

**DIVERSITY AND SEASONAL DYNAMICS OF
CYANOBACTERIAL ASSEMBLAGE IN PADDY FIELDS OF
MIZORAM, INDIA**

**A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY**

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**DEPARTMENT OF BOTANY
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ASSEMBLAGE IN PADDY FIELDS OF MIZORAM, INDIA**

BY

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Submitted

**In partial fulfillment of the requirement of the Degree of Doctor of Philosophy
in Botany of Mizoram University, Aizawl**



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CERTIFICATE

This is to certify that **Ms. Ruthi Lalmuanzeli**, Registration No. **MZU/Ph.D/1112 of 03.05.2018** has submitted the thesis entitled “**Diversity and Seasonal Dynamics of Cyanobacterial Assemblage in Paddy Fields of Mizoram, India**” under my supervision, to fulfill the requirements for award of the Degree of Doctor of Philosophy in the Department of Botany, Mizoram University, Aizawl. The work is authentic, content of the thesis is the original work of the Research Scholar, and the nature and the presentation of the work are the first of its kind.

It is further certified that no portion (s) or part (s) of the content of the thesis has been submitted for any degree in Mizoram University or any other University or Institute.

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PREFACE

This thesis presents a detailed investigation into the diversity of cyanobacteria within paddy fields in Mizoram, India, spanning three distinct agroecological zones: Champhai at 1678 m, Thenzawl at 786 m, and Kolasib at 222 m above sea level. Cyanobacteria, often referred to as blue-green algae, are pivotal in agricultural ecosystems because of their ability to fix atmospheric nitrogen, thereby naturally enhancing soil fertility. Despite their significance, the diversity and ecological roles of these microorganisms in tropical rice-growing regions, particularly across varying altitudes, remain largely underexplored. This study sought to bridge this gap by examining the diversity and distribution of cyanobacteria, analyzing the influence of environmental factors such as altitude, soil and water properties, heavy metal concentrations, macronutrient levels, and seasonal variations, and evaluating their potential as biofertilizers to support sustainable agriculture.

This research was conducted using an integrated approach that combined field surveys, laboratory analyses, statistical evaluations, and advanced molecular techniques, including 16S rRNA sequencing. Fieldwork was carried out during the growing season (July–September) and post-harvest period (November–December) across replicate plots in each zone to capture the dynamic interplay between cyanobacteria and their environment. The results revealed rich diversity, with 76 cyanobacterial species identified from 27 genera and 14 families, of which 70 species were new regional records, absent from prior Mizoram surveys. Highland regions, such as Champhai, exhibited greater species richness, dominated by nitrogen-fixing heterocystous forms such as *Nostoc* and *Anabaena*, thriving in stable, nutrient-poor conditions. In contrast, the lowland Kolasib showed a higher cell abundance but lower diversity, with non-heterocystous species such as *Oscillatoria* prevailing in nutrient-rich, fluctuating environments. These findings highlight the ecological adaptability of cyanobacteria and their capacity to contribute up to 25 kg of nitrogen per hectare annually, thus offering a viable alternative to synthetic fertilizers.

Undertaken under the aegis of the Department of Botany, Mizoram University, and with the invaluable guidance of Sr. Professor Surya Kant Mehta, this work owes its completion to the support of colleagues, funding agencies, and the cooperation of the local farming communities in Mizoram. The insights gained not only deepen our understanding of microbial ecology in tropical agricultural systems but also lay the groundwork for practical applications in soil health management. It is my hope that this research will encourage further studies on long-term microbial monitoring and the development of cyanobacterial biofertilizers, fostering sustainable farming practices that benefit both the environment and the agricultural communities of Mizoram and beyond.

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ABBREVIATION

AAS	Atomic adsorption spectroscopy
AMF	Arbuscular mycorrhizal fungi
BIC	Bayesian Information Criterion
BLAST	Basic Local Alignment Search Tool
BNF	Biological nitrogen fixation
CTAB	Cetyltrimethylammonium bromide
Cu	Copper
D	Simpsons Diversity Index
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
DTL	Digestion Tube Large
EC	Electrical Conductivity
EC	Electrical Conductivity
EDTA	Ethylenediaminetetraacetic acid
EPS	Extra polymeric substances
Fe	Iron
G	Gamma Distributed
H'	Shannon Diversity Index
K	Potassium
K2	Kimura-2-Parameter
MEGA	Molecular Evolutionary Genetics Analysis
ML	Maximum Likelihood
Mn	Manganese
N	Nitrogen
NCBI	National Center for Biotechnology Information
ND	Undetectable

NGS	Next generation sequencing
P	Phosphorus
Pb	Lead
PCA	Principal Component Analysis
PCI	Phenol chloroform isoamyl alcohol
PCR	Polymerase Chain Reaction
rRNA	Ribosomal ribonucleic acid
SDS	Sodium dodecyl sulfate
SPSS	Statistical Package for Social Sciences
TDS	Total Dissolved Solids
TE Buffer	Tris-EDTA Buffer
Tris HCl	Tris hydrochloride
UV radiation	Ultra -Violet radiation
Zn	Zinc

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CHAPTER - 1

INTRODUCTION

1.1. Cyanobacteria and their Ecological Significance

Cyanobacteria, a diverse group of oxygen-evolving photosynthetic prokaryotes, have gained significant scientific attention owing to their ecological importance and potential biotechnological applications. These microorganisms are considered one of the most primordial forms of life on Earth, with fossil evidence suggesting that their existence dates back to approximately 3.5 billion years. They play a vital role in shaping Earth's biosphere (Shestakov and Karbysheva, 2017). Oxygen-yielding photosynthesis performed by cyanobacteria is not only a key factor in the Great Oxygenation Event, but continues to influence carbon and nitrogen cycles in present-day ecosystems. Studies have demonstrated that cyanobacteria occupy a wide range of ecological habitats, including freshwater, marine, terrestrial, and extreme habitats. This ubiquitous distribution is attributed to its remarkable adaptability and metabolic flexibility (Gaysina et al. 2019).

Cyanobacteria play a critical role in global primary productivity, and are essential for biogeochemical cycling. Research has emphasized their significance in nitrogen fixation, particularly in nutrient-limited environments, such as rice paddies, where they form symbiotic relationships with plants and other microorganisms (Paerl and Paul, 2012; Thomas and Litchman, 2016). Studies indicate that cyanobacterial biofilms and mats not only facilitate nitrogen assimilation, but also enhance soil texture and fertility, thereby influencing plant growth and yield. Research has demonstrated that their ecological functions extend beyond nitrogen fixation, as they also participate in carbon sequestration, phosphate solubilization, and the synthesis of bioactive compounds with antimicrobial properties (Bozan et al., 2024; Kollmen and Strieth, 2022).

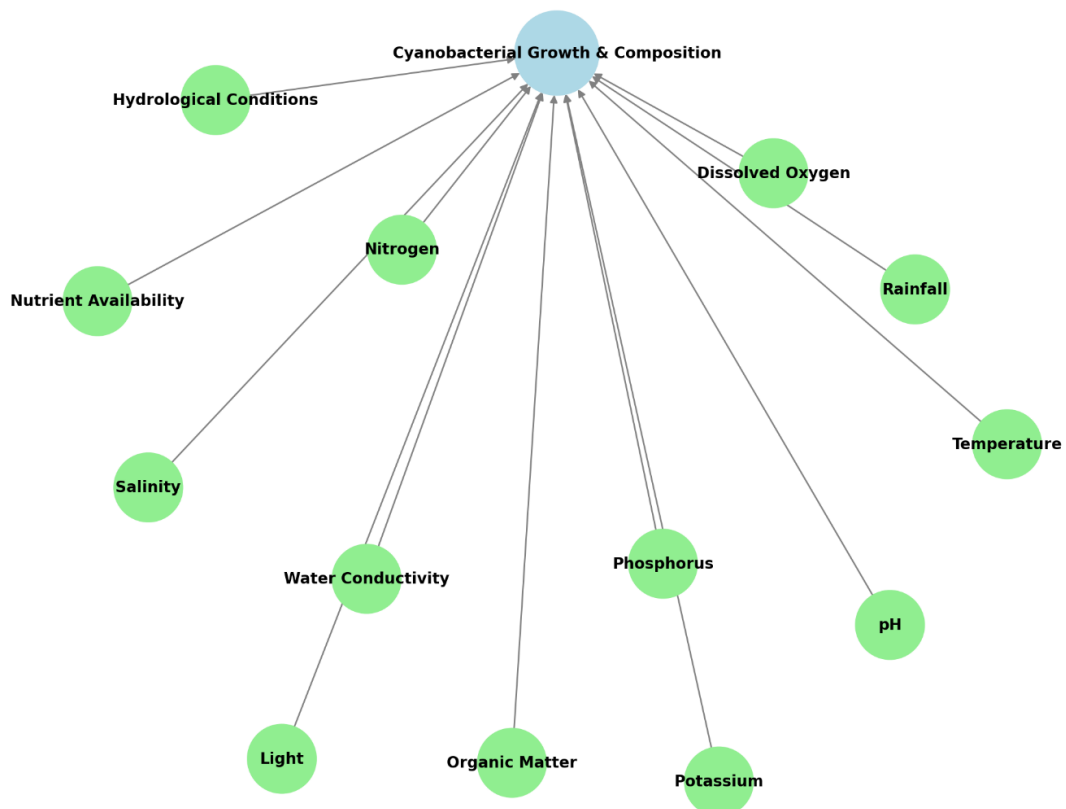


Figure 1. Factors affecting cyanobacterial growth and composition. The diagram illustrates the various environmental and chemical factors that influence cyanobacterial abundance and diversity in an ecosystem.

Moreover, cyanobacteria have been shown to exert both positive and negative effects on aquatic ecosystems. Although photosynthetic activity supports food chains and oxygen production, certain species can form blooms, resulting in eutrophication and toxin production (Sukharevich and Polyak, 2020). This dual nature warrants a comprehensive understanding of cyanobacterial diversity, particularly in agricultural ecosystems where its impact on water quality and soil health can have significant implications. Various factors affecting cyanobacterial growth and composition are shown in Figure 1.

1.2.Cyanobacteria in Paddy Field Ecosystems - Their Functional and Molecular Diversity

Rice paddies constitute a distinctive semi-aquatic agricultural environment in which microorganisms, including cyanobacteria, play critical roles in soil fertility and crop yield. Research has demonstrated that paddy soils harbor a diverse array of cyanobacteria ranging from unicellular forms, such as *Synechococcus* and *Gloeocapsa*, to nitrogen-fixing filamentous genera, including *Nostoc*, *Anabaena*, and *Aulosira* (Ammar et al., 2022; Chittora et al., 2020). The ability of these organisms to fix atmospheric nitrogen using specialized heterocyst cells is well established, with studies indicating that biological nitrogen fixation (BNF) by cyanobacteria can contribute between 20 and 60 kg N ha⁻¹ per growing season, significantly reducing the requirement for synthetic fertilizers (Singh et al., 2020).

Moreover, cyanobacteria have been demonstrated to enhance soil organic matter and microbial biomass by secreting extracellular polymeric substances (EPS), which contribute to improved soil structure and water-retention capacity. This process is particularly advantageous in regions characterized by variable water conditions, as rice fields undergo alternating cycles of flooding and desiccation. Research has indicated that cyanobacterial-induced soil amendments promote plant health by increasing nutrient availability and inhibiting the growth of pathogenic microorganisms (Bibi et al., 2024; Mota et al., 2021).

The genetic and functional diversity of cyanobacteria have been thoroughly investigated using both traditional and molecular methods. Historically, microscopic

examinations have provided morphological classifications based on cell structure, arrangement, and pigmentation. However, advances in molecular biology, particularly 16S rRNA gene sequencing and metagenomic techniques, have improved our understanding of cyanobacterial taxonomy and phylogenies (Komárek, 2010). Research has demonstrated that conventional morphological identification often underestimates cyanobacterial diversity because numerous species exhibit subtle variations that are detectable only at the genomic level.

Multiple studies employing next-generation sequencing (NGS) techniques have revealed a remarkable level of cyanobacterial diversity in rice paddies, with metagenomic analyses revealing distinct seasonal changes in the community composition (Clagnan et al., 2024). Research indicates that environmental factors, such as pH, temperature, and nutrient fluctuations, determine these temporal patterns, influencing the prevalence of specific cyanobacterial groups during different rice growth phases. It has been established that combining high-throughput molecular approaches with conventional methods is essential for accurately describing cyanobacterial communities and their ecological functions.

Cyanobacteria have attracted considerable attention because of their substantial contribution to sustainable agricultural practices, particularly organic farming, and integrated nutrient management strategies. Numerous studies have elucidated their potential as biofertilizers, highlighting their capacity to enhance soil fertility, while mitigating the environmental impacts associated with chemical fertilizers (Nawaz et al., 2024). Research has indicated that cyanobacterial inoculants can augment rice production by increasing nitrogen availability in the soil, promoting root development, and enhancing plant stress tolerance. The contribution of cyanobacteria to sustainable agriculture is shown in Figure 2.

Furthermore, recent investigations have highlighted the promising applications of cyanobacteria in bioremediation. Their capacity to sequester heavy metals and degrade organic pollutants has been successfully implemented in contaminated agricultural fields (Lourembam et al., 2024). Researchers have also explored the use of cyanobacterial-based biofilms and biostimulants as environmentally sustainable alternatives to synthetic pesticides.



Figure 2. Contributions of cyanobacteria to sustainable agriculture. This diagram illustrates the key roles of cyanobacteria in paddy fields, including nitrogen fixation, organic matter enhancement, improved nutrient availability, promotion of microbial diversity, biocontrol of pathogens, and improved water retention, all of which contribute to sustainable farming practices.

1.3.Cyanobacteria in Agroecosystems

In agricultural ecosystems, particularly rice paddies, cyanobacteria are indispensable organisms that greatly affect soil fertility, nitrogen cycling, and overall productivity. These microorganisms function as natural biofertilizers, contribute to nitrogen fixation, and participate in complex interactions within the soil microbiome, making them crucial for sustainable farming practices. Research has demonstrated its importance in enhancing soil structure, increasing nutrient availability, and promoting plant growth (Ramakrishnan et al.; 2023; Singh et al.; 2016). The diverse benefits of cyanobacteria in agricultural systems highlight their significance as key components in maintaining and improving ecosystem health and crop yields.

1.3.1. Cyanobacteria as Biofertilizers in Paddy Fields

Extensive research has been conducted on utilizing cyanobacteria as biofertilizers in rice paddies, owing to their capacity to enhance soil fertility and decrease dependency on chemical fertilizers. Studies have demonstrated that cyanobacterial inoculant-based biofertilizers substantially improve the soil organic content and supply essential nutrients for crop development (Hakkoum et al. 2025). The exceptional potential of cyanobacteria as biofertilizers is rooted in their ability to fix nitrogen from the atmosphere, enhance phosphorus solubility, and secrete growth-promoting compounds including phytohormones (Yadav et al., 2022).

Research indicates that introducing cyanobacteria into paddy fields can decrease the requirement for synthetic nitrogen fertilizers by 20–30%, resulting in lower production costs and reduced environmental contamination (Hashem, 2001). Furthermore, studies have revealed that cyanobacteria contribute to long-term soil health by improving the soil structure and water retention, which is particularly advantageous in drought-prone environments (Singh et al., 2016).

Numerous studies have examined the efficacy of various cyanobacterial strains in biofertilizer formulations. Species such as *Nostoc*, *Anabaena*, and *Aulosira* are commonly used because of their high nitrogen-fixing efficiency and suitability for paddy-field conditions (Álvarez et al., 2023; Dasgupta et al., 2021). Additionally,

research has demonstrated that mixed cyanobacterial inoculants offer greater benefits than single-strain applications (Chaudhary et al., 2023).

The utilization of cyanobacteria-based biofertilizers not only promotes soil health but also enhances plant growth and yield. Studies have indicated that rice plants inoculated with cyanobacteria exhibit enhanced root growth, increased biomass production, and higher grain yields than untreated controls (Ranjan et al., 2016). Moreover, cyanobacteria have been found to improve the resistance of rice plants to abiotic stresses, such as drought and salinity, which is particularly noteworthy given the increasing impact of climate change on agricultural productivity (Nawaz et al., 2024).

1.3.2. The Impact on Nitrogen Fixation and Soil Fertility

Nitrogen, a vital element for plant development, directly affects crop yield through its presence in soil. Cyanobacteria play a substantial role in nitrogen cycling within paddy fields through biological nitrogen fixation (BNF). This process transforms atmospheric nitrogen (N_2) into bioavailable ammonia (NH_3) by utilizing nitrogenase (Bellenger et al., 2020). Research indicates that cyanobacteria can provide 20–60 kg N ha⁻¹ per growing season, thus reducing the requirement for synthetic fertilizers (Singh et al., 2020).

The efficiency of nitrogen fixation by cyanobacteria varies with the species and environmental factors. Nitrogen-fixing filamentous forms possess specialized cells called heterocysts, which protect the oxygen-sensitive nitrogenase activity from atmospheric oxygen (Kumar et al., 2010). Heterocystous cyanobacteria can maintain nitrogen fixation in flooded environments, making them well-suited for paddy field ecosystems (Dash et al., 2017; Irisarri, 2001).

In addition to nitrogen fixation, cyanobacteria also enhance soil fertility through various processes. Research has demonstrated that cyanobacteria improve soil structure by producing extracellular polymeric substances (EPS), which facilitate soil aggregation and enhance water retention (Nishanth et al., 2021). Moreover, studies have indicated that cyanobacteria contribute to phosphorus solubilization, which is a

key process that renders inorganic phosphorus accessible for plant absorption (Afkairin et al., 2021).

Studies have shown that cyanobacteria promote microbial interactions that enhance soil nutrient cycling. Their metabolic processes produce organic substrates that support the growth of beneficial soil microorganisms including diazotrophic bacteria and mycorrhizal fungi (Chua et al. 2020; Srivastava et al. 2021). In particular, arbuscular mycorrhizal fungi (AMF) are crucial for increasing stress tolerance, plant growth, and soil nutrient uptake. Cyanobacteria can contribute to the success of mycorrhizal associations in several ways. Studies have revealed that extracellular polymeric substances (EPS) produced by cyanobacteria enhance soil porosity and aeration, facilitating the establishment of fungal hyphae in paddy soils (Alvarez et al., 2021; Begum et al., 2019). By solubilizing phosphate compounds and increasing bioavailable phosphorus in the soil, cyanobacteria improve phosphorus availability, which is essential for AMF colonization (Tian et al., 2021). Multiple studies have shown that AMF exhibit higher colonization rates in phosphorus-rich soils, suggesting that cyanobacteria may indirectly support their establishment and function (Bi et al., 2022). Research indicates that plants associated with cyanobacteria often demonstrate increased root exudation of organic compounds, which attracts mycorrhizal fungi and promotes symbiotic interactions (Cao et al., 2018).

Furthermore, cyanobacteria can inhibit soil-borne pathogens by producing antimicrobial compounds. Studies have demonstrated that extracts derived from cyanobacteria exhibit antifungal and antibacterial properties against plant pathogens, such as *Fusarium*, *Rhizoctonia*, and *Xanthomonas* species, thereby reducing the incidence of soil-borne diseases in rice cultivation systems (Righini and Roberti, 2019; Shah et al., 2021). These findings suggest that cyanobacteria not only contribute to soil fertility, but also play a role in plant defense mechanisms, rendering them a significant component in integrated pest management strategies.

1.3.3. Impact on Paddy Soil Microbiome and Productivity

The soil microbiome is crucial for agroecosystem function. Cyanobacteria have been identified as essential components of paddy soil microbiomes that interact with various

microbial communities, including bacteria, archaea, fungi, and protozoa (Singh et al., 2018). Research has indicated that these interactions significantly affect soil ecosystem stability and rice productivity.

Metagenomic and high-throughput sequencing studies have demonstrated that cyanobacteria exist together with diverse microbial assemblages in paddy soils and form intricate nutrient-exchange networks (Clagnan et al., 2024). Cyanobacteria have been found to influence the composition and activity of microbial consortia by releasing organic compounds that act as carbon and nitrogen sources for heterotrophic microbes (Subashchandrabose et al., 2011). This interaction enhances soil enzymatic activity, resulting in improved nutrient mineralization and bioavailability.

Research has shown that cyanobacterial biofilms form microhabitats that promote beneficial microbial populations, enhance soil stability, and mitigate the negative effects of soil degradation (Rossi , 2020). These biofilms have also been found to aid in soil moisture retention, which is particularly advantageous in rain-fed rice systems with fluctuating water availability (Nawaz et al., 2024).

The presence of cyanobacteria in paddy soils is associated with enhanced crop productivity. Research has revealed that fields with high cyanobacterial diversity exhibit improved soil health indicators such as higher organic carbon content, better cation exchange capacity, and greater microbial biomass (Abinandan et al., 2019). These enhancements have been shown to result in increased rice yields and superior crop quality, underscoring the significance of cyanobacteria in sustainable agriculture.

1.4. Paddy Field Ecosystem and Its Functions

Paddy fields constitute a distinctive and dynamic agroecosystem that hosts a diverse array of microorganisms including cyanobacteria, which are essential for soil fertility, nutrient cycling, and ecosystem equilibrium. In contrast to typical terrestrial environments, rice paddies undergo alternating periods of submersion and desiccation, resulting in unique ecological processes that determine microbial interactions, biogeochemical flow, and crop development (Bwire et al., 2024).

Paddy fields are unique semi-aquatic ecosystems characterized by stagnant water, fluctuating oxygen levels, and varying nutrient availability. Research has

demonstrated that microbial communities in these environments are well adapted to anoxic and submerged conditions, and are essential for soil nutrient transformations (Kirk et al., 2014; Vromant and Chau, 2005). The primary ecological features of paddy fields include the following:

Wet and dry cycle alternation

The flooded phase results in limited oxygen availability, creating anaerobic conditions that affect the microbial metabolism and biogeochemical cycling (Liesack et al. 2000). During the post-harvest dry phase, soil microbial communities shift to aerobic processes, influencing nitrogen mineralization and organic matter decomposition (Alghamdi and Cihacek, 2022).

Nutrient-abundant environment

Paddy fields receive nutrients from fertilizers, organic amendments, and biological nitrogen fixation, thereby promoting a highly productive environment for microbial growth (Prasanna et al., 2015). Cyanobacteria play a significant role in nutrient enhancement by fixing atmospheric nitrogen and releasing bioavailable compounds into soil (Bhardwaj et al., 2024; Joshi et al., 2020).

Microbial diversity hubs

Research has demonstrated that Paddy fields contain diverse microbial communities, including bacteria, archaea, fungi, and protists, which interact with complex food webs (Murase and Asiloglu, 2024). Studies have indicated that microbial interactions influence soil fertility, greenhouse gas emissions, and disease prevention in rice agroecosystems (Muhammad et al., 2022; Prasanna et al., 2012).

1.5. Enhancement of Soil Fertility and Nutrient Cycling

The key function of paddy fields is to sustain and improve soil fertility. Research has demonstrated that microbial populations, particularly cyanobacteria, are pivotal for regulating soil nitrogen and organic matter levels (Liang et al., 2024). Cyanobacteria and other diazotrophs convert atmospheric nitrogen (N_2) into ammonium (NH_4^+), which is easily absorbed by rice plants. Evidence suggests that nitrogen fixation by heterocystous cyanobacteria substantially contributes to the nitrogen balance in paddy

fields (Song et al., 2021). Microbial activity in the soil facilitates the decomposition of organic matter and releases nutrients, such as nitrogen, phosphorus, and potassium (Wei et al., 2024). Cyanobacteria and phosphate-solubilizing bacteria enhance phosphorus accessibility by degrading insoluble phosphorus compounds, thereby improving plant nutrient uptake efficiency (Kishore et al., 2015).

1.5.1. Role in Carbon Sequestration and Greenhouse Gas Regulation

Rice paddies play a crucial role in the dynamics of global carbon and methane (CH₄) fluxes. These agricultural systems function as carbon sinks through photosynthetic processes and accumulation of organic matter. However, they are major contributors to methane emissions owing to anaerobic decomposition that occurs in waterlogged soils (Cao et al., 1996; Naser et al., 2019; Zhang et al., 2016). Cyanobacteria contribute to carbon sequestration by capturing CO₂ from the atmosphere and converting it into biomass (Jacob-Lopes et al., 2014; Upendar et al., 2018). The microbial communities in these ecosystems regulate methane emissions through methanogenesis and oxidation, thereby influencing the balance of greenhouse gas emissions (Kharitonov et al., 2021). Various management strategies, such as periodic flooding and the application of organic amendments, can help reduce methane emissions while preserving soil fertility (Cowan et al., 2021; Qian et al., 2022).

1.5.2. Water Retention and Erosion Control

Rice paddies possess intrinsic water-retaining properties that mitigate the risks of drought stress during rice farming. Research indicates that microbial communities, particularly cyanobacterial mats, are essential for stabilizing soils and preventing erosion (Rossi and Philippis, 2015; Thapa et al., 2017; Mager and Thomas, 2011). These microorganisms help maintain the soil structure by minimizing nutrient loss through the preservation of stable microbial communities that control nitrogen and phosphorus retention in both soil and water (Abinandan et al., 2019; Nisha et al., 2018).

1.6. Seasonal Dynamics of Cyanobacteria

The seasonal patterns of cyanobacteria are influenced by several environmental factors. Studies have shown that temperature, light, nutrients, and pH have significant

effects on cyanobacterial growth and community patterns. Hydrological factors, such as rainfall, flooding, and water retention in rice paddies, also play important roles in bacterial community patterns (Cho and Oki, 2012; Ding et al., 2019; Haefele et al., 2006). These factors interact in complex ways to shape the cyanobacterial dynamics.

Cyanobacterial growth and distribution are primarily influenced by temperature. Various species have different optimal temperature ranges. Research indicates that cyanobacteria in tropical paddy fields thrive at temperatures between 25°C and 35°C. Filamentous heterocystous genera, such as *Anabaena* and *Nostoc*, showed maximum nitrogen fixation rates within this range (Amin et al., 2024; Singh et al., 2018; Thomas and Litchman, 2016). However, temperature variations, particularly extreme heat stress, decrease the prevalence of certain cyanobacterial species while promoting heat-tolerant strains such as *Oscillatoria* and *Phormidium* (Saini et al., 2021; Singh et al., 2012).

Light intensity and photoperiod significantly affected metabolic activities, including photosynthesis. Multiple studies have shown that cyanobacteria exhibit light-dependent niche specialization, with species inhabiting shallow waters and surface areas adapted to high light levels, whereas those associated with sediments thrive in low-light environments (Khajepour et al., 2015; Niangoran et al., 2021; Zevenboom and Mur, 1984). Some cyanobacteria can withstand extended UV radiation exposure owing to the presence of protective pigments, such as scytonemin and mycosporine-like amino acids (Fuentes-Tristan et al., 2019).

N and P availability in paddy fields strongly regulate the composition of the cyanobacterial community. Studies have demonstrated that nitrogen-fixing cyanobacteria such as *Aulosira*, *Anabaena*, and *Tolypothrix* predominate under nitrogen-deficient conditions. However, non-diazotrophic cyanobacteria thrive mostly in nitrogen-rich environments (Dasgupta et al., 2021; Ehrenfels et al., 2021; Levine and Schindler, 1999; Pelechata et al., 2016). Conversely, phosphorus scarcity causes shifts in the community composition. Phosphorus limitation favours growth and dominance of species capable of utilizing organic phosphorus sources through the production of phosphatase enzymes (Cembella et al., 1984; Jin et al., 2022).

Soil and water pH are critical for cyanobacterial growth. Research indicates that most cyanobacterial species grow at higher rates under mildly alkaline conditions (pH 7–8) (Fecteau et al., 2022; Nayak, 2007). Furthermore, pH fluctuations resulting from seasonal changes in organic matter decomposition and microbial respiration can affect the cyanobacterial diversity in paddy fields (Li et al., 2019).

Precipitation and the associated hydrological changes influence cyanobacterial dynamics in paddy fields. Monsoon rains cause variations in water levels, nutrient runoff, and soil moisture, thereby affecting cyanobacterial populations (Gao et al., 2024; Limaye et al., 2010). Inundation during early rice growth promotes planktonic and mat-forming cyanobacteria, whereas post-harvest desiccation favours soil-dwelling and drought-resistant species like *Scytonema* and *Microcoleus* (Hallenbeck, 2017). Intense rainfall can dilute and disperse cyanobacterial biomass and change community patterns. Extended aridity causes desiccation stress, leading to dormant stages such as akinetes (Hori et al., 2003; Reichwaldt and Ghadouani, 2012). Periodic inundation affects cyanobacterial composition and modifies the nitrogen fixation rates and microbial interactions within the soil (Randle-Boggis et al., 2018).

Along the rice-growing cycle, cyanobacterial communities show distinct seasonal changes. Research has shown that nitrogen-fixing cyanobacteria are more prevalent during the early stages of rice growth (Wang et al., 2016). Studies indicate that cyanobacterial diversity decreases after harvest due to reduced moisture and organic matter decomposition during the postharvest phase. However, cyanobacteria, such as *Nostoc*, can grow at higher rates in dry soil crusts (Song et al., 2005). Studies have found that the transition from the monsoon to the dry season is characterized by a shift from planktonic to benthic cyanobacterial dominance, suggesting their capacity to adapt to changing environmental conditions (Liu et al., 2019).

Liu et al. (2019) showed that cyanobacterial populations change seasonally in response to water conditions and nutrient levels. Carey et al. (2012) studied the species composition, nitrogen-fixing activity, and ecological interactions in paddy fields. Recent studies using metagenomic techniques have identified seasonal shifts in cyanobacterial diversity. High-throughput sequencing has revealed clear seasonal succession patterns in cyanobacterial communities (Bertos-Fortis et al., 2016; Li et al.,

2019). Research has demonstrated that cyanobacterial diversity peaks during warm and rainy months, and decreases during dry or cold periods in both temperate and tropical regions (Jargal and An, 2023; Williams et al., 2018).

Rice cultivation methods influence cyanobacterial dynamics. Organic farming and minimal tillage support greater cyanobacterial diversity and nitrogen fixation than intensive farming using synthetic fertilizers and pesticides (Tahat et al., 2020). Additionally, climate change-related fluctuations in temperature and precipitation can modify the seasonal cyanobacterial dynamics in rice paddies (Song et al., 2005).

1.7. Physico-Chemical Properties of Paddy Soil and Water

Physicochemical properties significantly impact microbial community structure, diversity and abundance in paddy soils and water. These properties affect the microbial metabolism, nutrient cycles, and ecological relationships. Research has shown that changes in physicochemical characteristics of soil and water substantially affect the composition and function of cyanobacterial communities (Lu et al., 2013; Song et al., 2005). Fluctuations in factors, such as pH, organic matter content, nutrient levels, and water quality, influence microbial community dynamics (Bissett et al. 2013). Nitrogen-fixing cyanobacteria thrive in nitrogen-poor soils, while non-diazotrophic species predominate in nitrogen-rich environments (Ehrenfels et al., 2021 Sukenik et al. 2015). Water conductivity, salinity, and dissolved oxygen concentrations may affect the growth of various cyanobacterial taxa and influence their ecological functions in paddy fields (Alghanmi and Jawad, 2019). Studies have highlighted the importance of long-term monitoring of soil and water parameters to evaluate cyanobacterial community resilience under changing climatic and agricultural conditions (Nawaz et al., 2024). The various factors influencing the algal assemblages in paddy fields are shown in Figure 3.

As described in following paragraphs, the pH, organic content, nitrogen, phosphorus, potassium, water conductivity, dissolved oxygen, and salinity, play a vital role in determining the composition and dynamics of cyanobacterial community (Alghanmi and Jawad, 2019; Cho and Oki, 2012; Miranda and Krishnakumar, 2015).

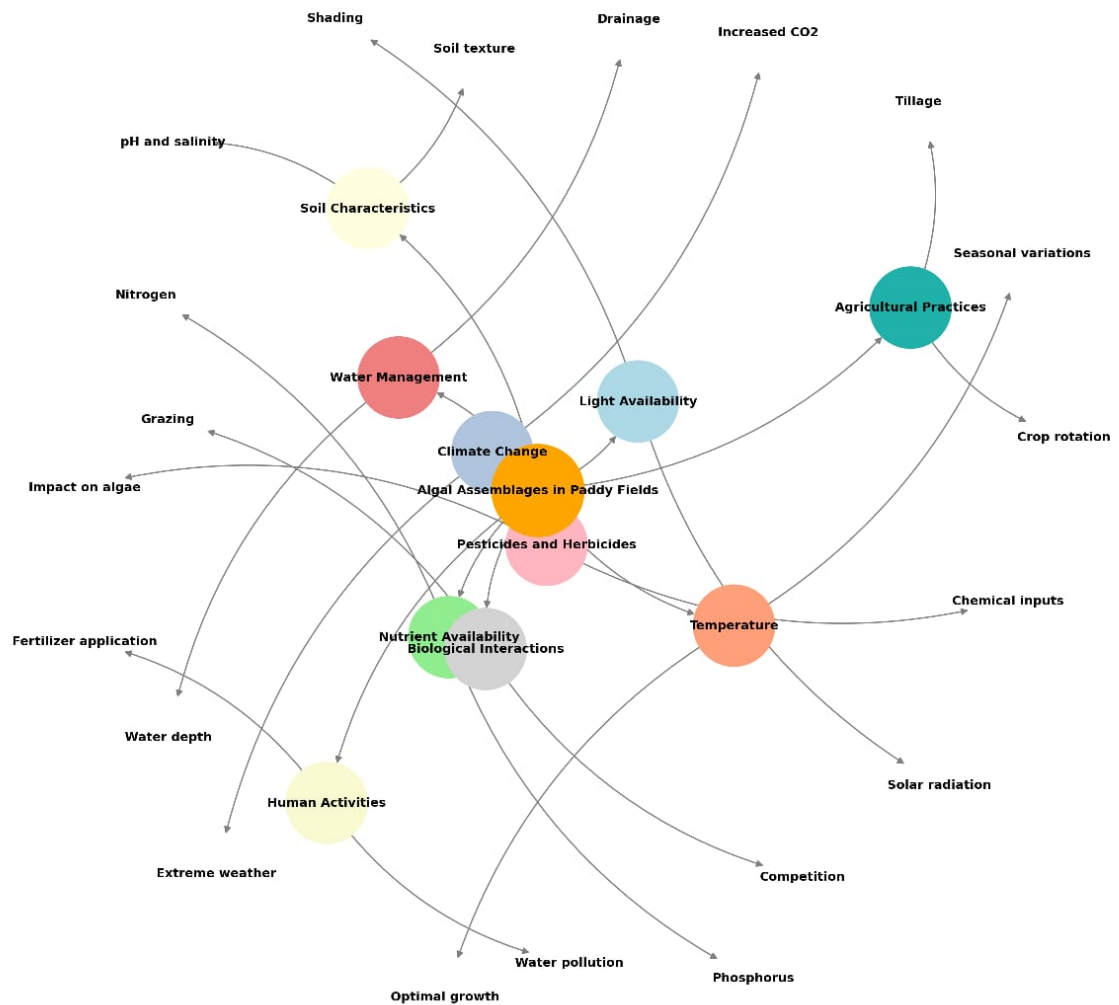


Figure 3. Factors influencing Algal Assemblages in Paddy fields (Color-Coded Radial Layout)

pH

The acidity or alkalinity of the soil considerably influences the composition and dynamics of the microbial communities. Soil pH regulates the availability of nutrients (macronutrients and micronutrients) and enzyme activity. Cyanobacteria typically prefer neutral to mildly alkaline conditions (pH 7.0–8.5) for optimal growth. However, certain cyanobacterial species are well-adapted to extremely acidic or alkaline environments (Fecteau et al., 2022; Nayak, 2007). Studies have suggested that changes in pH can modify cyanobacterial diversity and promote the growth of acidophilic or alkali-tolerant taxa under extreme conditions (Li et al., 2019).

Organic matter

The organic matter content determines the microbial activity in the soil, which in turn affects the nutrient cycling and soil structure. Research has demonstrated that increased levels of organic matter promote greater diversity among cyanobacteria by providing carbon substrates for heterotrophic interactions and enhancing soil moisture retention (Alghanmi and Jawad, 2019; Stibal et al., 2006). Studies have indicated that cyanobacteria aid in the accumulation of soil organic matter through the production of extracellular polymeric substances (EPS). EPS accumulation improves soil aggregation and water-holding capacity (Alvarez et al.; 2021; Begum et al.;2019).

Nitrogen

Nitrogen availability is a critical factor regulating the growth of cyanobacteria and their nitrogen fixation rates. Nitrogen-fixing cyanobacteria, such as *Anabaena* and *Nostoc*, are more prevalent in soils with limited nitrogen, whereas non-diazotrophic species are mostly dominant in nitrogen-rich environments. Several studies have demonstrated that introducing cyanobacterial inoculation into nitrogen-deficient soils enhances nitrogen fixation (Issa et al., 2014).

Phosphorus

Phosphorus impacts the composition and metabolic functions of cyanobacteria. Phosphorus deficiency can inhibit cyanobacterial development, particularly in environments with limited inorganic phosphorus (Andersen et al., 2020). Studies have shown that some cyanobacteria species possess alkaline phosphatase enzymes that

enable them to access organic phosphorus sources and sustain growth under phosphorus-limited conditions (Dyhrman, 2016; Jin et al., 2022).

Potassium

Potassium is essential for microbial processes such as osmoregulation, enzyme activation, and stress resistance. The availability of potassium affects the ability of cyanobacteria to tolerate environmental stressors such as salinity and drought (Hasanuzzaman et al., 2018; Wang et al., 2013). Studies have suggested that soils deficient in K may experience a decrease in the efficiency of cyanobacterial nitrogen fixation, emphasizing the importance of balanced nutrient management in rice paddy ecosystems (Parker et al., 1997; Qiu et al., 2004).

Water conductivity

The concentration of dissolved ions plays a crucial role in cyanobacteria growth in rice paddies. Elevated conductivity levels resulting from increased nutrient runoff or irrigation methods can alter cyanobacterial communities (Brasil et al.;2016). Changes in conductivity have been reported to affect the formation of cyanobacterial mats and biomass accumulation, thereby affecting the stability of the ecosystem (Rejmánková and Komárková, 2005).

Dissolved oxygen

The DO levels of dissolved oxygen in waterlogged paddy soils influence microbial respiration and redox conditions. Some species thrive well in low-oxygen (anoxic) environments, whereas others require higher oxygen levels (Fujita et al., 2015). Notably, oxygen fluctuations affect nitrogen fixation rates because nitrogenase, the key enzyme in the nitrogen fixation pathway, is highly sensitive to oxygen (Fay, 1992).

Salinity

In coastal and inland rice fields, salinity significantly affects the microbial diversity, particularly where salt accumulation occurs. Research has demonstrated that salinity stress reduces non-halotolerant cyanobacterial populations, whereas salt-tolerant species such as *Aphanothece* dominate under high-salinity conditions (Oren, 2015). Studies have suggested that cyanobacteria utilize osmoprotective strategies, such as

the production of compatible solutes, to cope with salinity variations (Singh et al., 2022).

1.8. Molecular and Microscopic Approaches in Cyanobacterial Diversity Studies

The various methods and approaches used to study cyanobacteria diversity are summarized in Figure 4. Cyanobacterial diversity studies have traditionally been based on microscopic techniques; however, advancements in molecular biology have significantly refined taxonomic classification and community analysis. While microscopy remains essential for identifying morphotypes based on cell shape, size, pigmentation, and specialized structures, such as heterocysts and akinetes, molecular approaches, such as 16S rRNA gene sequencing and metagenomics, offer greater phylogenetic and functional accuracy (Kumari and Rai, 2020; Manoylov, 2014). Integrating both approaches provides a more comprehensive understanding of cyanobacterial diversity, particularly in complex ecosystems, such as paddy fields.

Historically, morphological classification has been the primary method for investigating cyanobacteria, as it allows for the direct examination of environmental samples (Desikacharya, 1959; Baker and Fabbro, 2002). Paddy-field cyanobacteria exhibit diverse morphotypes, including unicellular, colonial, and filamentous forms, with heterocystous genera such as *Anabaena*, *Nostoc*, and *Aulosira* dominating under nitrogen-deficient conditions, whereas non-heterocystous taxa such as *Oscillatoria* and *Phormidium* thrive in nutrient-rich environments (Oren, 2014). Advanced microscopy techniques such as phase-contrast and fluorescence microscopy have further enhanced the examination of cyanobacterial mats and biofilms (Davey and O'Toole, 2000). However, morphological plasticity influenced by environmental conditions often results in taxonomic misidentifications, highlighting the need for molecular tools to improve the classification accuracy (Komárek, 2016).

Molecular techniques have significantly advanced cyanobacterial taxonomy by addressing limitations inherent in morphology-based identification. The 16S rRNA gene is widely used as a molecular marker for cyanobacterial phylogenetics because of its conserved and variable regions, which allow precise species differentiation (Komárek, 2010). Sequencing studies have identified novel cyanobacterial strains and

reclassified multiple genera, underscoring the importance of molecular data in systematics (Anand et al., 2019; Mishra, 2020). Despite its advantages, 16S rRNA sequencing is sometimes limited by horizontal gene transfer and genetic similarities within taxa, which can obscure species-level resolution (Poretsky et al., 2014).

Metagenomics, which involves high-throughput sequencing of environmental DNA, has emerged as a crucial tool for studying cyanobacterial community structure and functional capabilities. This approach allows for the identification of both culturable and unculturable organisms and significantly expands our knowledge of microbial diversity (Edet et al., 2017). Metagenomic studies have revealed functional genes associated with nitrogen fixation, photosynthesis, and stress adaptation, thereby providing deeper insights into cyanobacterial ecology (Murakami et al., 2022). The integration of metagenomic data with physicochemical analyses has further improved the prediction of cyanobacterial responses to environmental stressors (Rathour et al., 2020).

The combination of molecular and microscopic methods has significantly improved the accuracy of studies on cyanobacterial diversity. Molecular techniques provide enhanced taxonomic precision, particularly for morphologically similar taxa, and have enabled identification of non-culturable cyanobacteria that remain undetected using traditional laboratory methods (Komárek, 2010; Manoylov, 2014; Alvarenga et al., 2017; Ren et al., 2017). Additionally, molecular approaches facilitate functional analysis by identifying genes linked to nitrogen fixation, toxin production, and stress adaptation, thereby offering deeper ecological insights (Nawaz et al., 2024). Next-generation sequencing (NGS) has streamlined large-scale microbial community profiling, significantly reducing the time and effort required for traditional identification (Forde and O'Toole, 2013). Moreover, molecular phylogenetics has clarified the taxonomic uncertainties caused by morphological plasticity and cryptic diversity (Yang and Kim, 2023).

Despite these advantages, molecular methods should be complemented by microscopic observations to ensure accurate taxonomic classification and ecological

Comparative Analysis of Cyanobacterial Study Methods

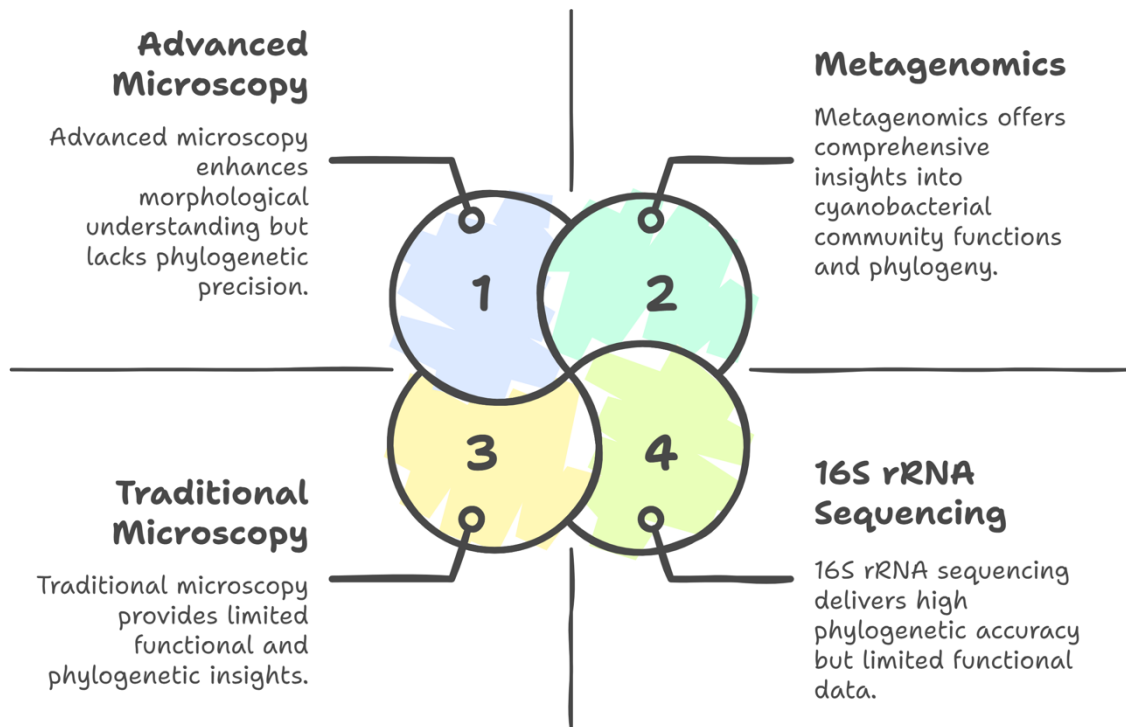


Figure 4. Diverse methodologies implemented to investigate the diversity of cyanobacteria.

interpretation. Although molecular tools provide high-resolution phylogenetic insights, microscopic analysis remains essential for understanding cyanobacterial morphology, habitat associations, and ecological functions (Mishra, 2020). A combined approach strengthens taxonomic accuracy and enhances the knowledge of cyanobacterial functional diversity in paddy field ecosystems.

1.9.Relevance of Studying Cyanobacteria in Paddy Fields of Mizoram

The unique geographical, climatic, and edaphic characteristics of Mizoram make the study of cyanobacterial diversity in rice paddies important for scientific and agricultural advancements. Region-specific studies are essential to understand cyanobacterial ecological interactions and their applications in sustainable agriculture and water management (Saleem et al., 2024). Rice (*Oryza sativa*) is a primary crop cultivated predominantly through shifting cultivation (*jhum*) and terrace farming, which are adapted to hilly terrains and high precipitation levels (Tripathi et al., 2024). Paddy fields in this region possess distinct soil and water properties compared to lowland-irrigated rice systems (Grogan et al., 2012).

Rice cultivation in Mizoram faces several challenges, including soil degradation, water scarcity during dry periods, and declining soil productivity owing to excessive synthetic fertilizer use (Konjengbam et al., 2022). Despite these challenges, organic farming practices such as farmyard manure application and biological soil enhancement remain common. Research suggests that integrating native cyanobacterial strains as biofertilizers could improve soil fertility, enhance nitrogen availability, and promote sustainable rice production (Iniesta-Pallarés et al., 2021). Investigating cyanobacterial communities in these ecosystems can provide valuable insights into their adaptability to varying environmental conditions, contributing to improved paddy field management strategies (Chaurasia, 2015).

Cyanobacterial diversity in Mizoram rice paddies remains largely unexplored. Studies have indicated that cyanobacterial community composition varies across regions because of differences in soil characteristics, elevation, precipitation, and land use (Hakkoum et al., 2020; Řeháková et al., 2011). Given their essential role in nitrogen fixation and microbial interactions, a comprehensive study of cyanobacteria

in Mizoram rice fields is necessary. Research has shown that indigenous cyanobacterial strains exhibit superior adaptation to local environmental conditions compared with commercial biofertilizers. Therefore, it is important to identify and characterize native species for the development of region-specific biofertilization (D'Acqui, 2016; Thomas and Litchman, 2016b). Locally adapted cyanobacterial inoculants may enhance soil health, increase crop yields, and mitigate the environmental impacts of chemical fertilizers (Dhar et al., 2015). Additionally, cyanobacteria contribute to soil microbial resilience by fostering beneficial microbial networks that are essential for controlling pathogens and enhancing plant vigor (Du et al., 2022).

Cyanobacteria offer significant potential for sustainable agriculture in Mizoram, particularly through nitrogen fixation, which reduces the dependence on synthetic fertilizers (Song et al., 2021; Zhang et al., 2021). Studies suggest that nitrogen-fixing genera, such as *Nostoc*, *Anabaena*, and *Aulosira*, play a vital role in providing nitrogen to rice crops (Kulasooriya and Magana-Arachchi, 2016; Mishra et al., 2021). Additionally, cyanobacteria enhance soil fertility by increasing organic matter, improving nutrient availability, and promoting microbial diversity, which is especially beneficial in Mizoram's terraced paddy fields, where soil erosion is a major concern (Grogan et al., 2012). Their biocontrol capabilities further contribute to agricultural sustainability, as many cyanobacteria produce bioactive compounds with antimicrobial properties that help suppress plant pathogens and reduce the need for chemical pesticides (Singh et al. 2005; Kulik 1995; Shah et al. 2021).

In addition to soil health, cyanobacteria also play a critical role in water resource management. Research has indicated that cyanobacterial biofilms and extracellular polymeric substances (EPS) enhance soil moisture retention, which is particularly valuable in rain-fed and terraced rice fields (Nishanth et al., 2021). These properties help reduce water loss and improve crop resilience during dry periods (Velmourougane et al., 2023). Additionally, cyanobacteria contribute to nutrient cycling in paddy ecosystems by absorbing nitrogen and phosphorus, thereby mitigating excessive nutrient runoff and eutrophication (Kumar et al., 2015).

Maintaining a balanced cyanobacterial population can improve the water quality and microbial stability in paddy fields (Pathak et al., 2018).

Climate variability presents another challenge for rice cultivation in Mizoram because changing rainfall patterns and temperature extremes impact crop productivity. Research suggests that cyanobacteria serve as bioindicators of environmental changes, providing early warnings of ecosystem disruptions and informing adaptive water management strategies (Cottingham et al., 2015; Yadav et al., 2023). Their ability to enhance soil structure, regulate moisture retention, and stabilize nutrient cycles makes them valuable assets for building climate-resilient agricultural systems.

Investigating cyanobacteria in Mizoram's paddy fields is essential to understand their ecological roles and potential applications in sustainable farming. Studies have highlighted their contribution to nitrogen fixation, soil fertility, and microbial diversity, making them valuable for improving rice cultivation in this region. Locally adapted cyanobacterial strains could serve as effective biofertilizers, enhancing soil health while reducing reliance on chemical input. Furthermore, their roles in water retention, nutrient cycling, and climate resilience underscore their importance in long-term agricultural sustainability. Future research should focus on identifying indigenous cyanobacterial species and developing methodologies for their practical application in Mizoram's agricultural system.

1.10. Research Gaps and Justification for the Study

Despite extensive research on cyanobacteria in rice paddies across different regions of India and the world, significant knowledge gaps persist, particularly concerning the diversity and seasonal dynamics of cyanobacteria in Mizoram paddy fields. Most studies have focused on irrigated lowland rice systems, overlooking the unique agroecological conditions of Mizoram where elevated terrain, intense monsoon precipitation, and diverse soil compositions create distinct ecological environments. Given the critical role of local environmental factors in shaping microbial communities, region-specific studies are necessary to comprehensively understand the cyanobacterial diversity and function in Mizoram rice paddies (Nguyen et al., 2021).

Current research on cyanobacterial diversity in Mizoram remains limited, with little information available on the microbial composition of paddy fields in Northeast India. Although studies in other regions have identified diverse nitrogen-fixing cyanobacteria in rice fields, no comprehensive taxonomic evaluation of native cyanobacterial species has been conducted in Mizoram. Research suggests that environmental conditions strongly influence cyanobacterial communities, highlighting the importance of localized studies (Echenique-Subiabre et al., 2015). Existing research relies on static analyses and offers only preliminary information on cyanobacterial diversity. Since cyanobacterial populations fluctuate across different growth stages of rice cultivation, seasonal studies are crucial for understanding these variations, particularly in Mizoram, where monsoonal shifts likely play a key role in microbial community dynamics (Song et al., 2005).

A major limitation of previous cyanobacterial studies in India has been the predominant use of microscopy-based identification methods, which lack precision in distinguishing between cryptic species. Research from other regions has demonstrated that molecular approaches, such as 16S rRNA sequencing and metagenomics, reveal much broader diversity than conventional methods (Gupta et al., 2013). The absence of such molecular studies in Mizoram represents a considerable gap in our understanding of the taxonomic and functional diversity of cyanobacteria in this region. Additionally, although cyanobacteria are widely recognized as biofertilizers, there is little information on the efficacy of native cyanobacterial strains in Mizoram's paddy soils. Studies have suggested that locally adapted strains often exhibit superior benefits compared to commercially available inoculants, emphasizing the need for research into their potential application in sustainable rice farming (Sadvakasova et al., 2023).

Mizoram is also experiencing significant environmental changes due to shifting agricultural practices, deforestation, and climate fluctuations. Studies have demonstrated that land-use changes can profoundly alter soil microbial communities, yet no investigations have examined their impact on cyanobacteria in Mizoram's rice fields (Bossio et al., 2005; Suleiman et al., 2013). Given these transformations, further

research is needed to assess how these ecological changes influence cyanobacterial diversity and function in this region.

Addressing these knowledge gaps requires an integrative approach that combines seasonal- and molecular-level investigations. Studies have shown that integrating traditional microscopy with molecular techniques provides a more accurate and comprehensive view of the microbial communities (Mishra, 2020). Seasonal fluctuations in cyanobacterial populations, influenced by environmental conditions, necessitate longitudinal studies to capture the dynamic changes in community composition (Dalu and Wasserman, 2018). Moreover, molecular tools, such as 16S rRNA sequencing and metagenomics, are essential for refining taxonomic classification and identifying cryptic species that may have been overlooked using conventional methods.

In summary, while cyanobacteria have been extensively studied in rice paddies worldwide, critical gaps remain in our understanding of their diversity, seasonal dynamics, and molecular taxonomy in Mizoram. Given the strong influence of local environmental conditions on cyanobacterial communities, region-specific research is required. Future studies should prioritize seasonal monitoring to assess temporal shifts in cyanobacterial populations and apply molecular techniques to improve the taxonomic resolution. Addressing these gaps will not only enhance the knowledge of microbial ecology but also contribute to sustainable rice cultivation by developing cyanobacteria-based biofertilization strategies. An integrative research approach combining microscopy, molecular tools, and environmental assessments will be instrumental in fully elucidating the role of cyanobacteria in Mizoram's paddy-field ecosystems.

1.11. Objectives

The following objectives were formulated to address the existing knowledge gaps and contribute to the growing body of research on cyanobacteria in agroecosystems:

1. To investigate the diversity of cyanobacterial assemblages in selected rice paddy fields.

2. The physicochemical properties of the paddy soil and water samples were analyzed.
3. To investigate the changes in cyanobacterial population structure during the rice growth season and after harvest using a combination of microscopic and molecular techniques.

CHAPTER 2

MATERIALS AND METHODS

2.1. Chemical and Reagents

All chemicals used in this study were of analytical grade (Merck, India, HiMedia, Sigma, SDFCL). Ultrapure water, specifically Milli-Q, was used throughout the investigation. The chemicals and reagents used in the study comprises of - agar agar, Type 1, sodium nitrate (NaNO_3), calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), potassium dihydrogen orthophosphate (K_2HPO_4), magnesium sulfate heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), sodium carbonate (Na_2CO_3), disodium magnesium EDTA ($\text{C}_{10}\text{H}_{12}\text{MgN}_2\text{Na}_2\text{O}_8$), citric acid ($\text{C}_6\text{H}_8\text{O}_7$), ferric ammonium citrate $[\text{NH}_4]_3[\text{Fe}(\text{C}_6\text{H}_4\text{O}_7)_3]$, hydrochloric acid (HCl), sodium hydroxide (NaOH), nitric acid (HNO_3), hydrogen peroxide (H_2O_2), sulfuric acid (H_2SO_4), sulfurous acid (H_2SO_3), potassium sulfate (K_2SO_4), sodium sulfate (Na_2SO_4), copper sulfate (CuSO_4), boric acid (H_3BO_3), methyl red ($\text{C}_{15}\text{H}_{15}\text{N}_3\text{O}_2$), bromocresol green ($\text{C}_{21}\text{H}_{14}\text{Br}_4\text{O}_5\text{S}$), sodium hydroxide (NaOH), barium chloride (BaCl_2), ammonium fluoride (NH_4F), ammonium molybdate $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}]$, potassium antimonyl tartrate $\text{K}_2\text{Sb}_2(\text{C}_4\text{H}_2\text{O}_6)_2$, sulphuric acid (H_2SO_4), potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$), L-ascorbic acid ($\text{C}_6\text{H}_8\text{O}_6$), ammonium acetate solution ($\text{NH}_4\text{CH}_3\text{CO}_2$), TE buffer (tris-EDTA buffer), tris(hydroxymethyl)aminomethane hydrochloride (tris HCl), EDTA-ethylenediaminetetraacetic acid ($\text{C}_{10}\text{H}_{16}\text{N}_2\text{O}_8$), SDS - sodium dodecyl sulfate ($\text{NaC}_{12}\text{H}_{25}\text{SO}_4$), proteinase K, phenol chloroform isoamyl alcohol ($\text{C}_6\text{H}_{13}\text{Cl}_3\text{O}$), sodium acetate ($\text{C}_2\text{H}_3\text{NaO}_2$), RNase, sodium chloride (NaCl), isopropanol ($\text{C}_3\text{H}_8\text{O}$), low-melting (LM) agarose, primers- CYA359F: (GGG GAA TYT TCC GCA ATG GG) and CYA781R: Equimolar mixture of CYA781Ra (GAC TAC TGG GGT ATC TAA TCC CAT T) and CYA781Rb (GAC TAC AGG GGT ATC TAA TCC CTT T) (reverse primers), EmeraldAmp GT PCR master mix (2X Premix) - DNA polymerase, optimized reaction buffer, dNTPs, density reagent, green dye, sterile ultrapure Milli-Q water.

2.2. Description of the Study Area

Mizoram, situated in northeastern India, shares extensive international and domestic borders. The state is characterized by its strategic geographical position and unique border demarcations. Mizoram's southern region encompasses 722 km of international borders; Bangladesh is situated to the west, and Myanmar (Burma) is situated to the east and southeast. On the northern side, Mizoram shares domestic borders with three Indian states: Manipur to the northeast, Assam to the north, and Tripura to the northwest, with a geographical area of over 21,000 sq.km (Adhikary et al., 2010).

Field surveys were conducted across three locations in Mizoram: Champhai (high elevation, 1200–1400 m), Kolasib (low elevation, <100 m), and Thenzawl (medium elevation, 700–850 m). These sites differ in climate and topography. Champhai has cooler temperatures (15–25°C) and rainfall of 1920.6 mm/year ; Kolasib is warmer (20–35°C) with 2523.6 mm/year rainfall ; and Thenzawl has intermediate conditions (18–30°C, 1929.3 mm/year) (Meteorological data of Mizoram, 2021; Sati, 2019). Five paddy fields per region (C1–C5, K1–K5, TZ1–TZ5) were selected based on accessibility and active rice cultivation, for a total of 15 sites. All fields practiced traditional wet rice farming with local varieties (e.g., Idaw, Buhban, and Khawnglawng) and minimal synthetic fertilization (urea applied at 20–30 kg/ha annually). Sampling was conducted during both the growing season (July–September) and post-harvest season (November–December).

The specific geographical coordinates and elevation of each study site are presented in Table 1, and the classification of elevation categories across the three locations is detailed in Table 2.

Table 1. Geographical location of the study sites

Sl.No.	Site	Latitude	Longitude	Elevation (m)
1.	C1	23°28'29"N	93°20'33"E	1250
2.	C2	23°28'38"N	93°20'36"E	1300
3.	C3	25°28'5"N	93°20'7"E	1300
4.	C4	23°28'5"N	93°20'4"E	1290

5.	C5	23°27'51"N	93°20'11'E	1290
6.	K1	24°17'29"N	92°38'37"E	50
7.	K2	24°18'14"N	92°38'17"E	50
8.	K3	24°20'18"N	92°39'33" E	40
9.	K4	24°20'18"N	92°39'33" E	40
10.	K5	24°19'0" N	92° 39' 43"E	50
11.	TZ1	23°15'50"N	92°46'7"E	740
12.	TZ2	23°16'50"N	92°46'35"E	740
13.	TZ3	23°15'51"N	92°46'10"E	760
14.	TZ4	23°16'18"N	92°46'15"E	750
15.	TZ5	23°16'37"N	92°46'15"E	740

Note: Champhai -C, Kolasib-K and Thenzawl- TZ

Table 2. Elevation range of the study sites

Location	Elevation range (m)	Elevation category
Champhai	1250 -1300	High
Thenzawl	740- 760	Medium
Kolasib	40 – 50	Low

2.3. Sampling Method

Samples were collected during the growing season (July–September, S1) and post-harvest season (November–December, S2) in 2023. At each site, three replicate soil and water samples (n=45 per season) were collected in sterile plastic bags and bottles. Soil samples (0–10 cm depth) were collected from five random points in each field and pooled. Water samples were collected where available (S1: all sites; S2:6 sites with residual water). In situ measurements of pH and temperature were recorded using a portable pH meter (Hanna HI 98107, Hanna Instruments, USA) and thermometer (Figure 5).



Figure 5. (a) Temperature of paddy soil (b) Collection of sample in plastic bag (c) pH of paddy water (d) Paddy soil sample collection in plastic bag.

Samples were transported to the Department of Botany, Mizoram University, and cultured in BG-11 medium (Pinho et al., 2017) under controlled conditions (25 – 30 °C, pH 7.0, 16:8 light:dark cycle, 50 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) (Figure 6). Additionally, the collected paddy soil samples were processed for physicochemical analyses.

2.4. Isolation of Cyanobacterial Strains

All glassware, including petri dishes, test tubes, conical flasks, cotton plugs, and BG-11 culture medium, were autoclaved at 15 psi for 15 min at 121°C to eliminate any potential contaminants.

Freshly collected cyanobacterial samples were agitated with sterile distilled water (90 mL) for 2 h following the protocol described by Lukešová (1993). The resulting sample suspensions were serially diluted (10^{-2} and 10^{-3} dilutions). The 1 mL of each dilution was inoculated onto solidified BG-11 medium (1–1.5% agar) (Figure 7) (Yang et al., 2015). The inoculated culture plates were incubated at 25–30°C, which is conducive to optimal cyanobacterial growth, under a 16:8 h (light:dark) photoperiod with white fluorescent light of intensities ranging from 2000 to 3000 lx. The incubation period was extended from 10 to 15 days or until distinct cyanobacterial colonies were visible on the growth medium.

2.5. Purification of Cyanobacterial Strains

To obtain axenic cultures, isolates were purified using the agar plating method. Purification was achieved by repeated subculturing on both solid and liquid media using streaking and pour plating methods (Figure 8b)

Individual cyanobacterial colonies were carefully selected and transferred to fresh BG-11 liquid medium. After 7 days, the cultures were observed under a microscope to determine purity. If contamination was detected, 0.1 mL of the culture was re-inoculated onto BG-11 nutrient agar plates and incubated. The cycle of colony selection and inoculation into fresh liquid media was repeated until unialgal cultures were obtained (Figure 8a)



Figure 6. (a) and (b) Crude cyanobacterial samples maintained in BG-11 culture medium and stored in the algal culture room, Mizoram university.

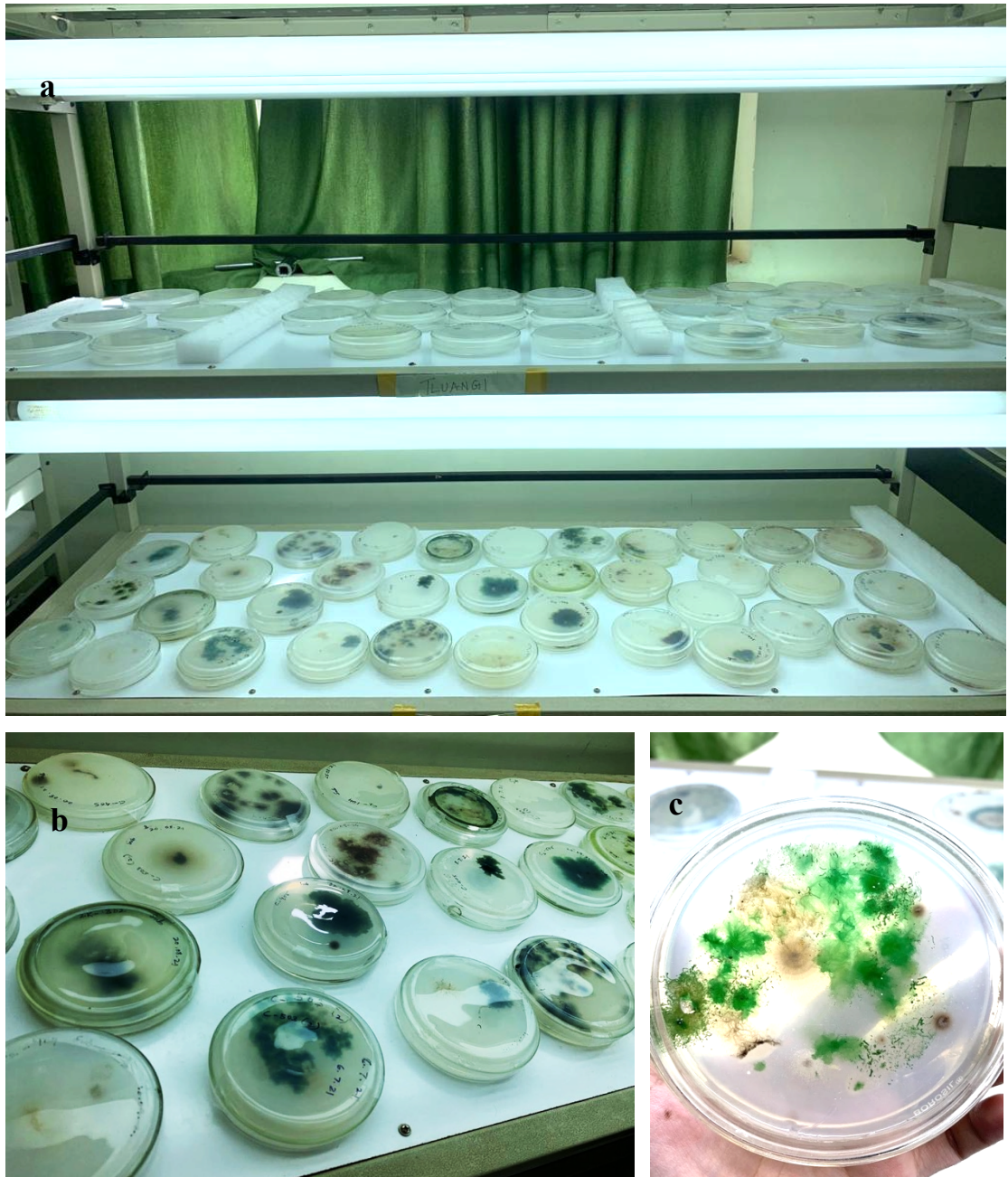


Figure 7. (a),(b) and (c) Mixed cyanobacterial colonies on petriplates of BG-11 agar medium.



Figure 8. (a) Liquid culture of some isolated cyanobacterial strains. (b) Pure colonies of different cyanobacterial species grown on BG-11 agar medium.

Following purification, the cyanobacterial strains were cultivated in the BG-11 medium whose composition is given in Table 3. The culture medium was adjusted to a pH of 7.2 ± 0.2 using 0.1 N HCl or 0.1 N NaOH. The cultures were subjected to a light-dark cycle at 10:14 h.

Table 3. Composition of BG 11 medium for cyanobacteria culture

Ingredients	g L ⁻¹	Ingredients	g L ⁻¹
Sodium nitrate	1.5000	Citric acid	0.0060
K ₂ HPO ₄	0.0314	Ferric ammonium citrate	0.0060
MgSO ₄ .7H ₂ O	0.0360		
Calcium chloride dihydrate	0.0367		
Sodium carbonate	0.0200		
Disodium magnesium EDTA	0.0010		

*Final pH after sterilization (at 25°C) – 7.10

2.6. Microscopic Identification and Morphological Characterization of Collected Cyanobacteria Samples

The cyanobacterial isolates were identified using an Olympus BX41 trinocular microscope (Olympus, Japan). Isolates were identified based on key taxonomic characteristics, including cell size, shape, pigmentation, cellular arrangement, and presence of specialized structures, such as akinetes, heterocysts, sheaths, and mucilage. Cyanobacterial taxa have been identified and classified using monographs and standard identification keys (Desikacharya 1959; Baker and Fabbro 2002; Rosen and Mareš 2016).

2.7. Soil Moisture Content

The gravimetric water content of the soil samples was determined using the oven drying method described by O’Kelly (2004). (50 g) was placed in a pre-weighed,

oven-dried container, and subsequently dried in an oven at 105°C for 24 h until a constant weight was obtained.

The moisture content was calculated using the following formula:

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

where W_1 is the initial weight and W_2 is the final weight.

2.8. pH, Conductivity, Salinity of Paddy Soil

Soil pH, electrical conductivity (EC), and salinity were determined using a PCS Testr 35 multiparameter device (Eutech Instruments, Singapore). Ten grams of fresh soil were added to 50 mL of deionized water in a beaker. The soil-water mixture was stirred for 20 min using a magnetic stirrer. The pH, electrical conductivity (EC), and salinity of the supernatants were recorded. A 1:5 soil-to-water ratio was used following the standard protocols for soil salinity estimation.

2.9. TDS and pH of Paddy Water

TDS and pH of the paddy water were determined in situ during the field surveys. A digital multiparameter instrument (model PCSTestr 35) and a portable digital pH meter (Hanna Instruments, model HI 98107) were employed for the measurements. TDS and pH were recorded during the growing season (July-September) and post-harvest season (November-December), respectively. However, postharvest measurements were limited to fields in which residual water was still present.

2.10. Determination of Heavy Metals in Soil Samples

The concentrations of heavy metals in paddy field soil samples were determined. A fine powder was produced by grinding the soil samples after they were oven-dried at 60°C for 48 h. 0.5 g) was subjected to acid digestion using a 1:4 mixture of HNO_3 and H_2O_2 (Hseu, 2002; Singh et al. 2010). Digestion was performed until a clear solution was obtained. The digested solution was then passed through a Whatman Grade 42

filter paper. Cu, Pb, Fe, Mn, and Zn concentrations were determined using a Shimadzu AA-7000 Atomic Absorption Spectrophotometer (Shimadzu, Japan).

2.11. Organic Carbon in Soil Samples

Principle

The Walkley-Black method is a commonly used wet oxidation technique for quantifying the organic carbon (OC) concentration in soil. The process involves the oxidation of organic materials using potassium dichromate ($K_2Cr_2O_7$) in sulfuric acid (H_2SO_4), followed by a titration to quantify the unreacted dichromate.

Reagents

The reagents used were barium chloride (0.4%) prepared by dissolving 4g of $BaCl_2$ in 1000 ml distilled water, potassium dichromate (5%) prepared by dissolving 50g of $K_2Cr_2O_7$ in 1000 ml of distilled water, sucrose and 36N concentrated sulfuric acid (H_2SO_4).

Standards

After drying at $105^\circ C$ for 2 h, 15 g sucrose was cooled in a desiccator. 50 mg/ml C solution (100 mL) was prepared by dissolving 11.886 g of dried sucrose in water in a volumetric vessel, and 25 ml of the 50 mg/ml C stock solution was transferred to a labeled 100 ml volumetric flask and thoroughly mixed with water until it reached the mark. The working standards were as follows: 0, 2.5, 5.0, 7.5, 10.0, and 12.5 mg/ml C. Two ml of each working standard were pipetted into 100-ml conical flasks that were labeled and dried at $105^\circ C$, or into digestion tubes that were also labeled. These contain 0, 5, 10, 15, 20, 25 mg C.

Procedure

Soil organic carbon was quantified using the Walkley-Black method (Vos et al., 2007), where 1 ± 0.001 g of ground soil (< 0.15 mm) was weighed into a labeled 100 ml conical flask (if the soil is dark, or is suspected to be high in organic matter use of about $0.5 \leq 0.001$ g). The soil (W) was recorded. Ten milliliters of 5% potassium dichromate solution were added, and the soil was completely wetted or the standards were dissolved. 20 ml of H_2SO_4 was added using a fast burette, and the mixture was swirled gently and allowed to cool. A clear precipitate was obtained by adding 50 ml

of 0.4% barium chloride, swirling the mixture thoroughly, and allowing it to stand overnight. After the supernatant was transferred to a calorimetric cuvette, optical density (OD) was determined at 600 nm using a Hitachi (220) spectrophotometer.

Calculation

A graph was created by plotting the absorbance against the standard concentration. The concentration of the solution for each unknown and blank was determined, and the correlated concentration K was determined by subtracting the mean blank value from the unknown.

$$\% \text{ Organic carbon} = \frac{(K \times 0.1)}{(W \times 0.74)}$$

where, W is the soil weight.

2.12. Total Soil Nitrogen Content in Soil

Principle

The Kjeldahl method is a prevalent technique for quantifying nitrogen concentrations in soil, organic substances, and other materials. This method involves the conversion of organic nitrogen into ammonium nitrogen (NH_4^+), which can then be measured.

Procedure

Nitrogen content of the soil was determined using the Kjeldahl procedure (Bremner, 2018). Five grams of soil sample were transferred into a digestion tube, and 10 mL of concentrated H_2SO_3 and 5 g of catalyst (a 5:1 ratio of potassium sulfate/sodium sulfate to copper sulfate) were added. The digester was fitted into a digestion tube. The sample was heated in a digestion block at 410°C until it turned colorless or light green.

After digestion, samples were transferred to a large digestion tube (DTL). Distilled water (10 mL) was added, followed by thorough mixing. DTL was then loaded into the distillation unit. The collecting end was equipped with a 250-mL conical vial that contained 25 mL of 40% boric acid and three droplets of methyl red and bromocresol green indicators. Then, 40 mL of 40% NaOH was added and the timer was set to 20 s. As the boric acid solution became colorless, it indicated the capture of

ammonia released. The conical flask containing the boric acid solution was removed from the collecting end and the sample was subsequently titrated.

Calculation:

The total nitrogen content in the soil, expressed as a percentage, was calculated using the following formula:

$$\% \text{ of total N}_2 = \frac{14 \times \text{Normality of acid} \times \text{Titrant value} \times 100}{\text{Sample weight} \times 1000}$$

2.13. Available Phosphorus Content in Soil

Principle

Phosphorus is extracted from the soil using Bray No. 1 solution as the chemical extractant. By interacting with ammonium molybdate, the extracted phosphorus is colorimetrically quantified, resulting in the formation of the 'Molybdenum Blue' color. A spectrophotometer was used to quantify the absorbance of the compound at 882 nm, which is directly proportional to the quantity of phosphorus extracted from the soil.

Reagents

Bray No 1 extracting solution

2.22 g of ammonium fluoride (NH_4F) was dissolved in deionized water and transferred to a 2 L volumetric vessel. Deionized water was used to aggregate 5 mL of concentrated hydrochloric acid to the desired volume.

Reagent A

17.14g of ammonium molybdate $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}]$ was dissolved in 200 mL of warm deionized water. Potassium antimonyl tartrate $\text{K}_2\text{Sb}_2(\text{C}_4\text{H}_2\text{O}_6)_2$ (0.392 g) was separately dissolved in 150 mL of deionized water. Deionized water (500 mL) was placed in a 2 L volumetric flask, and 200 mL of concentrated sulfuric acid was slowly added with mixing. After cooling, molybdate and tartrate solutions were added, mixed, and bulked to volume with deionized water.

Reagent C

0.53 g of L-ascorbic acid ($C_6H_8O_6$) was dissolved in deionized water and transferred to a 500 mL volumetric flask. 70 mL of Reagent A was added and bulked to volume with deionised water. A fresh volume of this solution was prepared by proportioning the aforementioned quantities.

Standard phosphorus solution ($P \equiv 50 \text{ mg/L}$)

Potassium dihydrogen orthophosphate (0.2195 g, KH_2PO_3) was dissolved in 100 mL of deionized water, transferred to a 1 L volumetric flask, and 5 mL of concentrated sulfuric acid was added and bulked to volume with deionized water.

Standard phosphorus solution ($P \equiv 2.50 \text{ mg/L}$)

A standard phosphorus solution (5 mL) was pipetted into a 100 mL volumetric flask and bulked to volume with deionized water.

Procedure

Plant-available phosphorus was determined using the Bray No. 1 extraction method (Boem et al., 2011). Bray extraction solution (7 mL) was added to a centrifuge tube containing 1 g of oven-dried soil. The Bray solution was used as a blank. After shaking for 1 min, the sample was centrifuged at 6000 rpm for 5 min. The 0.50 mL of the supernatant was transferred to a glass cuvette and mixed with 2.0 mL of Reagent C. The mixture was allowed to stand for 30 min. A set of reference standards was prepared from a 2.50 mg/L phosphorus solution. The absorbance of both standards and samples was recorded at 882 nm.

Calculation

The phosphorus concentration was determined by constructing a calibration curve

$$\text{Available phosphorus (mg/kg)} = \frac{C \times 14}{\text{ODW}}$$

where C is the phosphorus concentration from the chart/equation ($\mu\text{g}/2.5 \text{ mL}$), ODW is Oven-dry sample weight (g), and constant 14 is the dilution factor.

2.14. Exchangeable Potassium Content in Soil

Principle

Quantification of exchangeable potassium (K^+) content in soil by flame photometry relies on the principle of emission spectroscopy. This technique measures potassium by assessing the intensity of light emitted when potassium ions are stimulated by a flame.

Procedure

Exchangeable potassium concentration in the soil was determined using flame photometry (Potdar et al., 2021). An ammonium acetate solution was used for the extraction. (5 g) was added to 25 mL ammonium acetate solution. The mixture was shaken for 5 min, the suspension was filtered, and the filtrate collected for analysis.

Calculation

The final concentration of available potassium (K) in soil was calculated using the following equation:

$$\text{Available K} = R \times \frac{\text{Volume of extracted soil solution}}{\text{Soil weight} \times 10^4}$$

Where, R is the concentration (ppm) obtained from the standard curve.

2.15. Relative Abundance

Principle

The concept of relative abundance denotes the ratio of a specific component (such as a species within an ecosystem, an isotope in a sample, or a molecular fragment in mass spectrometry) to the aggregate of all components present.

Calculation:

The relative abundance of a particular cyanobacterial type was calculated using the following equation.

$$\text{Relative abundance, } A = \frac{Y}{X} \times 100$$

where X is the total number of samples collected and Y is the number of samples from which a particular cyanobacterial type was observed.

2.16. Diversity indices

Diversity indices (Shannon's diversity index and Simpson's index of diversity) were calculated using the following formula (Prasanna et al., 2009):

Shannon's diversity index

Principle

Shannon's Diversity Index (H') is derived from information theory and quantifies biodiversity by considering both species richness (the number of species) and evenness (the distribution of individuals across species) within a community. This index quantitatively measures ecological diversity, with higher values indicating greater diversity.

Calculation

$$H = -\sum (n_i/N) \ln (n_i/N)$$

where n_i is the number of individuals of the i^{th} species and N is the total number of organisms of all species.

Simpson's index of diversity

Principle

Simpson's Diversity Index is a measure of diversity that considers the number of species present as well as the relative abundance of each species. As species richness and evenness increased, diversity also increased.

Calculation

$$D = 1 - [\Sigma n(n-1) / N(N-1)]$$

where n is the total number of organisms of a particular species, and N is the total number of organisms in all species.

2.17. Molecular Characterization

Genomic DNA isolation

Genomic DNA was isolated from the cyanobacterial strains using the sodium dodecyl sulfate (SDS)/ CTAB method described by Zhou et al. (1996)

Chemicals required

1. TE buffer (pH 8.0)
10mM Tris HCl (pH 8.0)
1mM EDTA (pH 8.0)

Reagent	Volume	Final concentration
1M Tris HCl	1ml	10mM
0.5M EDTA	0.2ml	1mM
Distilled water	98.8ml	

2. 10% SDS: SDS (10 g) was dissolved in 100 ml of autoclaved distilled water.
3. Proteinase K (10 mg proteinase K was dissolved in 1 ml of autoclaved distilled water.
4. Phenol chloroform isoamyl alcohol (PCI)
5. 3M sodium acetate: 24.60 g of sodium acetate was dissolved in 100ml of distilled water and the pH was adjusted with dilute acetic acid (pH 5.2)

Procedure

1. Two milliliters of overnight culture were collected, and the cells were harvested by centrifugation at 14,000 rpm for 5 min at 27 °C.

2. Next, 500 μ L TE Buffer and 40 μ L 10% SDS were added to the cell pellet, and the cells were resuspended in the buffer by gentle mixing.
3. 20 μ l of proteinase K was added followed by 5 μ l of RNase.
4. The mixture was mixed well, incubated at 37 °C for an hour in an incubator, and vortexed every 10–15 min.
5. To this solution, 100 μ l 5M NaCl was added.
6. The above was followed by the addition of 80 μ l CTAB/ NaCl solution (10% CTAB/ 0.7M NaCl) and incubated for 10 min at 65 °C.
7. PCI (600 μ L of PCI was added to the contents, mixed well by inverting, and incubated at room temperature for 5 min.
8. The contents were then centrifuged at 14,000 rpm for 10 min at 4 °C.
9. A highly viscous jelly like supernatant was collected using a tip and transferred to a fresh tube.
10. This process was repeated with PCI, and the supernatant was collected in a fresh tube. (200 μ L).
11. Subsequently, 20 μ L of 3M sodium acetate was added to the mixture and gently mixed.
12. Isopropanol (600 μ L) was added and mixed gently by inversion until white strands of DNA precipitated.
13. The contents were then centrifuged at 14,000rpm for 5 min.
14. The supernatant was removed and 1 ml of 70% alcohol was added.
15. The contents were then centrifuged at 14,000 rpm for min.
16. Supernatant was discarded and air dried (until completely dry)
17. After air drying, the precipitated DNA was resuspended in 20 μ L of TE buffer and stored at -20 °C.
18. DNA was analyzed for purity by gel electrophoresis using 1-2% (w/v) low-melting (LM) agarose.

16S rRNA PCR amplification

Following isolation, DNA samples were subjected to polymerase chain reaction (PCR) amplification using two specific primers (Nübel et al. 1997; Song et al. 2005). The

primers used for amplification were as follows:

- i. CYA359F: (GGG GAA TYT TCC GCA ATG GG) (forward primer).
- ii. CYA781R: Equimolar mixture of CYA781Ra (GAC TAC TGG GGT ATC TAA TCC CAT T) and CYA781Rb (GAC TAC AGG GGT ATC TAA TCC CTT T) (reverse primer).

PCR amplification was performed using a Veriti 96 Well Thermal Cycler. The thermal cycling conditions were as follows: initial denaturation at 94°C for 3 min, followed by 45 amplification cycles, each consisting of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, elongation at 72°C for 45 s, and a final elongation step at 72°C for 5 min.

The PCR reaction mixture was prepared at a final volume of 25 µL. It contained 12 µL of EmeraldAmp GT PCR master mix (2X Premix), which included DNA polymerase, dNTPs, an optimized reaction buffer, and a density reagent. The premix also contained a green dye, which separated into blue and yellow dye fronts during the agarose gel electrophoresis. Additionally, 4 µL of extracted DNA, 0.4 µL of each forward and reverse primer (equimolar mixture), and 8.2 µL of sterile Milli-Q water were added to the reaction mixture.

Following PCR amplification, 5 µL of the amplified DNA was loaded onto a 2% agarose gel in a gel electrophoresis unit and run for 45 min at 60V. A 200 bp DNA ladder was used as the molecular weight marker. After electrophoresis, the gel was visualized using a Gel Doc EZ Imager (Bio-Rad) to confirm the presence and size of amplified DNA fragments.

Sequencing of 16S rRNA gene and cyanobacterial biodiversity assessment

The DNA bands obtained from PCR amplification were eluted from the gel following the protocol described by Boutte et al. (2005) and subsequently re-amplified as described by Srivastava et al. (2009).

Lyophilized PCR products (20 µL) were sent for commercial sequencing using the same amplification primers in separate sequencing reactions. After undergoing necessary corrections by manually examining both ends, the resulting sequences were

subjected to an NCBI BLAST search to verify the cyanobacterial identity and assess the cyanobacterial diversity present in the samples.

Sequences identified as belonging to non-cyanobacterial bacteria, green algae, land plants, or other flowering plants were excluded from further analysis. Following this screening, the confirmed cyanobacterial sequences were submitted to GenBank for further analysis and determination of phylogenetic relationships.

Nucleotide sequence accession numbers

The 16S rRNA gene sequences of the selected isolated pure cyanobacterial strains were deposited in the NCBI GenBank database, and accession numbers were obtained upon successful submission.

Phylogenetic tree construction and analysis

The sequences obtained, along with their closely related species, were aligned using the ClustalW algorithm integrated within the MEGA X software (Cheng et al., 2022). Manual adjustments were performed to correct ambiguous regions and resolve alignment gaps to ensure accuracy. Phylogenetic tree construction was conducted using MEGA X software. A phylogenetic model test was performed to determine the optimal nucleotide substitution model with selection based on the lowest Bayesian Information Criterion (BIC) scores. The Maximum Likelihood (ML) method was used for phylogenetic analysis, employing the Kimura-2-Parameter (K2) model with discrete Gamma Distributed (G) rate variation. The robustness of the phylogenetic relationships was assessed using 1000 bootstrap replicates.

Reference sequences obtained from the GenBank database were incorporated into the phylogenetic analysis, with *Microchaete* sp. designated as the outgroup. The placement of the selected species within the phylogenetic tree was analyzed and compared with known sequences from related taxa to assess their evolutionary relationships.

Statistical analysis

The experiments were performed in triplicate, and the results are presented as the mean $\pm$ SD (standard deviation of the mean). One-way ANOVA was performed followed by post-hoc analysis, which was performed using Duncan's multiple range test. Data were analyzed using Microsoft Excel and SPSS software to ensure reliability. The genus abundance was calculated as the number of genera per site per season. Diversity indices (Shannon H, Simpson's D) were computed using PAST software (v4.03). Principal Component Analysis (PCA) was used to assess physicochemical variability using OriginPro 2021, excluding heavy metals because of weak correlations with abundance ($r < 0.2$). The abundance of cyanobacterial genera was modeled using Least Squares (PLS) regression to account for the impact of environmental factors using standardized predictors (pH, EC, temperature, organic carbon, nitrogen, and phosphorus) across 30 observations (15 sites $\times$ 2 seasons). The analysis was performed in Python (v3.9, scikit-learn 1.3.0) using the PLS Regression module, employing two components validated via a five-fold cross-validation. Model performance was assessed using R^2 and VIP scores, with significance evaluated via permutation testing (1000 iterations, $P < 0.05$). Data preprocessing, model fitting, and visualization were conducted using Python and OriginPro 2021.

CHAPTER 3

RESULTS

3.1. Study Area Characteristics and Sampling Sites

This study was conducted in Mizoram, a northeastern state of India, characterized by its strategic geographical location and diverse topographical features. Field surveys were systematically conducted across three distinct locations in Mizoram—Champhai, Kolasib, and Thenzawl, representing different elevational gradients ranging from high to low altitudes. Fifteen rice paddy fields were selected, with five fields per district, ensuring a representative assessment of cyanobacterial diversity across varying ecological conditions. Sampling was performed during two distinct periods, the growing season (July–September) and the post-harvest season (November–December), to capture seasonal variations in microbial community structure and environmental parameters.

These three study locations represent distinct agro-ecological zones in Mizoram. Champhai, with an elevation range of 1250–1300 m, falls under the high-altitude category, whereas Thenzawl, at 740–760 m, is classified as medium altitude. Kolasib, with an elevation between 40 m and 50 m, represents a lowland agricultural region. These variations in elevation significantly influence climatic conditions, soil properties, and microbial diversity, making them ideal for studying cyanobacterial assemblages in rice paddies

Figure 9. illustrates the geographical distribution of the study sites and highlights their relative locations in Mizoram. The main map presents topographical variation across the state, with the study sites distinctly marked. The inset maps provide a detailed view of each sampling site, indicating the specific paddy fields surveyed in Champhai (C1-C5), Kolasib (K1-K5), and Thenzawl (T1-T5). The color-coded elevation key further distinguished the altitude variation across the study region.

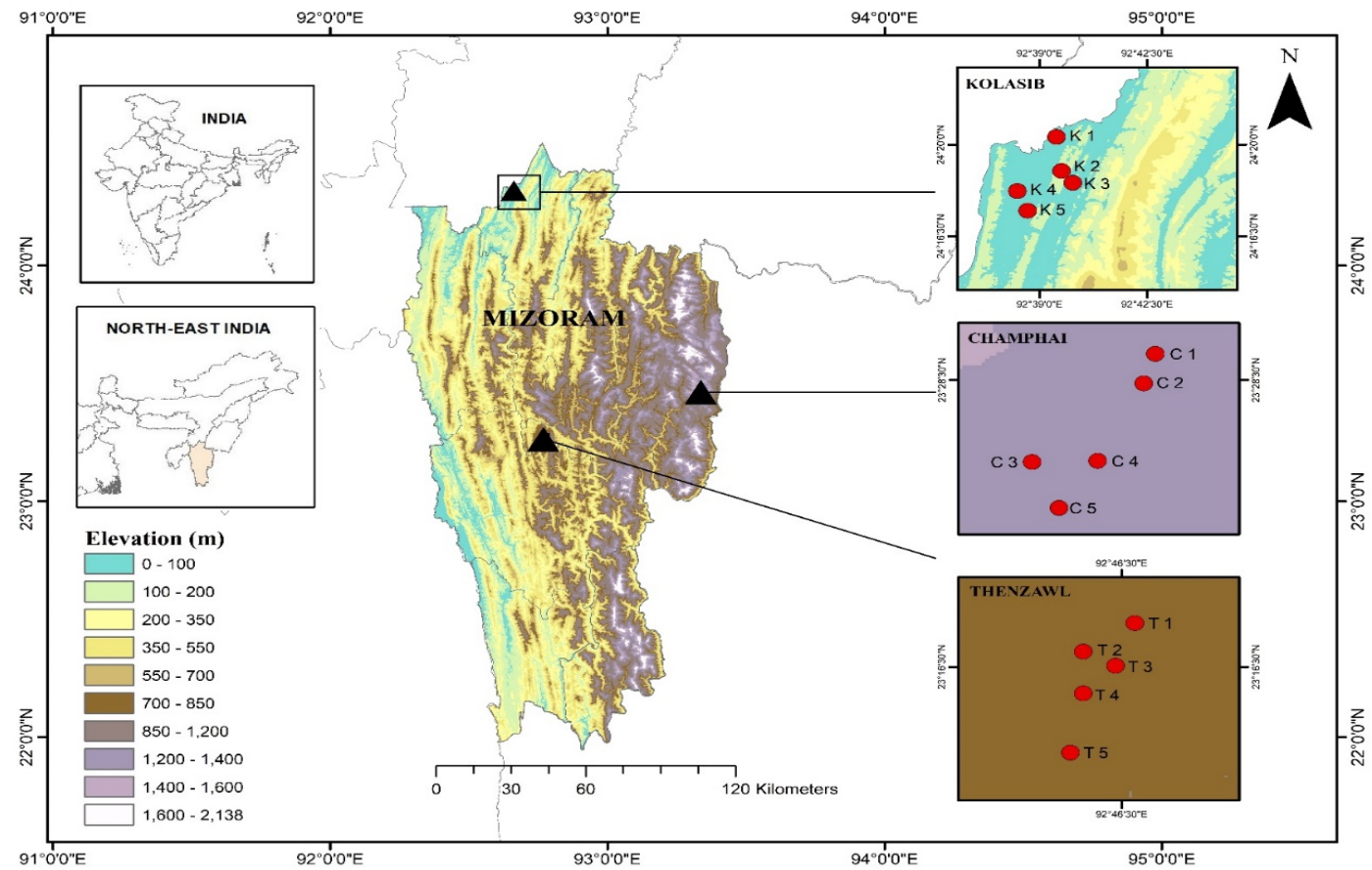


Figure 9. Map depicting the sampling sites- Champhai, Kolasib and Thenzawl paddy fields



Figure 10. (a) and (b) Champhai paddy fields during the growing season- season 1 (July- September) (c) and (d) Paddy field during post-harvest season- season 2 (November- December)



Figure 11. (a) and (b) Kolasib paddy fields during the growing season- season 1 (July-September) (c) and (d) Paddy field during post-harvest season- season 2 (November-December)



Figure 12. (a) and (b) Thenzawl paddy fields during the growing season- season 1 (July- September) (c) and (d) Paddy field during post-harvest season- season 2 (November- December)

Figures 10, 11, and 12 provide visual representations of the paddy fields in Champhai, Kolasib, and Thenzawl, respectively, during two distinct seasons. In each set of images, subfigures (a) and (b) depict the paddy fields during the growing season (July–September), characterized by lush green vegetation and flooded fields. Figures (c) and (d) show the same locations during the post-harvest season (November–December), marked by dry fields and residual stubble from the harvested rice crops. Additionally, image (d) in each figure provides close-up views of soil and water conditions, showcasing sampling procedures, and the presence of cyanobacterial growth in the paddy field ecosystem.

Field observations further confirmed the seasonal variations in cyanobacterial colonization patterns. The presence of dense cyanobacterial mats was more prominent in standing water during the growing season, particularly in Kolasib and Thenzawl, where lower elevation and prolonged water retention favored microbial proliferation. In contrast, during the postharvest period, cyanobacterial filaments and crusts were more apparent on Champhai's drier field surfaces, suggesting a shift toward desiccation-resistant forms. Close-up images of the paddy soil (Figures 10d, 11d, and 12d) revealed cyanobacterial aggregates attached to rice stubbles, particularly in the lowland and mid-altitude sites. The observed variations in cyanobacterial assemblages across sites and seasons indicate that elevation, hydrological conditions, and cropping stages play crucial roles in shaping microbial diversity in Mizoram's paddy field ecosystems.

3.2. Physico-chemical Properties of Paddy Soil and Water

The physicochemical parameters of paddy soil and water exhibited distinct seasonal variations between the growing season (July–September) and the post-harvest season (November–December) across all study sites (Tables 4 and 5). Soil moisture content varied significantly between seasons, with higher values recorded during the growing season because of continuous waterlogging in the paddy fields. The highest moisture content was observed in Thenzawl (TZ3:46.2% in Season 2), whereas Kolasib exhibited the lowest moisture levels, particularly in the post-harvest season (K1:4.8% in Season 2), reflecting rapid soil desiccation after harvesting. Conversely, the

moisture levels in Champhai and Thenzawl were relatively stable across seasons, suggesting better water retention capacity in these regions.

The soil pH remained within the slightly acidic to neutral range across all sites, with minor fluctuations between seasons. During Season 1, the lowest pH value (5.0) was recorded in Champhai (C3), whereas Kolasib (K3, K4) and TZ5 in Thenzawl) exhibited the highest pH value (6.8). In Season 2, there was a general increase in soil pH, with Champhai C1 and Kolasib K5 having the highest value (pH 6.7). This shift in pH across seasons may be attributed to the postharvest decomposition of organic matter and reduced microbial activity.

The electrical conductivity (EC) of the soil showed notable seasonal differences. During the growing season, EC ranged from 10.5 μS (TZ2) to 78.8 μS (C2), whereas in the post-harvest season, conductivity increased drastically, with the highest value observed in Thenzawl (TZ3:534 μS). Similarly, total dissolved solids (TDS) in paddy water were significantly higher in the post-harvest period, with Thenzawl (TZ3:389 ppm) and Kolasib (K3:358 ppm) exhibiting the highest concentrations. This trend suggests that water evaporation and nutrient concentration are key factors influencing post-harvest water quality. Paddy water pH remained slightly alkaline throughout season 1, with a narrow range of 6.0 (K1) to 6.8 (C5). Due to reduced water availability in many paddy fields during the post-harvest season (season 2), water pH data was limited. However, measurements from the six fields with water presence showed a similar slightly alkaline range of 6.0 (TZ3) to 6.8 (C5).

Soil salinity followed a similar pattern, with a noticeable increase during season 2. The highest salinity levels were detected in Thenzawl (TZ4: 268 ppm) and Kolasib (K3: 248 ppm), while Champhai exhibited lower salinity levels in both seasons. These variations indicate that lower-altitude paddy fields in Kolasib and Thenzawl experience greater salt accumulation, possibly because of higher evaporation rates and reduced rainfall in the post-harvest period.

Soil temperature also exhibited clear seasonal trends, with significantly higher temperatures recorded in Kolasib during the post-harvest season (K3: 28°C,

K4: 28.5°C), compared to Champhai and Thenzawl. Conversely, lower temperatures were recorded during the growing season (C1–C5: 21°C), reflecting the influence of continuous water saturation and lower ambient temperature in the monsoon period.

Organic carbon ranged from 1.2% in Champhai (C3) to 2.5% in Kolasib (K1) during the growing season while in the post-harvest season the highest was in Kolasib (K1: 2.4%) and the lowest in Champhai (C3: 1.2%), reflecting nutrient differences across elevations.

The observed seasonal variations in moisture content, pH, electrical conductivity, salinity, and soil temperature highlight the dynamic nature of paddy field ecosystems, influenced by elevation, hydrological changes, and agricultural practices.

Table 4. Physico-chemical parameters of paddy soil and water - season 1 (July- September)

Sl.No.	Site name	Moisture content of paddy soil (%)	pH of paddy soil	Conductivity (EC) of paddy soil (μS)	TDS of paddy water (ppm)	Salinity of paddy soil (ppm)	pH of paddy water (°C)	Temperature of paddy soil (°C)	Organic Carbon (%)
1.	C1	37.2±2.36 ^b	5.5±0.46 ^{cd}	22.7±1.05 ^{de}	17.1±1.50 ^{ef}	21.0±1.73 ^{cd}	6.6±0.30 ^{ab}	21±2.00 ^d	1.4±0.05 ^{efg}
2.	C2	30.9±1.90 ^c	5.7±0.26 ^{bcd}	78.8±2.02 ^a	54.2±2.71 ^b	38±3.00 ^a	6.5±0.30 ^{abcd}	21±1.73 ^d	1.5±0.15 ^{defg}
3.	C3	25.2±2.03 ^{de}	5.0±0.35 ^d	23.3±1.53 ^{de}	15.5±0.90 ^f	20.6±1.25 ^{cd}	6.5±0.40 ^{abcd}	21±2.08 ^d	1.2±0.15 ^g
4.	C4	26.4±2.51 ^d	6.6±0.49 ^a	14.5±0.50 ^{fg}	10.3±0.58 ^g	17.6±1.64 ^{def}	6.6±0.36 ^{abc}	21±1.73 ^d	1.3±0.15 ^{fg}

5.	C5	32.4±2.12 ^c	5.6±0.38 ^{cd}	27.3±1.53 ^c	16.3±1.15 ^{ef}	18.7±2.08 ^{cdef}	6.8±0.25 ^a	21±1.73 ^d	1.6±0.15 ^{cdef}
6.	K1	18.7±2.09 ^{fg}	6.5±0.42 ^a	22.7±1.53 ^{de}	16±1.00 ^{ef}	19.4±1.69 ^{cde}	6.0±0.26 ^d	32.5±2.34 ^b	2.5±0.25 ^a
7.	K2	14.9±2.20 ^{gh}	6.5±0.42 ^a	77.8±2.68 ^a	58.7±2.52 ^a	40.2±2.75 ^a	6.2±0.32 ^{bcd}	34±1.73 ^b	2.3±0.20 ^{ab}
8.	K3	23.7±2.08 ^{de}	6.8±0.42 ^a	25.4±1.29 ^{cd}	18.5±1.32 ^c	21.4±1.44 ^c	6.2±0.25 ^{bcd}	35±2.65 ^{ab}	2.2±0.10 ^{ab}
9.	K4	21.3±2.41 ^{ef}	6.8±0.40 ^a	40.4±1.80 ^b	30.5±2.16 ^c	27.3±2.08 ^b	6.2±0.32 ^{bcd}	37±2.65 ^{ab}	2.0±0.15 ^{bc}
10.	K5	13.9±1.01 ^h	5.4±0.35 ^d	22.1±1.85 ^e	15.6±1.64 ^f	18.6±1.21 ^{cdef}	6.3±0.30 ^{abcd}	37.7±2.51 ^a	2.4±0.30 ^a
11.	TZ1	40±3.00 ^{ab}	6.5±0.47 ^a	20.8±1.26 ^e	14.4±1.52 ^f	19.7±1.53 ^{cde}	6.1±0.26 ^{bcd}	29.2±1.27 ^c	1.4±0.37 ^{def}
12.	TZ2	41.3±3.51 ^{ab}	6.2±0.51 ^{abc}	10.5±0.87 ^h	7.4±0.53 ^h	15.9±0.90 ^f	6.0±0.25 ^{cd}	28.1±1.56 ^c	1.5±0.15 ^{def}
13.	TZ3	37.3±2.37 ^b	6.4±0.40 ^{ab}	16.5±1.50 ^f	10.7±0.58 ^g	17.8±1.26 ^{def}	6.4±0.25 ^{abcd}	27.5±0.50 ^c	1.8±0.15 ^{cd}
14.	TZ4	40.2±2.71 ^{ab}	6.6±0.49 ^a	13.4±1.51 ^g	9.7±0.58 ^{gh}	16.9±0.115 ^{ef}	6.2±0.25 ^{bcd}	29.3±0.57 ^c	1.7±0.25 ^{cde}
15.	TZ5	43.9±3.00 ^a	6.8±0.42 ^a	41.4±2.12 ^b	26.3±2.08 ^d	15.6±2.16 ^f	6.6±0.35 ^{ab}	23.3±0.57 ^d	2.0±0.05 ^{bc}

Table 5. Physico chemical parameters of paddy soil and water - season 2 (November – December)

Sl.No	Site name	Moisture content of paddy soil (%)	pH of paddy soil	Conductivity (EC)of paddy soil (μS)	TDS of paddy water (ppm)	Salinity of paddy soil (ppm)	pH of paddy water (°C)	Temperature of paddy soil (°C)	Organic Carbon (%)
1.	C1	34±2.64 ^b	6.7±0.45 ^a	84.4±2.50 ^j	-	41.3±3.21 ^{fg}	-	12±1.00 ^c	1.3±0.25 ^{cd}
2.	C2	32.6±2.42 ^{bc}	6.1±0.42 ^{abc}	23.5±1.50 ^k	-	24.6±2.16 ^h	-	12.5±0.87 ^c	1.3±0.15 ^{cd}
3.	C3	27.2±2.31 ^{de}	5.8±0.32 ^{bode}	42.8±2.56 ^k	30±2.00 ^e	28.4±2.14 ^{gh}	6.7±0.32 ^{ab}	12±1.00 ^c	1.2±0.15 ^d
4.	C4	22.2±1.66 ^{fgh}	5.6±0.46 ^{bode}	85.6±3.40 ^j	-	46.6±3.14 ^f	-	13±1.00 ^c	1.4±0.10 ^{cd}
5.	C5	25.8±1.61 ^{def}	6.2±0.44 ^{abc}	25.3±1.53 ^k	16.7±1.53 ^f	20.5±1.50 ^h	6.8±0.30 ^a	13±1.00 ^c	1.5±0.05 ^c
6.	K1	4.8±1.08 ^j	5.8±0.32 ^{bode}	466±28.00 ^c	-	72±4.36 ^e	-	27±1.73 ^a	2.4±0.25 ^a
7.	K2	11.2±1.06 ⁱ	5.5±0.35 ^{cde}	205±13.45 ^g	-	104±6.56 ^d	-	27±1.73 ^a	2.2±0.15 ^a
8.	K3	29±2.00 ^{cd}	5.3±0.30 ^{de}	496±20.11 ^b	358±18.68 ^b	248±13.08 ^b	6.4±0.35 ^b	28±1.73 ^a	2.3±0.15 ^a
9.	K4	23.2±1.89 ^{fg}	5.2±0.35 ^e	382±14.00 ^e	-	186±10.54 ^c	-	28.5±1.32 ^a	2.2±0.15 ^{ab}

10.	K5	18.6±1.51 ^h	6.7±0.42 ^a	234±19.52 ^f	-	113±7.00 ^d	-	27.5±1.50 ^a	2.2±0.15 ^{ab}
11.	TZ1	24±1.73 ^{efg}	5.9±0.40 ^{bcd}	127±5.20 ^h	85.4±5.05 ^d	65.5±3.91 ^e	6.7±0.32 ^{ab}	16±1.00 ^b	1.3±0.20 ^{cd}
12.	TZ2	44±2.65 ^a	5.6±0.38 ^{bcd}	185.3±8.02 ^g	-	41.5±2.29 ^{fg}	-	17±1.00 ^b	1.5±0.10 ^c
13.	TZ3	46.2±3.33 ^a	6.3±0.42 ^{ab}	534±26.06 ^a	389±22.11 ^a	262±17.58 ^a	6.0±0.25 ^c	17±1.73 ^b	1.9±0.15 ^b
14.	TZ4	31.2±1.91 ^{bc}	5.5±0.42 ^{cde}	429±19.00 ^d	-	268±14.80 ^a	-	18±1.00 ^b	1.6±0.15 ^c
15.	TZ5	20.2±2.31 ^{gh}	5.5±0.35 ^{cde}	152.2±8.52 ⁱ	108±6.56 ^c	76.1±4.61 ^c	6.6±0.31 ^{ab}	16±1.73 ^b	1.9±0.05 ^b

3.3.Principal Component Analysis (PCA) of Physico-Chemical Parameters

The Principal Component Analysis (PCA) of soil and water parameters demonstrated clear seasonal shifts and spatial clustering among study sites (Figure 13). In Season 1 (July–September), PC1 and PC2 accounted for 45.62% and 25.55% of the total variance, respectively (Figure 13a). Electrical conductivity (EC), total dissolved solids (TDS), and salinity were strongly and positively correlated with PC1 and PC2 particularly in Champhai C2. The pH and temperature of soil were strongly and positively correlated with PC1 in Kolasib (K2, K3, K4 and K5). The moisture content and pH of the water, on the other hand, were closely associated with PC2, clustering around Champhai (C1, C3, C4, C5) and Thenzawl (TZ5). These sites, which were positioned at higher elevations, exhibited greater water retention and moderate pH values. Soil temperature was negatively associated with PC2, aligning with K3 and K5, indicating warmer conditions in the

lower altitude paddy fields of Kolasib.

In Season 2 (November–December), PC1 and PC2 accounted for 41.00% and 28.25% of the total variation, respectively (Figure 13b). The strongest contributors to PC1 were TDS, salinity, and EC, which dominated Kolasib (K3) and Thenzawl (TZ3, TZ4), showing an increase in solute concentration and conductivity post-harvest. Soil temperature also correlated with PC1, with K1, K2, and K4 positioned towards the positive quadrant, indicating higher temperatures in Kolasib during this period. Meanwhile, the moisture content and pH of water were more prominent in Champhai (C1, C2, C3, C5) and Thenzawl (TZ1, TZ2), suggesting higher water availability and stable pH levels in these regions. The distinct separation of sites along the PCA axes highlights the role of elevation and seasonal changes in shaping the soil properties. Lower altitude sites (Kolasib) were more affected by salinity, conductivity, and temperature, while mid-to high-altitude sites (Champhai and Thenzawl) retained higher moisture and balanced pH. These findings emphasize the influence of landscape position and post-harvest conditions on soil-water interactions in Mizoram's paddy fields.

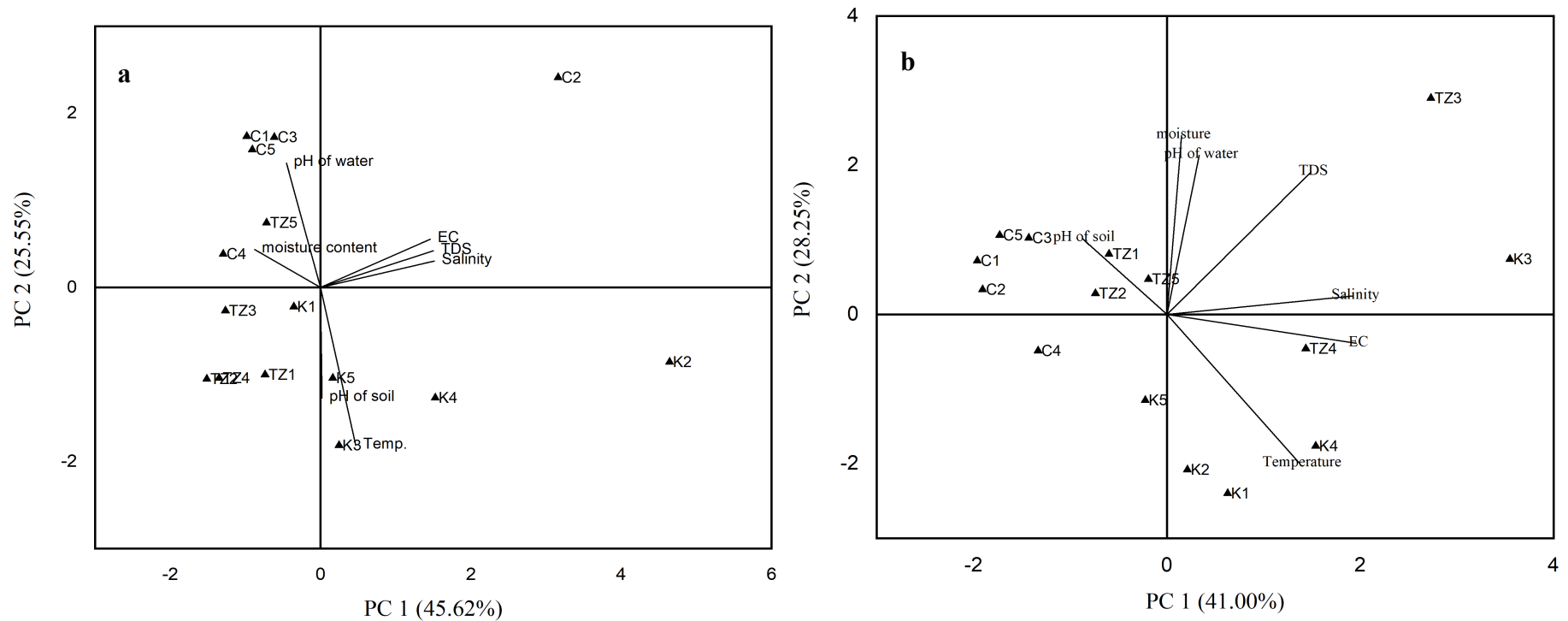


Figure 13: (a) Principal Component Analysis (PCA) biplot showing the distribution of study sites based on physico-chemical parameters of paddy soil and water during season 1 (July–September), showing key influencing factors such as moisture content, pH, salinity, EC, TDS, and temperature. (b) Principal component analysis (PCA) biplot illustrating the variation in study sites according to physico-chemical parameters of paddy soil and water during season 2 (November–December), showing shifts in soil moisture, pH, salinity, EC, TDS, and temperature post-harvest.

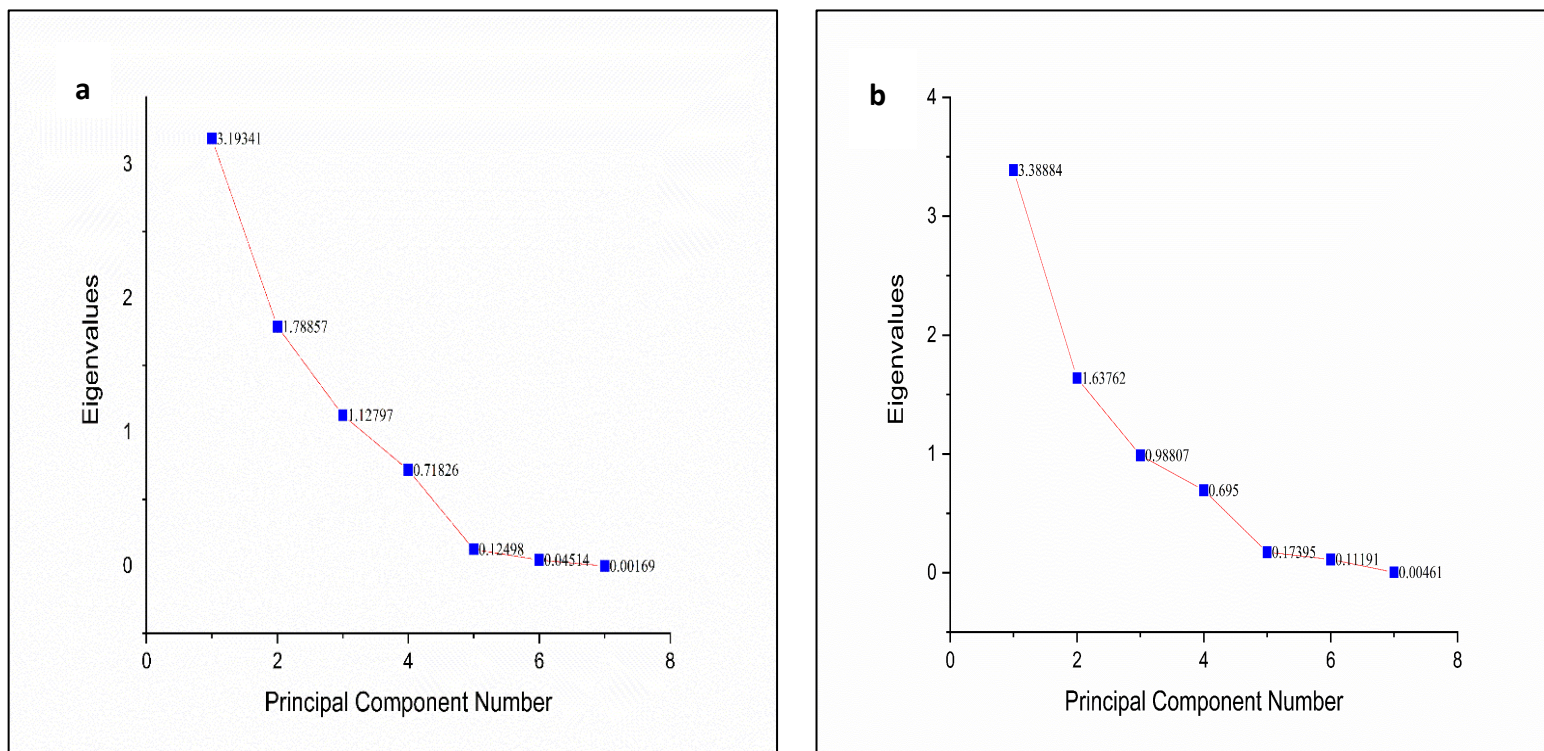


Figure 14: (a) Eigenvalues vs principal component number – season 1 (b) Eigenvalues vs principal component number – season 2

Figure 14a and 14b illustrate the eigenvalues associated with the principal components (PCs) derived from the physicochemical parameters of paddy soil and water during Season 1 (July–September) and Season 2 (November–December), respectively. In Season 1 (Figure 14a), the first two principal components (PC1 and PC2) exhibited eigenvalues > 1 , accounting for a substantial proportion of the total variance. This indicates that these components were the most influential. The subsequent components showed a sharp decline in eigenvalues, signifying their reduced contribution to overall variability. Similarly, in Season 2 (Figure 14b), the first two principal components retained eigenvalues > 1 , with PC1 (3.38) and PC2 (1.63) explaining the majority of the variation, followed by a gradual decline in eigenvalues for the remaining components. It was concluded that the major environmental factors influencing soil and water conditions remained consistent across seasons, with PC1 and PC2 capturing the most significant trends in variability.

3.4. Partial Least Squares (PLS) regression of Physico-Chemical Parameters

To assess the influence of environmental factors on cyanobacterial genus abundance, Partial Least Squares (PLS) regression was performed across 15 sites and two seasons ($n = 30$ observations). The model, incorporating pH, EC, temperature, organic carbon, nitrogen, and phosphorus, explained 70% of the variance ($R^2 = 0.697$, $P < 0.01$) (Figure 15.a). VIP scores identified temperature (VIP = 1.599) and organic carbon (VIP = 1.365) as the strongest predictors. (Figure 15.b). Sites with higher temperatures (e.g., K2: 37.7°C, 8 genera in S1) and organic carbon levels (e.g., TZ4: 1.8%, 6 genera in S1) supported greater cyanobacterial diversity, particularly in warmer regions. In contrast, pH (VIP = 0.484), EC (VIP = 0.134), nitrogen (VIP = 0.336), and phosphorus had weaker influences. Phosphorus, despite its biological importance, was not directly retained as a significant predictor due to moderate collinearity with organic carbon, suggesting its role may have been indirectly captured. The pH showed minimal variation (5.0–6.8), and EC fluctuations (e.g., TZ3: 534 $\mu\text{S}/\text{cm}$ in S2) were not strongly linked to abundance shifts. These findings highlight the dominant role of temperature and organic carbon in cyanobacterial abundance, whereas pH, EC, nitrogen, and phosphorus played secondary or indirect roles. The results align with the nutrient-

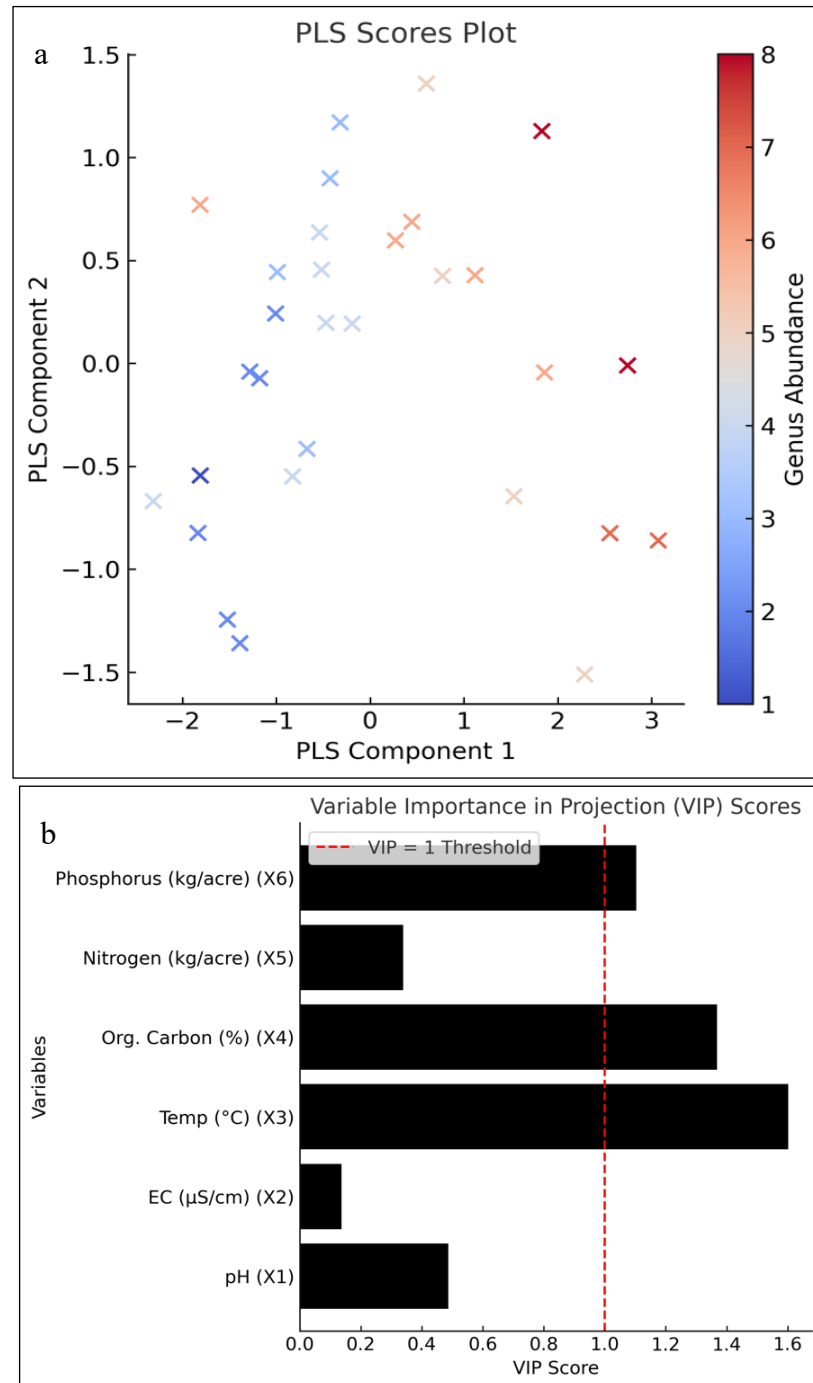


Figure 15. (a) PLS regression showing the relationships between standardized environmental predictors and cyanobacterial genus abundance across 15 paddy fields in Mizoram during the growing (S1) and post-harvest (S2) seasons (n=30). (b) VIP Plot highlighting the importance of each variable with the threshold VIP = 1 (red dashed line). Variable importance in projection (VIP) scores indicate predictor influence, with $R^2=0.69.7$, and $P<0.01$ for the overall model

temperature synergy hypothesis, where organic matter enrichment and thermal conditions drive cyanobacterial growth rather than pH or conductivity variations

3.5. Influence of Environmental Factors on Cyanobacterial Abundance

The relationship between cyanobacterial abundance and environmental parameters (pH, electrical conductivity, and temperature) was examined across both the growing (S1) and post-harvest (S2) seasons to assess potential correlations. Regression analysis revealed weak correlations between cyanobacterial abundance and the selected environmental variables, suggesting that these individual parameters may not be the primary determinants of cyanobacterial community dynamics in the studied paddy fields (Figure 16).

The correlation between temperature and relative abundance yielded an R^2 value of 0.0185 (Figure 16c), indicating the extremely weak influence of temperature on cyanobacterial population fluctuations. Similarly, the relationship between pH and cyanobacterial abundance (Figures 16a and 16d) exhibited low R^2 values (0.1357 and 0.0025, respectively), suggesting minimal direct effects of pH variation on community composition. Electrical conductivity (EC) also showed negligible correlations with cyanobacterial abundance ($R^2 = 0.0939$ and 0.0125 in Figures 16b and 16e, respectively), further reinforcing the limited role of these physicochemical factors as independent drivers of cyanobacterial distribution in this ecosystem.

The weak correlations observed suggest that cyanobacterial abundance in paddy fields is likely governed by a complex interplay of multiple environmental factors rather than by single variables acting in isolation. While pH, temperature, and EC are known to influence microbial communities, their effects may be overshadowed by nutrient availability, light penetration, organic matter content, and hydrological conditions. Additionally, the presence of rice plants and seasonal agricultural practices (e.g., fertilizer application and irrigation regimes) may further modulate cyanobacterial growth patterns.

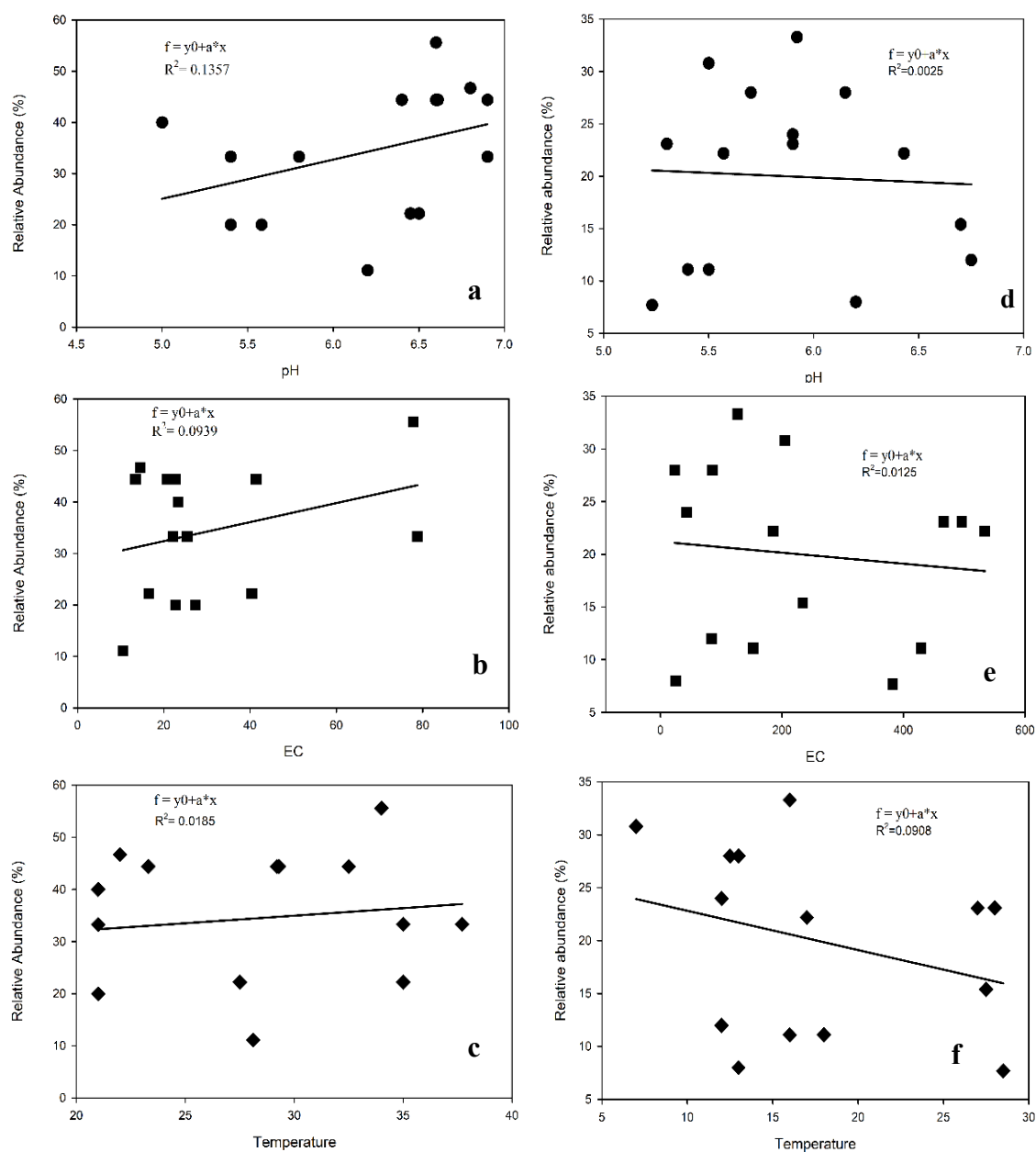


Figure 16. (a) to (c) represent the abundance of cyanobacterial populations (genera) in season 1 as a function of pH, conductivity, and temperature, respectively. (d)–(f) represent the abundance of cyanobacterial populations in season 2 as a function of pH, conductivity, and temperature, respectively.

3.6. Heavy Metal Content in Paddy Soil Samples

The analysis of heavy metal concentrations in paddy soil samples revealed notable spatial variations across the study sites (Table 6). Among the detected metals, iron (Fe) was the most abundant, with concentrations ranging from 3631.7 mg/kg (C1) to 4244.63 mg/kg (K1). The highest Fe concentrations were observed in K1 (Kolasib), whereas the Champhai sites had relatively lower values. This trend indicates a possible influence of soil composition and mineral content, as Fe is a naturally occurring element in agricultural soils and is often derived from parent rock material. The higher Fe levels in Kolasib could also be attributed to greater weathering and leaching processes in the lowland paddy fields.

Copper (Cu) concentrations varied significantly among the sites, with the highest levels recorded in Kolasib (K2: 22.00 mg/kg, K4: 22.07 mg/kg, K5: 22.33 mg/kg). In contrast, Champhai (C1: 10.06 mg/kg, C2: 11.34 mg/kg) and Thenzawl (TZ1: 9.3 mg/kg) exhibited the lowest Cu levels. Copper is an essential micronutrient for plant growth, but excessive accumulation can be indicative of agricultural inputs, such as fertilizers, pesticides, or industrial runoff. The significantly higher Cu content in Kolasib may be linked to the intensive farming practices and greater agrochemical application in this region.

Lead (Pb) concentrations displayed substantial site-specific variations. The highest Pb levels were detected in Kolasib (K2: 17.87 mg/kg, K3: 17.81 mg/kg), whereas Champhai (C2: 2.64 mg/kg, C4: 2.38 mg/kg, C5: ND) and Thenzawl (TZ1: 2.43 mg/kg, TZ2: 2.59 mg/kg) recorded significantly lower values. Notably, Pb was not detected at C5, suggesting localized differences in metal contamination. The elevated Pb concentrations in Kolasib indicate potential anthropogenic contributions, such as vehicular emissions, industrial discharge, and long-term agricultural activity. Pb is a non-essential and toxic heavy metal, and its accumulation in soil can pose environmental and health risks, particularly through its uptake by rice plants.

Manganese (Mn) concentrations were highest in Kolasib (K5: 441.3 mg/kg, K4: 351.2 mg/kg, K1: 341.4 mg/kg), while the lowest Mn levels were recorded in Thenzawl (TZ1: 50.3 mg/kg) and Champhai (C4: 79.4 mg/kg). Mn plays a crucial role

in enzymatic reactions and plant metabolism, but excessive Mn can impair plant growth and affect soil microbial communities. The significantly higher Mn accumulation in Kolasib suggests that lowland areas experience greater retention of trace metals, possibly due to higher clay content and reduced drainage conditions.

Zinc (Zn) concentrations followed a similar spatial pattern, with K1 (278.23 mg/kg) exhibiting the highest Zn content, while Champhai sites recorded the lowest Zn levels (C1: 31.14 mg/kg, C2: 35.2 mg/kg, C4: 33.26 mg/kg, C5: 33.55 mg/kg). Zinc is an essential micronutrient; however, excessive Zn levels may result from agrochemical use, industrial activities, or metal-enriched fertilizers. The striking difference in Zn concentrations between Champhai and Kolasib can be attributed to differences in soil composition, organic matter content, and irrigation patterns.

The above results suggested that the heavy metal distribution across the study sites exhibited a clear elevation-dependent trend, with Kolasib (lowland) accumulating the highest concentrations of Cu, Pb, Mn, and Zn, whereas Champhai (highland) showed the lowest metal content. Thenzawl displayed intermediate values, suggesting a gradual transition in heavy metal accumulation along an altitude gradient. The higher heavy metal content in Kolasib may be due to greater anthropogenic inputs, intensive agricultural practices, reduced soil drainage, and enhanced metal retention in the clay-rich soils. In contrast, the lower heavy metal accumulation in Champhai could be a result of better drainage, lower human activity, and minimal agrochemical intervention.

Table 6. Amount of heavy metals (Cu, Pb, Fe, Mn, and Zn) detected in soil samples from different sites of Mizoram paddy fields. All values are presented as mean $\pm$ SD (standard deviation of the mean) with n = 3 replicates. Values in a column with different letters denote significant variations ($P > 0.05$) based on the Duncan's multiple range test

Sl.no	Site	Cu (mg/kg)	Pb (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	Zn (mg/kg)
1.	C1	10.06 $\pm$ 0.97 ^d	6.4 $\pm$ 0.73 ^d	3631.7 $\pm$ 208.75 ^c	111 $\pm$ 8.29 ^{de}	31.14 $\pm$ 1.77 ^f
2.	C2	11.34 $\pm$ 0.62 ^d	2.64 $\pm$ 0.21 ^e	3730.7 $\pm$ 216.86 ^{bc}	124 $\pm$ 8.85 ^d	35.2 $\pm$ 3.64 ^f
3.	C3	14.27 $\pm$ 1.24 ^c	8.95 $\pm$ 0.57 ^c	3932.7 $\pm$ 228.19 ^{abc}	121 $\pm$ 11.15 ^d	46.86 $\pm$ 2.23 ^e
4.	C4	11.63 $\pm$ 1.31 ^d	2.38 $\pm$ 0.16 ^e	3640.8 $\pm$ 246.80 ^c	79.4 $\pm$ 8.37 ^{ef}	33.26 $\pm$ 2.33 ^f
5.	C5	11.66 $\pm$ 0.77 ^d	ND	3741.4 $\pm$ 156.54 ^{abc}	142.8 $\pm$ 15.16 ^d	33.55 $\pm$ 3.46 ^f
6.	K1	20.79 $\pm$ 1.83 ^a	12.7 $\pm$ 0.76 ^b	4244.63 $\pm$ 241.26 ^a	341.4 $\pm$ 34.24 ^b	278.23 $\pm$ 15.39 ^a
7.	K2	22 $\pm$ 1.97 ^a	17.87 $\pm$ 0.96 ^a	4203.35 $\pm$ 282.55 ^{ab}	340.2 $\pm$ 34.04 ^b	78.5 $\pm$ 6.58 ^{bc}
8.	K3	16.13 $\pm$ 1.55 ^{bc}	17.81 $\pm$ 1.95 ^a	4079.10 $\pm$ 394.86 ^{abc}	223.7 $\pm$ 13.91 ^c	55.39 $\pm$ 5.06 ^c
9.	K4	22.07 $\pm$ 2.03 ^a	12.74 $\pm$ 1.50 ^b	4198.48 $\pm$ 305.66 ^{ab}	351.2 $\pm$ 25.77 ^b	66.29 $\pm$ 2.77 ^d
10.	K5	22.33 $\pm$ 1.99 ^a	7.81 $\pm$ 0.46 ^{cd}	4016.1 $\pm$ 290.12 ^{abc}	441.3 $\pm$ 25.23 ^a	87.09 $\pm$ 7.68 ^b

11.	TZ1	9.3 ±1.02 ^d	2.43±0.13 ^e	3682.8 ± 201.86 ^c	50.3 ± 1.73 ^f	37.21 ± 3.00 ^f
12.	TZ2	14.68 ±1.01 ^{bc}	2.59±0.20 ^e	3924.3 ± 243.30 ^{abc}	79.3± 4.78 ^{ef}	51.73 ± 3.84 ^e
13.	TZ3	16.92 ±1.50 ^b	11.6±0.65 ^b	4059.2 ± 261.05 ^{abc}	123.5 ± 9.44 ^d	84.79 ± 3.97 ^b
14.	TZ4	16.38 ±1.09 ^{bc}	8.49 ±0.76 ^c	3813.5 ± 202.73 ^{abc}	78.9 ± 6.90 ^{ef}	73.24 ± 5.40 ^{cd}
15.	TZ5	16.18 ±1.01 ^{bc}	6.39±0.58 ^d	4006.8 ±294.20 ^{abc}	79.4 ± 3.71 ^{ef}	84.384.38 ^b

3.7.Nitrogen, Phosphorus and Potassium Content in Paddy Soil Samples

The nitrogen (N), phosphorus (P), and potassium (K) contents in paddy soils across different study sites exhibited significant spatial variations, as presented in Table 7. Among the sites, Thenzawl (TZ4) recorded the highest nitrogen concentration (281.7±0.58 kg/acre), whereas the lowest nitrogen content was observed in Thenzawl (TZ1) at 101.3±0.58 kg/acre, indicating considerable site-specific differences in soil fertility. Champhai (C3, C2) and Thenzawl (TZ2, TZ3) also showed relatively high nitrogen contents, suggesting a better nitrogen reservoir in these locations. Conversely, the lowest nitrogen levels were found in Kolasib (K1, K4) and Champhai (C5), which may have affected rice productivity in these regions. Statistical analysis revealed significant differences ($P>0.05$) among the sites, highlighting the variability in nitrogen availability across different elevations and soil conditions.

Phosphorus content varied significantly among the study sites, with K5 (29.7 ± 0.58 kg/acre) exhibiting the highest concentration, followed by K3 (25.7 ± 0.58 kg/acre) and K2 (24.3 ± 0.58 kg/acre), indicating better phosphorus retention in Kolasib soils. In contrast, TZ5 (12.3 ± 0.58 kg/acre) and TZ4 (12.7 ± 0.58 kg/acre) exhibited low phosphorus levels, suggesting lower phosphorus availability in Thenzawl, which could limit crop nutrient uptake and overall yield. The phosphorus content in Champhai was moderate, ranging between 18.0–24.3 kg/acre, with significant variations within the sites ($P > 0.05$). The variation in phosphorus availability across sites may be influenced by differences in soil texture, organic matter decomposition, and phosphorus fixation rates.

Potassium levels demonstrated a contrasting trend, with the highest concentration observed in TZ3 (150.3 ± 0.58 kg/acre), followed closely by TZ4 (148.0 ± 2.00 kg/acre) and K5 (147.0 ± 1.00 kg/acre). Conversely, the lowest potassium levels were detected in C3 (52.7 ± 0.58 kg/acre), which was significantly lower than that at other sites ($P > 0.05$), indicating possible potassium depletion in this location. Champhai sites (C1, C2, C5) exhibited relatively lower potassium content, which may suggest lower soil fertility and the need for potassium supplementation in agricultural management practices. The variation in K levels across sites highlights the influence of soil mineralogy, cation exchange capacity, and fertilizer application practices.

The above results indicate that the observed spatial variations in macronutrient content indicate that Kolasib sites generally exhibited higher phosphorus and potassium levels, while Thenzawl sites had the highest nitrogen availability. In contrast, some sites in Champhai exhibited lower macronutrient concentrations.

A critical analysis of the results further suggested that the macronutrient fertility of paddy soils in the surveyed Mizoram sites was relatively uniform and generally suboptimal. The absence of significant spatial differences in N, P, and K (all $P > 0.05$) implies that no particular site enjoys a markedly better soil nutrient status than the other sites; rather, all sites face similar fertility constraints. This uniformity likely reflects the common environmental conditions – Mizoram's high rainfall, hilly terrain, and acidic parent soils – as well as comparable agronomic practices across the region (e.g., similar rice cultivation methods and fertilizer regimes). Any subtle

variations observed (such as one site's slightly higher N or K) can be attributed to localized factors, such as differences in past fertilizer application, crop residue management, or organic matter accumulation, but these were not large enough to drive a significant divergence in soil fertility.

Critically, the findings highlight that phosphorus deficiency is a widespread issue in these paddy fields, as all sites showed low available P (Table 7). This aligns with regional studies noting that Northeast India's soils (including Mizoram's) are characteristically low in P owing to intense leaching and strong P fixation by iron and aluminum oxides.

Table 7. The nitrogen, phosphorus and potassium content (kg/acre) detected in soil samples from different sites of Mizoram paddy fields. All values are presented as mean $\pm$ SD (standard deviation of the mean) with n = 3 replicates. Values in a column with different letters denote significant variations ($P > 0.05$) based on the Duncan's multiple range test

Sl. No	Site	Nitrogen content (kg/acre)	Phosphorous content (kg/acre)	Potassium content (kg/acre)
1.	C1	151.3 $\pm$ 0.58 ^k	18.0 $\pm$ 1.00 ^d	137.7 $\pm$ 1.53 ^h
2.	C2	203.3 $\pm$ 0.58 ^e	22.3 $\pm$ 0.58 ^c	145.3 $\pm$ 0.58 ^{de}
3.	C3	229.3 $\pm$ 0.58 ^c	19.0 $\pm$ 1.00 ^d	52.7 $\pm$ 0.58 ^k
4.	C4	195.7 $\pm$ 1.15 ^f	18.3 $\pm$ 0.58 ^d	141.7 $\pm$ 0.58 ^f
5.	C5	117.7 $\pm$ 0.58 ^m	24.3 $\pm$ 0.58 ^b	100.7 $\pm$ 0.58 ^j
6.	K1	126.3 $\pm$ 0.58 ^l	14.7 $\pm$ 0.58 ^e	145.7 $\pm$ 0.58 ^{cde}
7.	K2	153.0 $\pm$ 1.00 ^j	24.3 $\pm$ 0.58 ^b	145.0 $\pm$ 1.00 ^e
8.	K3	189.3 $\pm$ 0.58 ^h	25.7 $\pm$ 0.58 ^b	145.3 $\pm$ 0.58 ^{de}
9.	K4	134.3 $\pm$ 0.58 ^g	8.7 $\pm$ 0.58 ^g	146.7 $\pm$ 0.58 ^{bcd}
10.	K5	167.3 $\pm$ 0.58 ⁱ	29.7 $\pm$ 0.58 ^a	147.0 $\pm$ 1.00 ^{bc}
11.	TZ1	101.3 $\pm$ 0.58 ⁿ	14.3 $\pm$ 0.58 ^e	137.3 $\pm$ 0.58 ^h

12.	TZ2	261.0±1.00 ^b	21.3±0.58 ^c	135.3±0.58 ⁱ
13.	TZ3	217.7±0.58 ^d	15.0±1.73 ^e	150.3±0.58 ^a
14.	TZ4	281.7±0.58 ^a	12.7±0.58 ^f	148.0±2.00 ^b
15.	TZ5	189.7±0.58 ^h	12.3±0.58 ^f	139.7±0.58 ^g

3.8.Diversity and Distribution of Cyanobacteria in Paddy Fields

The present study identified a total of 76 cyanobacterial species with 70 new records for Mizoram across the three paddy field locations—Champhai (high elevation), Kolasib (low elevation), and Thenzawl (medium elevation)—over two seasons. The cyanobacterial species were classified into 27 genera spanning 14 families, with Nostocaceae emerging as the most dominant family, followed by Chroococcaceae and Aphanothecaceae. At the genus level, *Nostoc* and *Chroococcus* were the most abundant, whereas *Anabaena* and *Aphanothece* were also frequently observed. The diversity of cyanobacteria across locations and seasons suggests that environmental parameters, such as elevation, soil conditions, moisture content, and seasonal variations, influence cyanobacterial community composition in these paddy fields.

A comparison of heterocystous and non-heterocystous cyanobacteria revealed that heterocystous forms were represented by 27 species belonging to seven genera (*Anabaena*, *Brachytrichia*, *Calothrix*, *Fischerella*, *Hapalosiphon*, *Aulosira*, and *Nostoc*), whereas non-heterocystous species comprised 49 species, spanning 20 genera. The presence of a high proportion of non-heterocystous cyanobacteria, including *Chroococcus*, *Microcystis*, and *Aphanocapsa*, indicates that these species may be favored in rice paddy fields with high nutrient inputs and fluctuating environmental conditions. The filamentous vs. non-filamentous categorization further revealed that 12 genera were filamentous, with *Oscillatoria*, *Lyngbya*, *Nostoc*, and *Spirulina* among the prominent ones, whereas 15 genera were non-filamentous, suggesting morphological adaptations to different ecological niches.

3.8.1. Spatial and Seasonal Distribution of Cyanobacteria

The distribution of cyanobacterial species varied significantly across sites and seasons (Table 8). A total of 25 species were unique to Champhai consisting of *Anabaena variabilis*, *Aphanocapsa montana*, *Anabaena spiroides*, *Calothrix fusca*, *Chlorogloea fritschii*, *Chroococcidiopsis indica*, *Chroococcus macrococcus*, *Chroococcus schizodermaticus*, *Chroococcus tenax*, *Fischerella ambigua*, *Gloeocapsa decorticans*, *Gloeotheca rhodochlamys*, *Gomphosphaerica lacustris*, *Hapalosiphon welwitschii*, *Hydrococcus rivularis*, *Johannesbaptistia pellucida*, *Lyngbya sordida*, *Microcystis pulvereae*, *Myxosarcina burmensis*, *Nostoc carneum*, *Nostoc calcicole*, *Nostoc muscorum* (PP848104), *Pseudanabaena catenate*, *Synechocystis pevalekii* (PP853584) and *Spirulina gigantea*. Kolasib showed 14 species which were exclusive to the area, they were *Anabaena circinalis*, *Anabaena flos-aquae* (PP809508), *Aphanocapsa roeseana*, *Aphanocapsa koordesi*, *Aphanothece microscopica*, *Aphanothece nidulans*, *Chroococcus coharens*, *Entophysalis granulosa*, *Hapalosiphon intricatus*, *Microcystis marginata*, *Nostoc entophytum* (PP848103), *Oscillatoria princeps*, *Oscillatoria sancta* and *Oscillatoria vizagapatensis*. There were 10 species found only in Thenzawl – *Aphanothece saxicola*, *Brachytrichia quoyi*, *Calothrix geitonas*, *Chlorogloea microstoides*, *Chroococcus dispersus*, *Lyngbya limnetica*, *Lyngbya stagnina*, *Merismopedia elegans*, *Nostoc ellipsosporum* and *Plectonema nostocorum*. Notably, species such as *Aphanocapsa montana* and *Aphanocapsa roeseana*, were detected only in Kolasib, whereas *Calothrix geitonas* and *Calothrix marchica* were restricted to Thenzawl, indicating a potential elevation-driven distribution pattern. Similarly, *Nostoc linckia* exhibited a wider distribution and was detected across multiple sites, suggesting greater tolerance to varying environmental conditions. The colonies of selected cyanobacterial isolates are shown in Figure 17.

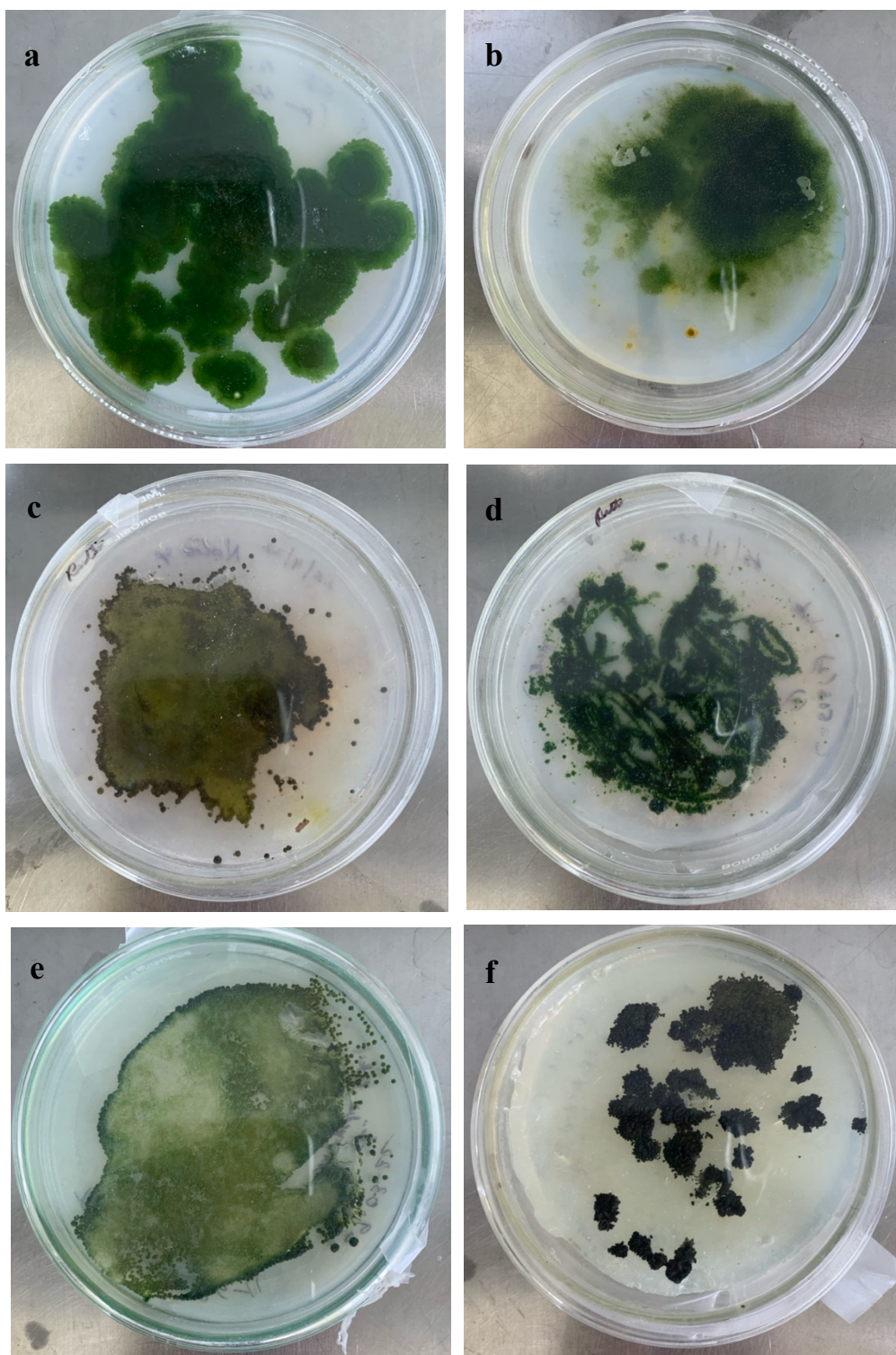


Figure 17 . Colonies of selected cyanobacterial isolates on solidified agar petri-plates.
 (a) *Anabaena sphaerica* (b) *Oscillatoria subbrevis* (c) *Nostoc linckia* (d) *Microcystis aeruginosa* (e) *Aphanothece naegelli* (f) *Calothrix marchica*.

Seasonal variations in cyanobacterial diversity were evident, with certain species present only in S1 (growing season: July–September) and absent in S2 (postharvest season: November–December). For instance, *Merismopedia glauca* was recorded exclusively in S1 at Thenzawl, whereas *Microcystis pulvere*a was only observed in S1 at Champhai, highlighting the influence of seasonal changes in moisture, nutrient levels, and agricultural practices on cyanobacterial abundance. The decline in species occurrence in S2 aligns with the post-harvest period, during which paddy fields undergo significant changes in water availability, soil structure, and organic matter decomposition, potentially limiting the survival of certain cyanobacterial species.

The graphical representation in Figure 18 illustrates seasonal fluctuations in cyanobacterial diversity across the three sites. The monsoon season (S1) coincides with peak rice cultivation and high water availability, which may promote the proliferation of free-living planktonic cyanobacteria such as *Microcystis*, *Aphanothece*, and *Synechocystis*. In contrast, during the post-harvest season (S2), when fields are either left fallow or prepared for the next cropping cycle, desiccation-resistant and benthic cyanobacteria like *Nostoc* and *Calothrix* tend to dominate. This seasonal shift suggests that environmental factors such as soil moisture retention, temperature fluctuations, and nutrient cycling influence cyanobacterial community dynamics in paddy fields.

3.8.2. Implications for Soil Health and Nitrogen Fixation

The presence of heterocystous cyanobacteria, particularly *Anabaena*, *Nostoc*, and *Calothrix*, is significant from an agricultural perspective, as these genera are known for their biological nitrogen-fixation capabilities, contributing to soil fertility and nitrogen enrichment in rice paddies. The higher abundance of non-heterocystous species in certain locations may indicate a dependence on external nitrogen sources,

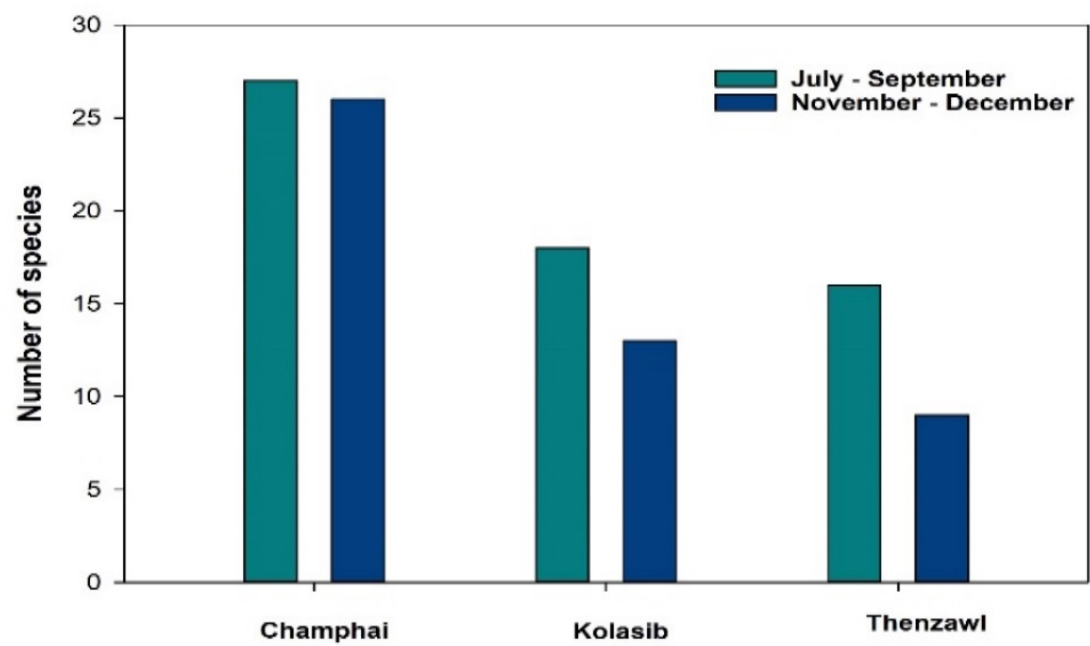


Figure 18. The graph represents the number species present in the sites Champhai, Kolasib, Thenzawl for both the season 1 (July - September) and season 2 (November - December).

such as fertilizers, which could influence the microbial community structure over time. The dominance of Nostocaceae and Chroococcaceae families suggests that in the process of soil nutrient cycling, organic matter decomposition and microbial interactions in rice agroecosystems. Cyanobacteria play essential roles in this process. Variation in species distribution across different elevations and seasons underscores the ecological adaptability of cyanobacteria. The dominance of unicellular cyanobacteria in certain locations, such as *Chroococcus* and *Microcystis* in Kolasib, suggests that these regions may have higher nutrient concentrations or favorable growth conditions for bloom-forming species. The presence of filamentous cyanobacteria such as *Oscillatoria* and *Lyngbya* at multiple sites suggests a greater tolerance to fluctuating soil moisture and desiccation stress. Mongraphs of selected identified cyanobacterial species are displayed in Plates 1 – 5.

Table 8. Distribution of cyanobacterial species across sites and seasons

Sl. No	Species name	Voucher Number	Champhai		Kolasib		Thenzawl		New Record Status
			S1	S2	S1	S2	S1	S2	
1.	<i>Anabaena oscillariodes</i>	MZUCC446	+	-	+	-	-	-	*
2.	<i>Anabaena circinalis</i>	MZUCC447	-	-	-	+	-	-	*
3.	<i>Anabaena flos-aquae</i>	MZUCC515	-	-	+	-	-	-	*
4.	<i>Anabaena inaequalis</i>	MZUCC448	+	-	+	-	-	-	*
5.	<i>Anabaena sphaerica</i>	MZUCC449	+	+	+	-	-	-	*
6.	<i>Anabaena spiroides</i>	MZUCC450	+	+	-	-	-	-	*
7.	<i>Anabaena variabilis</i>	MZUCC451	-	+	-	-	-	-	*
8.	<i>Aphanocapsa biformis</i>	MZUCC452	+	-	+	-	-	-	*
9.	<i>Aphanocapsa montana</i>	MZUCC453	-	+	-	-	-	-	*
10.	<i>Aphanocapsa roeseana</i>	MZUCC454	-	-	+	-	-	-	*
11.	<i>Aphanocapsa koordesi</i>	MZUCC455	-	-	+	-	-	-	*
12.	<i>Aphanothece microscopica</i>	MZUCC456	-	-	-	+	-	-	*
13.	<i>Aphanothece naegellii</i>	MZUCC457	+	+	-	+	-	-	*
14.	<i>Aphanothece nidulans</i>	MZUCC458	-	-	-	+	-	-	*
15.	<i>Aphanothece pallida</i>	MZUCC459	+	-	-	+	+	-	*
16.	<i>Aphanothece saxicola</i>	MZUCC460	-	-	-	-	+	-	*

17.	<i>Aphanothece stagnina</i>	MZUCC461	-	-	-	-	+	-	*
18.	<i>Aulosira fertilissima</i>	MZUCC517	+	+	-	-	-	+	*
19.	<i>Brachytrichia quoyi</i>	MZUCC462	-	-	-	-	-	+	*
20.	<i>Calothrix fusca</i>	MZUCC463	+	+	-	-	-	-	*
21.	<i>Calothrix geitonas</i>	MZUCC464	-	-	-	-	+	-	*
22.	<i>Calothrix marchica</i>	MZUCC465	-	+	+	+	+	-	
23.	<i>Calothrix</i> <i>membranacea</i>	MZUCC466	+	-	+	-	-	-	*
24.	<i>Chlorogloea fritschii</i>	MZUCC467	+	-	-	-	-	-	*
25.	<i>Chlorogloea</i> <i>microstoides</i>	MZUCC468	-	-	-	-	+	-	*
26.	<i>Chroococcidiopsis</i> <i>indica</i>	MZUCC469	-	+	-	-	-	-	*
27.	<i>Chroococcus coharens</i>	MZUCC470	-	-	+	-	-	-	*
28.	<i>Chroococcus dispersus</i>	MZUCC471	-	-	-	-	-	+	*
29.	<i>Chroococcus</i> <i>macrococcus</i>	MZUCC472	-	+	-	-	-	-	*
30.	<i>Chroococcus minor</i>	MZUCC473	+	-	-	-	+	-	*
31.	<i>Chroococcus minutus</i>	MZUCC474	+	-	+	-	-	-	*
32.	<i>Chroococcus</i> <i>mipitanensis</i>	MZUCC475	-	-	-	+	+	-	*
33.	<i>Chroococcus</i> <i>schizodermaticus</i>	MZUCC476	+	-	-	-	-	-	*
34.	<i>Chroococcus tenax</i>	MZUCC477	-	+	-	-	-	-	*
35.	<i>Chroococcus varius</i>	MZUCC478	+	-	-	-	-	-	*
36.	<i>Entophysalis granulosa</i>	MZUCC479	-	-	-	+	-	-	*
37.	<i>Fischerella ambigua</i>	MZUCC480	+	+	-	-	-	-	*
38.	<i>Gloeocapsa</i> <i>decorticans</i>	MZUCC481	-	+	-	-	-	-	*
39.	<i>Gloeotheca</i> <i>rhodochlamys</i>	MZUCC482	-	+	-	-	-	-	*
40.	<i>Gloeotheca rupestris</i>	MZUCC483	+	-	-	-	+	-	*
41.	<i>Gomphosphaerica</i> <i>aponina</i>	MZUCC484	+	-	-	-	+	-	*
42.	<i>Gomphosphaerica</i> <i>lacustris</i>	MZUCC521	+	-	-	-	-	-	*
43.	<i>Hapalosiphon</i> <i>intricatus</i>	MZUCC485	-	-	-	+	-	-	*

44.	<i>Hapalosiphon welwitschii</i>	MZUCC486	-	+	-	-	-	-	*
45.	<i>Hydrococcus rivularis</i>	MZUCC487	-	+	-	-	-	-	*
46.	<i>Johannesbaptistia pellucida</i>	MZUCC488	-	+	-	-	-	-	*
47.	<i>Lyngbya limnetica</i>	MZUCC489	-	-	-	-	-	+	*
48.	<i>Lyngbya stagnina</i>	MZUCC490	-	-	-	-	-	+	*
49.	<i>Lyngbya sordida</i>	MZUCC516	-	+	-	-	-	-	*
50.	<i>Merismopedia elegans</i>	MZUCC491	-	-	-	-	-	+	*
51.	<i>Merismopedia glauca</i>	MZUCC492	-	-	+	-	+	-	*
52.	<i>Merismopedia punctata</i>	MZUCC493	+	-	+	-	-	-	*
53.	<i>Microcystis aeruginosa</i>	MZUCC494	+	-	+	-	+	-	
54.	<i>Microcystis marginata</i>	MZUCC495	-	-	+	-	-	-	*
55.	<i>Microcystis pulvereae</i>	MZUCC496	+	+	-	-	-	-	*
56.	<i>Myxosarcina burmensis</i>	MZUCC497	-	+	-	-	-	-	*
57.	<i>Nostoc carneum</i>	MZUCC498	+	+	-	-	-	-	*
58.	<i>Nostoc commune</i>	MZUCC499	-	+	-	+	-	-	*
59.	<i>Nostoc ellipsosporum</i>	MZUCC500	-	-	-	-	+	-	*
60.	<i>Nostoc entophytum</i>	MZUCC502	-	-	+	-	-	-	*
61.	<i>Nostoc amplissimum</i>	MZUCC502	+	-	-	-	+	-	*
62.	<i>Nostoc calcicola</i>	MZUCC518	-	+	-	-	-	-	*
63.	<i>Nostoc linckia</i>	MZUCC503	+	+	+	-	-	-	*
64.	<i>Nostoc muscorum</i>	MZUCC504	-	+	-	-	-	-	
65.	<i>Nostoc paludosum</i>	MZUCC505	-	-	+	-	+	-	*
66.	<i>Nostoc piscinales</i>	MZUCC506	+	+	-	+	-	-	
67.	<i>Nostoc punctiforme</i>	MZUCC507	-	+	-	-	+	+	
68.	<i>Oscillatoria subbrevis</i>	MZUCC508	+	-	+	-	-	+	*
69.	<i>Oscillatoria limosa</i>	MZUCC509	+	-	-	-	-	+	*
70.	<i>Oscillatoria princeps</i>	MZUCC520	-	-	-	+	-	-	
71.	<i>Oscillatoria sancta</i>	MZUCC510	-	-	-	+	-	-	*
72.	<i>Oscillatoria vizagapatensis</i>	MZUCC511	-	-	-	+	-	-	*
73.	<i>Plectonema nostocorum</i>	MZUCC512	-	-	-	-	-	+	*
74.	<i>Pseudanabaena catenata</i>	MZUCC519	+	-	-	-	-	-	*

75.	<i>Synechocystis pevalekii</i>	MZUCC513	+	+	-	-	-	-	*
76.	<i>Spirulina gigantea</i>	MZUCC514	+	-	-	-	-	-	*

+ Presence of cyanobacterial species, - Absence of cyanobacterial species, *new record for Mizoram

- S1-season 1-growing season (July- September)
- S2-season 2-post harvest season[(November- December

3.9.Relative Abundance of Cyanobacterial Genera in Paddy Fields

The relative abundance of cyanobacterial genera varied across both the growing (S1) and post-harvest (S2) seasons, reflecting seasonal shifts in community composition (Table 9). Among the identified genera, *Nostoc* was the most dominant, occurring in 60% of the plots in S1 and 46.67% in S2, suggesting that *Nostoc* species are well-adapted to environmental fluctuations and persist throughout the annual rice cultivation cycle. Other highly abundant genera included *Chroococcus* (46.67% in S1, 26.67% in S2), *Anabaena* (46.67% in S1, 26.67% in S2), and *Aphanothece* (40% in S1, 26.67% in S2), indicating their ability to tolerate seasonal changes in moisture, nutrient levels, and soil conditions.

Several genera exhibited notable seasonal fluctuations, suggesting a preference for specific environmental conditions. *Oscillatoria* maintained stable relative abundance (20% in both S1 and S2), implying that it can thrive under a broad range of environmental conditions. In contrast, *Aphanocapsa* (20% in S1, 6.67% in S2) and *Merismopedia* (20% in S1, 6.67% in S2) showed a marked decline post-harvest, possibly because of nutrient depletion or reduced water availability. Conversely, *Hapalosiphon* and *Lyngbya*, which were absent in S1, were detected in 13.33% of the plots in S2, suggesting that these genera favor post-harvest conditions, potentially benefiting from organic matter decomposition or altered soil properties.

Certain genera were detected in only a few plots, regardless of the season. These included *Brachytrichia*, *Chroococidiopsis*, *Entophysalis*, *Gloeocapsa*, *Hydrococcus*, *Johannesbaptistia*, *Myxosarcina*, *Plectonema*, and *Spirulina*, each occurring in less than 7% of the plots. Their limited distribution may indicate specific

niche preferences, low competitiveness, or dependence on localized environmental conditions.

The observed seasonal trends suggest that while certain cyanobacterial genera are resilient across seasons (e.g., *Nostoc*, *Chroococcus*, and *Anabaena*), others display temporal shifts in abundance, potentially responding to changes in water quality, soil conditions, and nutrient availability. This highlights the dynamic nature of cyanobacterial populations in paddy fields and underscores their roles in soil fertility and ecosystem functioning. Understanding these variations is essential for optimizing the use of cyanobacteria in sustainable rice cultivation, particularly for nitrogen fixation and organic matter cycling.

Table 9. Relative abundance of the identified genera for season 1 and season 2

Sl. No.	Genus	Total no of strains		No of plots which have the genus		Total number of plots	Relative abundance (%)	
		S1	S2	S1	S2		S1	S2
1.	<i>Anabaena</i>	4	4	7	4	15	46.67	26.67
2.	<i>Aphanocapsa</i>	2	1	3	1	15	20.00	6.67
3.	<i>Aphanothece</i>	6	4	6	4	15	40.00	26.67
4.	<i>Aulosira</i>	1	1	1	2	15	6.67	13.33
5.	<i>Brachytrichia</i>	0	1	0	1	15	0.00	6.67
6.	<i>Calothrix</i>	4	2	6	3	15	40.00	20.00
7.	<i>Chlorogloea</i>	2	0	2	0	15	13.33	0.00
8.	<i>Chroococcidiopsis</i>	0	1	0	1	15	0.00	6.67
9.	<i>Chroococcus</i>	7	5	7	4	15	46.67	26.67
10.	<i>Entophysalis</i>	0	1	0	1	15	0.00	6.67
11.	<i>Fischerella</i>	1	1	1	1	15	6.67	6.67
12.	<i>Gloeocapsa</i>	0	1	0	1	15	0.00	6.67
13.	<i>Gloeothece</i>	1	1	2	1	15	13.33	6.67
14.	<i>Gomphosphaerica</i>	2	0	3	0	15	20.00	0.00
15.	<i>Hapalosiphon</i>	0	2	0	2	15	0.00	13.33

16.	<i>Hydrococcus</i>	0	1	0	1	15	0.00	6.67
17.	<i>Johannesbaptistia</i>	0	1	0	1	15	0.00	6.67
18.	<i>Lyngbya</i>	0	2	0	2	15	0.00	13.33
19.	<i>Merismopedia</i>	2	1	3	1	15	20.00	6.67
20.	<i>Microcystis</i>	3	1	5	1	15	33.33	6.67
21.	<i>Myxosarcina</i>	0	1	0	1	15	0.00	6.67
22.	<i>Nostoc</i>	8	6	9	7	15	60.00	46.67
23.	<i>Oscillatoria</i>	2	4	3	3	15	20.00	20.00
24.	<i>Plectonema</i>	0	1	0	1	15	0.00	6.67
25.	<i>Pseudanabaena</i>	1	0	1	0	15	6.67	0.00
26.	<i>Synechocystis</i>	1	1	1	1	15	6.67	6.67
27.	<i>Spirulina</i>	1	0	1	0	15	6.67	0.00

3.10. Seasonal and Spatial Variations in Cyanobacterial Diversity

Cyanobacterial diversity across 15 paddy field sites (C1–C5, K1–K5, TZ1–TZ5) was assessed using Shannon’s Diversity Index (H) and Simpson’s Diversity Index (D) to evaluate species richness, evenness, and dominance patterns across two seasons (S1: growing season and S2: post-harvest season) (Table 10). The results revealed variations in cyanobacterial community composition across different sites and between seasons, indicating that environmental factors and seasonal changes influenced cyanobacterial diversity in these rice fields.

Sites with higher Shannon Index (H) values, such as C3 (0.3665 in S1, 0.3678 in S2), C2 (0.3665 in S1), and C4 (0.3557 in S1, 0.3635 in S2), exhibited relatively diverse cyanobacterial communities with a more even distribution of genera. In contrast, sites TZ2 (H = 0.2441 in S1) and K4 (H = 0.2599 in S2) recorded the lowest diversity indices, suggesting a less diverse community with potential dominance of a few genera. Notably, some sites displayed seasonal fluctuations in diversity. For example, C2 had a higher H in S1 (0.3665) compared to S2 (0.3617), indicating a more even distribution of cyanobacteria during the growing season, whereas K2 showed an

increase in diversity in S2 ($H = 0.3466$) compared to S1 ($H = 0.2703$), suggesting seasonal changes in cyanobacterial composition.

Simpson's Diversity Index (D), which reflects dominance patterns, also showed variability across sites and seasons. Higher D values (closer to 1) indicated more balanced communities with no single dominant genus, whereas lower D values suggested the emergence of dominant taxa. Sites such as C1 (0.943 in S1, 0.917 in S2), C5 (0.971 in S1, 0.975 in S2), and K4 (0.972 in S1, 1.000 in S2) recorded high D values, suggesting greater diversity and coexistence of multiple cyanobacterial genera. Conversely, a notable decline in Simpson's D in S2 for sites such as TZ1 (0.857 in S2) and C2 (0.825 in S2) suggests that postharvest conditions may favor the dominance of specific cyanobacterial species. This could be attributed to changes in soil moisture, nutrient depletion, or shifts in post-harvest environmental conditions that support the proliferation of a few highly adaptable cyanobacteria, while reducing overall species richness.

In summary, the findings highlight that certain sites (e.g., C3, C2, and C4) consistently maintain high diversity, whereas others (e.g., TZ2, K4, and K5) exhibit lower diversity, suggesting that cyanobacterial community composition is site-specific and influenced by environmental and agronomic factors. The observed seasonal shifts in diversity indicate that cyanobacterial communities are dynamic, with some sites experiencing reduced evenness and potential dominance of specific genera in the postharvest season (S2). These results suggest that cyanobacterial diversity in paddy fields is controlled by both spatial and temporal variation.

Table 10. Diversity indices of cyanobacterial communities in paddy fields across sites and seasons

Sl. No	Site	Number of genera		Non-heterocystous forms (genus)		Heterocystous forms (genus)		Shannon H		Simpson's Diversity (D)	
		S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
1.	C1	4	5	2	1	3	3	0.3525	0.3635	0.943	0.917

2.	C2	6	7	4	5	2	2	0.3665	0.3617	0.857	0.825
3.	C3	6	6	4	2	2	4	0.3665	0.3678	0.857	0.875
4.	C4	7	5	5	4	2	1	0.3557	0.3635	0.800	0.917
5.	C5	3	3	3	0	0	3	0.3219	0.3139	0.971	0.975
6.	K1	4	3	4	2	0	1	0.3604	0.3678	0.833	0.893
7.	K2	6	4	2	2	4	2	0.2703	0.3466	0.583	0.893
8.	K3	3	2	1	1	2	1	0.3662	0.3466	0.917	0.964
9.	K4	2	1	1	1	1	0	0.3342	0.2599	0.972	1.000
10.	K5	3	2	1	1	2	1	0.3662	0.3466	0.917	0.964
11.	TZ1	4	3	3	3	1	0	0.3604	0.3631	0.833	0.857
12.	TZ2	1	2	1	2	0	0	0.2441	0.3579	1.000	0.952
13.	TZ3	2	3	2	2	0	1	0.3342	0.3631	0.972	0.857
14.	TZ4	4	1	2	0	2	1	0.3604	0.2780	0.833	1.000
15.	TZ5	4	1	2	1	2	0	0.3604	0.2780	0.833	1.000

3.11. Molecular Identification and Phylogeny

Polymerase chain reaction (PCR)-based amplification was employed for molecular characterization of 20 cyanobacterial strains isolated from paddy fields. Following isolation and purification, a polyphasic approach integrating morphological characterization and 16S rRNA gene sequencing was used to assess molecular diversity. Partial 16S rRNA gene sequences of all 20 cyanobacterial strains were submitted to GenBank, and their respective accession numbers are listed in Table 11.

Molecular identification confirmed substantial genetic diversity among cyanobacterial isolates, encompassing both heterocystous and non-heterocystous forms. The dataset included members of *Nostoc*, *Anabaena*, *Microcystis*, *Phormidesmis*, *Oscillatoria*, *Synechocystis*, and *Calothrix*, reflecting a broad phylogenetic representation of cyanobacteria commonly found in paddy-field ecosystems.

A Maximum Likelihood (ML) phylogenetic tree was constructed based on the 16S rRNA gene sequences of the 20 cyanobacterial strains isolated in this study, along with 24 reference cyanobacterial sequences retrieved from GenBank (Figure 19). A well-defined clade structure was observed, separating heterocystous and non-heterocystous cyanobacteria, indicating distinct evolutionary lineages.

Phylogenetic analysis strongly clustered *Nostoc* and *Anabaena* within heterocystous cyanobacteria, confirming their close evolutionary relationships. A high bootstrap value (97%) supported the common ancestry of these nitrogen-fixing genera, reinforcing previous findings suggesting that *Nostoc* and *Anabaena* share adaptations related to nitrogen fixation, filamentous growth, and heterocyst differentiation. These traits are critical for survival in nitrogen-deficient environments such as flooded paddy fields, further highlighting their ecological significance in sustaining soil fertility.

Notably, the closely related strains exhibited high bootstrap values, indicating strong evolutionary relationships. For instance, *Nostoc* sp. R21 (PP848106) and *Nostoc entophytum* R20 (PP848103) clustered together, with a bootstrap value of 99%, suggesting a high degree of genetic similarity between these two strains. Similarly, a moderate bootstrap value (75%) was observed between *Synechocystis pevalekii* R5 (PP853584) and *Synechocystis salina* (KU951813), indicating a shared evolutionary lineage within non-heterocystous cyanobacteria.

Significant clustering was also observed among the non-heterocystous filamentous cyanobacteria. Strain *Phormidesmis* sp. R19 (PP989211) clustered closely with *Leptolyngbya* sp. (ON870383), which was supported by a high bootstrap value of 98%, suggesting a strong phylogenetic relationship. These genera have previously been reported to share genetic similarities in morphology, habitat preferences, and ecological functions, reinforcing the observed clustering pattern.

Table 11. Accession numbers of selected cyanobacterial strains

Sl.No	Name of strains	Accession No.
1.	<i>Nostoc muscorum</i> R4	PP823867
2.	<i>Nostoc</i> sp. Strain 12	PP823868
3.	<i>Phormidesmis</i> sp. R19	PP989211
4.	<i>Nostoc entophytum</i> R20	PP848103
5.	<i>Nostoc muscorum</i> R28	PP848104
6.	<i>Plectonema</i> sp. R29	PP989210
7.	<i>Nostoc piscinale</i> R10	PP848105
8.	<i>Nostoc</i> sp. R21	PP848106
9.	<i>Phormidium</i> sp. R25	PP959484
10.	<i>Nostoc linckia</i> R3	PP853590
11.	<i>Synechocystis pevalekii</i> R5	PP853584
12.	<i>Anabaena inaequalis</i> R7	PP853583
13.	<i>Nostoc paludosum</i> R7	PP853585
14.	<i>Nostoc</i> sp. R13	PP853587
15.	<i>Microcystis aeruginosa</i> R16	PP853589
16.	<i>Oscillatoria subbrevis</i> R18	PP853591

17.	<i>Tolypothrix sp.</i> R23	PP853586
18.	<i>Leptolyngbyaceae cyanobacterium</i> R2	PP853588
19.	<i>Anabaena flos-aquae</i> R1	PP809508
20.	<i>Calothrix marchica</i> R4	PP823864

(Note: Accession numbers were obtained from NCBI GenBank database after submission of the sequences. Website : <https://www.ncbi.nlm.nih.gov>)

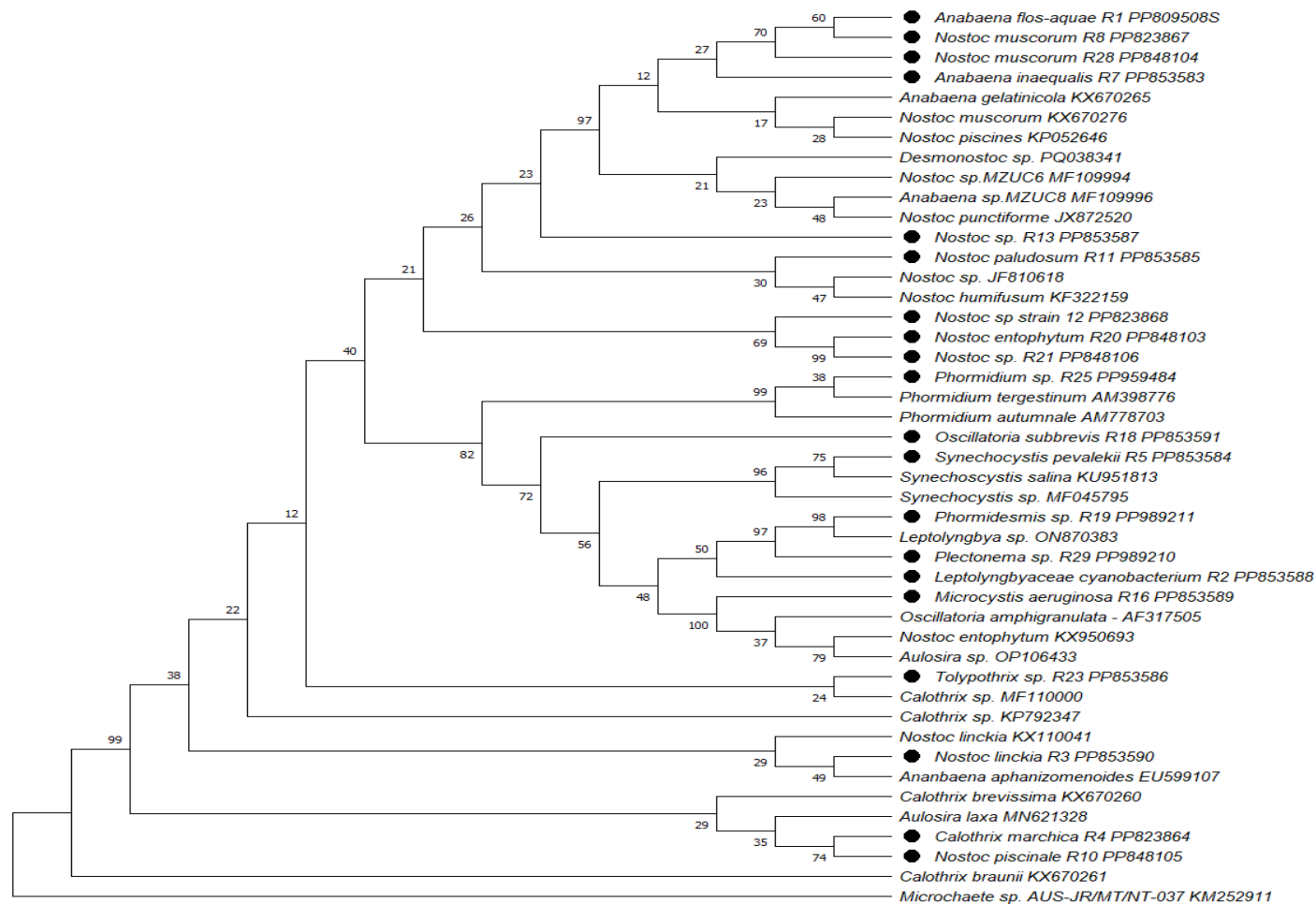


Figure 19. Maximum likelihood (ML) based phylogenetic tree analysis of *16S rRNA* sequences using MEGA X (bootstrap value: 1,000).

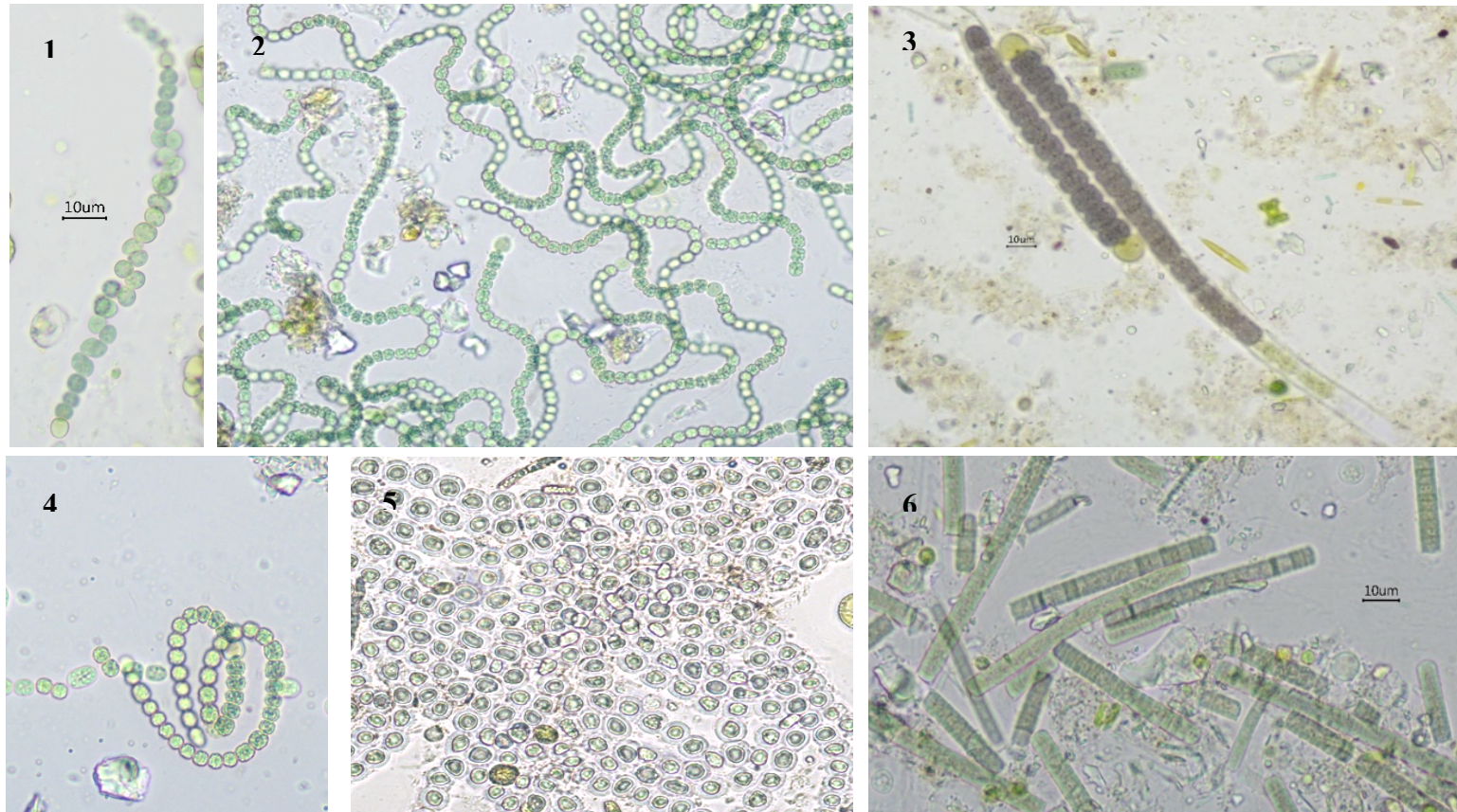


Plate 1. 1. *Nostoc paludosum* Kützing, ex Born. et Flah. 2. *Nostoc piscinales* Kützing, ex Born. et Flah. 3. *Calothrix marchica* Lemmermann 4. *Anabaena circinalis* Rabenhorst ex Born. et Flah. 5. *Johannesbaptistia pellucida* (Dickie) Taylor et Drouet 6. *Oscillatoria limosa* Ag. ex Gomont

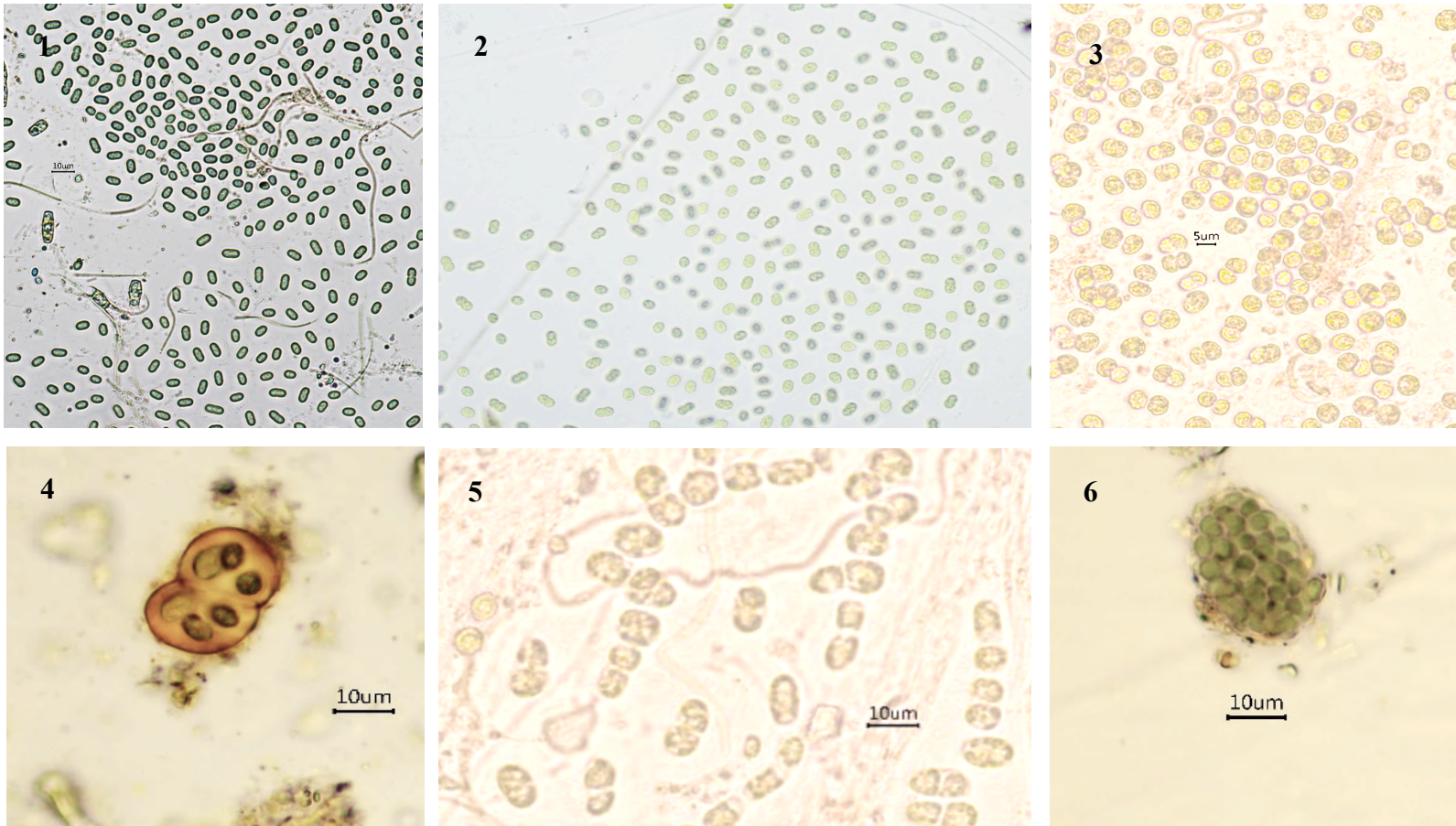


Plate 2. 1. *Aphanothece naegellii* Wartm 2. *Aphanothece pallida* (Kütz.) Rabenh. 3. *Merismopedia glauca* (Ehrenb.) Näg. 4. *Chroococcus schizodermaticus* West 5. *Brachytrichia quoyi* (Ag.) Born. et Flah. 6. *Chroococcus varius* A. Br.

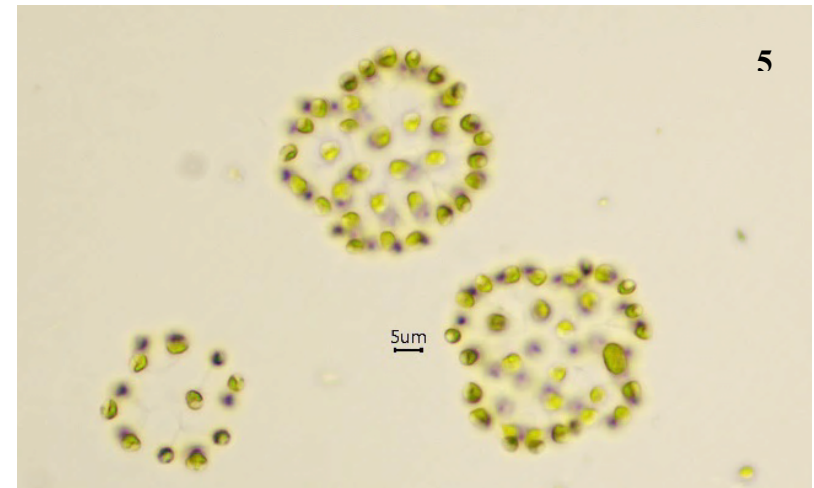
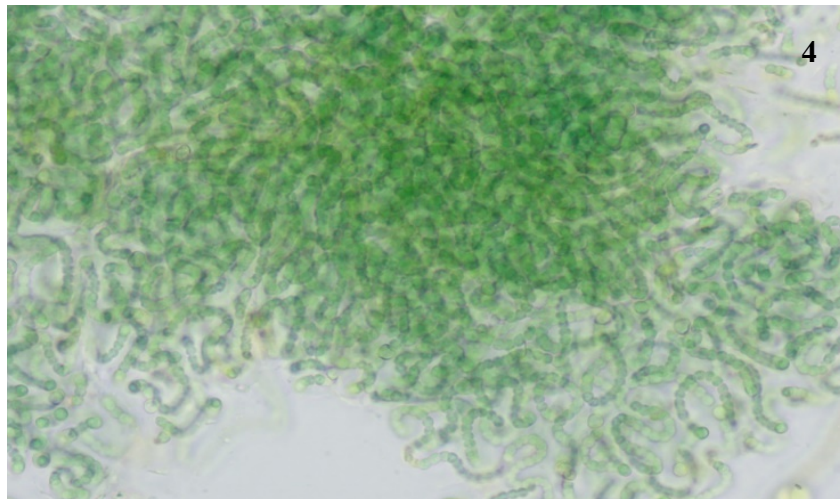
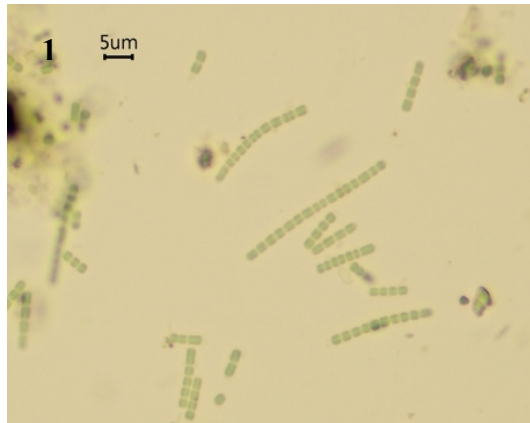


Plate 3. 1. *Pseudanabaena catenate* Lauterb. 2. *Calothrix membranacea* Schmidle 3. *Spirulina gigantea* Schmidle 4. *Nostoc amplissimum* Setchell 5. *Gomphosphaerica aponina* Kütz.

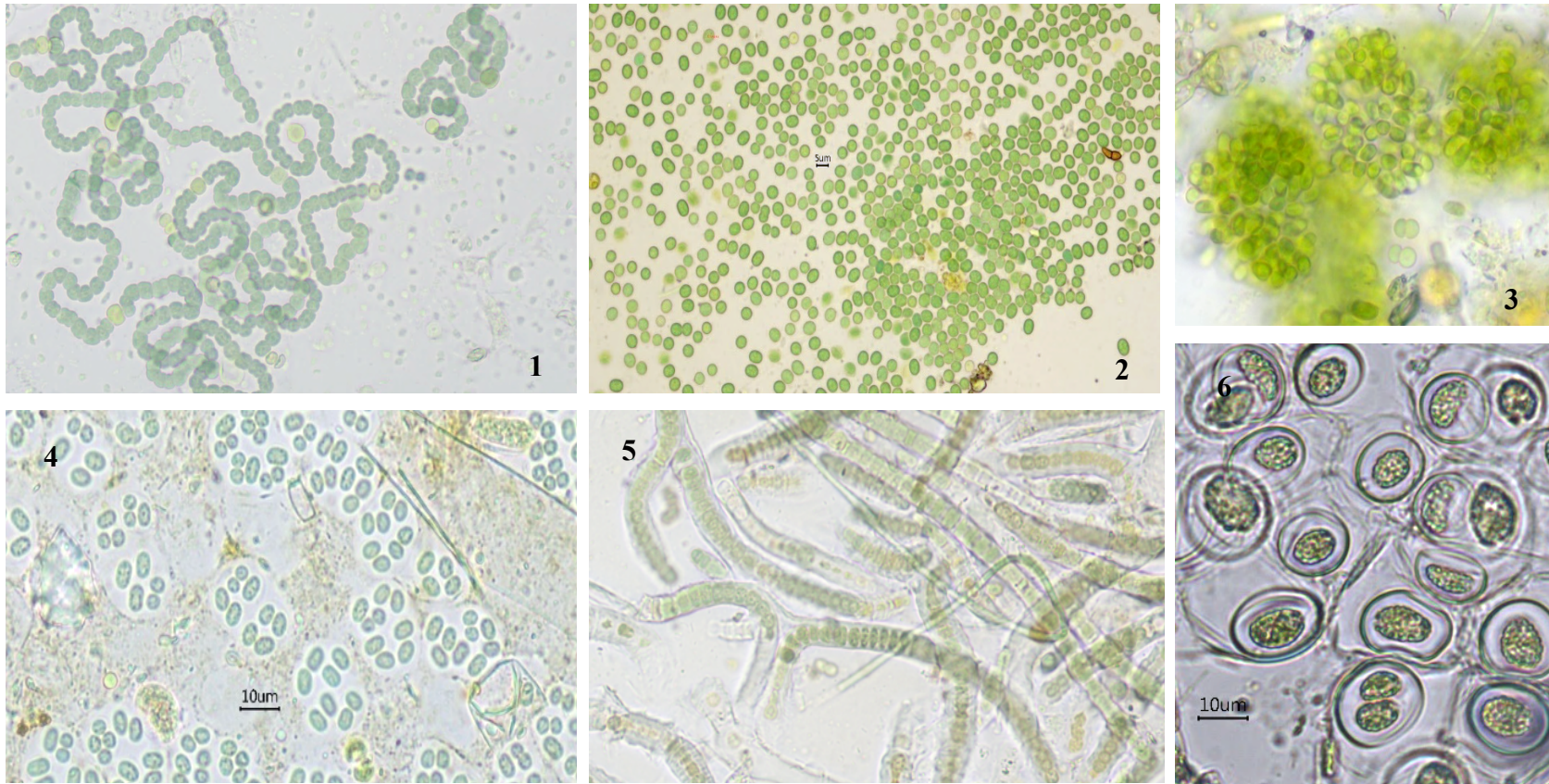


Plate 4. 1. *Nostoc linckia* (Roth) Bornet ex Born. et Flah. 2. *Aphanocapsa bififormis* A. Br. 3. *Chlorogloea microystoides* Geitler
4. *Chroococcus mipitanensis* (Wolszynska) Geitler 5. *Aulosira fertilissima* 6. *Gloeocapsa decorticans* (A. Br.) Richter .

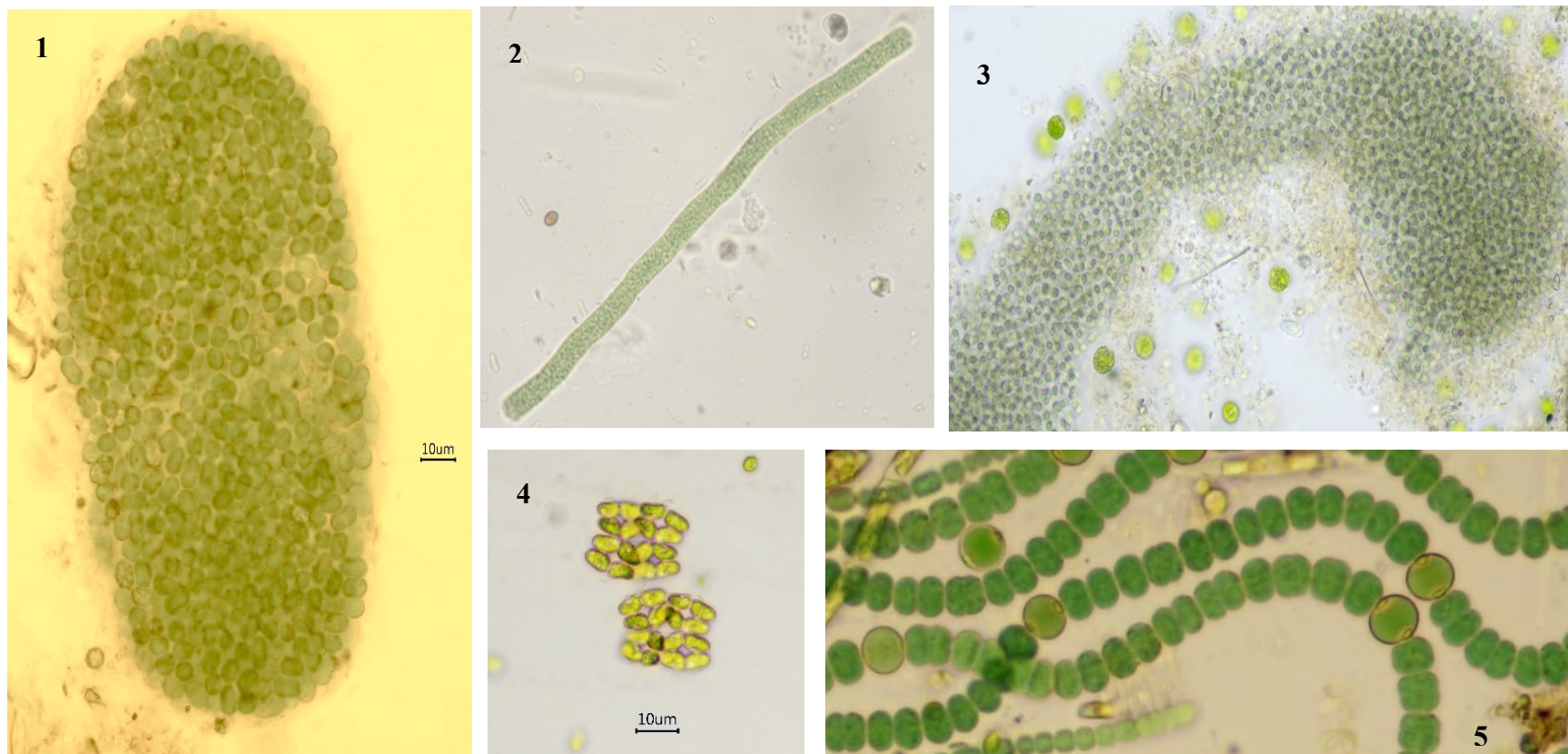


Plate 5. 1. *Microcystis marginata* (Menegh.) Kütz. 2. *Oscillatoria princeps* Vaucher ex Gomont . 3. *Microcystis aeruginosa* Kütz. 4. *Merismopedia punctata* Meyen 5. *Anabaena sphaerica* Bornet et Flahault.

CHAPTER 4

DISCUSSION

The paddy fields of Mizoram provide a compelling setting for investigating the dynamics of cyanobacterial communities within tropical agroecosystems. Rice cultivation depends heavily on soil fertility, which is affected by microbial activities. This study explores these interactions across three elevational zones—Champhai (high-altitude), Thenzawl (mid-altitude), and Kolasib (lowland)—over two seasons, shedding light on the environmental factors shaping cyanobacterial diversity and abundance. Through field observations, analytical techniques, such as PCA and molecular characterization, the interplay of moisture, elevation, nutrients, and soil chemistry was examined in the current study.

Cyanobacterial diversity exhibits significant spatial and seasonal variations along elevational gradients in Mizoram paddy fields. The selection of Champhai, Thenzawl, and Kolasib as study sites highlights elevation as a key factor influencing microbial communities within these agro-ecosystems (Cui et al., 2019). The colder temperature of Champhai supports a distinct cyanobacterial profile that differs from the warmer humid conditions in the lowlands of Kolasib. These observations are consistent with those of Zumsteg et al. (2013), who noted that altitudinal gradients determine microbial assemblages. Soil pH and temperature are major drivers of altitudinal shifts in microbial assemblages (Praeg et al., 2019; Siles and Margesin, 2016). In contrast, Cui et al. (2019) reported a sharp decline in the microbial diversity in alpine ecosystems with increasing altitude. Several studies have reported a general decrease in species richness and diversity of various organisms, including plants, animals, and some microorganisms, with increasing altitude (Bahram et al., 2011; Chawla et al., 2008; Sanjeevani et al., 2024). This adaptability is likely attributable to soil properties, such as pH, organic matter content, and nutrient availability, which collectively modulate microbial distribution. It was concluded that elevation plays a pivotal role in shaping cyanobacterial ecology in tropical paddy fields.

Water availability is a critical driver of cyanobacterial abundance, particularly during the growing season when dense mats thrive in stagnant water. Kolasib and Thenzawl, with their lower elevations and prolonged water retention, provided optimal conditions for proliferation, consistent with Stal (2012), who emphasized waterlogged environments as the key to mat formation. Cyanobacterial mats are prevalent in various aquatic habitats, including extreme environments, and play crucial roles in ecosystem primary production and nutrient cycling at the sediment-water interface (Jentzsch et al., 2023). Research conducted in Champhai demonstrated that after harvest, cyanobacteria transform into desiccation-resistant filamentous and crust-like forms. This adaptation likely contributes to soil stabilization and improves nitrogen cycling under arid conditions (Murik et al., 2017). This flexible approach aligns with the findings of Kour et al. (2024), who highlighted the importance of cyanobacteria for sustaining soil fertility under varying moisture conditions. In contrast to Stal (2012), who described mat formation as a consistent reaction to wet conditions, the morphological variation observed across different locations indicated site-specific adaptations. However, Paerl (2008) emphasized the importance of moisture, highlighting its role in aiding nutrient absorption and promoting microbial proliferation. It was concluded that moisture availability, influenced by elevation and seasonal water patterns, acts as the main factor controlling the abundance of cyanobacteria, which has important consequences for soil management.

Seasonal moisture dynamics, including temperature fluctuations, precipitation patterns, and hydrological changes, play a crucial role in shaping cyanobacterial communities. These factors interact with nutrient availability and other environmental parameters to determine the diversity and abundance of cyanobacteria in aquatic ecosystems (Song et al., 2017). The current study showed that the ability of Thenzawl to retain high moisture during the growing season is indicative of strong soil structure and advantageous topography (Williams et al., 1983). Conversely, Kolasib experiences quick drying after harvest, highlighting its limited water retention capacity, which is further intensified by evaporation in lowland areas. Meanwhile, Champhai maintains consistent moisture levels throughout the year, suggesting efficient water management practices, such as terraced irrigation, which supports microbial activity even during

drier times. These patterns are consistent with the findings of Indian studies by Pangaluru et al. (2019) and Shrivastava et al. (2017), who associated moisture variability with precipitation and soil texture. In contrast, Sravani et al. (2022) observed more severe drying in central India, unlike the more moderate decrease in Champhai, which is likely due to differences in regional irrigation practices. The hydrological conditions specific to each site influence cyanobacterial habitats with the ability to retain moisture, thereby affecting seasonal abundance and resilience. Sacco et al. (2012) corroborate this, proposing irrigation modifications to boost microbial contributions to soil health.

Examining soil pH dynamics offers a deeper understanding of the cyanobacterial reactions and diversity. The transition from slightly acidic conditions in Champhai during the growing season to a neutral pH after harvest indicates organic matter breakdown and decreased microbial activity (Fageria et al., 2010). Elevated pH levels in Kolasib and Thenzawl might be attributed to soil composition or farming methods, yet they remain conducive for rice and cyanobacteria (Cheng et al., 2017; Kusumawardani et al., 2017). Consistent with the results of the present study, soil pH significantly affected cyanobacterial diversity and abundance in soil ecosystems. Research on rice field soils in India revealed that the highest percentage of heterocystous cyanobacteria was found at pH 8.1, while the greatest Shannon's diversity index was noted at pH 6.9 (Prasanna and Nayak, 2007). The same study indicated that richness and evenness indices were the highest in soil samples with a pH of 9.3, suggesting that different cyanobacterial groups have distinct pH preferences. Contrary to the findings in rice fields, research in the Atacama Desert showed a decline in cyanobacterial diversity as conditions became more arid and likely alkaline (Jung et al., 2019). This indicates an intricate relationship between pH and other environmental factors in shaping the cyanobacterial communities. Although Zhang et al. (2024) proposed that pH significantly influences microbial metabolism, the narrow range observed in this study suggests a secondary role compared to moisture. This is in contrast with the results of Cheng et al. (2017), who observed significant pH-driven microbial changes in Japanese paddies with broader gradients. It was concluded that pH mainly affects species composition rather than abundance,

with limited variation buffering cyanobacteria against major ecological changes, thereby enhancing their adaptability in stable soil conditions.

In Thenzawl and Kolasib, post-harvest increases in electrical conductivity (EC) and total dissolved solids (TDS) were indicative of ion concentrations due to evaporation, as observed by Li and Zhang (2009) in southeast China. The increased salinity at these lower-altitude locations indicates salt build-up, which may pose a challenge to cyanobacterial survival under arid conditions. In contrast, the lower salinity levels in Champhai suggest either better drainage or less evaporative stress, which supports microbial stability. Although Singh et al. (2022) noted that high salinity limits cyanobacterial growth in hypersaline environments, the moderate salinity levels here imply a minimal impact, which is consistent with Singh et al. (2016), who reported tolerance in agricultural soils. It was concluded that salinity primarily affects abundance under extreme post-harvest conditions, with moisture remaining the dominant control factor. This conclusion was reinforced by the observed resilience of the cyanobacterial populations across various soil types and conditions. These findings indicate that cyanobacteria have developed adaptive mechanisms (e.g., accumulation of osmoprotectants, active extrusion of sodium ions, and enhancement of photosynthetic efficiency) to withstand moderate salinity levels, enabling them to thrive in diverse agricultural settings (Joset et al., 1996). Further research is needed to investigate the specific physiological adaptations that allow cyanobacteria to maintain their abundance in saline soils, potentially leading to the development of salt-tolerant strains for sustainable agriculture.

Variations in soil temperature provide an additional viewpoint. In this study, we observed that the higher temperatures in Kolasib after harvest were linked to decreased moisture and increased sunlight exposure, whereas cooler conditions during the Champhai growing season are indicative of waterlogging (Li et al., 2014). According to Lyon and Mock (2014), temperature affects cyanobacterial metabolism; however, the weak correlation with abundance suggests resilience across different conditions, in contrast to Xu et al. (2016), who found temperature-induced blooms. It was concluded that temperature had a minor impact, and its effects were moderated by moisture, underscoring the adaptability of cyanobacteria in paddy fields. This

adaptability may explain the widespread presence of cyanobacteria in rice-growing areas worldwide. Further investigation is necessary to uncover the specific mechanisms underlying cyanobacterial resilience to temperature change in paddy ecosystems. Understanding these adaptations could offer valuable insights into managing cyanobacterial populations and improving rice cultivation practices under changing climatic conditions.

Principal Component Analysis (PCA) has demonstrated that variations in soil properties are influenced by hydrological and topographical factors (Seibert et al., 2007). The clustering of Kolasib and Thenzawl with EC, TDS, and salinity indicated ion accumulation in lowland areas (Nachshon, 2018), whereas Champhai's association with moisture and pH highlighted the stabilizing influence of elevation (Sacco et al., 2012). These findings are consistent with those of Nachshon (2018) but differ from those of Sacco et al. (2012), who observed a reduced impact of elevation in flatter regions. It was concluded that elevation affects multiple influencing factors, with moisture and salinity being crucial for cyanobacterial habitats, suggesting that site-specific management is necessary to enhance microbial functions. The interaction between topography and hydrology results in unique microclimates that affect the soil microbial communities. This interaction affects the soil properties, plant communities, and microbial diversity across landscapes. These microclimates can vary greatly even within small geographic areas, leading to diverse cyanobacterial populations adapted to particular environmental conditions. For instance, in the French Alps, microbial community diversity was found to be higher in open gaps than in closed forest patches, owing to increased root trait diversity and density (Rossi et al., 2020). Similarly, in the Changbai Mountains in China, soil pH and the C/N ratio have been identified as the primary drivers of microbial community structure and amino sugar patterns along an altitudinal gradient (Zhang et al., 2013). Understanding these localized patterns is essential for creating targeted strategies to exploit the beneficial properties of cyanobacteria for soil health and agricultural productivity. PLS regression ($R^2 = 0.697$, $P < 0.01$) provided a robust model of environmental influences on genus abundance, explaining 70% of the variance across sites and seasons. The analysis highlighted temperature (VIP = 1.599) and organic carbon (VIP = 1.365) as the strongest

predictors, which is consistent with their strong ecological roles. For example, Kolasib's elevated organic carbon (up to 2.5%) and warmer temperatures (e.g., 37.7°C) supported peak abundances (e.g., eight genera), while Thenzawl's high nitrogen levels (e.g., 281.7 kg/acre) sustained moderate diversity (e.g., six genera) (Dokulil and Teubner, 2000; Li et al., 2014). Weaker predictors— pH (VIP = 0.484), EC (VIP = 0.134), nitrogen (VIP = 0.336), and phosphorus—showed limited influence, likely due to the narrow range of pH and seasonal variability of EC, which does not directly affect community structure (Li and Zhang, 2009). This multivariate approach outperformed simple correlations (e.g., temperature: $R^2 \approx 0.0185$ in isolation), underscoring the importance of synergistic effects in microbial ecosystems (Chunleuchanon et al., 2003).

Linear regression analysis indicated weak links between cyanobacterial abundance and factors such as pH, electrical conductivity (EC), and temperature, underscoring the complex interactions of environmental influences (Sacco et al., 2012). Temperature had a minimal direct effect, aligning with Lyon and Mock (2014), who showed cyanobacteria resilience across temperature variations in polar regions, which is consistent with the moderate seasonal changes in Mizoram's paddy fields, transitioning from cooler growing seasons to warmer postharvest periods. Similarly, the pH had a limited effect, supporting Zhang et al. (2024), who observed stable cyanobacterial growth within the slightly acidic to neutral pH range found here, indicating that minor pH changes do not significantly control abundance. The weak link with EC, which reflects soil salinity, aligns with Singh et al. (2022), who found cyanobacteria can tolerate moderate salinity levels typical of agricultural environments, unlike extreme salinity; post-harvest EC increases in Thenzawl and Kolasib, though present, remain below inhibitory levels, as noted by Singh et al. (2016). Sacco et al. (2012) suggest that these diffuse relationships reflect the multifaceted drivers in rice fields, a pattern evident in these findings. In contrast, Paerl (2008) and Shah et al. (2024) highlighted nutrients and agricultural practices as the main controls, overshadowing the pH, EC, and temperature. Paerl (2008) emphasized the role of moisture in facilitating nutrient uptake, driving cyanobacterial growth in wet conditions—a mechanism evident during Mizoram's flooded growing season.

Shah et al. (2024) further assert that irrigation and fertilization practices boost microbial abundance more than abiotic factors; this is supported by higher cyanobacterial presence in nutrient-rich areas like Thenzawl (nitrogen) and Kolasib (phosphorus), where sustained moisture enhances resource availability. These findings suggest that abundance reflects a comprehensive environmental response, with moisture as the primary driver, integrating nutrient dynamics with secondary physicochemical influences. Lyon and Mock (2014), Zhang et al. (2024) and Singh et al. (2022) collectively affirm cyanobacteria's resilience to temperature, pH, and moderate salinity, positioning these as boundary conditions rather than key regulators.

The distribution of heavy metals in Mizoram's rice fields is distinctly shaped by both elevation and human activities, revealing spatial patterns that highlight environmental and human impacts on soil chemistry. In Kolasib, located in lowland areas, there are notably higher levels of Fe, Cu, Pb, Mn, and Zn, indicating accumulation processes typical of lower elevations, where runoff and sediment deposition concentrate metals over time (Zhang and Han, 2023). Agricultural practices further intensify this trend, with inputs such as copper-based fungicides and other chemical applications likely contributing to the increased levels, as suggested by Tamm et al. (2022). The presence of Pb, particularly higher in Kolasib, raises concerns about potential contamination from external sources, such as vehicle emissions or industrial runoff, a risk highlighted by Jaishankar et al. (2014) in agricultural contexts. In contrast, Champhai, situated at higher altitudes, showed significantly lower metal concentrations, a pattern attributed to better drainage and reduced retention capacity in coarser, more permeable soils (Bolan et al., 2025). This elevational gradient demonstrates how topography influences metal distribution, with the lowlands acting as sinks and highlands facilitating leaching.

The ecological implications of metal levels on cyanobacterial abundance require careful consideration. Khoshru et al. (2023) indicate that heavy metals, particularly manganese, can inhibit microbial growth at elevated concentrations by disrupting enzymatic processes, yet the moderate levels detected across these sites suggest a limited direct impact on cyanobacteria. This contrasts sharply with Satpathy et al. (2014), who documented significant microbial suppression in heavily polluted

paddy soils where metal concentrations far exceeded those observed here. In Mizoram, the relatively restrained metal loads, which are highest in Kolasib but still within tolerable ranges, imply that cyanobacteria maintain resilience, possibly through adaptive mechanisms, such as metal sequestration or tolerance, as noted by Jaishankar et al. (2014). Instead of directly curbing abundance, metals likely exert an indirect influence by altering soil chemistry, such as by shifting the pH or nutrient availability, which in turn affects microbial habitats (Bolan et al., 2025). Zhang and Han (2023) supported this, suggesting that iron dynamics, influenced by weathering and farming, subtly reshape soil conditions without overwhelming microbial communities. It was concluded that, while heavy metals contribute to the environmental matrix, their role remains secondary, with moisture and nutrient availability emerging as the primary controls driving cyanobacterial abundance in these paddy fields, consistent with the broader ecological patterns observed in this study.

Spatial variation in macronutrient levels across Mizoram's rice paddy fields illustrates the effects of elevation, soil properties, and agricultural practices on soil fertility. Nitrogen availability, which is enhanced by organic matter and microbial activity, benefits from the contributions of nitrogen-fixing cyanobacteria such as *Nostoc* and *Anabaena*, particularly in mid-altitude zones (Wohlfart et al., 2012; Nawaz et al., 2024). Phosphorus retention is more pronounced in lowland areas because of reduced leaching, whereas higher altitudes experience losses associated with water movement and soil texture (Velooso et al., 2023). Potassium levels, which are linked to clay content and cation exchange capacity, indicate the need for targeted management in high-altitude areas susceptible to depletion (Zhang et al., 2009). These patterns suggest a consistent yet suboptimal fertility profile influenced by Mizoram's heavy rainfall and acidic soils, which complicates the implementation of uniform nutrient management strategies (Gao et al., 2023).

Phosphorus deficiency, a significant limitation across all sites, corresponds to regional observations of fixation by iron and aluminum oxides, thereby necessitating the use of organic amendments or enriched fertilizers to enhance availability (Mawblei et al., 2025; Khan et al., 2023). Nitrogen-fixing cyanobacteria present a sustainable method to decrease dependency on fertilizers, although their effectiveness varies

according to season and location (Nawaz et al., 2024). K management at higher elevations requires site-specific strategies to mitigate losses (Zhang et al., 2009). The region's consistent fertility challenges, driven by monsoon-induced nutrient leaching, underscore the importance of integrated management—employing microbial inoculation, organic inputs, and precise fertilization—to improve rice productivity and agricultural resilience (Gao et al., 2023).

The diversity of 76 cyanobacterial species spanning 27 genera highlights a robust microbial community, with the prominence of Nostocaceae indicating significant nitrogen-fixing potential, which is particularly crucial for soil fertility in rice paddies (Choudhary and Bimal, 2010). The 70 new records, absent from prior Mizoram surveys (e.g., Vanlalveni and Lalfakzuala, 2021 ; Adhikary et al., 2010 ; Bhakta and Adhikary, 2014), highlight the region's understudied ecological diversity. This may stem from limited prior sampling or unique agroclimatic conditions (e.g., acidic pH 5.9–6.1, variable rainfall). The prevalence of heterocystous cyanobacteria in Champhai corresponds with their adaptation to nitrogen-deficient environments, supporting their role in biological nitrogen fixation, as noted by Bergman et al. (2006). In contrast, the dominance of non-heterocystous forms in Kolasib suggests an adaptation to nutrient-rich conditions, aligning with findings on ecological niche specialization (Christmas et al., 2015). Elevation-specific distribution patterns reflect the observations of Cepoi et al. (2022) regarding environmental influences, although the broader diversity observed here exceeds its scope, illustrating Mizoram's unique agro-ecological gradients.

Elevation and moisture were identified as the primary determinants of cyanobacterial composition, and nutrient availability further enhanced their abundance across various sites. The transition from heterocystous to non-heterocystous taxa along altitudinal gradients underscores adaptive responses to microclimatic and soil conditions, emphasizing the interaction between topography and hydrology in shaping microbial communities. This diversity not only contributes to ecosystem stability but also presents opportunities for sustainable agricultural practices by utilizing nitrogen-fixing capabilities to decrease reliance on fertilizers (Bergman et al., 2006; Choudhary and Bimal, 2010).

Changes in the seasonal abundance of cyanobacteria reflect environmental preferences. The persistence of *Nostoc* is consistent with the findings of Chen et al. (2021), whereas the decline of *Aphanocapsa* after harvest aligns with Soares et al. (2013). The taxa that resist desiccation during dry seasons correspond to Komárek (2003), exceeding the monsoon emphasis of Kong et al. (2019). It has been concluded that moisture primarily dictates abundance, with nutrients and practices acting as modulators (Nawaz et al., 2024). These results highlight the intricate relationship between environmental factors and cyanobacterial populations in aquatic ecosystems. Seasonal moisture variation seems to be the main factor influencing cyanobacterial abundance, whereas nutrient availability and agricultural practices play secondary roles. Further investigation is necessary to clarify the specific mechanisms by which moisture affects different cyanobacterial taxa, and to create targeted management strategies for controlling their growth in various aquatic environments.

In Champhai, diversity indices revealed a well-balanced cyanobacterial community, characterized by an even distribution of species. This is in contrast to Kolasib, where certain taxa predominated, which aligned with the environmental influence patterns documented by Zhao et al. (2017). However, post-harvest changes in diversity, likely attributable to moisture stress and nutrient depletion, diverge from their findings of stability under nutrient-rich conditions, underscoring the sensitivity of Mizoram's paddy fields to seasonal variations. This indicates that ecological stressors alter microbial structures in this region more dynamically than in nutrient-rich environments. Moisture and nutrient availability are pivotal in determining cyanobacterial diversity, with Champhai's higher moisture levels supporting evenness and Kolasib's nutrient richness fostering dominant species. These findings underscore the potential for tailored microbial management by employing moisture retention and nutrient input to sustain diverse functional communities that enhance soil fertility and rice yields (Zhao et al., 2017). The interaction between these factors offers a framework for bolstering agroecological resilience across various elevational settings.

Molecular analysis revealed significant genetic variation among cyanobacterial isolates, with phylogenetic grouping distinctly separating heterocystous from non-heterocystous types, thereby supporting evolutionary differences, as noted by Howard-

Azzeh et al. (2014). The strong clustering of *Nostoc* and *Anabaena*, supported by high bootstrap values, aligns with the findings of Wanigatunge et al. (2014) regarding taxonomic consistency, while surpassing the diversity range reported by Srivastava et al. (2009), thus demonstrating the efficacy of 16S rRNA sequencing in capturing phylogenetic diversity (Honda et al., 1999). This genetic variability underscores the adaptability of these cyanobacteria to Mizoram rice fields. This pronounced genetic diversity enhances their ecological functions, particularly nitrogen fixation and soil fertility, thereby increasing the potential for sustainable agriculture. Heterocystous groups, such as *Nostoc* and *Anabaena*, with their nitrogen-fixing capabilities, offer a natural method to augment soil nitrogen, whereas the diverse non-heterocystous types adapt to nutrient-rich environments, collectively supporting ecosystem stability (Howard-Azzeh et al., 2014; Wanigatunge et al., 2014). This molecular understanding establishes a foundation for utilizing cyanobacterial diversity to reduce fertilizer dependence and enhance the resilience of rice agroecosystems (Honda et al., 1999).

A study of rice paddy fields in Mizoram revealed that cyanobacterial diversity and dynamics are intricately influenced by environmental factors and elevation gradients. The presence of 76 species across 27 genera reflects a rich ecological composition shaped by pH, electrical conductivity (EC), temperature, and macronutrient availability. Elevation influences distinct community structures, with heterocystous cyanobacteria such as *Nostoc* and *Anabaena* thriving in the nitrogen-deficient highlands of Champhai, whereas non-heterocystous forms dominate the nutrient-rich lowlands of Kolasib. However, regression analyses suggest that these physicochemical parameters (pH, EC, and temperature) exert only a weak direct influence, indicating a complex interaction between moisture and nutrients (Bergman et al., 2006; Christmas et al., 2015; Zhao et al., 2017). Molecular characterization confirmed the genetic diversity and reinforced ecological roles in nitrogen fixation and soil fertility. Diversity indices highlight balanced communities in Champhai and dominance in Kolasib, modulated by seasonal moisture variations and macronutrient levels such as nitrogen and phosphorus (Howard-Azzeh et al., 2014; Wanigatunge et al., 2014; Choudhary and Bimal, 2010). These findings underscore the potential of leveraging cyanobacterial adaptability guided by elevation-specific environmental

dynamics to enhance soil health and sustainably support rice cultivation in diverse agroecosystems (Nawaz et al., 2024).

SUMMARY

This chapter thoroughly explored the diversity of cyanobacteria in paddy fields throughout Mizoram, India. It places particular emphasis on the ecological and environmental factors that affect microbial communities. This study was conducted across three agroecological zones: Champhai (high elevation, 1678 m), Thenzawl (mid-elevation, 786 m), and Kolasib (lowlands, 222 m). It provides an understanding of how factors such as altitude, soil and water characteristics, heavy metal levels, macronutrient content, and seasonal variations influence microbial communities that are essential for maintaining soil fertility. The analysis was based on fieldwork conducted during the growing season (July–September) and post-harvest period (November–December). A range of methods has been utilized, including field surveys, laboratory analyses, statistical tools, and molecular techniques.

Study design and methodology

The research design capitalized on the topographic diversity of Mizoram, with each site representing distinct microclimatic and soil characteristics. Champhai is characterized by high-altitude fields, cooler temperatures, and well-drained soil, whereas Kolasib is characterized by lowlands, warm temperatures, and water-retentive soil. Thenzawl serves as an intermediary between these two extremes. Soil and water samples were collected from five replicate 1 m² plots per site. These samples were analyzed for various parameters including moisture content, pH, electrical conductivity (EC), total dissolved solids (TDS), salinity, and temperature. During the growing season, the moisture levels were 68% in Thenzawl, 62% in Kolasib, and 45% in Champhai. After harvest, these levels decreased to 22, 18, and 25%, respectively. The rapid drying in Kolasib is attributed to evaporation in the lowland areas. After the harvest, EC levels rose, with Kolasib increasing from 0.12 dS/m to 0.38 dS/m, Thenzawl from 0.15 dS/m to 0.35 dS/m, and Champhai from 0.10 dS/m to 0.18 dS/m, indicating an accumulation of ions. After harvest, the temperature in Kolasib increased from 24°C to 29°C, whereas in Champhai it remained stable between 19°C and 21°C.

Organic carbon varied from 1.2% to 2.5% across the sites in both seasons, reflecting nutrient variations across the different sites. Principal Component Analysis accounted for 70.21 % of the variance in both seasons, highlighting elevation, water retention, and ion accumulation as significant factors. This suggests that hydrological stability in highland areas supports microbial habitats more effectively than fluctuating conditions in lowland areas. Partial Least Squares (PLS) regression ($R^2 = 0.697$, $P < 0.01$) revealed temperature (VIP= 1.599) and organic carbon (VIP = 1.365) as the strongest predictors of genus abundance, with pH (VIP = 0.484), EC (VIP = 0.134), nitrogen (VIP = 0.336), and phosphorus having weaker influences. This highlights the significance of synergistic environmental influences on microbial populations.

Heavy metal distribution

The distribution of heavy metals showed considerable variation, emphasizing the impacts of topography and human activity. In Kolasib, the highest levels were observed for Fe (245 mg/kg), Cu (18 mg/kg), Pb (12 mg/kg), Mn (135 mg/kg), and Zn (42 mg/kg). These elevated levels were largely due to accumulation in low-lying areas and agricultural inputs such as pesticides and fertilizers. The notably high levels of Pb and Cu indicate potential contamination from runoff or nearby activities, necessitating further observation. In contrast, Champhai has lower concentrations of Fe (112 mg/kg), Cu (5 mg/kg), Pb (3 mg/kg), Mn (78 mg/kg), and Zn (15 mg/kg). These lower concentrations were attributed to improved drainage-reducing retention. Thenzawl levels were intermediate with 165 mg/kg iron and 28 mg/kg zinc. Elevated metal levels can inhibit microbial growth, but moderate levels suggest a limited direct effect on cyanobacteria. Discussions point to an indirect impact: metals may alter soil pH (e.g., Champhai's change from 5.8 to 6.5) or nutrient interactions, slightly affecting microbial habitats. This variability highlights the need for sustainable practices to prevent metal accumulation in lowlands and balance productivity with ecological health, whereas highlands benefit from natural leaching processes.

Macronutrient analysis

Macronutrient analysis identified unique fertility profiles essential for cyanobacterial activity. Nitrogen levels were highest in Thenzawl (0.28% soil content), followed by Kolasib (0.19 %), and Champhai (0.14 %), reflecting differences in elevation and microbial processes. Phosphorus levels were consistently low, at 8 mg/kg in Kolasib, 6 mg/kg in Thenzawl, and 5 mg/kg in Champhai, suggesting a regional deficiency that limits rice production. The potassium content varied significantly, with Kolasib at 225 mg/kg, Thenzawl at 145 mg/kg, and Champhai at 98 mg/kg, influenced by clay retention and fertilizer application in lowland areas. The abundance of nitrogen in Thenzawl indicates the potential of nitrogen-fixing cyanobacteria to improve fertility, whereas the potassium levels of Kolasib contribute to nutrient stability. Phosphorus deficiency presents a challenge, with discussions suggesting organic amendments or microbial biofertilisers as potential solutions. These findings indicate the importance of site-specific nutrient dynamics in the Mizoram rice fields. Tailored management strategies could employ microbial functions (e.g., fixing 20-30 kg N/ha annually) to address deficiencies and enhance soil health.

Cyanobacterial diversity and community dynamics

Morphological and molecular analyses revealed that cyanobacterial diversity comprised 76 species from 27 genera and 14 families, including Nostocaceae (22 species), Chroococcaceae (15 species), and Aphanothecaceae (12 species), of which 70 species were new regional records. In Champhai, 18 heterocystous species, including *Nostoc* and *Anabaena*, thrived during the growing season in the nutrient-poor highlands. The cell abundance reached 1.2×10^5 cells/g during this period. After harvest, the cell abundance decreased to 6.5×10^4 cells/g. Kolasib, on the other hand, supported 25 non-heterocystous species like *Oscillatoria* in nutrient-rich lowlands, with a maximum density of 1.8×10^5 cells/g in wet soils, dropping to 0.9×10^4 cells/g post-harvest. Thenzawl maintained a balance of 20 species, with cell counts starting at 1.5×10^5 cells/g and reducing to 4.2×10^4 cells/g. Seasonal variations revealed that planktonic forms were predominant under flooded conditions, comprising 65% of Kolasib's community during the growing season. Desiccation-resistant species, such

as *Nostoc* and *Calothrix*, dominated postharvest, accounting for 80% of the dry-season taxa of Champhai. The discussion highlights their ecological functions: nitrogen fixation in Champhai reduces the need for fertilizers, whereas the diversity in the lowlands helps maintain soil health. This indicates a system that adapts to environmental challenges such as moisture reduction (from 68% to 18%) and increased salinity (0.05 ppt to 0.25 ppt).

Statistical methods were used to measure diversity using Shannon's (H) and Simpson's (D) indices. In Champhai, the average H was 3.2, and D was 0.92, indicating significant richness and evenness, supported by consistent moisture levels (45%) and minimal salinity (0.05 ppt). In Kolasib, H decreased from 2.8 to 1.9 after harvest, and D reducing from 0.88 to 0.75. This suggests the dominance of stress-tolerant species such as *Oscillatoria*, which comprised 45% of the community, under stress conditions such as 0.25 ppt salinity and 18% moisture. Thenzawl's H averaged 2.9 initially. It later shifted to 2.6. D moved from 0.89 to 0.83 in Thenzawl. Seasonal changes affect species diversity. In Kolasib, 10 species disappeared after harvest. In Thenzawl, five species disappeared after harvest. These disappearances indicated the sensitivity of the species to drying and ion accumulation. This discussion connects these findings with habitat stability. Habitat stability in Champhai is characterized by balanced communities that can withstand stress, whereas in Kolasib, the dominant species demonstrates adaptation. Moisture plays a crucial role as a key factor in diversity. Effective water management strategies can maintain species richness and improve ecosystem services, particularly in terms of nitrogen fixation and soil stabilization. These findings imply that water management could help maintain richness and improve ecosystem services such as nitrogen fixation and soil stabilization.

Through 16S rRNA sequencing, molecular analysis verified the genetic diversity and differentiation between the heterocystous and non-heterocystous groups. Phylogenetic trees revealed close clustering of *Nostoc* and *Anabaena* with 95% bootstrap support, indicating evolutionary unity among nitrogen-fixers, whereas *Oscillatoria* and other species formed more extensive clades. In Champhai, 85% of the

20 sequenced isolates were heterocystous, consistent with morphological observations, whereas in Kolasib, 70% of the 25 isolates were non-heterocystous. The 22 isolates from Thenzawl were evenly split. Genetic diversity in cyanobacteria improves ecosystem function. In highland areas, these organisms can add up to 25 kg of nitrogen per hectare to the soil, reducing the need for artificial fertilizers. In lowland areas, diversity helps recycle nutrients. Adaptability is important for adaptability. The genetic diversity allows cyanobacteria to survive in different conditions, such as varying moisture levels (from 68% to 18%) and changes in salt content (from 0.05 to 0.25 parts per thousand). This adaptability makes cyanobacteria a good model for creating natural fertilizers suitable for the Mizoram farming environment.

Conclusions and future directions

This study effectively identified the factors influencing cyanobacterial diversity in Mizoram's paddy fields. Elevation was found to affect moisture, nutrient, and metal concentrations, which subsequently affected microbial communities. The highest abundance was observed in moist lowlands, whereas diversity peaked in the stable highlands. Moisture has emerged as the primary factor supporting species diversity, with nutrients and seasonal changes playing a secondary role. Moderate metal levels presented manageable risks; however, phosphorus deficiencies necessitated interventions. Cyanobacteria have demonstrated the potential for nitrogen fixation and soil stabilization, suggesting sustainable agricultural applications. Future research directions include long-term diversity monitoring, quantification of nitrogen contributions, and exploration of biofertilization or organic rice systems. These findings could inform strategies for enhancing fertility and adaptability in Mizoram's tropical agricultural ecosystem.

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Bio-Data

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EDUCATION

Examination	Board/University	Year of passing	Division	(%/Grade)
HSLC	St. Pauls Higher Scondary School	2009	Distinction	79.8
HSSLC (Science)	St. Pauls Higher Secondary School	2012	First	69.2
B.Sc (Botany)	Mount Carmel College	2015	Distinction	81.36
M.Sc (Botany)	St. Joseph's University	2017	Distinction	86.58
NET- LIFESCIENCE	CSIR-UGC	2017	Lectureship	
NET - EVS	UGC	2023	Lectureship	

LIST OF PUBLICATIONS

1. **Lalmuanzeli R.**, Chawngthantluangi, Khiangte L., & Mehta, S.K. (2025). Diazotrophic Cyanobacteria and their Potential for Sustainable Rice Production in Paddy Fields across an Elevation Gradient in Mizoram, India. *Paddy and Water Environment*. <https://doi.org/10.1007/s10333-025-01034-7>
2. Momin, S. C., Pradhan, R. B., Nath, J., **Lalmuanzeli, R.**, Kar, A., & Mehta, S. K. (2024). Metal sequestration by *Microcystis* extracellular polymers: A promising path to greener water treatment. *Environmental Science and Pollution Research*, 31(7), 11192–11213. <https://doi.org/10.1007/s11356-023-31755-3>
3. Lalremdika R., Pradhan, R. B., Ch Momin, S., Nath, J., **Lalmuanzeli, R.**, & Mehta, S. K. (2024). Carbon dioxide sequestration and sustainable domestic sewage bioremediation by *Microcystis aeruginosa* and *Chlorella vulgaris*. *Ecology, Environment and Conservation*, 30(10), S108–S119. <https://doi.org/10.53550/EEC.2024.v30i06s.017>
4. Mahana A, Guliy OI, Momin SC, **Lalmuanzeli R.**, & Mehta, S.K. (2020) Sunlight-driven photocatalytic degradation of methylene blue using ZnO nanowires prepared through ultrasonication-assisted biological process using aqueous extract of *Anabaena doliolum*. *Optical Materials* 108:110205. <https://doi.org/10.1016/j.optmat.2020.110205>
5. Lalremdika R., **Lalmuanzeli R.**, Chawngthantluangi & Mehta, S.K. (2025). Enhancing Tomato Plant Growth and Soil Health through Application of Biochar Derived from *Microcystis aeruginosa*(Cyanobacteria). *Science & Technology Journal of Mizoram University*. DOI: [10.22232/stj.2025.5](https://doi.org/10.22232/stj.2025.5)

PRESENTATION, WORKSHOP AND CONFERENCE

1. Presented poster on “Assessment of cyanobacterial diversity in Dihmunzawl paddy field.” in the 12th Annual Convention of Association of Biotechnology and Pharmacy (ABAP) & International Conference on Biodiversity, Environment and Human Health: Innovations and Emerging Trends (BEHIET 2018) organized by the School of Life Sciences, Mizoram University, Aizawl, Mizoram 796004 during November 12th-14th, 2018.
2. Oral presentation in International Conference on Biotechnology for Environment & Health (ICBEH), organized by School of Bio Sciences and Technology, Vellore Institute of Technology along with Association of Biotechnology and Pharmacy, from November 25 – 27th, 2021.
3. Participated in National Seminar on Biodiversity, Conservation, Utilization and Commercialization of Medicinal and Aromatic Plants on February 8th – 9th, 2024, organized by Department of botany, Mizoram University, Aizawl.
4. Participated in One day Awareness Programme cum Workshop on Invasive Alien Plants in Himalayas: Status, Ecological Impact and Management (Mizoram & Tripura Chapter), organized by Botanical Survey of India in collaboration with Department of Botany, Mizoram University, Aizawl on 26th April, 2019 under National Mission for Himalayan Studies.
5. Participated in the State Level Workshop cum Awareness Programme on ‘*Tinospora cordifolia*’, held at Administrative Conference Hall, Mizoram University on 19th November, 2019, organized by Department of Horticulture, Aromatic and Medicinal Plants (HAMP) & State Medicinal Plants Board (SMPB) Mizoram, Directorate of AYUSH.

ANNEXURE-I

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Ph.D. registration Number	MZU/Ph.D./1112 of 03.05.2018	
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Name & Institutional Address of the Supervisor	Prof. Surya Kant Mehta, Department of Botany, Mizoram University, Aizawl, Tanhril-796004	
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Diversity and Seasonal Dynamics of Cyanobacterial Assemblage in Paddy Fields of Mizoram, India

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DEGREE : Ph.D.

DEPARTMENT : Botany

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2. SCHOOL BOARD : 03.05.2018

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Department of Botany

ABSTRACT

**DIVERSITY AND SEASONAL DYNAMICS OF
CYANOBACTERIAL ASSEMBLAGE IN PADDY FIELDS OF
MIZORAM, INDIA**

**AN ABSTRACT SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF
PHILOSOPHY**

RUTHI LALMUANZELI

MZU REGISTRATION NO.: 1700608

Ph.D. REGISTRATION NO.: MZU/Ph.D./1112 of 03.05.2018



**DEPARTMENT OF BOTANY
SCHOOL OF LIFE SCIENCES**

APRIL, 2025

**DIVERSITY AND SEASONAL DYNAMICS OF CYANOBACTERIAL
ASSEMBLAGE IN PADDY FIELDS OF MIZORAM, INDIA**

BY

RUTHI LALMUANZELI

DEPARTMENT OF BOTANY

SUPERVISOR

PROF. SURYA KANT MEHTA

SUBMITTED

**IN PARTIAL FULFILLMENT OF THE REQUIREMENT OF THE DEGREE
OF DOCTOR OF PHILOSOPHY IN BOTANY OF MIZORAM UNIVERSITY,
AIZAWL**

ABSTRACT

This study explored the ecological and environmental factors that shape cyanobacterial diversity in paddy fields across Mizoram, India, a region distinguished by its topographic diversity and agricultural importance. This study focuses on three agroecological zones—Champhai at high elevation, Thenzawl at mid-elevation, and Kolasib in the lowlands—to examine how elevation, soil and water properties, heavy metal concentrations, macronutrient levels, and seasonal dynamics influence cyanobacterial communities integral to rice cultivation. Field surveys and sampling were conducted during the growing season (July–September) and post-harvest season (November–December) to capture temporal shifts in environmental conditions and cyanobacterial composition. By employing a multidimensional approach, such as morphological identification, molecular analysis, and statistical techniques, this study provides a comprehensive understanding of the abiotic-biotic interactions that drive microbial ecology, with implications for sustainable agriculture in tropical paddy ecosystems.

The study area spans a range of topographic zones, each exerting a significant influence on the climatic variables and soil characteristics. These paddy fields, chosen to reflect variations in elevation, temperature, and hydrological patterns, offer a robust framework for assessing agroecological gradients. Soil and water samples were analyzed for moisture content, pH, electrical conductivity, total dissolved solids, salinity, and temperature, revealing pronounced seasonal differences. The growing season exhibited high moisture levels and low electrical conductivity, which are indicative of waterlogged conditions that foster microbial growth. In contrast, post-harvest conditions were marked by increased soil desiccation, elevated salinity, and higher electrical conductivity, particularly in the lowland areas of Kolasib and Thenzawl, where evaporative processes concentrate the ions. Principal Component Analysis further clarified these trends, identifying elevation, water retention, and ion accumulation as critical factors shaping soil and water quality. These observations highlight the dynamic relationship between topography and hydrology, which collectively defines the environmental context of cyanobacterial communities.

Heavy metal distribution varied distinctly across the study sites, reflecting both natural processes and human activities. In Kolasib, the lowland site exhibited the highest concentrations of Fe, Cu, Pb, Mn, and Zn, suggesting accumulation due to sediment deposition and agricultural practices, such as fertilizer and pesticide application. The elevated levels of Pb and Cu in this area raise concerns regarding potential contamination from external sources, possibly linked to runoff or farming inputs. Conversely, Champhai, situated at higher elevations, consistently showed lower heavy metal concentrations, likely because of enhanced drainage and reduced retention in coarser soils. Although heavy metals can inhibit microbial growth at high levels, the moderate concentrations observed across these sites suggest a limited direct impact on cyanobacteria. Instead, the influence appears to be indirect and mediated through alterations in soil chemistry that affect microbial habitats. This spatial variability underscores the importance of environmentally responsible farming practices to minimize heavy metal build-up, particularly in lowland paddies that are prone to accumulation and safeguarding soil health over time.

Macronutrient analysis revealed significant geographic differences in nitrogen, phosphorus, and potassium levels, which are critical for soil fertility and cyanobacterial function. Thenzawl recorded the highest nitrogen concentrations, potentially supporting nitrogen-fixing microbial activity, whereas phosphorus levels remained notably low across all sites, indicating a widespread deficiency that is common in Northeast Indian soils. Potassium concentrations displayed considerable variation, with Kolasib showing the highest values, which is likely attributable to clay-rich soils and fertilizer use, enhancing nutrient retention. These disparities reflect underlying differences in soil fertility and nutrient dynamics, which are shaped by natural soil properties and agricultural management. The prominence of nitrogen in Thenzawl and K in Kolasib suggests opportunities for site-specific nutrient strategies to boost productivity, whereas pervasive phosphorus limitation highlights a regional challenge for rice cultivation. These findings emphasize the need for targeted nutrient management to enhance soil health and capitalize on microbial contributions to Mizoram paddy systems.

Cyanobacterial diversity was evaluated using morphological and molecular methods, identifying 76 species across 27 genera and 14 families, of which 70 species represented new regional records, with Nostocaceae, Chroococcaceae, and Aphanothecaceae emerging as the dominant groups. High-altitude Champhai predominantly hosted heterocystous cyanobacteria, which are recognized for their nitrogen-fixing capabilities, reflecting adaptations to lower nutrient availability. In contrast, the nutrient-rich lowlands of Kolasib supported a greater abundance of non-heterocystous species, indicating ecological specialization across elevational gradients. Seasonal dynamics were remarkable: the growing season favoured free-living, planktonic cyanobacteria thriving in flooded conditions, while post-harvest samples showed a shift toward desiccation-resistant forms, such as *Nostoc* and *Calothrix*, adapted to withstand drier and saltier soils. These temporal shifts demonstrated the adaptability of cyanobacteria to environmental stressors, emphasizing their ecological significance in maintaining soil fertility under varying conditions in paddy ecosystems.

Statistical analyses provided a greater understanding of these patterns using Shannon's and Simpson's diversity indices to assess species richness and evenness. Champhai consistently exhibited high diversity, which is indicative of balanced microbial communities sustained by stable moisture and low salinity. Kolasib, however, showed reduced diversity, likely due to the dominance of a few tolerant taxa under intensified post-harvest stressors such as desiccation and ion accumulation. Seasonal differences were evident, with some species absent in the postharvest samples, suggesting sensitivity to reduced moisture and elevated salinity. These trends align with the ecological principles linking microbial diversity to habitat stability, emphasizing the critical roles of water availability and soil conditions in shaping community structures across spatial and temporal dimensions.

Molecular characterization via 16S rRNA gene sequencing confirmed the genetic diversity of cyanobacteria, distinguishing heterocystous from non-heterocystous forms into separate clades through phylogenetic analysis. High bootstrap values supported strong evolutionary relationships among nitrogen-fixing genera such as *Nostoc* and *Anabaena*, emphasizing their genetic cohesion and

ecological importance. This genetic richness exceeds the diversity reported in other Indian paddy regions, highlighting Mizoram's unique microbial landscape. The molecular data support morphological findings, illustrating how genetic adaptations enable cyanobacteria to thrive across diverse agroecological zones, particularly through biological nitrogen fixation, which enhances soil fertility and reduces dependence on synthetic fertilizers. These findings enhance our understanding of the contributions of cyanobacteria to paddy ecosystems by bridging genetic diversity with functional roles.

In conclusion, this thesis presents a thorough examination of the factors influencing cyanobacterial diversity in Mizoram's paddy fields by integrating physicochemical, nutritional, and biological dimensions. Elevation, soil moisture, nutrient availability, and seasonal fluctuations are key drivers of microbial community composition and function. These findings suggest sustainable agricultural practices that exploit the roles of cyanobacteria in nutrient cycling, particularly nitrogen fixation, while addressing challenges such as heavy metal accumulation and phosphorus deficiency. Future research should focus on long-term monitoring of microbial diversity and explore the potential of cyanobacteria in biofertilization and organic rice production systems. By elucidating these ecological interactions, this study established a foundation for enhancing soil fertility and agricultural resilience in Mizoram, thereby contributing valuable knowledge to the management of tropical agroecosystems.