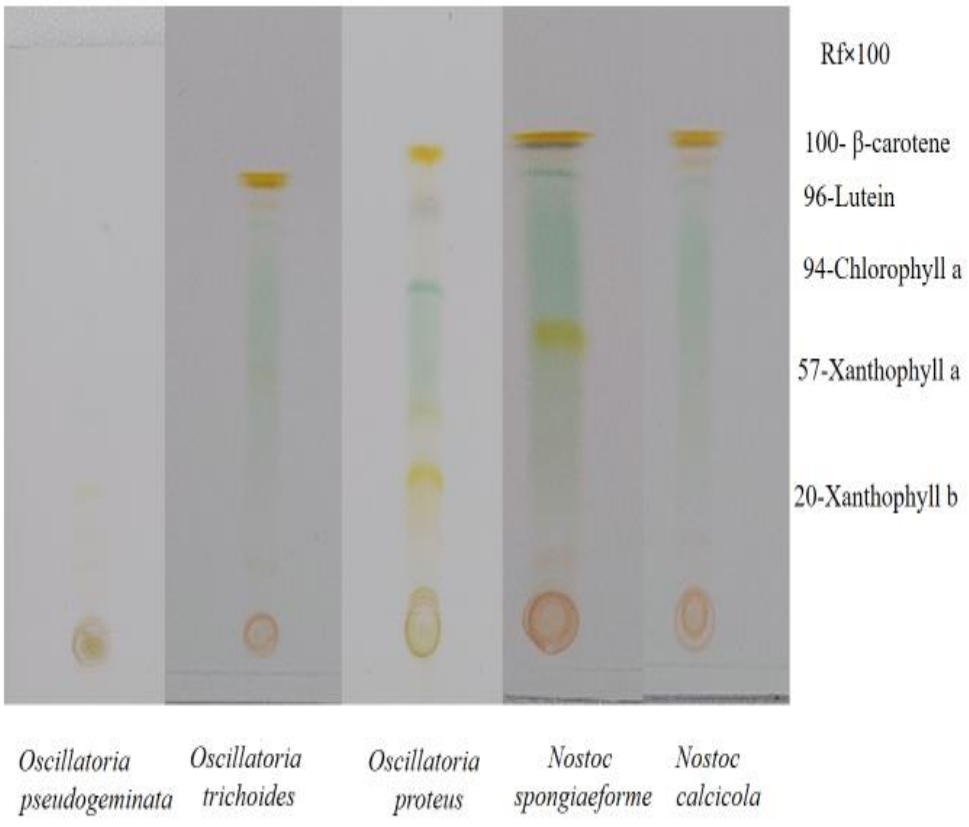


Fig. 8: TLC profile of photosynthetic pigments of the five different cyanobacteria.

4.2.1 d. Phycobilins:

The presence of different phycobilins was studied using a spectrophotometer. Three different types of phycobilins, phycocyanin, allophycocyanin, and phycoerythrin, were observed in five cyanobacteria. Phycocyanin concentration present in *Oscillatoria pseudogeminata* is 1 mg, *Osciilatoria trichoides* 0.48 mg mL^{-1}, *Oscillatoria protecus* 0.62 mg mL^{-1}g, *Nostoc spongiforme* 1.63 mg mL^{-1} and *Nostoc calcicola* 0.45 mg mL^{-1}. The allophycocyanin pigment present in *Oscillatoria pseudogeminata* is 0.26 mg mL^{-1}, *Osciilatoria trichoides* 0.1 mg mL^{-1}, *Oscillatoria protecus* 0.15 mg mL^{-1}, *Nostoc spongiforme* 0.74 mg $mL^{-1}$and *Nostoc calcicola* 0.21 mg mL^{-1}. The phycoerythrin concentration in *Oscillatoria pseudogeminata* is 0.41 mg mL^{-1}, *Osciilatoria trichoides* 0.26 mg mL^{-1}, *Oscillatoria protecus* 0.23 mg mL^{-1}, *Nostoc spongiaeforme* 0.56 mg mL^{-1} and *Nostoc calcicola* 0.4 mg mL^{-1}. The results are shown in tabular form in Table 6 and in bar grapgh in Figure 9.

Table 6. The concentration of phycobilins in the five different cyanobacteria mgmL^{-1}

Algae	Phycocyanin	Allophycocyanin	Phycoerythrin
Oscillatoria pseudogeminata	1	0.26	0.41
Oscillatoria trichoides	0.48	0.1	0.26
Oscillatoria protecus	0.62	0.15	0.23
Nostoc spongiaeforme	1.63	0.74	0.56
Nostoc calcicola	0.45	0.21	0.4

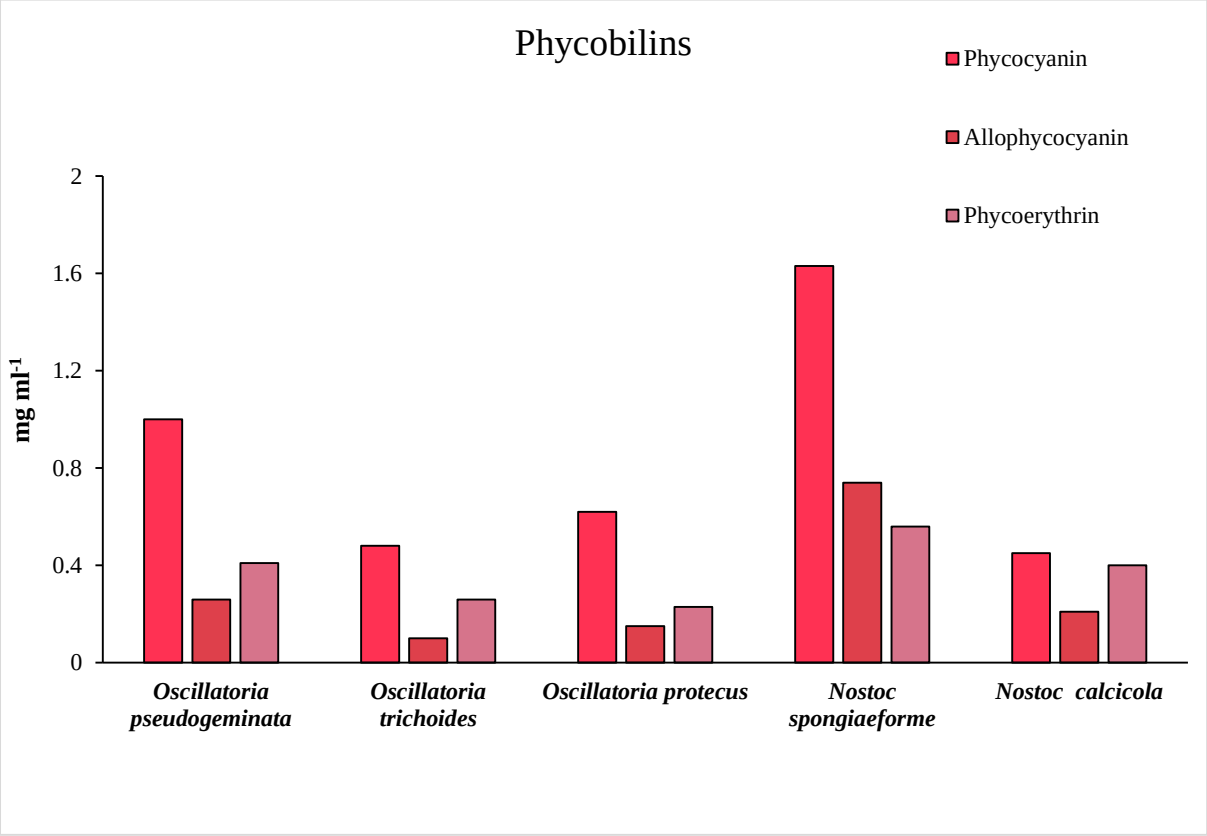


Fig 9: Concentration of different phycobilins in the five cyanobacteria.

4.3. Lipids

4.3.1. Phosphoglycolipids

Phosphoglycolipids separation was carried out by thin-layer chromatography of the five cyanobacteria studied. These spots were identified according to Rf values by comparing them with standards. In *Oscillatoria pseudogeminata*, three different spots were observed with Rf values 100, 92, and 80, identified as lipid Monogalactosyldiglyceride. In *Oscillatoria trichoides*, three spots were observed with Rf values 100, 92, and 79, identified as lipid Monogalactosyldiglyceride. In *Oscillatoria proteus*, four spots were observed with Rf values 96, 80, 64, and 32, and they were identified as lipids Monogalactodiglyceride, Sulfoguinosylglycerol, and Digalactosyldiglyceride. In *Nostoc spongiaeforme*, only 1 spot was observed with an Rf value of 96, identified as lipid Monogalactodiglyceride. In *Nostoc calcicola*, only 1 spot was observed with an Rf value of 96, identified as lipid Monogalactodiglyceride. Table 7 shows the different phosphoglycolipids present in five cyanobacteria and the observed TLC image of lipids is shown in Figure 10.

Table 7: Thin layer chromatography profile of phosphoglycolipids in five cyanobacteria.

Sr. N.	**Rf× 100**	***Oscillatoria pseudogeminata***	***Oscillatoria trichoides***	***Oscillatoria protecus***	***Nosctoc spongiaeforme***	***Nostoc calcicola***
1	**100**	Pigments	Pigments	Pigments	Pigments	Pigments
2	**92**	Monogalactosyl diglyceride	Monogalactosyldiglyceride	Monogalactosyldiglyceride	Monogalactodiglyceride	Monogalactodiglyceride
3	**80**	Monogalactosyl diglyceride	Monogalactosyldiglyceride	Monogalactosyldiglyceride	-	-
4	**64**	-	-	Sulfoquinosylglycerol	-	-
5	**32**	-	-	Digalactosyldiglyceride	-	-

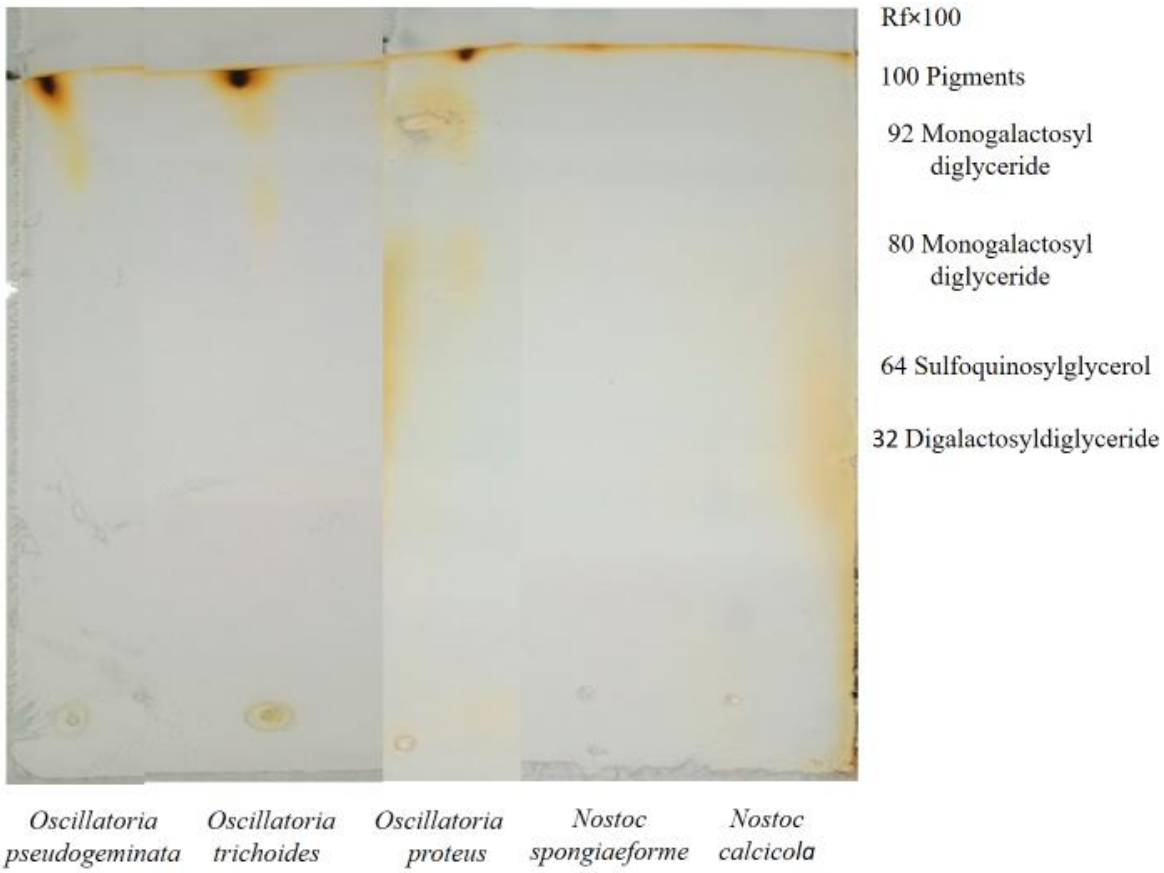


Fig. 10 Thin layer chromatography (TLC) profile of phosphoglycolipids of the five cyanobacteria.

4.3.2. Neutral lipids

Neutral lipids separation was carried out by thin-layer chromatography in five cyanobacteria.Identification of these spots was done according to Rf values compared with their standards. In *Oscillatoria pseudogeminata*, three different spots were observed, which were identified as esters with an Rf value of 96 as triglyceride, an Rf value of 36 as diglyceride, and an Rf value of 15 as monoglyceride. In *Oscillatoria trichoides*, three spots were observed with Rf values 96 as esters and monoglyceride with R values 23, 15. In *Oscillatoria proteus*, three spots were observed with Rf value 93 as esters, Rf value 88 as triglyceride, and Rf value 15 as monoglyceride. In *Nostoc spongiaeforme*, only 1 spot was observed with an Rf value of 93, and it is identified as an ester. In *Nostoc calcicola*, only 1 spot was observed with an Rf value of 93, and it is identified as esters .The TLC images are shown in Figure 11 and the corresponding table is given in Table 8.

Table 8: Thin layer chromatography profile of Neutral lipids of five cyanobacteria.

Sr No	**Rf× 100**	***Oscillatoria pseudogeminata***	***Oscillatoria trichoides***	***Oscillatoria proteus***	***Nostoc spongiaeforme***	***Nostoc calcicola***
1	**96**	Esters	Esters	Esters	Esters	Esters
2	**88**	-	-	Triglyceride	-	-
3	**36**	Diglyceride	-	-	-	-
4	**23**	-	Monoglyceride	-	-	-
5	**15**	Monoglyceride	Monoglyceride	Monoglyceride	-	-

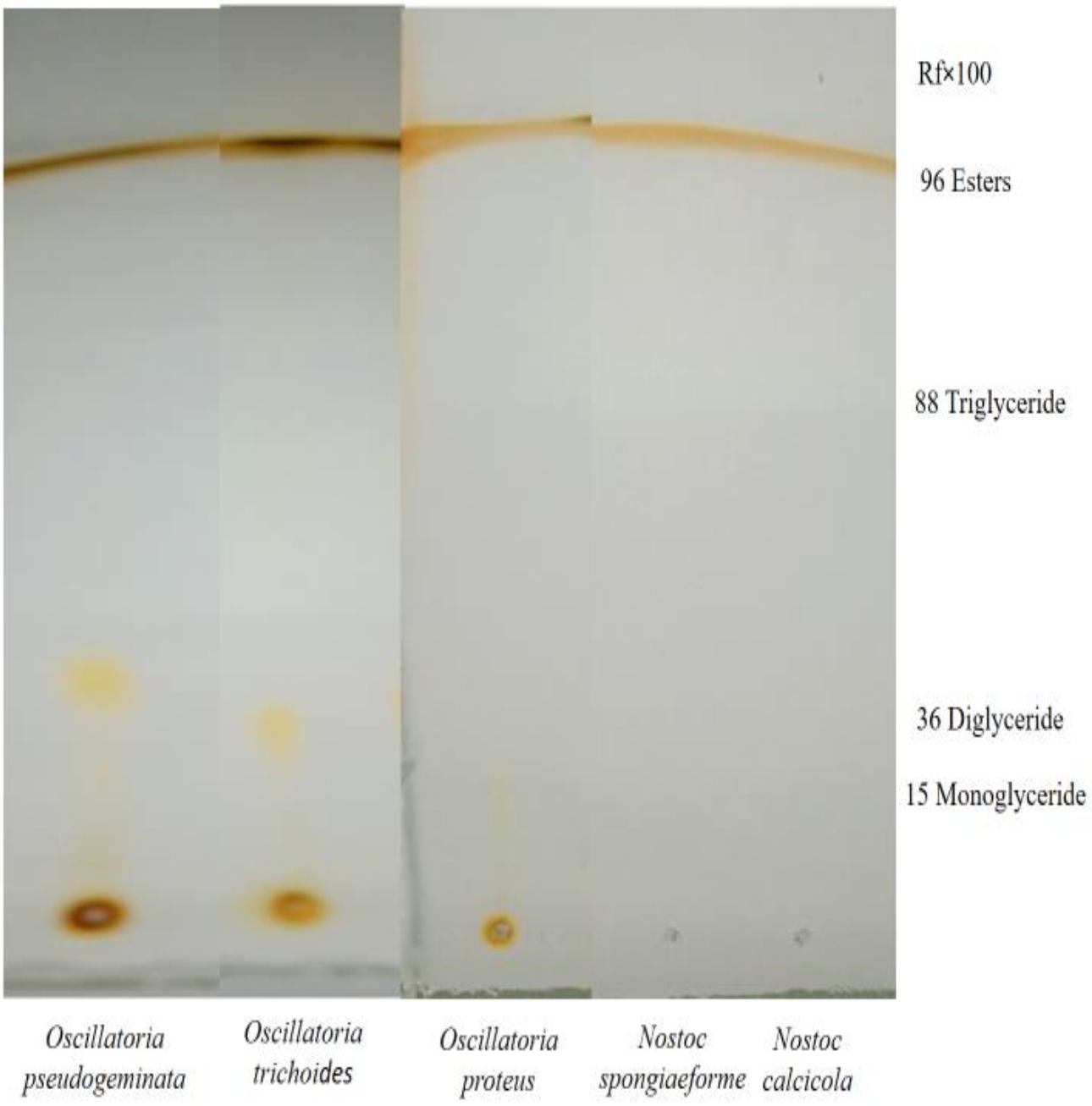


Fig. 11 Thin layer chromatography profile of neutral lipids of five different cyanobacteria.

5. DISCUSSION

5.1. Characterization of Photosynthetic pigments

In the present investigation, photosynthetic pigments were isolated from fresh water and marine water cyanobacteria such as *Oscillatoria pseudogeminata, Oscillatoria trichoides, Oscillatoria proteus, Nostoc spongiaeforrme and Nostoc calcicola*. In these different cyanobacteria, the concentration of chlorophyll a, carotenoids, and phycobilins was found using spectrophotometric and TLC techniques. A large variation in concentrations was observed across different cyanobacterial samples (Fig 6 & Table 3, Fig 7 & Table 4, Fig 9 & Table 6).

Our study reported that *Oscillatoria pseudogeminata* showed higher chlorophyll a and carotenoid concentrations while the same species showed lower concentrations of phycobilins. Earlier studies show that through spectrophotometric analysis, it was confirmed that the alga *Chlorella vulgaris* contains more chlorophyll a, chlorophyll b, total chlorophylls, and total carotenoids compared to the cyanobacteria *Spirulina platensis* and the food supplements derived from them (Hynstova et al., 2018). Kuhl & Fenchel (2000) observed strong photosynthesis in synthetic cyanobacterial mats.

The pigment extract analysis through TLC was validated using a UV-Vis spectrophotometer. Filamentous algae were observed to possess substantial quantities of Chlorophyll a, Chlorophyll b, and Carotenoids (Maneesh et al., 2022). Our studies show significant value in TLC measurement for mats and filamentous cyanobacteria due to the presence of β- carotene, lutein, chlorophyll a, xanthophyll 1, and xanthophyll 2. The result shows that the photosynthetic pigments from five different cyanobacteria, *Oscillatoria pseudogeminata, Oscillatoria trichoides, Oscillatoria proteus, Nostoc spongiaeforrme and Nostoc calcicola.,*

which were β- carotene, xanthophylls, chlorophyll a and carotene. The pigments in microalgae and cyanobacteria hold significant promise for industrial use due to commercial demand for compounds like β-carotene, astaxanthin, and phycocyanin (Pagels et al., 2021). Tomer et al. (2018) The type and amount of pigments can vary between different strains of cyanobacteria. When exposed to normal fluorescence light, all the cyanobacterial strains studied showed a single peak at 339 nm in their pigment spectra. This indicates that these strains have the ability to produce compounds that protect them from light damage.

We studied the concentration of phycobilins using a spectrophotometer. Three different kinds of phycobilins were phycocyanin, allophycocyanin, and phycoerythrin. Earlier studies have shown that the spectrophotometric and spectrofluorometric characteristics of individual phycobilin pigments vary in shape, wavelength maximum (λ max), and location. In terms of absorbance spectra, phycocyanin shows a single peak at 620 nm, while phycoerythrin has two peaks at 495 nm and 565 nm, and allophycocyanin displays peaks at 615 nm and 650 nm. In emission spectra, phycocyanin, phycoerythrin, and allophycocyanin exhibit a single peak at 644 nm, 576 nm, and 660 nm, respectively (Sasim et al., 2014).

5.2. Characterization of Lipids

Phosphoglycolipids and neutral lipids in five cyanobacteria, namely *Oscillatoria pseudogeminata, Oscillatoria trichoides, Oscillatoria proteus, Nostoc spongiaeforme and Nostoc calcicola* (Table 7 and Fig 10) were studied. Our studies in all five cyanobacteria examined lipid classes, which are monogalactosyldiglycerol, sulphoquinovosyldiglycerols and digalactosyldiacylglycerols. Earlier studies showed that many freshwater cyanobacteria have lipid classes commonly found in photosynthetic prokaryotes and eukaryotes. These lipids include monogalactosyldiacylglycerols, digalactosyldiacylglycerols, sulphoquinovosyldiglycerols,

and phosphatidylglycerols (Quinn & Williams, 1983). A lipid extraction method involving sonication followed by solvent extraction (using a 1:1 volume ratio of chloroform to methanol) yielded the highest lipid content at 18.58%. The fatty acids obtained from the extraction were primarily polyunsaturated, including linoleic acid and α-linolenic acid (Arporn et al., 2012).

Our result also shows the presence of neutral lipids such as triglyceride, diglyceride, and monoglycerides common in all five samples (Table 8 and Fig. 11). Neutral lipids, primarily triacylglycerols, and esters, have been recognized as the primary way carbon is stored in microalgae (Deng et al., 2010). The triacylglycerol levels in *Monodus subterraneus* were observed to rise when phosphate was absent. Similar outcomes were noted in *Chlorella sp.,* where a decrease in phosphorus concentration from 240 to 32 μM increased neutral lipid content (Khozin-Goldberg and Cohen 2006). Lynch et al., (2015) showed that the native green alga had a notably higher accumulation of neutral lipids than the cyanobacteria.

6. CONCLUSION

In conclusion, the assessment of photosynthetic pigments and lipids in five different cyanobacteria species, *Oscillatoria pseudogeminata, Oscillatoria trichoides, Oscillatoria proteus, Nostoc spongiaeforme, and Nostoc calcicola,* involving a combination of quantitative and qualitative techniques were performed using thin-layer chromatography and spectrophotometry. Spectrophotometry is valuable for quantitative analysis, as it measures the absorbance of specific pigments at known wavelengths. This data can be used to calculate the concentration of pigments in the cyanobacterial sample. Results revealed that the *Oscillatoria pseudogeminata* shows higher chlorophyll a and carotenoid concentrations.*Nostoc spongiaforme* shows higher concentration of Phycocyanin,,Phycoerythrin and Allophycocyanin.

Thin-layer chromatography allows for separating and visualizing different pigments and lipids, providing qualitative information about their presence and distribution in the sample. The studies showed that photosynthetic pigments such as β –carotene, Lutein, Chlorophyll a, Xanthophyll 1, and Xanthophyll 2 are present in the samples we studied. .

Phosphoglycolipids, such as monogalactosyldiglycerol, sulphoquinovosyldiglycerols, and digalactosyldiacylgycerol, were present in the samples. The qualitative analysis found neutral lipids such as esters, triglyceride, diglyceride, and monoglyceride in some of the samples, which measured the intensity or area of each separated band. Both photosynthetic pigments and lipids play vital roles in cyanobacterial physiology. They are of interest for various biotechnological and environmental applications, hence the importance of efficient extraction methods to study their composition and potential uses.

REFERENCES

Abed, R. M., Dobretsov, S., & Sudesh, K. (2009). Applications of cyanobacteria in biotechnology. Journal of Applied Microbiology, 106 (1), 1-12.

Adir, N., Bar-Zvi, S., & Harris, D. (2020). The amazing phycobilisome. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *1861* (4), 148047.

BabuB,WuJT.Production of natural butylated hydroxyl toluene as an antioxidant by fresh water phytoplankton. Journal of Phycology. 2008;44 (6):1447-1454.

Banack, S. A., Metcalf, J. S., Jiang, L., Craighead, D., Ilag, L. L., & Cox, P. A. (2012). Cyanobacteria produce N-(2-aminoethyl) glycine, a backbone for peptide nucleic acids which may have been the first genetic molecules for life on Earth. *PLoS One*, *7*(11), e49043.

Bartley GE, Scolnick PA. Plant carotenoids: Pigments for photoprotection, visual attraction and human health. The Plant Cell, 1995; 1:1027-1038.

Björn, L. O., Papageorgiou, G. C., Blankenship, R. E., & Govindjee. (2009). A viewpoint: why chlorophyll a. *Photosynthesis research*, *99*, 85-98.

Chaiklahan, R., Chirasuwan, N., Loha, V., & Bunnag, B. (2008). Lipid and fatty acids extraction from the cyanobacterium *Spirulina*. *Sci Asia*, *34*(3), 299-305.

Chini Zittelli, G., Lauceri, R., Faraloni, C., Silva Benavides, A. M., & Torzillo, G. (2023). Valuable pigments from microalgae: phycobiliproteins, primary carotenoids, and fucoxanthin. *Photochemical & Photobiological Sciences*, 1-57.

Chini Zittelli, G., Lauceri, R., Faraloni, C., Silva Benavides, A. M., & Torzillo, G. (2023). Valuable pigments from microalgae: phycobiliproteins, primary carotenoids, and fucoxanthin. *Photochemical & Photobiological Sciences*, *22*(8), 1733-1789.

Chittora, D., Meena, M., Barupal, T., Swapnil, P., & Sharma, K. (2020). Cyanobacteria as a source of biofertilizers for sustainable agriculture. *Biochemistry and biophysics reports*, *22*, 100737.

Cohen, Y. E. H. U. D. A., & Gurevitz, M. I. C. H. A. E. L. (2006). The cyanobacteria—ecology, physiology and molecular genetics. *The prokaryotes*, *4*, 1074-1098.

Colyer, C. L., Kinkade, C. S., Viskari, P. J., & Landers, J. P. (2005). Analysis of cyanobacterial pigments and proteins by electrophoretic and chromatographic methods. Analytical and bioanalytical chemistry, 382, 559-569.

Criscuolo, A., & Gribaldo, S. (2011). Large-scale phylogenomic analyses indicate a deep origin of primary plastids within cyanobacteria. *Molecular biology and evolution*, *28*(11), 3019-3032.

da Silva, J. C., & Lombardi, A. T. (2020). Chlorophylls in microalgae: Occurrence, distribution, and biosynthesis. *Pigments from Microalgae Handbook*, 1-18.

de Loura, I. C., Dubacq, J. P., & Thomas, J. C. (1987). The effects of nitrogen deficiency on pigments an d lipids of cyanobacteria. Plant physiology, 83(4), 838-843.

De Marsac, N. T., & Cohen-Bazire, G. (1977). Molecular composition of cyanobacterial phycobilisomes. *Proceedings of the National Academy of Sciences*, *74*(4), 1635-1639.

Deng XD, Fei XW, Li YJ (2010) The effects of nutritional restriction on neutral lipid accumulation in *Chlamydomonas* and *Chlorella*. Afr J Microbiol Res 5:260–270.

Dillon, J. G., Tatsumi, C. M., Tandingan, P. G., & Castenholz, R. W. (2002). Effect of environmental factors on the synthesis of scytonemin, a UV-screening pigment, in a cyanobacterium (*Chroococcidiopsis* sp.). Archives of microbiology, 177, 322-331.

Dwivedi, S., & Ahmad, I. Z. (2023). Evaluation of the effect of UV-B radiation on growth, photosynthetic pigment, and antioxidant enzymes of some cyanobacteria. *Environmental Research*, *218*, 114943.

El-Sheekh, M. M., Alwaleed, E. A., Ibrahim, A., & Saber, H. (2021). Detrimental effect of UV-B radiation on growth, photosynthetic pigments, metabolites and ultrastructure of some cyanobacteria and freshwater chlorophyta. *International Journal of Radiation Biology*, *97*(2), 265-275.

Elumalai, S., Rajesh, K. G., Jegan, G., Sangeetha, T., & Roop, S. D. (2014). Extraction of phycocyanin an important pharmaceutical phycobiliproteins from cyanobacteria. *International Journal of Pharmaceutical Research and Development*, *6*(4), 67-74.

Eungrasamee, K., Zhu, Z., Liu, X., Jantaro, S., & Lindblad, P. (2024). Lipid metabolism in cyanobacteria: biosynthesis and utilization. In *Cyanobacteria* (pp. 85-116). Academic Press.

Glazer, A. N. (1977). Structure and molecular organization of the photosynthetic accessory pigments of cyanobacteria and red algae. *Molecular and cellular biochemistry*, *18*, 125-140.

Gonzalez-Medina, M., & Medina-Franco, J. L. (2019). Chemical diversity of cyanobacterial compounds: A chemoinformatics analysis. *ACS Omega*, *4*(4), 6229-6237.

Hirata K, Yoshitomi S, Dwi S, Iwabe O, Mahakhant A, Polchai J, Miyamoto K (2003) Bioactivities of nostocine a produced by a freshwater cyanobacterium *Nostoc spongiaforme* TISTR 8169. J Biosci Bioeng 95:512–517.

Hotos, G. N., & Antoniadis, T. I. (2022). The effect of colored and white light on growth and phycobiliproteins, chlorophyll and carotenoids content of the marine cyanobacteria *Phormidium* sp. and *Cyanothece* sp. in batch cultures. *Life*, *12*(6), 837.

Hu, J., & Wang, Q. (2018). Regulatory sRNAs in cyanobacteria. *Frontiers in microbiology*, *9*, 2399.

Hynstova, V., Sterbova, D., Klejdus, B., Hedbavny, J., Huska, D., & Adam, V. (2018). Separation, identification and quantification of carotenoids and chlorophylls in dietary supplements containing Chlorella vulgaris and Spirulina platensis using high performance thin layer chromatography. *Journal of pharmaceutical and biomedical analysis*, *148*, 108-118.

Ishihara, J. I., & Takahashi, H. (2023). Raman spectral analysis of microbial pigment compositions in vegetative cells and heterocysts of multicellular cyanobacterium. Biochemistry and Biophysics Reports, 34, 101469.

Jaiswal, A., Koli, D. K., Kumar, A., Kumar, S., & Sagar, S. (2018). Pigments analysis of cyanobacterial strains. Int. J. Chem. Stud, 6(2), 1248-1251.d lipids of cyanobacteria. *Plant physiology*, *83*(4), 838-843.

Jodłowska, S., & Latała, A. (2011). The comparison of spectrophotometric method and high-performance liquid chromatography in photosynthetic pigments analysis. OnLine Journal of Biological Sciences, 11(2), 63-69.

Juttner F, Todorova AK, Walch N, von Philipsborn W (2001) Nostocyclamide M: a cyanobacterial cyclic peptide with allelopathic activity from Nostoc 31. Phytochemistry 57:613–619.

Juttner F, Todorova AK, Walch N, von Philipsborn W, Nostocyclamide M (2001) A cyanobacterial cyclic peptide with allelopathic activity from *Nostoc* 31. Phytochem 57:613–619.

K. Sallal, N. A. Nimer,& S. S. Radwan .(1990) . Lipid and fatty acid composition of freshwater cyanobacteria volume 136, 10.

Kaiwan-arporn, P., Hai, P. D., Thu, N. T., & Annachhatre, A. P. (2012). Cultivation of cyanobacteria for extraction of lipids. biomass and bioenergy, 44, 142-149.

Khajepour, F., Hosseini, S. A., Ghorbani Nasrabadi, R., & Markou, G. (2015). Effect of light intensity and photoperiod on growth and biochemical composition of a local isolate of *Nostoc calcicola*. *Applied biochemistry and biotechnology*, *176*, 2279-2289.

Khatulistiani, T. S., Oktavia, D. A., Munifah, I., & Marraskuranto, E. (2023) Antioxidant and Anti-tyrosinase Activities of *Halymenia durvillei* Water Extract Containing R-Phycoerythrin Before and After microencapsulation.

Khozin-Goldberg I, Cohen Z (2006) The effect of phosphate starvation on the lipid and fatty acid composition of the fresh water eustigmatophyte *Monodus subterraneus*. Phytochemistry 67:696–701.

Kleigrewe, K., Gerwick, L., Sherman, D. H., & Gerwick, W. H. (2016). Unique marine derived cyanobacterial biosynthetic genes for chemical diversity. *Natural product reports*, *33*(2), 348-364.

Krauspe, V., Fahrner, M., Spät, P., Steglich, C., Frankenberg-Dinkel, N., Maček, B. & Hess, W. R. (2021). Discovery of a small protein factor involved in the coordinated degradation of phycobilisomes in cyanobacteria. *Proceedings of the National Academy of Sciences*, *118*(5), e2012277118.

Krumbein, W. E., Cohen, Y., Shilo, M. 1977. Solar Lake (Sinai) 4. Stromatolitic cyanobacterial mats. Limnology and Oceanography 22:635–656.

Kühl, M., & Fenchel, T. (2000). Bio-optical characteristics and the vertical distribution of photosynthetic pigments and photosynthesis in an artificial cyanobacterial mat. Microbial Ecology, 40, 94-103.

Lall, A., & John, S. A. (2017). Cyanobacterial pigments analysis extracted from different sources of District Allahabad. *Journal of Pharmacognosy and Phytochemistry*, *6*(6), 1413-1415.

Lara, Y. J., McCann, A., Malherbe, C., François, C., Demoulin, C. F., Sforna, M. C., ... & Javaux, E. J. (2022). Characterization of the Halochromic Gloeocapsin Pigment, a Cyanobacterial Biosignature for Paleobiology and Astrobiology. *Astrobiology*, *22*(6), 735-754.

Lau, N. S., Matsui, M., & Abdullah, A. A. A. (2015). Cyanobacteria: photoautotrophic microbial factories for the sustainable synthesis of industrial products. *BioMed research international*, *2015*.

Lemasson, C., Marsac, N. T. D., & Cohen-Bazire, G. (1973). Role of allophycocyanin as light-harvesting pigment in cyanobacteria. *Proceedings of the National Academy of Sciences*, *70*(11), 3130-3133.

Liljenberg, C. and Kates, M. 1985. Changes in lipid composition of Oat root membranes as a function of water-deficit stress. Can J Biochem Cell Biol. 63: 77-84.

Liljenberg, C. and Von Arnold, S. 1987. Effects of physiological and ontogenetical ageing on membrane lipid levels in pea leaves (Pisum sativum). J Plant Physiol. 130: 497-509.

Lynch, F., Santana-Sánchez, A., Jämsä, M., Sivonen, K., Aro, E. M., & Allahverdiyeva, Y. (2015). Screening native isolates of cyanobacteria and a green alga for integrated wastewater treatment, biomass accumulation and neutral lipid production. Algal Research, 11, 411-420.

Mandal, M. K., Chanu, N. K., & Chaurasia, N. (2020). Cyanobacterial pigments and their fluorescence characteristics: applications in research and industry. In Advances in cyanobacterial biology (pp. 55-72). Academic Press.

Maneesh, G., & Siva, G. V. Extraction & Analysis of Algal Pigments by Thin Layer Chromotography & Spectrophotometric Analysis.

Meena, M., Swapnil, P., Zehra, A., Dubey, M. K., & Upadhyay, R. S. (2017). Antagonistic assessment of *Trichoderma* spp. by producing volatile and non-volatile compounds against different fungal pathogens. *Archives of Phytopathology and Plant Protection*, *50*(13-14), 629-648.

Mehnert, G., Rücker, J., Nicklisch, A., Leunert, F., & Wiedner, C. (2012). Effects of thermal acclimation and photoacclimation on lipophilic pigments in an invasive and a native cyanobacterium of temperate regions. European Journal of Phycology, 47(2), 182-192.

Nowruzi, B., & Porzani, S. J. (2021). Toxic compounds produced by cyanobacteria belonging to several species of the order Nostocales: A review. *Journal of Applied Toxicology*, *41*(4), 510-548.

Osório, C., Machado, S., Peixoto, J., Bessada, S., Pimentel, F. B., C. Alves, R., & Oliveira, M. B. P. (2020). Pigments content (chlorophylls, fucoxanthin and phycobiliproteins) of different commercial dried algae. *Separations*, *7*(2), 33.

Pagels, F., Pereira, R. N., Vicente, A. A., & Guedes, A. C. (2021). Extraction of pigments from microalgae and cyanobacteria—A review on current methodologies. Applied Sciences, 11(11), 5187.

Pagels, F., Salvaterra, D., Amaro, H. M., Lopes, G., Sousa-Pinto, I., Vasconcelos, V., & Guedes, A. C. (2020). Bioactive potential of *Cyanobium* sp. pigment-rich extracts. Journal of Applied Phycology, 32, 3031-3040.

Pagels, F., Vasconcelos, V., & Guedes, A. C. (2021). Carotenoids from cyanobacteria: Biotechnological potential and optimization strategies. *Biomolecules*, *11*(5), 735.

Palinska, K. A., Deventer, B., Hariri, K., & Lotocka, M. (2011). A taxonomic study on Phormidium–group (cyanobacteria) based on morphology, pigments, RAPD molecular markers and RFLP analysis of the 16S rRNA gene fragment. *Fottea*, *11*(1), 41-55.

Pandey, V. D., Pandey, A., & Sharma, V. (2013). Biotechnological applications of cyanobacterial phycobiliproteins. International Journal of Current Microbiology and Applied Sciences, 2(9), 89-97.

Patel, V. K., Das, A., Kumari, R., & Kajla, S. (2023). Recent progress and challenges in CRISPR-Cas9 engineered algae and cyanobacteria. *Algal Research*, 103068.

Prasanna, R., Sood, A., Jaiswal, P., Nayak, S., Gupta, V., Chaudhary, V., ... & Natarajan, C. (2010). Rediscovering cyanobacteria as valuable sources of bioactive compounds. Applied Biochemistry and Microbiology, 46, 119-134.

Pulz, O. (2001). Photobioreactors: production systems for phototrophic microorganisms. *Applied microbiology and biotechnology*, *57*, 287-293.

Puzorjov, A., & McCormick, A. J. (2020). Phycobiliproteins from extreme environments and their potential applications. *Journal of experimental botany*, *71*(13), 3827-3842.

Quinn, P. J., & Williams, W. P. (1983). The structural role of lipids in photosynthetic membranes. *Biochimica et Biophysica Acta (BBA)-Reviews on Biomembranes*, *737*(2), 223-266.

Renaudin, M., Darnajoux, R., & Bellenger, J. P. (2021). Quantification of moss-associated cyanobacteria using phycocyanin pigment extraction. *Frontiers in Microbiology*, *11*, 611792.

Řezanka, T., & Dembitsky, V. (2006). Metabolites produced by cyanobacteria belonging to several species of the family Nostocaceae. Folia Microbiologica, 51(3), 159–182.

Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M., & Stanier, R. Y. (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Microbiology*, *111*(1), 1-61.

Sankhalker, S. 2000. Photoinhibition of photosynthesis and possible role of xanthophyll cycle in protection against photodamage in sorghum seedlings. Ph. D. thesis, Goa University, Goa

Sanmartín, P., Aira, N., Devesa-Rey, R., Silva, B., & Prieto, B. (2010). Relationship between color and pigment production in two stone biofilm-forming cyanobacteria (*Nostoc* sp. PCC 9104 and *Nostoc* sp. PCC 9025). Biofouling, 26(5), 499-509.

Sharma, P. K., & Hall, D. O. (1996). Effect of photoinhibition and temperature on carotenoids in sorghum leaves. *Indian journal of biochemistry & biophysics*, *33*(6), 471-477.

Simis, S. G., Ruiz-Verdú, A., Domínguez-Gómez, J. A., Peña-Martinez, R., Peters, S. W., & Gons, H. J. (2007). Influence of phytoplankton pigment composition on remote sensing of cyanobacterial biomass. *Remote Sensing of Environment*, *106*(4), 414-427.

Singh, N. K., Dhar, D. W., & Tabassum, R. (2016). Role of cyanobacteria in crop protection. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *86*, 1-8.

Smith GD, Doan NT (1999) Cyanobacterial metabolites with bioactivity against photosynthesis in cyanobacteria, algae and higher plants. J Appl Phycol 11:337–344.

Smith, G. D., & Thanh Doan, N. (1999). Cyanobacterial metabolites with bioactivity against photosynthesis in cyanobacteria, algae and higher plants. Journal of Applied Phycology, 11, 337-344.

Sobiechowska-Sasim, M., Stoń-Egiert, J., & Kosakowska, A. (2014). Quantitative analysis of extracted phycobilin pigments in cyanobacteria—an assessment of spectrophotometric and spectrofluorometric methods. Journal of Applied Phycology, 26, 2065-2074.

Sorrels, C. M., Proteau, P. J., & Gerwick, W. H. (2009). Organization, evolution, and expression analysis of the biosynthetic gene cluster for scytonemin, a cyanobacterial UV-absorbing pigment. Applied and Environmental Microbiology, 75(14), 4861-4869.

Stal, LJ (1995) Physiological ecology of cyanobacteria in microbial mats and other communities. New Phytol 131, 1–32.

Strieth, Dorina, Judith Stiefelmaier, Björn Wrabl, Julia Schwing, Andrea Schmeckebier, Sarah Di Nonno, Kai Muffler, and Roland Ulber. "A new strategy for a combined isolation of EPS and pigments from cyanobacteria." *Journal of Applied Phycology* 32 (2020): 1729-1740.

Thevarajah, B., Nishshanka, G. K. S. H., Premaratne, M., Wasath, W. A. J., Nimarshana, P. H. V., Malik, A., & Ariyadasa, T. U. (2023).Cyanobacterial pigment production in wastewaters treated for heavy metal removal: Current status and perspectives. *Journal of Environmental Chemical Engineering*, *11*(1), 108999.

Tomer, A. K., Neelam, D. K., & Dadheech, P. K. (2018). Pigments profiling of non-heterocystous filamentous cyanobacterial taxa (oscillatoriales) inhabited in biological crusts and soda lake. *Vegetos*, *31*(1), 43-50.

Tomitani, A., A.H. Knoll, C.M. Cavanaugh and Ohno, T. 2006. The evolutionary diversification of cyanobacteria: molecular-phylogenetic and paleontological perspectives. PNAS 103(14): 5442-5447.

Turnham, E. and Northcote, D. H. 1984. The incorporation of (1-14C) acetate in to lipids during embryogenesis in oil palm tissue cultures. Phytochem. 23: 35-39.

Vermaas, W. F., Timlin, J. A., Jones, H. D., Sinclair, M. B., Nieman, L. T., Hamad, S. W., ... & Haaland, D. M. (2008). In vivo hyperspectral confocal fluorescence imaging to determine pigment localization and distribution in cyanobacterial cells. Proceedings of the National Academy of Sciences, 105(10), 4050-4055.

Watanabe, S., Stazic, D., Georg, J., Ohtake, S., Sakamaki, Y., Numakura, M. & Hess, W. R. (2023). Regulation of RNase E during the UV stress response in the cyanobacterium *Synechocystis* sp. PCC 6803. *Life*, *2*(1), 43-57.

Waterbury, J. B., S.W. Watson, R.R.L. Guillard and Brand, LE 1979. Widespread occurrence of a unicellular, marine, planktonic cyanobacterium. Nature 227: 293-294.

Wynn-Williams, D. D., H. G. M. Edwards, E. M. Newton, and J. M. Holder. 2002. Pigmentation as a survival strategy for ancient and modern photosynthetic microbes under high ultraviolet stress on planetary surfaces. *Int. J. Astrobiol.*1:39-49.

Zavarzin, G. A. (2001). The role of biota in global climate change. *Russian Journal of Plant Physiology*, *48*(2), 265-272.