ASSESSMENT OF CYANOBACTERIAL DIVERSITY OF PLAINS AND NORTH HILLS OF CHHATTISGARH BY USING POLYPHASIC APPROACH

THESIS

SUBMITTED FOR THE AWARD OF DEGREE OF

DOCTOR OF PHILOSOPHY

IN

Botany

IN THE SCHOOL OF STUDIES IN LIFE SCIENCE

TO

GURU GHASIDAS VISHWAVIDYALAYA
BILASPUR (C.G)

BY

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UNDER THE SUPERVISION OF

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2018


Dedicated
To
My
Beloved
Mother
And Family

CANDIDATE'S DECLARATION

I, **Robin Anigo Minj,** certify that the work embodied in this Ph.D. thesis is my own bonafide work carried out by me under the supervision of **Dr. Satya Shila Singh** for a period of **four years** from **31/10/2014 to 30/10/2018** at **Department of Botany, Guru Ghasidas Vishwavidyalaya, Bilaspur (C.G.).** The matter embodied in this Ph.D. thesis has not submitted for the award of any other degree/diploma.

I declare that I have faithfully acknowledged, given credit to and referred to the research workers wherever their works have been cited in the text and the body of the thesis. I further certify that I have not wilfully lifted up some other's work, Para, text, data, result, etc. reported in the journals, books, magazines, reports, dissertations, theses, etc., or available at web-sites and included them in this Ph.D. thesis and cited as my own work.

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Place: Bilaspur

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This is to certify that the above statement made by the candidate is correct to the best of my knowledge. The research work embodied in the thesis was carried out under my supervision and that the candidate has worked under me for the period required under the regulation.

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Robin Anigo Minj

LIST OF ABBREVIATIONS

µ	Micro
µg ml^{-1}	Microgram per milliliter
µl	Micro liter
µM	Micromolar
APC	Allophycocynin
C_2H_4	Ethylene
cm	Centimeter
DO	Dissolve oxygen
EC	Enzyme Commission
GC-MS	Gas chromatography-mass spectrometery
gL^{-1}	Gram per liter
GS	Glutamine synthetase
H.F.	Heterocyst frequency
M	Molar
mg L^{-1}	Milligram per liter
mM	Milimolar
n mol	Nano mol
NH_4^+	Ammonium
O. D.	Optical density
^oC	Degree centigrade
PC	Phycocyanin
PE	Phycoerythrin
ppm	Parts per million
PS II	Photosystem II
TDS	Total dissolve solid
UV	Ultra violet

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PREFACE

Cyanobacteria are the most abundant organisms inhabiting planet Earth. They were the first organisms to contribute oxygenic photosynthesis and initiated a drastic change in the earth's atmosphere by creating aerobic condition from the anaerobic condition. Cyanobacteria, popularly known as blue green algae, belong to the domain bacteria. Due to their morphology, pigmentation and oxygen evolving Photosystem, they also act as a link between bacteria and algae. Cyanobacteria are basically nitrogen fixer (presence of nitrogenase enzyme) and carbon fixer (presence of CCM mechanism) in nature and in spite of this; they also contribute as the primary producer for the ecosystem which plays a significant role in balancing the aquatic and terrestrial ecosystems. They are found in a wide range of habitats (hyper saline, brackish waters, soda lakes, freshwater, paddy fields, soils, deserts, cave walls, hot springs, polar regions and more importantly in marine habitats).

Cyanobacteria have become increasingly popular because of their high morphological plasticity and therefore they create the confusion for the proper cyanobacterial systematic. In recent years, the development of polyphasic approach has arisen with the new possibilities offered to the molecular biologists to help in the genetic identity as well as the specific ecological roles of unexplored cyanobacteria species.

In present study, the cyanobacterial diversity has been properly evaluated by adopting the modern technique "Polyphasic approach" based on the morphological, physiological and biochemical attributes.

The entire research work has been performed using the 40 cyanobacterial species which was isolated from the different sites of Plain regions as well as North Hills of Chhattisgarh. The morphological identification of the cyanobacterial species has been done by following the keys of T.V. Desikachary, whereas molecular identification has been performed by analyzing the 16S rRNA gene sequences.

Moreover, 40 cyanobacterial species was grown in standard BG11 culture medium and identified based on phenotypic characters including the shape and size of vegetative cells, heterocyte and Heterocyte frequency and shape and size of akinetes. Subsequently, growth was measured at every alternate day in terms of absorbance at 663 nm.

Physiological attributes have been also analyzed in terms of cellular constitutes such as Chlorophyll a, carotenoid and phycocyanin, total carbohydrate content, total protein content and different enzymatic activites i.e. nitrogenase activity, nitrate reductase activity and glutamine synthetase activity. Similarly, the biochemical characterization of 40 cyanobacterial species have also been investigated through lipid profiling (FAME analysis) and whole cell protein pattern analysis (SDS- PAGE). Finally, molecular characterization using 16S rRNA gene sequences have also been performed to inferred the genetic relatedness among the cyanobacterial strains isolated from different habitats.

CHAPTER-1

Introduction

1.1 CYANOBACTERIA

Cyanobacteria are commonly known as blue green algae and are believed to be evolved nearly 3.5 billion years ago. They were responsible for a great event of the initial oxygenation of the earth's atmosphere (Adams, 2000; Hamilton *et al*., 2016; Schirrmeister *et al*., 2015). They were the first organisms to contribute oxygenic photosynthesis and initiated a drastic change in the earth's atmosphere by creating an aerobic condition from the anaerobic condition (Schirrmeister *et al*., 2013). Cyanobacteria are the simple and primitive group of microorganisms which act as a link between both bacterial and algal community. They also belong to a large and diversified group of gram negative prokaryotic organisms exhibit unique taxonomic status with incipient nucleus, loosely arranged thylakoids, hexagonal carboxysomes, gas vesicles, phycobilisomes, ribosomes and variety of storage granules, glycogen, cyanophycin, lipids or polyphosphate (Liberton and Pakrasi, 2008). They are the remarkable group of prokaryotes, comprising of more than 150 genera and 2000 species, which play a significant role in balancing the aquatic and terrestrial ecosystems. In contrast to other prokaryotes, presence of *Chl* a and accessory pigments make cyanobacteria as the eminent primary producers of the ecosystems as well as the skeleton of the food web (Rai, 1990; Thajuddin, and Subramanian, 1992; Kanagasabapathi and Rajan, 2010). Cyanobacteria are the ubiquitous organisms found at all the possible habitats and follow the similar distribution pattern and morphological characters as that of the bacterial community. They are the successor in hyper saline and brackish water, alkaline lakes, freshwater, paddy fields, soils, deserts, cave walls, hot springs, polar regions and more importantly in marine habitats. A wide range of environmental conditions of such as light, temperature, pH and nutrients may induced alterations in differential combinations of physiological and biochemical characters for high adaptive strategy (Tandeau de Marsac and

Houmard, 1993; Narayan *et al.,* 2006; Nayak *et al*., 2007; Sonak *et al*., 2009). Though the bacteria and cyanobacteria show an almost similar type of origin but they are very different in their ecological, biological and morphological behavior (Komarék, 2010). The cyanobacterial phycobilisomes consist of accessory pigments *i.e.* Phycocyanin (PC), Allophycocyanin (APC) and Phycoerythrin (PE) and play as a primary light harvesting antennae for PS II in cyanobacteria (Glazer, 1984). But other major pigments are also important for oxygenic photosynthesis such as Chl a and carotenoid. They have a number of bioactive compounds (Stevens and Bauer, 1991; Six *et al.,* 2004) Considering the evolutionary prospects, cyanobacteria are the progenitors of chloroplast and ultimately contributed a major role in evolution of algae and land plants.

According to Fritsch (1945), the class cyanophyceae is comprised of five orders *i.e.* Chroococcales, Chamaesiphonales, Pleurocapsales, Nostocales and Stigonematales The order Chroococcales include the simplest, unicellular and ancient form of cyanobacteria, that never exhibit polarity in their structural organization whereas order Chamaesiphonales include unicellular cyanobacteria that having polarity in their structural orientation. The other order Pleurocapsales include heterotrichous and filamentous cyanobacteria that are devoid of heterocyte. The order Nostocales consist of the heterocytous and filamentous cyanobacteria but some are of comprised false branching. The fifth order Stigonematales contain heterocytous and filamentous cyanobacteria along with true branching system. However, a new classification of cyanobacteria was introduced recently having different orders of the cyanobacterial community *i.e.* Gloeobacterales, Synechococcales, Oscillatoriales, Chroococcales, Pleurocapsales, Spirulinales, Chroococcidiopsidales and Nostocales (Komárek *et al*., 2014).

The whole community of the cyanobacterial diversity exhibit both unicellular, colonial and filamentous forms (Whitton and Potts, 2000). Generally cyanobacterial colonial forms contain definite or indefinite nucleus of cells with different specific patterns such as radial (*Oscillatoria* sp.), rectangular (*Aphanocapsa* sp.), globular

(*Gloeocapsa* sp.) or irregular (*Microcystis* sp.). The evolution of filamentous morphology is the result of repeated cell divisions that occur in a single plane at right angles with respect to the main axis of the filament. The filamentous cyanobacteria show the great variability in terms of cell size and shape. The member of order Nostocales and Stigonematales have a heterogeneous cellular composition. The members of the order Nostocales have generally unbranched and pseudobranched multicellular trichome while members of the order Stigonematales have filamentous cyanobacteria as was observed in order Nostocales but they are identified by true and distinct branching pattern. Furthermore, cyanobacteria of order Nostocales and Stigonematales also possess specialized cell such as heterocyte, akinetes and hormogonia which are differentiated from the vegetative cells under unfavorable conditions (Kantz and Bold, 1969; Komárek, 1994). In spite of presence of chlorophyll *a* and several accessory pigments that provide them with oxygenic photosynthetic ability, cyanobacterial group are also protected with polysaccharide sheath from an unfavorable conditions. The cell wall and cell membrane in the cyanobacteria provide a structural integrity and mediate an acquisition of the selective nutrients respectively (Flores *et al*., 2006; Flores and Harrero, 2010; Kalaitzis *et al*., 2009; Komarék, 2010; Shukla *et al*., 2012; Mishra *et al*., 2013; Singh *et al*., 2014).

Due to their prokaryotic nature, cyanobacteria reproduce either by cell division or by the formation of hormogonia or by the baeocytes. The members of Chroococcales reproduce by means of cell division (fission) whereas the members of Chamaesiphonales multiply through the production of both exospores and endospores. Apart from this, the representatives of the order Pleurocapsales reproduce *via* generation of endospores only. Furthermore, cyanobacteria belonging to the order Nostocales and Stigonematales produce number of hormogonia and akinetes to complete their reproductive cycle (Srivastava *et al*., 2011; Singh *et al*., 2012).

1.2 DISTRIBUTION PATTERN OF CYANOBACTERIA

Cyanobacterial communities are found in the mutual association with the paddy fields. The paddy field ecosystem provides the suitable condition for

influencing the growth of cyanobacterial communities. However, the condition is also suitable for influencing the enormous and diversified habitats for other microorganisms. These habitats may be micro-environmentally or physico-chemically different to each other and may also make organisms to exhibit biologically distinct properties. Such heterogeneity of the habitats may also play a significant role in influencing the structure and diversity of microbial communities. The paddy field ecosystem as a whole may support various microbiological processes which are agronomically or biogeochemical very important (Kimura, 2000; Kirk, 2004). Cyanobacteria are widespread throughout the world and contribute majorly to the nitrogen economy in ecosystem. They are found as a free-living organism or symbiotically in terrestrial and aquatic environments. They are found in common symbiosis with diatoms, sponges and dinoflagellates. In terrestrial environments, cyanobacteria form symbiosis with a wide range of different hosts, including fungi, bryophytes (liverworts and hornworts), *Azolla* (water fern), *Cycads* (gymnosperm) and *Gunnera* (angiosperm) (Rai *et al*., 2000; Carpenter and Foster 2002; Janson, 2002).

It is oftenly considered that they are the first organism group that colonies the bare areas of rocks and soils. The cyanobacterial community is not restricted up to the terrestrial environment but they are also found in fresh water as well as marine ecosystem also. In marine condition, cyanobacteria are often distributed in shallow regions of sea and ocean, soft or porous rocks such as soft stones in the benthic region. The basic mode of energy metabolism is through photosynthetic activity. They bore hollows into the sandstone and limestone and act as an excavators (Weber *et al*., 1996).

They flourish in salty, brackish or fresh aquatic habitats but sometimes they also grow at any extreme habitats e.g. cold and hot springs where no other microalgae can survive. Most of the marine cyanobacterial planktons such as *Trichodesmium*, *Crocosphaera, Prochlorococcus* contribute to the widespread oxygenation of the open oceans and grow along the shore as the benthic vegetation in the zone between

the high and low tide marks (Humm and Wicks, 1980; Gallon *et al.,* 2002; Sanchez-Baracaldo, 2015).

Cyanobacteria are also known to sustain in a number of extreme environments i.e. temperature variation regions, land contaminated with heavy metals, xenobiotic compounds as well as salt enriched areas etc. Cyanobacteria are regular facing serious stress in heavy metal contaminated areas. Apart from this, some cyanobacterial strains are also found in hyper saline condition and generally they appear in coastal environments e.g. *Halospirulina tapeticola*, *Aphanothece halophytica*, *Anabaena torulosa* (Apte *et al*., 1997; Nübel *et al.,* 2000; Waditee *et al*., 2001; Bhuvaneshwari *et al*., 2017). They play an essential role in various fields of biotechnology (Margesin and Schinner, 2001).

1.3 CLASSIFICATION OF CYANOBACTERIA

Earlier, the most widely accepted classification introduced the cyanobacteria in a separate class cyanophyceae which is comprised of five orders i.e. Chroococcales, Chamaesiphonales, Pleurocapsales, Nostocales and Stigonematales (Fritsch, 1945). Recent trends of classifications has also suggested 8 different orders of cyanobacterial community i.e. Gloeobacterales, Synechococcales, Oscillatoriales, Chroococcales, Pleurocapsales, Spirulinales, Chroococcidiopsidales and Nostocales (Rippka *et al*., 1979; Rippka, 1988; Castenholz, 2001; Komarék and Johansen, 2014; Singh *et al*., 2017).

Previously the cyanobacterial taxonomy was based on Bacteriological Code of Nomenclature (Rippka *et al*., 1979; Castenholz & Warterbury, 1989; Oren and Tindall, 2005). The botanical name was based on morphological characteristics which remains the initial step in the new bacteriological approach. The botanical system emphasized a study of natural material. On the other hand, bacteriological systematic is based on genomic information's of the bacterial system as well as cyanobacterial system which is transformed from the former one to the later one. The bacteriological system creates a phylogenetic relationship among cyanobacteria and bacteria.

However, it never assures the placement of those cyanobacteria which are yet to be cultured and identified (Laamanen, 1996). The bacteriological classification of pure cultured cyanobacteria basically based on the phenotypic, chemotypic and genotypic characteristics and such type of taxonomical study where a holistic approach of several dimensions is considered as polyphasic approach. But to resolve the confusion of taxonomical status of cyanobacteria, the nomenclature of cyanobacteria was based on botanical approach whereas the descriptions and identification was done according to the bacterial taxonomy (Hoffmann *et al*., 2005). However, it was a tough criterion to follow both traditional morphological classification and the classification based on molecular methods (Hoffmann, 2005; Oren and Tindall, 2005).

When the bacteriological approach was considered for cyanobacterial classification it was found that the cyanobacterial system was divided into five sections *i.e*. I, II, III, IV and V. Section I and II consist of unicellular cyanobacteria whereas the filamentous, non-heterocytous cyanobacteria were placed under Section III. Section IV and V exclusively comprised of heterocytous filamentous cyanobacterial species that reproduces by hormogonia and also have the ability to develop heterocyte and akinetes. In the section IV sometimes cyanobacteria show the false branching whereas order V shows true branching cyanobacteria divided in two or more plane respectively (Hoffman and Castenholz, 2001; Komarék and Johansen, 2014). Further studies also revealed that Section IV is divided into two subsections: subsection IV. I represents those strains that never show polarity in their trichomes. Whereas subsection IV.II have distinct polarity in their trichomes, tapering from the apex to the base (Castenholz, 2001; Rippka *et al*., 2001).

1.4 SPECIFITY OF CYANOBACTERIA FROM OTHER MICROBIAL COMMUNITY

Since cyanobacterial emergence from bacterial community was known from the very beginning but it has its own specificity such as divergent morphology, physiology, biochemical and molecular approach. It possess similarity with algae by having specific pigments (chlorophyll a), carotenoid and accessory pigment

(Phycobilisomes) etc. These characters are sufficient to assess genetic relatedness between the bacteria, algae and cyanobacterial strains (Nayak *et al*., 2006; Wang *et al*., 2010; Ashokkumar and Anand, 2010; Wang *et al*., 2010; de-Figueiredo *et al*., 2011). Various biochemical characters have also proved to be useful for the taxonomic evaluation of cyanobacteria. Some chemotaxonomic markers such as fatty acids, proteins, pigments *etc.* are also employed nowadays for assessing the genetic diversity of the natural as well as laboratorically grown species of cyanobacteria (Lyra *et al*., 1997; Abed *et al*., 2006; Jungblut *et al*., 2009; Ezhilarasi and Anand, 2010; Galhano *et al*., 2011). Nowadays FAME profiling of a species is also employed as the species markers for assessing phylogenetic relationships (Tamina *et al*., 2007; Shukla *et al*., 2012). It has been successfully studied among the members of the order Nostocales and Stigonematales (Kenyon *et al*., 1972; Murata *et al*., 1992; Sood *et al*., 2007). Sometimes, protein profiling based on SDS-PAGE is also successfully implicated to differentiate between thirteen different species of *Azolla.* Based on the above studies, it was also concluded that protein profiling may be used as a useful biochemical tool for providing identification and classification at the species level or below (Lyra *et al*., 1997). It was also helpful in characterizing filamentous cyanobacterial strains at genera level. However, a many loopholes have been reported during protein pattern analysis and such was failed to produce a reliable grouping of the cyanobacterial community. Therefore, this biochemical tool is not solely considered for an identification of new strains of cyanobacteria. Even though, it can be used for screening the cyanobacterial relationship at the genus level only (Lyra *et al*., 1997).

1.5 ECOLOGICAL PROSPECTS OF CYANOBACTERIA

Cyanobacteria are abundantly present in the paddy fields and play an important role in maintaining the rice field's fertility through nitrogen fixation. A high density of heterocytous cyanobacteria are reported in rice fields. The flooded paddy field during early stage of sowing are the best suitable environment for the growth of cyanobacteria (Nayak *et al*., 2009). Due to proper fertilization and flooded condition

in the soil of paddy field, the eco-physicochemical factors as the pH, temperature, oxygen concentration and nutrient availability are prevailed continuously during the period of paddy growth which is suitable environment for growth and survival of the cyanobacterial species (Quesada and Valiente, 2004; Saadatnia and Riahi, 2009). In return, cyanobacteria provide enormous amount of nitrogen and phosphorus which are the most required nutrients at the time of rice cultivation (Roger and Reynaud, 1982; Wilson, 2006; Saadatnia and Riahi, 2009). Cyanobacteria are the global nitrogen fixer and they fix at the rate of approximately 80 kg N ha^{-1} $year^{-1}$. The mechanism of nitrogen fixation occurs in a specialized cell (modification of vegetative cell) of the cyanobacterial species known as heterocyte. The heterocyte have a special enzyme complex *i.e.* Nitrogenase which is responsible for the nitrogen fixation. Nitrogenase enzyme consists of two component *i.e.* component I (Dinitrogenase reductase), responsible for reducing molecular nitrogen (N_2) in to the form of ammonia. In this way, they have the capability to convert atmospheric nitrogen (unavailable form into the form of ammonia. The enzyme nitrogenase is an extremely oxygen sensitive enzyme (Capone and Carpenter, 1982; Capone, 1982; Postgate, 1987). Cyanobacteria are also an important contributors in global carbon and nitrogen budgets (Stewart and Falconer, 2008). Phototrophic nitrogen assimilation by planktonic cyanobacteria contribute a major part of the total nitrogen demand. Bloom forming diazotrophic cyanobacteria make the phosphorus limited and nitrogen rich system. Nitrogen fixing cyanobacteria are greatly influenced the community production processes and also impact on the coupling of C-N-P cycles.

Heterocyte are the unique specialized structure that are only confined to the cyanobacteria but are absent in unicellular cyanobacteria (Castenholz, 2001). During nitrogen deprived condition, approximately 5 to 10 % of the vegetative cells of the entire filamentous cyanobacterial species are converted into the specialized cells *i.e.* heterocyte (Wolk *et al*., 1994; Wolk, 2000). The heterocyte is formed by dismantling of PSII, reducing oxygen level in the vegetative cells, the concentration of light harvesting chlorophyll- protein complex in the vegetative cells (Carr, 1983; Bradley and Carr, 1976; Gupta and Carr, 1981; Alberte *et al*., 1980). Further, extra protective

layer is also synthesized around the vegetative cell to protect the nitrogenase complex against oxygen inactivation by creating a micro-oxic intracellular environment (Wolk, 1991; Fay, 1992; Buikema and Haselkorn, 1993). Some other important alterations in vegetative cell was also mentioned for development to support nitrogen fixation *i.e.* synthesis of nitrogenase proteins (dinitrogen enzyme complex), enhanced synthesis of oxidative pentose phosphate pathway enzymes and heterocyte-specific ferredoxin to provide reductant to nitrogenase, enhanced levels of glutamine synthetase for rapid assimilation of NH_4^+ (ammonium) (Apte, 1992). Cyanobacteria generally have the capability to utilize nitrate, nitrite and ammonium as combined nitrogenous source for their growth (Flores and Herrero, 1994). The very popular GS-GOGAT pathway is quantitatively the most important primary pathway for the assimilation of ammonia (Wolk *et al*., 1994; Böhme, 1998).

1.6 AIM AND OBJECTIVES

The main purpose of my research work was to analyze the diversity and taxonomical status of the cyanobacteria isolated from different sites of Chhattisgarh by using the polyphasic approach *i.e.* the combination of the morphological, physiological and biochemical aspects with molecular phylogeny. Nowadays, polyphasic framework is commonly supported by many taxonomists but numerous taxonomic problems are emerged from the lack of correspondence between data inferred from genotypes and phenotypes (Suda *et al*., 2002; Komárek and Kástovský, 2003; Lokmer, 2007; Galhano *et al*., 2011; Singh *et al*., 2017; Singh *et al*., 2016; Singh *et al*., 2016; Jahodářová *et al*., 2018). Despite of all the modern options and large gaps in the knowledge of 16S rRNA gene, 16S rRNA remains the most extensively used tool for Phylogenetic studies. Every new sequence adds an additional information and helps to improve cyanobacterial taxonomy. Thus, the next aim of my work was to get new sequences of important cyanobacteria to fill the gaps in tree topology. However, many discrepancies between traditional classification and phylogenetic relationships are still unresolved. Present study are also focused on the isolation of cyanobacterial species from different types of plains as well as north hills

of Chhattisgarh soil and also to access the diversity which are remained unexplored. So, our preference of interest was to develop a suitable criterion for the identification and characterization of unexplored diazotrophic cyanobacteria from the hills and plain regions of Chhattisgarh. Based on the above mentioned gap in the knowledge following objectives were taken into consideration.

- Identification and characterization of the cyanobacterial strains collected from different sites of Chhattisgarh (based on microscopic observations).
- To study the physiological diversity among the cyanobacterial strains isolated from different site of Chhattisgarh.
- Biochemical characterization based on protein and lipid profiling.
- Molecular phylogeny of the selected cyanobacterial strains using 16S rRNA gene sequences and bioinformatics tools.

CHAPTER-2

Review of Literature

2.1 CYANOBACTERIA: AN OVERVIEW

Cyanobacteria are the first and primitive photoautotrophic prokaryotes which are morphologically most diverse organisms on our Earth (Hedges *et al*., 2001; Degan *et al.,*2012; Schirrmeister *et al*., 2013; Thomazo *et al;* 2018). They play a significant role in the development of an oxygenic environment due to their photosynthetic activity. Though, their origin and cellular organization are bacterial type but their ecological, biological and morphological features are very specific which cause cyanobacteria to place themselves apart from bacteria (Flores *et al*., 2006; Kalaitzis *et al*., 2009; Flores and Herrero, 2010).

Cyanobacteria possess certain entrancing characters such as buoyancy and fixation of atmospheric carbon and nitrogen (Walsby, 1994; Burja *et al*., 2001; Berman- Frank *et al*., 2003; Herrero *et al*., 2004; Picossi *et al*., 2014). They play a significant role as a primary producer in the natural ecosystems (Field *et al*., 1998; Bryant, 2003). The cyanobacteria contain chlorophyll *a,* carotenoid and other accessory blue green pigments. Accessory pigments are present in phycobilisomes which serve as the primary light-harvesting antennae for Photosystem II in cyanobacteria (Glazer 1984; Sidler, 1994; Six *et al*., 2004; Mullineaux 2008., Ho *et al.,* 2017; Plooy *et al.,*2018). These supramolecular complexes are primarily composed of phycobiliproteins, a brilliantly colored family of water-soluble proteins (C-phycocyanin, C-phycoerythrin and allophycocyanin) bearing covalently attached, open-chain tetrapyrroles known as phycobilin. The chlorophylls and accessory pigments efficiently capture specific wavelengths of light, transferring the light energy to the cell. (Klementiev *et al.,* 2018; Khatoon *et al.,*2018)

In filamentous cyanobacteria, cells are differentiated into specialized thick-walled cells called heterocyte at regular intervals along the filament that protects the cell from physical damage **(**Wolk, 1996; Adams, 2000; Xu *et al*., 2008; Valladares *et al*. 2016) and they contribute a major role in nitrogen economy by fixing unavailable dinitrogen into the available form of nitrogen (NH_3). Due to nitrogen fixation efficiency of heterocytes, the vegetative cells depend on the heterocytes for fixed nitrogen and in return the vegetative cells provide photosynthate as the source of the reductant (Ramaswamy *et al*., 1996; Meeks and Elhai, 2002). The vegetative cells generate energy through photophosphorylation and oxidative phosphorylation (Flores and Herrero, 2010; Haselkorn, 2007; López-Igual *et al*.,2010; Flaherty *et al*., 2014).

Presence of combined nitrogen *i.e.* Nitrate, ammonia and glutamine repress the heterocyte formation in cyanobacteria (Wolk 2000). In some filamentous cyanobacteria, heterocyte is developed under anaerobic and nitrogen deficient condition where nitrogen fixation is regulated and can operate efficiently (Rajagopalan and Callahan, 2010; Corrales-Guerrero *et al*., 2015). The combined nitrogen (nitrate and ammonium) are excellent nitrogen source for cyanobacterial growth.

The assimilation of nitrate takes place by an active nitrate transport system and then intracellular nitrate is reduced to ammonium through two sequential reactions catalyzed by nitrate reductase (NR) and nitrite reductase (NiR) whereas ammonium is further assimilated by the glutamine synthetase-glutamine 2-oxoglutarate amidotransferase (GS-GOGAT) pathway to generate diverse metabolites containing nitrogen in the cell (Herrero *et al*., 2001). The gene products of *nrt* ABC, *nir* and *nar* B are involved for the reduction of nitrate to nitrite (Luque *et al*., 1993; Rubio *et al*., 1996; Aicchi *et al.,* 2001; Flores and Herrero, 2005). Previous studies showed that the nitrate uptake is inhibited due to the presence of ammonium which operates through the feed-back inhibition exerted by the products of the ammonium assimilation pathway via Glutamine Synthetase (Flores *et al*., 1980; Flores and Herrero, 1994; Mura-Pastor *et al*., 2005).

Nitrate reductase enzyme is a high molecular weight complex (MW about 75,000 Da), consisted of three prosthetic groups, FAD-heme (*cytb*557) and Mo-protein that uses NADH or NADPH as an electron donor (Crawford, 1995; Lopes *et al*., 2002). Nitrate reductase is an inducible enzyme and presence of nitrate induced the enzyme activity. Martín-Nieto *et al*. (1992) reported biphasic kinetic behavior in NR activity of filamentous, heterocyte-forming cyanobacteria with respect to nitrate. Similarly ammonium ions repress the expression of the cyanobacterial genes involved in nitrate uptake and its assimilation (Herrero *et al*., 2001; Muro-Pastor *et al*., 2005).

In cyanobacteria, ammonium assimilation takes place mainly by the sequential action of GS and glutamate synthase (GOGAT). This pathway represents a connecting step between C and N metabolism and is also tightly regulated in cyanobacteria. Several promoters have been found for the structural GS gene. High level of GS activity is reported in heterocyte which catalyses the ATP dependent amidation of glutamate and produces glutamine. Heterocyte lack glutamine-2-oxoglutarate amidotransferase (GOGAT**)** activity which is responsible for the reductive trans amidation of 2-oxoglutarate, producing two glutamate molecules (Merida *et al*.,1991; Wolk *et al*., 1994; Martín-Figueroa *et al*., 2000). Three distinguishable forms of GS have been reported *i.e.* GS type I (GSI) and GS type III (GSIII) have been reported in cyanobacteria (Garcia-Domínguez *et al*., 1997). The *glnA* gene lies within the operon *glnA-ntrBC* and is responsible for GSI structure which is composed of 2 identical layers of 6 subunits (*i.e*.12 identical subunits) (MW about 50,000) arranged in two hexagonal rings (Flores and Herrero, 1994; Merrick and Edwards, 1995; Eisenberg *et al*., 2000).

When a nitrogen is added in the medium, GS activity is higher in heterocyte as compared to vegetative cell (Haselkorn *et al.,*1980; Bargman *et al.,* 1985). Presence of ammonium modulates the intercellular pools of amino acids (Tapia *et al.,* 1996). In cyanobacteria ratio of carbon and nitrogen modulate the transcriptional/post-transcriptional level of GS (Mura-Pastor *et al.,* 2005).

There are certain microbes which have the ability to fix unavailable form of Nitrogen (N_2) into available from ammonium and the process is known as biological N_2 fixation. This process occurs in an anoxic environment or under intracellular micro-oxic conditions and is catalyzed by the enzyme, Nitrogenase which is highly oxygen sensitive (Stacey *et al*., 1992; Frank *et al*., 2002; Church *et al.,*2005).

Nitrogenase is highly conserved enzyme complex and found in more diverse group of Bacteria, Archaea as well as cyanobacteria. Nitrogenase enzyme complex consists of two components, an iron protein encoded *nif* H (Fe-protein) and an iron-molybdenum protein (MoFe-protein) encoded by *nif* KD The Fe-protein is a homodimer, composed of a single Fe_4S_4 cluster bound between identical ~32–40 kDa subunits. The MoFe-protein is heterotetrameric consisting of two types of polypeptides ($\alpha_2\beta_2$) with approximately molecular weight 250 kDa. Each unit contains two types of clusters, a P cluster (Fe_8S_7) and a FeMo center an inorganic structure of $Fe_7Mo_1S_9$ and an organic component, homocitrate, the P cluster functions as a conduit for electron transfer, accepting electrons from the Fe_4S_4 cluster of the iron protein linked with ATP hydrolysis and donating them to the FeMoCo center, the site of substrate reduction **(**Zeher *et al.,* 2000; Zeher *et al.,* 2001).

$$\mathbf{N_2 + 8H^+ + 8e^- + 16MgATP \rightarrow 2NH_3 + H_2 + 16MgADP + 16Pi}$$

Overall biological nitrogen fixation were energy depending process that requires approximately 16 ATP so as to reduce one molecule of dinitrogen (Gallon and Chaplin, 1987).

2.2 CYANOBACTERIAL DIVERSITY

Cyanobacteria, the most extraordinary species and rich group of algae, which are ecologically wide spread and have global significance in nitrogen and carbon fixation and their uses are continuously increasing for ecological monitoring and biotechnological significances (Tiwari *et al.,* 2005; Dey *et al.,*2010; Asadi *et al*., 2011). Although, cyanobacteria are ubiquitous in nature and found at all possible

habitats. They have efficiency to survive/grow at any extreme conditions where no other microalgae can survive (Mur *et al*., 1999).

Many cyanobacterial species are able to found in the terrestrial habitats whereas some are confined as a planktonic in the benthic habitat (Usher *et al*., 2007; Thajuddin *et al*., 2010). They play a significant role in the functional processes of ecosystems and nutrient cycling (Whitton, 1992).

Cyanobacteria have also an ability to colonize and grow on the substrates such as volcanic ashes, deserts and rocks (Dor and Danin, 1996). Another remarkable feature which makes them more valuable is to survive at extremely high and low temperatures (Roy *et al.,* 2014). Cyanobacteria are also very tolerant to extreme conditions such as hot springs, mountain streams, Arctic and Antarctic lakes, snow and ice (Castenholz, 1973; Kol, 1968; Kann, 1988; Laamanen, 1996).

Overall the occurrence of cyanobacteria in fresh water blooms, marine ecosystems, rice fields, within limestone, salt affected lands, deserts, polar environments and in symbiotic associations highlight their ability to survive in the above niches (Rai, 1990; Vagnoli *et al.,* 1992; Elster *et al*., 1999; Sigler *et al*., 2003; Carpenter and Foster, 2002; Adams, 2000; Rajaniemi *et al*., 2005; Convey and Stevens, 2007; Bergman *et al*., 2007; Convey *et al*., 2008; Kumar *et al.,* 2013). Apart from this cyanobacteria grow luxuriantly in the paddy field**.** Due to their nitrogen fixing ability, they are used as a biofertilizer (Singh, 1961; Vaishampayan *et al.,* 2001; Singh *et al.,* 2016; Chittapun *et al.,* 2018; Pathak *et al.,* 2018). Due to divers habitats and physiological elasticity, cyanobacteria are used as an excellent experimental organism for plant ecologist, physiologist, microbiologist and biotechnologist (Nayak and Prasanna, 2007; Saadatnia and Riahi, 2009; Choudhary, 2009; Srivastava *et al*., 2009; Shariatmadari *et al.,*2011; Maheshwari, 2013; Chakraborty *et al*., 2014; Singh *et al*., 2014; Roy *et al*., 2014; Adhikari and Buarah, 2015; Indrama 2015; Joseph and Saramma, 2016; Zhu *et al*., 2016; Thajamanbi, 2016). Previous report also revealed that heterocytous cyanobacteria are successfully colonized in rice field soil and they significantly enhance microbial activity and

growth of the rice plant. Finally, they play a significant role in sustainable management of the rice ecosystem.

Earlier it was also reported that the interaction among physiological activities, abiotic and biotic factors decide the distribution and diversity of cyanobacteria. (Jeyachitra *et al.,* 2013; Faldu *et al*., 2014; Singh 2014; Rishi and Awasthi, 2015; Keithellakpam *et al.,* 2015; Tiwari *et al*., 2015; Liu *et al*., 2015; Sharma and Jain, 2016). Sometimes, they establish symbiotic association with fungi, bryophytes, pteridophytes, gymnosperm and angiosperm (Rai, 1990). They also form biofilms and microbial mats on shores, stones, plants and artificial objects (Stahl, 2000).

2.3 TAXONOMY OF CYANOBACTERIA

The Systematic arrangement of any microorganisms into their group according to their structural and functional behavior is termed as classification. Such system should reflect the evolutionary relationships between the organisms (Komárek, 2010). Due to plastic behavior of cyanobacteria, their classification based on phenotypic characterization do not always provide a true and phylogenetically reliable taxonomy. Furthermore, the cyanobacteria have more complex morphology as compared to other prokaryotic microbes (Giovanni *et al*., 1988; Wilmotte, 1994).

The reclassification of cyanobacteria have been considered several times but recently it has been depicted in two ways –(1) Bacteriological classification system and (2) Botanical classificatory system (Rippka *et al.,* 1979; Komarek and Anagnostidis 1989, 1999, 2005; Oren, 2004).

Traditional classificatory system (International Code of Botanical Nomenclature) of cyanobacteria was based on morphological features *viz.* Shape, color and dimension of the vegetative, heterocyte and akinetes, presence of sheath, constriction at cross walls, branching pattern and gas vacuoles (Greuter *et al.,* 2000). But this classification does not reflect a true Phylogenetic relationship and reliable taxonomy (Giovannoni *et al.,* 1988; Wilmotte, 1994).

According to Rippka *et al.,*1979, cyanobacterial classification has been mentioned in tabulated form.

S.N.	Order	Characters	References
1	Chroococcales (Section I)	Unicellular cyanobacteria	Geitler, 1932
2	Pleurocapsales (Section II)	large cells, subdivide in to baeocytes	Geitler,1925 Waterbury and Rippka , 1989
3	Oscillatoriales (Section III)	Uniseriate filaments, without heterocytes	Geitler,1932; Castenholz and Waterbury 1984; Anagnostidis and Komarek 1988
4	Nostocales (Section IV)	Filamentous unbranched cyanobacteria with heterocyte	Geitler, 1925
5	Stigonematales (Section V)	True branching and multiseriate forms, heterocytous	Geitler, 1925

Later on, this classification was modified in Bergey's manual of Bacteriological systematic (Boone and Castenholz, 2001). Furthermore, an extensive revision was done in the classification of cyanobacteria under the botanical code of nomenclature where cyanobacteria (Cyanoprokaryota) was classified into five orders: Chroococcales, Pleurocapsales, Oscillatoriales, Nostocales and Stigonematales which are further divided into families, sub families, genera and species (Anagnostidis and Komarek, 1985, 1990; Komarek and Anagnostidis 1989, 2005; Komarek and Johansen, 2014; Komarek, 2016). The members of Chroococcales and Pleurocapsales are unicellular or if multicellular are found in groups whereas the members of order Oscillatoriales contains non heterocytous and filamentous cyanobacteria.

The members of Nostocales posses trichomes where cells are differentiated into thick walled heterocyte for nitrogen fixation. Similarly, members of Stigonematales cells are multicellular, heterocytous, trichomes but have true branch (Komarek, 1994). Based on heterogeneous cellular composition of Nostocales and Stigonematales, the branching pattern is of the two types *i.e.* If the cells in a filament divide in more than one plane generates true branch e.g. *Hapalosiphon*, *Westiellopsis*

(Komarek *et al.,* 2013). Similarly, a division occurs in a filament occurs in a such way in a common sheath appeared like two separate filament known as false branching (e.g. *Tolypothrix*, *Scytonema*).

Due to morphological plasticity in cyanobacterial behavior an identification of natural cyanobacteria is complicated as well as prolonged maintenance of culturing standard laboratory conditions ultimately enhance the level of misidentification evaluating a true evolutionary relationships within the lineage (Mollenhauer, 1988; Rippka, 1988; Komárek and Anagnostidis, 1989; Rippka and Herdman, 1992; Ward *et al*., 1998; Komárek and Anagnostidis, 2005; Marquardt & Palinska, 2007; Steven *et al*., 2012; Palinska and Surosz, 2014). Therefore, only consideration of morphological attributes for the study of cyanobacterial taxonomy has been a matter of debate (Turner, 1997; Otsuka *et al.,* 2000; Lyra *et al*., 2005; Rajaniemi *et al*., 2005; Oren, 2014).

Nowadays, modern approaches have been used for proper cyanobacterial systematics (Hoffmann, 2005; Oren and Tindall,2005). A number of new techniques have been used especially molecular tools *viz.* PCR based DNA finger printing and non PCR-based like DNA-DNA hybridization, guanine-cytosine ratio for the assessment of cyanobacterial taxonomy (Komárek and Cáslavská, 1991; Moore *et al.,* 1998; Komárek and Kaštovský, 2003; Hoffmann *et al*., 2005). In spite of these, morphological, physiological and biochemical markers are also used for the study of cyanobacterial diversity and evolutionary relationships (Oren, 2004; Oren and Tindall, 2005; Hoffmann, 2005; Oren, 2011; Mishra *et al.,* 2013; Singh *et al*., 2015).

2.4 CYANOBACTERIAL DIVERSITY BASED ON BIOCHEMICAL ATTRIBUTES

The Morphological and physiological attributes are solely not sufficient to determine the proper phylogenetic relationships and taxonomic classification within the lineage (Stanier *et al*., 1978; Rippka *et al*., 1979; Castenholz and Waterbury, 1989; Komárek *et al*., 2014). So, no classificatory system is true until and unless it is

based on more than one criterion. That's why morphological, physiological, biochemical and molecular attributes have been considered for taxonomical classification and phylogenetic relationship among the cyanobacteria (Viti *et al*., 1997; Lyra *et al.,* 1997; Gugger *et al*., 2002; Henson *et al*., 2002; Rajaniemi *et al*., 2005).

So in this way polyphasic approach that is the combination of morphological, physiological and biochemical including the molecular attributes are frequently used to resolve the problems of cyanobacterial taxonomy (Komárek and Cáslavská, 1991; Komárek and Kaštovský, 2003; Hoffman et al., 2005; Mishra *et al.,* 2012; Singh *et al.,* 2014; Singh *et al.,* 2015; Minj *et al.,* 2016; Singh *et al*., 2017).

Several physiological parameters such as cellular constituents (protein, carbohydrates and pigments), enzymatic activities such as nitrogenase activity, nitrate and nitrite reductase activity, glutamine synthetase etc. have been tested for the study of cyanobacterial diversity (Rippka *et al*., 1979; Narayan *et al*., 2006; Prasanna *et al*., 2006; Wang *et al*., 2010; Mishra *et al*., 2013).

2.4.1 Fatty Acid Methyl Esters (FAME) as Chemotaxonomic Marker

Fatty acids are evolutionarily old and conservative group of structurally distinct organic compounds including fats, waxes, phospholipids, glycolipids *etc.* (Petkov and Garcia, 2007; Galhano *et al*., 2011). Fatty acids are also used as valuable biochemical markers for providing an information for the evulation of the taxonomic positions of the cyanobacteria (Romano *et al*., 2000; Li and Watanabe, 2001; Gugger *et al*., 2002a; Řezenka *et al*., 2003; Li and Liu, 2003; Liu *et al*., 2005; Temina *et al*., 2007; Jungblut *et al*., 2009; Shukla *et al.,*2012).

Nowadays, Fatty acids have been used in complementary approaches in the assessment of cyanobacterial diversity and Phylogenetic relationship based on polyphasic approach (Otsuka *et al*., 1999; Li and Watanabe, 2004; Li *et al*., 2008; Galhano *et al*., 2011; Rajeshwari and Rajashekhar, 2011; Los and Mironov, 2015;

Zimba and Haung, 2016). It was also known that the variation in the total fatty acid composition of cyanobacteria reflects the physiological state of their genotype.

The cellular fatty acids are also considered as the key signature for taxonomical studies of the phyla or class, species and below (Brocks *et al*., 2003; Los and Murata, 2004; Jungblut *et al*., 2009; Bergé and Barnathan, 2005; Rossi *et al*., 2006; Temina *et al*., 2006; Maulucci *et al.,* 2016). Different combination of fatty acids have been reported in cyanobacterial strains such as Palmitic, palmitoleic, Hexadecadienoic, Stearic and oleic, Linolenic acid Erucic acid and α-Linolenic acid etc. (Kenyon, 1972; Cohen *et al*., 1995; Gugger *et al;* 2002; Galhano *et al*., 2011; Thomas *et al.*, 2017).

Recently, an unique fatty acid composition was reported in membrane lipids of cyanobacterium sp. strain IPPAS B-1200 which is enriched with myristic and myristoleic acids (Zimba *et al.,* 2015; Starikov *et al.*, 2017).

Temina *et al.* (2006) identified several *Nostoc* species based on fatty acid composition. Though genera *Anabaena* and *Aphanizomenon* showed a high degree of dissimilarity on the morphological basis but cellular FAME profiling showed similarly between both the genera (Gugger *et al*., 2002a).

Gugger *et al.* (2002b) used fatty acids profiling as a marker and concluded that the strain *Nostoc* sp. 152 member of order Nostocales shows similarity with *Planktothrix* NC/128/R, member of order Oscillatoriales. Fatty acid 16:1 ω 9C and 16:0 fatty acids which form hepatotoxin are found among *Anabaena* as well as in *Aphanizomenon* and *Cylindrospermum* which are nontoxic genera. A differentiation was observed among *Nostoc flagelliforme, Anabaena, Microcystis and Synechococcus* on the basis of fatty acid profiling (Lieu *et al*., 2003). Li *et al*. (2004) studied the lipid composition of 13 strains of *Anabaena* and grouped it two types, type 2A and type 2B. Shukla *et al.,* (2011) studied twelve cyanobacterial strains of the two orders Nostocales and Stigonematales by using FAME profiling as a chemotaxonomic marker and reported some major fatty acids i.e. palmatic acid, hexadecadienoic acid,

stearic acid, oleic acid, linoeic and linolenic acid. FAME profiling study shows similarity among *Anabaena cylindrica* Lemm, *Nostoc muscorum* and another strain *Aphanizomenon gracile* all belong to the same order but morphologically they are different (Galhano *et al.,* 2010).

The fatty acid profiling was done among the filamentous genera of the subsection III, IV and V (Kenyon *et al*., 1972; Sato and Murata, 1981; Sallal *et al*., 1990; Ahlgren *et al*., 1992; Caudales and Wells, 1992; Cohen *et al*., 1993). The fatty acid composition of the six different genera of the order Pleurocapsales was investigated (Caudales *et al*., 2000). The study of the Fatty acid composition among six genera of Pleurocapsales revealed high content of saturated and unsaturated fatty acids but trace amount of polyunsaturated fatty acid. A high percentage of PUFA and SAFA but low level of MUFA was investigated in nitrogen fixing cyanobacteria (Vargas *et al*., 1998). A high in PUFA content and also have MUFA: PUFA ratios were reported in free living strains of genera *Anabaena* and *Nostoc* (Caudales and Wells 1992; Guedes *et al.,* 2011).

The cyanobacteria were divided into five sections on the basis of Fatty acid composition (Kenyon *et al*., 1972; Murata *et al*., 1992; Cohen *et al*. 1995). The strains of the group I are devoid of polyunsaturated fatty acid (PUFA) but contain only saturated and monounsaturated fatty acids (Cohen *et al*., 1995). Group II includes such cyanobacterial strains containing 18:3 Δ 9, 12, 15 (18:3 ω 3) as the only C18 PUFA. Whereas 18: 3 Δ 6,9,12 (18:3 ω 6) as the major C18 PUFA were reported in group III strain. But Strains of group IV have either 18:3 Δ 9,12,15 or 18:3 Δ 6,9,12 or both but sometimes may produce 18:4 Δ 6,9,12,15 (18:4ω 3,octadecatetraenoic acid).

2.4.2 Whole Cell Protein Profiling based on SDS-PAGE

Several types of biomarkers are used for the study of biological processes but the proteins are more advantageous over other parameters. It is the major portion of cell mass. SDS Polyacrylamide gel electrophoresis is the most widely used method

for analyzing protein pattern (Wilson and Walker, 2010). Whole Cell Protein Profiling based on SDS-PAGE is one of the most important criterions for the identification and differentiation of the microorganisms and offers a better taxonomic resolution at species or subspecies level (Derbyshire and Whitton, 1968; Elliot *et al*., 1993; Vandamme *et al*., 1996; Sood *et al*., 2007; Mishra *et al.,* 2013).

Genetic diversity was also assessed on the basis of SDS-PAGE and 2D gel electrophoresis in the number of other microorganism's such as bacteria, rhizobacteria and *Frankia s*trains (Noel and Brill, 1980; Benson and Hanna, 1983; Benson *et al*., 1984; Woes, 1987; Berber *et al*., 2003; Singh *et al*., 2010). Protein profiling based on SDS-PAGE analysis was also used for differentiating 13 species of *Azolla* (Sood *et al.,* 2007). Again, Palinska *et al*. (1996) studied *Merismopedia* on the basis of SDS-PAGE and 16S rRNA gene sequences.

Similarly, different strains of *Anabaena* were characterized on the basis of SDS-PAGE profiling and heterogeneous cluster formation was reported (Ezhilarasi and Anand, 2010). Lyra *et al*. (1997) also observed similar observation by using SDS PAGE and PCR / RFLP of 16S rRNA. Nayak *et al*. (2007) evaluated inter and intra-specific relationships among the different *Anabaena* isolates based on SDS-PAGE, morphological and physiological characters. But due to huge requirement of culture for protein profiling, SDS-PAGE still not a widely applied technique for the taxonomical study of cyanobacteria.

2.5 CYANOBACTERIAL DIVERSITY BASED ON MOLECULAR ATTRIBUTES

In the case of microorganisms, the taxonomy based on morphological or the morphological diversitification is not always represent a true evolutionary relationships (Stackebrandt and Woese, 1981). So large number of highly useful DNA markers are commonly used for the identification of genetic polymorphism. Most common molecular marker is used for the taxonomical and Phylogenetic study of cyanobacteria *i.e*. 16S rRNA (Wilmotte, 1994; Vandamme *et al*., 1996; Komárek and

Johansen, 2014). Due to its nine conserved and nine hypervariable region, 16S rRNA gene is the most commonly used marker gene for the identification of bacteria and is also played a central role in inferring Phylogenetic relationships (Stackebrandt and Goebel, 1994; Komarek and Johansen, 2014; Shariatmadari *et al.,* 2017). Molecular diversity based on 16S rRNA has also observed among the cyanobacteria of different habitats (Wanigatange *et al*., 2014; Singh *et al*., 2016; Singh *et al*., 2017b; Singh *et al*., 2017a).

In case of molecular study of *Bacillus*, 16S rRNA gene is found to be one of the most informative marker (Ludwig and Schleifer, 1994; Ludwig and Klenk, 2001; Mahasneh *et al*., 2011; Sacchi *et al*., 2012).

Cyanobacterial specific 16S rRNA gene primers have been designed and used for taxonomical and diversity studies (Urbach *et al*., 1992; Nelissen *et al*., 1996; Nübel *et al*., 1997; Lepère *et al*., 2000; Iteman *et al*; 2000). A large database of 16S rRNA gene sequences are available which help in recognition of novel taxa based on 16S rRNA gene sequences (Tindall *et al*., 2010; Dom´ınguez-Escobar *et al.,* 2011; Singh *et al*., 2017).

The phylogenetic investigations based on 16S rRNA sequences revealed that some of the unicellular and non heterocytous filamentous cyanobacterial genera are probably polyphyletic in origin whereas heterocytous cyanobacteria belong to subsection IV and V are monophyletic in origin (Castenholz, 2001; Lyra *et al*., 2001; Rippka *et al*., 2001; Iteman *et al*., 2002; Rajaniemi *et al*., 2005; Willame *et al*., 2006; Sahu *et al*., 2012; Kilgore *et al*., 2018).

Based on 16S rRNA gene sequences, *Anabaena* and *Aphanizomenon* strains were not monophyletic. (Gugger *et al*., 2002., Gulhana *et al*., 2010., Minj *et al*., 2016). Whereas the heterocytous cyanobacteria of Nostocales and Stigonematales showed ambiguous evolutionary relationship (Gugger and Hoffmann 2004; Henson *et al.,*2004; Sahu *et al.,* 2012., Mishra *et al*., 2013). Phylogenetic studies based on 16S rRNA gene sequences revealed that the cyanobacteria are diversified from one

another very recently (Giovannoni *et al*., 1988; Wilmotte and Herdman, 2001; Zapomělová *et al.,* 2008; Yarza *et al*., 2012; Hentschke and Komarek, 2014; Shestakov and Karbysheva, 2017). Probably, the origin of these false attractions is due to the combination of different factors and it is linked to the fact that these false similarities might arise by chance between the groups without real phylogenetic affinities (Ludwig *et al*., 1998; Korelusová, 2008; Dom´ınguez-Escobar *et al*., 2011). Similar to bacteria for differentiating cyanobacterial species and genera of 95% similarity was established for identification of cyanobacterial genera based on the identity derived from nucleotide or protein database sequence analysis (Stackebrandt and Goebel, 1994). But it is not always a standard key criterion for separating biological taxa although may be used as the first marker for generic differentiation. Nowadays, the characterization of genera is usually based on about 95 % or less genetic similarity combined with at least one diacritical, autapomorphic, cytomorphological characters (Garcia-Pichel *et al*., 1998; Hrouzek *et al*., 2005). Molecular analysis of new strains of *Scytonema* revealed that the false branching cyanobacteria represent polyphyletic group (Singh *et al.,* 2016., Singh *et al*., 2017b; Minj *et al.,* 2016).

Recently, many workers work on the molecular diversity of cyanobacteria by using 16s rRNA as a marker (Nübel *et al.,* 2002; Lopes et al., 2012). Many novel taxa of cyanobacteria were also reported on the basis of 16S rRNA gene sequences including other molecular attributes (Mares, 2010). A polyphasic approach was used to revised the genus *Planktothrix, Cylindrospermopsis, Raphidiopsis, Nostoc* and its type species (Li *et al*., 2008; Haande *et al*., 2008; Moro *et al.,* 2010; Lamprinou *et al*., 2011; Nowruzi *et al.,* 2012; Hašler *et al*., 2014; Gaget *et al.,* 2015).

The potentiality and phylogenetic studies have done among different cyanobacteria species on morphological and physiological basis (Nayak *et al*., 2014; Keithellakpam *et al.,* 2015; Oinam *et al.,* 2015; Valerio *et al*., 2015).

Above all, 16S rRNA gene is a valuable tool for assessing the diversity and phylogeny of cyanobacteria with certain limitations. *i.e.* it is not divergent enough to

give good separation in close relation measure at species level (Fox *et al*., 1992; Normand *et al*., 1996; Casamatta *et al.,* 2005; Dvořák *et al.,* 2014). So, it is advisable to use polyphasic approach for cyanobacterial diversity and evolutionary relationship among cyanobacteria.

CHAPTER-3

Materials and Methods

This chapter includes the entire methodologies employed during the tenure of investigations.

3.1 SAMPLING SITES

The cyanobacterial samples were collected from plains and the northern hills of the state Chhattisgarh. The districts covered under the plains were Bilaspur, Raigarh, Korba, Janjgir Champa, Mahasmund, Durg, Raipur, Dhamtari, Kabirdham, and Rajnandgaon. On the other hand, Jashpur Nagar, Sarguja, Surajpur were covered under North Hills region of the state (Figure 1).

3.2 SAMPLE COLLECTION

Initially, the soil and water samples were collected from the selected paddy fields. The temperature of the water was taken immediately with the help of Water and Soil Analysis kit Model 161 (EI products, Panchkula, Haryana, India). For the analysis of physico-chemical attributes of the soil samples, soil samples were bought in the laboratory.

Apart from this, the cyanobacterial samples from different sites were also collected in the sample bags properly and carried to the laboratory.

3.3 ANALYSIS OF PHYSICO-CHEMICAL ATTRIBUTES OF THE SOIL

The physico-chemical properties of soil and water such as pH, temperature, conductivity, total dissolved solids, salinity and dissolved oxygen were measured as per the standard protocols. The soil temperature was measured using deluxe laboratory thermometer and while other soil physico-chemical test were performed by water and soil analysis kit 161 (EI products, Panchkula, Haryana, India) For soil

analysis, a homogenous soil solution was prepared by dissolving 15 gm soil sample in 30 ml of distilled water with rigorous shaking. The soil solution was filtered through Whatman's filter paper no. 1. To obtain the clear supernatant, filtrate was centrifuged at 5000rpm, supernatant was collected in pre-sterilized 200 ml borosil make beaker.

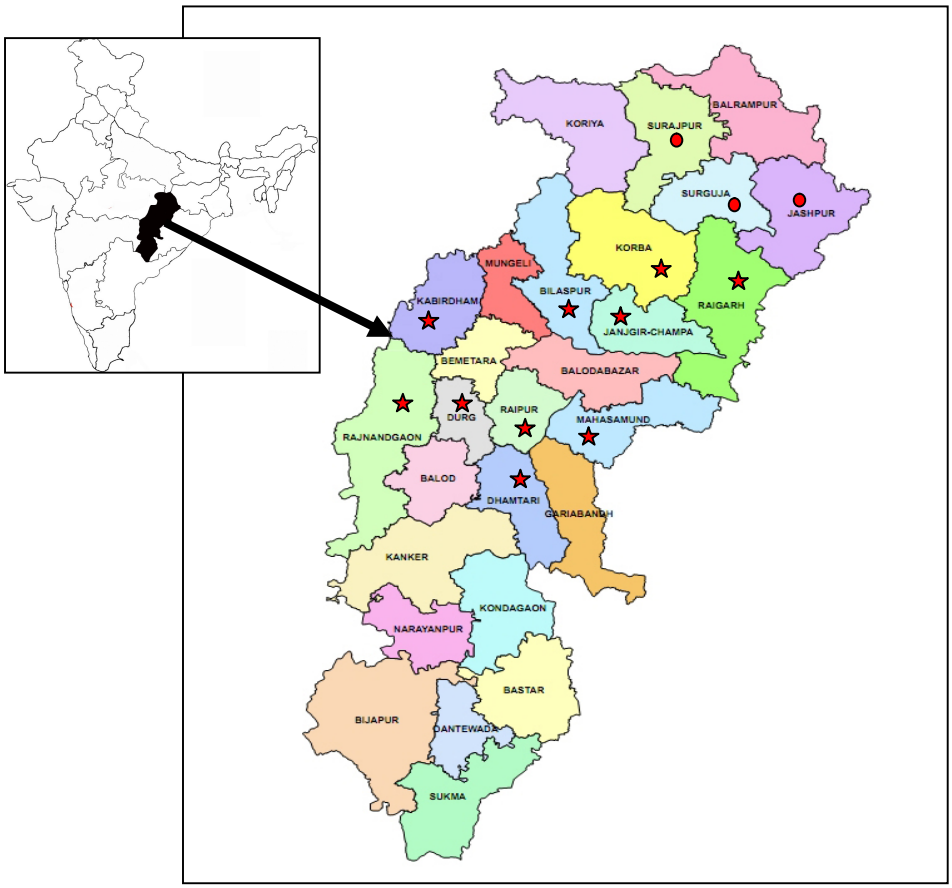

Figure 1. Map of Sampling Sites. **Plain regions of Chhattisgarh** (Bilaspur, Raigarh, Korba, Janjgir Champa, Mahasmund, Durg, Raipur, Dhamtari, Kabirdham, Rajnandgaon and **North hill regions (**Jashpur Nagar, Sarguja, Surajpur)

Legends show the explored sites ★Indicates Plain regions. ● Indicates North Hill regions

The collected supernatant was used for the following physicochemical analysis. pH of the soil was measured with the help of a Water and Soil Analysis Kit Model 161(EI Products, Panchkula, Haryana, India). Conductivity of the soil samples was measured with the help of conductivity cell which was provided with the Water and Soil analysis kit in terms of µSeimens cm^{-1} (Singh *et al*., 2014). Similarly, dissolved Oxygen (DO) of the soil sample was also calculated with the help of DO

probe supplemented with the kit. Before measuring the DO of the sample, the DO probe was calibrated with sodium sulphite solution. The DO of the soil samples were measured in terms of ppm (parts per million). Finally, total dissolved Solids (TDS) present in the soil samples were also measured and expressed in terms of mg per unit volume of water (mg L^{-1}). All the experiments were performed in triplicates.

3.4 GLASSWARES AND CHEMICALS

All the Glasswares used in the present study were of Riviera and Borosil make. Culture tubes and flasks (50, 100, 250, 500 and 100 ml capacities) were used for culturing and experimental purpose. All the Chemicals and reagents used in the present investigations were purchased from Sigma-Aldrich, USA, HiMedia, India and CDH (Central Drug House), Bangalore Genei (Merck, USA and Bio-Rad, USA.

3.5 STERILIZATION OF GLASSWARES

Before initiating the experiments, glasswares were dipped in the chromic acid solution (1% potassium dichromate in concentrated H_2SO_4 *i.e.* sulphuric acid) for 24 hours. After 24 hours, the glasswares were washed with detergent. After washing with the detergent the glasswares were rinsed with tap water followed by double distilled water and dried in hot air oven at 100° C for 4-5 hours. After complete drying, the glasswares were autoclaved at 121ºC and 15 psi pressure for 15 minutes. For cultivation of axenic culture of cyanobacteria, the laminar flow was fumigated with chloroform to avoid contamination. The laminar flow was completely wiped with 90 % alcohol and exposed to UV rays for 15 minutes.

3.6 ISOLATION, CHARACTERIZATION AND MAINTENANCE OF THE CYANOBACTERIAL STRAINS

The cyanobacterial strains were isolated from different paddy fields of Plains regions (Bilaspur, Raigarh, Korba, Janjgir-Champa, Mahasmund, Durg, Raipur, Dhamtari, Kabirdham and Rajnandgaon) as well as North hills region (Jashpur Nagar, Sarguja, Surajpur) of Chhattisgarh. For isolation and maintenance of these strains, the

cyanobacterial mats collected from different locations were kept in well polyethylene bags and brought into the laboratory. The morphological studies of the isolated cyanobacterial strain were done according to the standard keys of Diskachary, 1959; Rippka *et al*., 1979; Komarek and Anagnostidis, 1989 and Komarek and Johansen, 2014. Further, these cyanobacterial strains were characterized using molecular attributes *i.e.* 16S rRNA gene sequences. The 16S rRNA gene sequences of the cyanobacterial strains were submitted to the obtained NCBI database and accordingly accession numbers were obtained. All the isolated cyanobacterial strains were maintained in BG-11^0 medium (pH 7.2) and placed under standard growth condition *i.e.* 28 ± 2 ºC temperature and 50-55 µE m^{-2} s^{-1} light intensity with photoperiod of 14:10 h light dark cycle to maintain the axenic cultures (Rippka *et al*., 1979; Singh *et al*., 2014). The cultures were shaken twice a day for proper exchange of gases. The composition of $BG11^0$medium is given in Tabulated form.

Composition of BG-11^0 medium (Rippka *et al*., 1979)

Macronutrients	**(gL^{-1})**	**Micronutrients**	**(mgL^{-1})**
$K_2HPO_4.3H_2O$*	0.08	H_3BO_3	0.286
$MgSO_4.7H_2O$	0.15	$MnCl_2.4H_2O$	0.181
$CaCl_2.2H_2O$	0.072	$ZnSO_4.7H_2O$	0.022
$NaHCO_3$	0.084	$Na_2MoO_4.2H_2O$	0.039
Na_2-citrate	0.012	$CuSO_4.2H_2O$	0.007
Fe (III) $(NH_4)_3$citrate§		$CoCl_2.2H_2O$	0.004
Na_2-EDTA	0.002		

§Fe(III)$(NH_4)_3$citrate and *$K_2HPO_4.3H_2O$ were autoclaved separately and added to the pre-cooled sterilized liquid medium to avoid precipitation.

Procedure

Exponentially grow cyanobacterial strains were harvested and transferred to 250 ml freshly prepared $BG11^0$ nutrient medium for initiating the experiment. The cultures were timely suspended in the solid media propagated with 1.2 % agar in the liquid nutrient media.

3.7 INOCULATION

The cyanobacterial strains were inoculated in liquid and solid BG 11^0nutrient medium under pre sterilized laminar air flow chamber. The chamber was initially surface sterilized with alcohol followed by UV irradiation for 30 minutes

3.8 MORPHOLOGICAL CHARACTERIZATION

The selected cyanobacterial cells were morphologically characterized based on the shape and size of vegetative cells, heterocyte (if present), akinetes, spore, branching (true or false) using bright field microscope (Catscope, Catalyst Biotech) at 10X and 40 X resolution.

3.8. 1 *Heterocyte Frequency and Average filament length*

The average filament length and heterocytes frequency of the cyanobacterial strains were studied after placing the cyanobacterial strains in a clean and clear glass slide using a pre sterilized glass dropper. A single drop of glycerol and iodine was poured using the glass dropper on the top of the cyanobacterial strains. A cover slip was placed carefully on the top of the cyanobacterial culture. The heterocytes frequency of the cyanobacterial strains were calculated according to the given formula

$$\boldsymbol{Heterocyte\ frequency}(\%) = \frac{\boldsymbol{Total\ number\ of\ heterocyte}}{\boldsymbol{Totalnumber\ of\ vegetative\ cells + heterocyte}} \boldsymbol{x100}$$

3.9 PHYSIOLOGICAL CHARACTERIZATION

All the 40 cyanobacterial strains isolated from different paddy fields of Chhattisgarh, were to further characterized for their the physiological and biochemical attributes such as growth curve, chlorophyll estimation, carotenoid estimation,

phycobillin protein estimation, protein estimation, carbohydrate estimation and Nitrogenase activity, Nitrate reductase activity and Glutamine synthetase activity.

3.9.1 *Growth curve*

3 ml of exponentially grown homogenous culture was withdrawn from each flask containing cyanobacterial strains and was transferred to pre sterilized test tubes. Then after the absorbance were taken at 663 nm using UV-VIS Spectrophotometer, Elico Ltd., Bhopal (M.P).

3.9.2 *Estimation of Chlorophyll a and Carotenoid content*

Reagents

95 % Methanol

Sterilized double distilled water

Procedure

Exponentially grow cyanobacterial culture (5 ml) was centrifuged and the pellet obtained was re-suspended in 5 ml 95% methanol. After 45 minutes of incubation it was again centrifuged and the absorbance of supernatant read at 665 nm for Chl a and 420 nm for carotenoid. The amount of Chl a in the supernatant was calculated as per protocol of McKinney (1941) using following coefficient factor

$$\textbf{Chl a } (\mu g\ ml^{-1}) = 13.4 \times A_{665}$$

Similarly, total carotenoid content was calculated using following multiplication factor

$$\textbf{Carotenoid } (\mu g\ ml^{-1}) = 200 \times A_{420}$$

3.9.3 *Estimation of Phycobilin protein content*

Reagents

1 M Potassium Phosphate Buffer (pH6.8)

Procedure

The different cyanobacterial strains were centrifuged at 6000 rpm for 10 min. Then after the pellet obtained was suspended in potassium phosphate buffer (pH 6.8) and kept at 4 ºC. The pellets were then crushed with acid washed sand using mortar and pestle. The crushed samples were centrifuged at 8,000 rpm for 5 min. The supernatant were collected in different test tubes. The pellets were re-crushed in Potassium phosphate buffer (pH 6.8) and re-crushed using mortar and pestle. The process was repeated for 3 times to get maximum amount of phycobillin content. The extracts were combined and absorbance were taken at 562 for phycocyanin content, 615 nm for allophycocyanin content, 652 nm for phycoerythrin content. The phycobillin content was expressed in terms of µg $ml^{-1}$and calculation was done according to Bennet and Bogorad (1973).

$$\mathbf{Phycocyanin(PC)(\mu g\ ml^{-1})} = \frac{\boldsymbol{A615 - 0.474\ (A652)}}{\mathbf{5.34}}$$

$$\mathbf{Allophycocyanin(APC)(\mu g\ ml^{-1})} = \frac{\boldsymbol{A652 - 0.474\ (A615)}}{\mathbf{5.09}}$$

$$\mathbf{Phycocerythrin(APC)(\mu g\ ml^{-1})} = \frac{\boldsymbol{A562 - [2.14(PC) - 0.849\ (APC)}}{\mathbf{9.62}}$$

3.9.4 ***Estimation of Protein Content***

Total cellular protein content in the cyanobacteria was estimated using the protocol of Lowry *et al*. (1951).

Reagents

(a) NaOH (1N)

(b) Sodium carbonate (Na_2CO_3) (5 %)

(c) Copper Sulphate ($CuSO_4$) (0.5 %)

(d) Sodium -potassium tartarate (1 %)

(e) Folin and Ciocalteu's phenol reagent (1N)

Preparation of Reagent C

Reagent A: 5 gm of Na_2CO_3 was dissolved in 100 ml of water

Reagent B: 1 gm of sodium potassium tartarate was dissolved in 100 ml of distilled water and further 0.5 gm of $CuSO_4$ was also dissolved in sodium potassium tartarate solution. 50 ml of solution A and 2 ml of solution B were mixed freshly during the time of experiment.

Procedure

Protein was estimated by Lowry *et al.* (1951). First of all cyanobacterial cells were harvested by centrifugation at 10,000 rpm for 15 minutes. The pellet obtained was homogenized with sterile double distilled water. From this algal 1.0 ml culture was taken for estimating protein. To this 1N NaOH (1.0 ml) was added so as to hydrolyze the cells. Then the samples were incubated in boiling water bath for 10 minutes. After incubation, samples were taken out and kept at room temperature. Again, 5.0 ml of 5% Na_2CO_3 + 0.5% $CuSO_4$ in 1% sodium-potassium tartarate in the ratio 25:1 was added and kept at room temperature for 15 minutes. Now to this set up, 1.0 ml of Folin-Ciocalteau reagent was added and left for 15 minutes again. The absorbance was measured at 650 nm. The protein content was estimated using a standard protein assay curve of lysozyme and expressed in terms of $\mu g\ ml^{-1}$.

3.9.5 ***Estimation of Carbohydrate***

Reagents

Phenol 5% w/v

Concentrated H_2SO_4

Procedure

Carbohydrate was estimated by using the protocol of Dubois *et al*. (1956). Cyanobacterial sample (1ml) was taken in thick walled tubes and mixed with 1 ml of phenol (5% w/v). To this solution, 5ml of concentrated sulphuric was subsequently added through a fast flowing pipette and tubes were shaken simultaneously for fast and complete mixing. The tubes were kept in water bath for 10 minutes at 30º C. Thereafter, tubes were kept at room temperature for 5 minutes. Finally, the solution developed the characteristic yellow color. Absorbance was taken at 485 nm on a spectrophotometer against a reagent blank. Glucose was used as standard for estimating carbohydrate and expressed in terms of $\mu g\ ml^{-1}$.

3.9.6 *Estimation of Nitrate Reductase Activity* (EC 1.7.7.2)

Reagents

KNO_3 (20 µM)

Methyl viologen (4 µM)

$NaHCO_3$ (0.3 M)

$Na_2S_2O_4$ (10 µM) in $NaHCO_3$ (0.3 M)

Na_2CO_3 (0.1 M)

$NaHCO_3$ (1.0 M)

Sulphanilamide reagent

NEDH Reagent

NEDH reagent prepared by dissolving 1g sulphanilamide (1:4 v/v) in 100 ml sterilized double distilled water 0.2% (w/v) [N-(1-Napthyl)] ethelenediamine dihydrochloride reagent.

Procedure

The exponentially grown of cyanobacterial culture were centrifuged at 10,000 rpm for 5 min. The pellets obtained were permeabilized using 20 µl

toluene by shaken vigorously at 4°C. The cell suspensions were mixed with 1 ml reaction mixture containing 100 µmol $NaHCO_3$-Na_2CO_3buffer (pH 10.5), KNO_3, methyl viologen and $Na_2S_2O_4$ in 0.3M $NaHCO_3$ and incubated for 5 minutes at 30°C. Dithionite reduced methyl viologen was used as reductant. NO_2^- was formed as a result of nitrate reductase catalysed reaction (Herrero *et al*., 1981). To estimate NO_2^- by azocoupling method protocol of Snell and Snell, 1949 was followed. Diazotization was performed by mixing 1 ml of culture with 1 ml sulphanilamide and1 ml of NEDH reagent. A pink colour was developed and the absorbance was recorded at 540 nm. Nitrate reductase activity was calculated from the standard curve of sodium nitrite and was expressed in terms of µmol mg^{-1} protein.

3.9.7 *Estimation of Glutamine Synthetase Activity* (EC 6.3.1.2)

Reagents

Enzyme Extraction buffer (pH 7.0)

Toluene

1 M Imidazole HCl – buffer, pH 7.0

1 M L-glutamate (sodium salt), pH 7.0

1.67 M $MgCl_2$

Reaction mixture*, pH 7.0*

Glutamine (0.1M, pH7.0)

Potassium arsenate (1.0 M, pH adjusted to 7.0 with KOH)

Sodium ADP (0.01M, pH adjusted to 7.0 with NaOH)

Imidazole – HCl buffer (1.0 m, pH adjusted to 7.0 with HCl)

$MnCl_2$ (0.1M)

Hydroxylamine – HCl (2.0 M)

Stop mixture(in a ratio of 1:1:1)

10% $FeCl_3$ (4.0 ml)

24% Trichloroacetic acid

6N HCl

Procedure

One ml pelleted cyanobacterial cultures were toulenized for permeabilization at 4°C for 10 min. The aliquot was again centrifuged to remove the topmost toluene layer. 3 ml of reaction mixture containing 40 mM Imidazole HCl (pH 7.0), 30 mM L-glutamine, 3 mM $MnCl_2$, 0.4 mM ADP, 20 mM sodium arsenate, 60 mM hydroxylamine, was added to the permeabilized cells and the solution was incubated in water bath at 30°C for 30 min. The reaction was stopped by adding 1 ml mixture of $FeCl_3.6H_2O$ prepared in 0.2 N HCl, 24% TCA and 6 N HCl in 1:1:1 ratio. Glutamine synthetase (transferase) activity was expressed in terms of nanomol γ-glutamyl hydroxamate μg^{-1} protein min^{-1} as quantified by the reference to standard curve obtained with γ-glutamyl hydroxamate in the assay medium measured at 540 nm (Shapiro and Stadtman, 1970).

3.9.8 *Estimation of Nitrogenase activity* (EC 1.18.6.1)

Reagents

Acetylene gas: the acetylene gas was produced by reacting water and calcium carbide (CaC_2) in stoppered bottles. By mixing water and carbide the acetylene gas was produced inside the bottles and was collected in a sterile 20 ml syringe.

Trichloroacetic Acid (TCA) 50%

Procedure

To estimate nitrogenase activity, sterilized vacutainer tubes were used. 2 ml of exponentially cyanobacterial cultures were transferred in the 10 ml sterilized vacutainer tubes. 10% v/v of air present inside the vacutainer tubes was taken out using a sterile syringe and the empty space was then filled by injecting acetylene gas. The tubes carrying cyanobacterial cells as well as 10% acetylene gas were placed at standard growth condition for about 6 hours. For terminating the reaction, 50% of Trichloroacetic acid (TCA) was added to the tubes. Conversion of injected acetylene gas to ethylene denotes the occurrence of nitrogenase activity. The amount of

ethylene gas was measured by withdrawing 100 µl gas samples from the tubes and injecting it to a gas chromatograph (GC, Agilent 6890N and 7890A). Nitrogenase activity was expressed as n mol C_2H_4 mg^{-1} protein h^{-1} (Ernst *et al*., 1992).

The Nitrogenase activity was calculated using the following formula

$$Nitrogenase Activity = \frac{(Peak Area) x (Volume of Gas in tube)}{Kx\left(\frac{Quantity of}{sample injected}\right) x \left(\frac{Times of}{Incubation}\right) x \left(\frac{Quantity of total}{sample incubated}\right)}$$

Where,

K=peak area of 1 nmol standard ethylene gas

3.10 BIOCHEMICAL CHARACTERIZATION

3.10.1 *Fatty acid analysis*

Reagents

Sodium methoxide

Methanol

Chloroform

Boron tri- fluoride

n-Hexane

Preparation of reagents

Sodium methoxide - 45 g NaOH in methanol (150 ml), distilled water (150 ml)

Methanol: Chloroform- 1:2 (ratio)

Procedure

Changes in fatty acids in the cyanobacterial cells were detected through FAME (Fatty acid methyl ester) analysis by using the protocol of Kang and Wang (2005) with minor modifications. For that, cyanobacterial cells were harvested by centrifugation at 12,000 rpm for 15 min. Approximately, 1.0 g dried cell biomass

were taken and crushed using pestle and mortar in chloroform: methanol (2:1) reagents. The crushed biomass was filtered through Whatman's no.1 filter paper and was collected in different 15 ml screw cap tubes. Then after, the mouth of screw cap tubes was remained open till the filtrate became dried and concentrated at room temperature (25ºC). Further, an equal volume of methanol was added to all the tubes. A fixed volume of the extract was taken and 5 ml sodium methoxide was added for saponification. Later, few drops of boron triflouride-methanol reagent were added and placed in a boiling water bath (100°C) and incubated for 10 min. Further, it was immediately placed into the ice bath. 5 ml of n-Hexane was added to each cyanobacterial sample respectively and was further incubated at room temperature for 30 min. After some times, two distinct layers were visible. Two thirds of the upper layer containing methyl esters of fatty acid extract was transferred to the small gas chromatography (GC) vials. 3 µl of the extracted fatty acid methyl ester mixture were subjected to GC-MS analysis. The initial temperature of the column was set at 160°C and was increased at a rate of 2°C per minute to reach a final temperature of 270°C for 75 minutes.

3.10.2 *Biochemical analysis of whole cell protein based on SDS-PAGE*

To investigate the change in protein profiling pattern among the selected cyanobacterial strains, SDS-PAGE analysis was conducted. Whole cell protein estimation was done so that an overall and total picture of the protein profile could be drawn out.

Reagents

Protein extraction buffer

40 mM Tris HCl pH 7.5

2 mM EDTA

1% Triton X 2%

1 mM PMSF

Polyvinyl pyrrolidone (PVP)

Protein isolation and purification

Cells of cyanobacterial strains were centrifuged at 10,000 rpm for 10 minutes at 4ºC. The pellets obtained were extracted with extraction buffer for 2-3 times. To ensure complete lysis of the cell, the cells were recrushed with extraction buffer in presence of liquid nitrogen for 5-6 times using mortar and pestle. The crushed samples were further carefully resuspended in 5 ml of extraction buffer and centrifuged at 14,000 rpm for 45 min at 4ºC. The supernatants obtained were re-centrifuged at 6,500 rpm for 45 min at 4ºC to remove residual debris and contaminants from the supernatants. Then after, supernatants were transferred in a beaker containing 12.5% TCA dissolved in the acetone. The solutions were then incubated at -20ºC for overnight and the precipitated proteins were washed with 100% acetone for 3-4 times to remove TCA. The protein samples were again centrifuged at 10,000 rpm for 10 min at 4ºC. The obtained protein pellets were air dried completely and stored at -80 ºC. The protein content was estimated by using the Lowry *et al*., 1951 protocol**.**

Reagents for (SDS-PAGE) gel electrophoresis

Protein profiling of all the cyanobacterial strains were done by performing SDS-PAGE (Lyra *et al*., 1997). The stacking gel contained 5% (w/v) while the separating gel/resolving gel contained 12% (w/v) of acrylamide. The chemical composition for preparation of 12% resolving and 5% stacking gels are mentioned below:

30% acrylamide mix (29.0 g acrylamide + 1.0 g bisacrylamide)

1.5 M Tris-HCl buffer (pH 8.8)

1.0 M Tris-HCl buffer (pH 6.8)

Ammonium persulfate (APS) 10%

Sodium dodecyl sulfate (SDS) 10%

TEMED

Glycerol

Bromophenol blue

2% β-mercaptoethanol

Phosphate Buffer Saline (pH 7.4)

Acetic Acid

Methanol

Glycine

Trichloroacetic acid

Glycerol

Working solution for 12 % resolving gel (Total Volume 30 ml).

HPLC Grade Water (Merck) 9.3 ml

30 % acrylamide mix 12.5 ml

1.5 M Tris-Cl (pH 8.8) 7.5 ml

10% (Ammonium per sulfate) 0.3 ml

10% (SDS) 0.3 ml

TEMED 0.012 ml

Working solution for 5 % stacking gel (Total Volume 10 ml).

HPLC Grade Water (Merck) (6.88 ml)

30% Acrylamide mix (1.70 ml)

1.0 M Tris-Cl (pH 8.8) (1.25 ml)

10% (APS) (0.10 ml)

10% (SDS) (0.10 ml)

TEMED (0.006 ml)

(v) Sample Loading Buffer (1X)

Sample loading buffer was prepared by adding 1M Tris-HCl (pH 6.8), 10% (w/v) SDS, Glycerol 100% (v/v), 4 mg bromophenol blue, 2% β-mercaptoethanol. The protein sample was mixed with sample loading buffer in the ratio of 1:1

(vi) Phosphate Buffer Saline (pH 7.4)

Phosphate buffer saline (pH 7.4) was prepared by mixing sodium chloride (NaCl)-137mM, potassium chloride (KCl)-27 mM, disodium hydrogen phosphate (Na_2HPO_4)-10mM and 2 mM potassium dihydrogen phosphate (KH_2PO_4).

(vii) Preparation of electrophoretic gel and electrophoresis operation

SDS-PAGE was performed in a vertical slab gel electrophoretic apparatus (Bangalore, India) with 12% resolving gel and stacking gel of 5% acrylamide mixture. The gels made were 13 cms in height and 17 cms in width. The electrophoretic buffer used was Tris- glycine with the composition provided below. 1X Tris-Glycine electrophoretic buffer was prepared by mixing Tris-base (7.55 g), Glycine (47.0 g) and 10 % SDS prepared in 25 ml**.** The final volume was maintained to 500 ml with deionized water. For working condition the buffer was diluted to 1X.

The 1X Tris- glycine buffer was poured into the lower and upper electrophoresis tanks, so as to create a gradient. The protein samples were loaded in the gel and was allowed to run at 65V (15 mA) until the tracking dye reached the end of the resolving gel. The gel was run for 10-11 hrs and the temperature was maintained approximately to 10-15° C in the air conditioned chamber.

Gel fixing solution (acetic acid: methanol: double distill water) (10:20:70)

Chemical constituents	**Amount taken**
Acetic Acid	50 ml
Methanol	100 ml
DDW	350 ml

Gel staining solution (CBB R-250)* (acetic acid: methanol: double distill water)

Chemical constituents added	Amount taken
Acetic Acid	50 ml
Methanol	225 ml
DDW	225 ml
CBB R-250	1.25 gm added to the above solution
Total volume	500 ml

* *The CBB R-250 staining solution was filtered through Whatman filter paper no. 1 before use*

Gel distaining solution (acetic acid: methanol: double distill water).

Chemical constituents	Amount taken
Acetic Acid	50 ml
Methanol	150 ml
DDW	300 ml
Total volume	500 ml

(xii)Procedure for SDS-PAGE

Whole cell protein extraction was done as per the modified protocol of (Lyra *et al*., 1997). Exponentially grown cyanobacterial biomass (250 ml) was centrifuged at 7600 rpm for 10 minutes at 24ºC. The obtained pellets were sonicated (Branson 102CE, Shanghai) 5 times for 10 seconds in phosphate saline buffer (PBS). Homogenated cells were centrifuged at 15,000 rpm for 45 minutes at 4ºC. The supernatant so obtained was re- suspended in denaturation sample loading buffer in the ratio 1:1 [containing 1M Tris HCl (pH 6.8), SDS (10%), glycerol (100%), β-mercaptoethanol (2%), Bromophenol blue (4 mg) and double distilled water (1.4 ml)]. The samples were heated at 100ºC for 5 minutes before loading into the wells of the gel prepared by 12% acrylamide. Each 80 µl of samples containing100 µg ml^{-1} protein were loaded quantification of the protein was done as per Lowry *et al*., 1951. It was run at 65 V, 15 mA for 11 hours. After electrophoresis, gel was fixed in fixative for

10-15 minutes and washed twice with deionized water. The gel was then stained with Coomassie Brilliant blue (R-250) staining solution for 10-12 hours and then was transferred to destaining solutions and kept for 8-12 hours as per the requirement. Bands were visualized under gel documentation unit (Bio-Rad, USA) and were compared with the molecular weight marker (14.3-97.4 kDa) (Bangalore Genei) run on both the sides of the gel along with the sample. The analysis was repeated thrice so as to verify the reproducibility of the patterns.

3.11 MOLECULAR CHARACTERIZATION

The selected cyanobacterial strains were further characterized based on highly conserve 16S rRNA gene sequences.

3.11.1 *Reagents for DNA isolation*

Extraction buffer pH 8.0, (100 mM Tris-HCl, 20 mM EDTA, 1.4 M NaCl, 2% CTAB); lysozyme stock (50 mg ml^{-1}), proteinase K stock (10 mg ml^{-1}), 1N HCL, TE buffer (pH 8.0) (50mM Tris HCl, 10 mM EDTA), ammonium acetate, isopropanol, bromophenol blue, glycerol, absolute alcohol, Tris base, boric acid, EDTA, phenol, chloroform, isoamyl alchohol, ultrapure Water, etc. All the reagents used were of molecular grade from Merck, (Germany).

(i) Preparation of reagents

***Extraction Buffer* (pH 8.0) (200ml)**

100 mM Tris HCl	2.4228 g
20 mM EDTA	1.1689 g
1.4 M NaCl	16.3632 g
2 % CTAB	4.00 g

pH was adjusted with 1N HCl

(ii) TE Buffer (pH 8.0) (100ml)

50 mM Tris HCl	0.6057 g
10 mM EDTA	0.29224 g

(iii) DNA precipitation (added in the ratio 0.25:1 volume)

Ammonium acetate solution	3 M
Isopropanol	1 volume

Both the constituent were kept at -20^{o} C and added in chilled state

(iv) 70 % Ethanol

(v) Loading Buffer 6X (100 ml)

0.25% bromophenol blue	0.25 g in 100ml
30% glycerol in water	30 ml
Double Distilled Water	69.75 ml

For working condition it was diluted to 1X

(vi) Buffer for agarose gel electrophoresis (5X) (TBE) g ml^{-1}

Tris base	54.0 g
Boric Acid	27.5 g
0.5 M EDTA	20.0 ml

For working condition TBE stock was diluted to 0.5X

3.11.2 ***Isolation of Genomic DNA***

Genomic DNA of cyanobacterial cultures were isolated by using the modified protocol of Golden *et al*. (1988). For the isolation of genomic DNA, Pellets were harvested by centrifuging the culture at 13,000 rpm for 10 min. Thereafter, pellets were crushed in 500-µl extraction buffer (100 mM Tris HCl, pH 8.0, 20 mM EDTA, 1.4 M NaCl and 2% CTAB) and lysozyme (50 µl, 25 mg ml^{-1})

was added into it. The samples were incubated for 60 min at 37°C with a gentle mixing at every 10 min. Then 3 µl Proteinase K (25 mg ml^{-1}) was added and the samples were again incubated at 65°C for 60 min. The obtained mixture was extracted twice by saturated phenol: chloroform (25:24) and once by chloroform: isoamyl alcohol (24:1) through centrifugation at 12,000 rpm for 10 min. Samples were kept overnight at -20°C for the precipitation of the DNA. Again, 1 volume of chilled isopropanol and 0.25 volume of ammonium acetate (3 M) were added to the above samples. Precipitated DNA was centrifuged at 10,000 rpm for 10 min and the supernatant was discarded. The precipitated DNA was washed with 70% ethanol and again centrifuged at 10,000 rpm for 2 min. The obtained DNA pellets were vacuum dried and finally dissolved in TE buffer having pH 8.0 (Tris HCl 50 mM, EDTA 10 mM).

3.11.3 *Quantification of DNA content*

The concentration of DNA was quantified by measuring the extinction coefficient of the samples. The purity of DNA was checked by tacking ratio of absorbance at 260 and 280 nm. 1X TE was taken as a standard blank (Sambrook and Russel 2001). The optical density observed for the DNA samples was calculated using the equation given below :

Amount of DNA in ng ml^{-1} = O.D $_{260\ nm}$ x Dilution factor x 50

where, A_{260nm} of dsDNA = 50 µg ml^{-1}

3.11.4 *Gel electrophoresis of obtained DNA*

In most of the DNA samples, the electrophoresis separation were carried out using Agarose gels. This is because most genomic and larger DNA samples and fragments are larger than proteins and are unable to enter a polyacrylamide gel which has small pore size. All DNA fragments move towards anode with same mobility under electrical field due to their charge per unit length of any given fragment is same

(due to the presence of phosphoric groups). The following reagents were prepared for the separation of DNA.

TAE buffer

50 x stock solution

Tris base -242 g

Glacial acetic acid 57.1 ml

EDTA 500 mM pH 8.0 -100 ml

1x working solution

50 x stock diluted 50:1 with water

Ethidium bromide - 0.5 µg ml^{-1}

Agarose -0.8%

3.11.5 *Preparation of Agarose gel*

DNA molecules can be separated according to their size by agarose gel electrophoresis. Agarose (0.8%) was dissolved in 1X TAE buffer (50 ml) by heating in a microwave until boiling, this solution was allowed to cool and Ethidium bromide was added (0.05 µg ml^{-1}). Gels (0.8 %) were then carefully cast electrophoresis unit tank, any bubbles were removed with a pipette tip and then gel comb was added and the gel allowed to set. Furthermore, DNA was mixed with 1 /10 vol. bromophenolblue loading dye and loaded onto an agarose gel. A molecular weight marker was used for comparison to determine the size of the DNA fragments. 1x TAE was used as running buffer; running-conditions were 5-10V/cm. For visualization of the DNA, Ethidium bromide was added to the gel in a final concentration of 1 µg/ml. Observe the gel frequently to ensure that the dye front has not crossed the bottom of the gel Visualize the gel under ultraviolet light for DNA bands.

3.11.6 *PCR amplification of 16S rRNA gene sequences*

Extracted DNA was amplified using specific primers fd1 (5'-AGAGTTTGATCCTGGCTCAG-3') and rd1 (5′-AAGGAGGTGATCCAGCC-3′ (Weisburg *et al.* 1991). PCR was performed in a total volume of 50 µl reaction containing 50 ng of template DNA, 10× PCR buffer, $MgCl_2$ (25 mM), primers (50 pmol μl^{-1}), dNTP's mix (200 µM), and 1 U Taq DNA polymerase. PCR amplification was done using the Bio-Rad, DNA Engine (Peltier Thermal cycler).

The PCR program was setup as follows : initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 30s, annealing at 58°C for 30s and extension at 72°C for 1 min. A final extension at 72°C for 8 min was done. The PCR products were electrophorezed in 1.2% (w/v) horizontal agarose gel After electrophoresis the obtained (1,500 bp) specific bands were cut and eluted as per the manufacturer's protocol of Hi-Media Gel Elution kit. The products were sent for sequencing to Link Biotech, New Delhi. The determined sequences have been deposited to National Center for Biotechnology Information (NCBI) database and accordingly accession numbers have been assigned for the same.

List of primers along with target site used in the Analysis

Name	Target gene	5' - 3'	Reference
fD1	16S rRNA	(5'-AGAGTTTGATCCTGGCTCAG-3')	(Weisburg *et al*., 1991)
rD1	16S rRNA	(5'-AAGGAGGTGATCCAGCC-3')	(Weisburg *et al*., 1991)

List of Enzymes, Kits and primers for Genomic DNA isolation.

Items	Company
Taq DNA polymerase	Bangalore Genei
dNTP's	Bangalore Genei
$MgCl_2$	HiMedia
Lysozyme	HiMedia, Mumbai
Taq Buffer A	Bangalore genei
Genomic DNA Extraction Kit (MB505)	HiMedia, Mumbai
PCR purification and Gel Extraction Kit (MB511)	HiMedia Mumbai

DNA ladder (low range DNA ruler)	Bangalore Genei
Agarose	HiMedia

3.11.7 *Phylogenetic Analysis*

Phylogenetic analysis is defined as the study of evolutionary biology of organism with respect to its related neighbors. The more closely related species are more likely to share a recent common ancestor. The main aim was to discover rates of evolutionary change, predict the sequences function and population history. It helps to identify the changes occurred in the sequences of closely related organism over a period of their evolution.

Nucleotide sequences were compared with sequence information available in the National Center for Biotechnology Information database using BLAST (http://www.ncbi.nlm.nih.gov/BLAST) and multiple-sequence alignment was done with all partial and complete sequences using the MEGA 6.0 (Larkin et al. 2007). The Phylogenetic tree was constructed using the neighbor-joining algorithm provided in MEGA 6.0 (Tamura *et al.,* 2007). Distances for the NJ tree were estimated by using the algorithm of Tajima and Neil (1984) and nucleotide positions containing gaps and missing data were considered.

3.11.8 *Secondary structure of 16S rRNA gene sequences*

The 16S rRNA gene sequences of forty cyanobacterial strains were used for the construction of secondary structure models and were folded using RNAfold web server (Mathews *et al.,* 2004). These secondary structures were used to assess for the observed differences in 16S rRNA gene sequence data.

CHAPTER-4

Results

4.1 PHYSICO-CHEMICAL CHARACTERIZATION OF SOIL SAMPLES

The soil samples were collected from different paddy fields of plain regions of district Bilaspur, Raigarh, Korba, Janjgir–Champa, Mahasamund, Durg, Raipur, Dhamtari, Kabirdham, and Rajnandgaon of state Chhattisgarh (Figure 1). A total forty five sampling sites were decided to collect soils samples among the district of state Chhattisgarh. There were Uslapur, Ratanpur, Chilhati, Turkadih, Koni, Mopka, Sarangarh, Transport Nagar, Baramkela, Sariya Kharsiya, Sahaspur, Gevera, Kenda, Balod, Sivrinarayan, Pamgarh, Janjgir-Champa, Mulmula, Rahoud, Mahasamund, Basna, Sankra, Khallari, Ganjpara, Bhilai, Bhilaikala, Potiya, Rasmada, Birgaon, Arang, Sigma, Tilda, Lalpur, Navagaon, Siliyani, Dhamtari, Banjari, Sarangpuri, Kawardha, Pandriya, Papiriya, Dongargarh, Somri and Tilai (Table 1). Different parameters *i.e.* pH, Temperature, Salinity, Dissolve oxygen, Conductivity, Total dissolved solid were considered for the physicochemical characterization of the soil samples collected from different sampling sites. The maximum pH were observed in paddy field of Turkadih (9.30 ± 0.1) followed by (8.8 ± 0.23) Kenda. The lowest was recorded in Navagarh and Dhamtari (6.83 ± 0.12). Apart From this, rest of the soil samples collected from different sites showed pH range between (7.1 ± 0.65 to 8.67 ± 0.32) (Table 1)

Temperature of the different soils ranged from 26 °C to 37°C and the maximum temperature was reported in the soil of Sarangpuri (37.43 ± 1.55 °C) followed by Khallari (37.2 ± 0.67°C) where as the lowest was observed from Sivrinarayan (26.5 ± 1.19°C).

The maximum salinity was observed in the paddy field of Turkadih (142.83 ± 2.0 g L^{-1}) followed by Uslapur paddy field (131.1 ± 3.48 g L^{-1}). Whereas the lowest

was observed in Somri (31.27 ± 1.50 g L^{-1}) Apart from this, rest of the soil sample collected from different sites showed salinity range between 31.97 ± 0.95 g L^{-1} to 130.5 ± 2.30 g L^{-1}.

Maximum dissolved oxygen (DO) was observed in the paddy field of Uslapur (132.0 ± 4.36 ppm) followed by Chilhati paddy field (130.0 ± 3.17 ppm). Minimum dissolved oxygen was reported in the soil of Sivrinarayan (16.2 ± 0.61 ppm) Apart from this, rest of the soil samples collected from different sites showed the range of dissolved oxygen from 20.83 ± 1.35 ppm to 87.134 ppm.

Maximum conductivity was reported in the field of Sarangarh (152.0 ± 3.5µ Seimen cm^{-1}) followed by (151.40 ± 2.08 µ Seimen cm^{-1}) Birgaon. The lowest was observed in Uslapur (25.7 ± 1.15 µ Seimen cm^{-1}). Among other sampling sites, conductivity range of the soils was varied between 33.92 ± 0.93 µ Seimen cm^{-1} to 140.33 ± 2.5 µ Seimen cm^{-1}).

Maximum total dissolved solid (TDS) were observed in paddy field of Turkadih (568.67 ± 8.3 mg L^{-1}) followed by Uslapur (427.7 ± 10.02 mg L^{-1}) whereas lowest was observed in Ratanpur (83.0 ± 1.76 mg L^{-1}). The rest of the soil samples collected from different sites showed TDS range between 98.1 ± 1.42 mg L^{-1} to 204.10 ± 5.89 mg L^{-1}.

The physico-chemical characterization of the soil samples collected from different district of North hills region *i.e.* Jashpur Nagar, Sarguja and Surajpur of state Chhattisgarh were done. Total fifteen different area have been selected from different districts *i.e.* Jashpur Nagar, Deepatoli, Gholeng, Ranidah Ambikapur, Mainpath, Ramgarh, Sitapur, Surajpur, Prem Nagar, Ramanujganj, Pratapur (Table 2).

Different soil parameters have been considered for physicochemical characterization. The pH of the different soil samples ranged from 7.2 to 8.7 and the maximum was observed in the soil of paddy field of Ambikapur (8.7 ± 0.4) followed by Gholeng and Premnagar (8.6 ± 0.5) whereas lowest pH was recorded from the paddy field of Surajpur (7.2 ± 0.153). Apart from this, rest of the soil samples

collected from different sampling sites showed pH range between 7.3 ± 0.3 to 8.43 ± 0.50.

Maximum temperature was reported in the soils of Ranidah (34.8 ± 1.0 ºC) followed by the soil of Surajpur (32.6±0.569) whereas lowest was recorded in Mainpath (27.43 ± 0.60 ºC). Rest of the soil samples collected from different sites showed temperature range between 28.7 ± 1.6 ºC to 31.27 ± 0.35 ºC.

Maximum salinity was characterized in the soil of Mainpath (122.03 ± 2.57g L^{-1}) followed by field of Ramanujnagar (110.3 ± 1.539 g L^{-1}).The lowest level of salinity was observed in Jashpur Nagar (39.47 ± 1.29 g L^{-1}). Apart from this, rest of soil samples collected from different sites showed salinity range between 40.33 ± 0.58 g L^{-1} to 86.7 ± 1.1 g L^{-1}.

Maximum dissolved oxygen (DO) was observed in the paddy field of Gholeng (87.8 ± 1.1 ppm) followed by Mainpath (68.40 ± 1.32 ppm) and Pratapur (68.20 ± 1.47 ppm). Whereas lowest DO was observed in the soil of Surajpur (21.2 ± 1.021 ppm). Rest of the soil samples collected from different sites showed DO range between 32.17 ± 1.44 ppm to 67.47 ± 1.24 ppm.

Maximum conductivity was observed in the field of Ambikapur (152.1 ± 2.5 µ Seimen cm^{-1}) followed by Ramanujnagar (129.1 ± 1.3 µ Seimen cm^{-1}) Whereas lowest conductivity was observed in the soil of Premnagar (35.2 ± 0.493). The conductivity of the rest of the soil samples collected from the different sites was ranged between 36.9 ± 1.0 to 113.90 ± 5.54 µ Seimen cm^{-1}.

Maximum total dissolved solid was observed in the soil of Ranidah (204.1 ± 5.9mg L^{-1}) followed by Deepatoli (157.83 ± 1.70 mg L^{-1}); Surajpur (152.9 ± 2.052 mg L^{-1}) and Ramgarh (150.5 ± 3.1 mg L^{-1}). The lowest TDS was observed in the soil of Jashpur Nagar (101.7 ± 2.0 mg L^{-1}). Apart from this, rest of the soil samples collected from different sites showed TDS range between 102.7 ± 2.722 mg L^{-1} to 146.6 ± 1.9 mg L^{-1}.

Table 1. Physico-chemical characterization of soil and water sample of different paddy fields of Plain regions of Chhattisgarh

District	Area	pH	Temperature (ºC)	Salinity (g L^{-1})	Dissolved Oxygen (ppm)	Conductivity (µ Seimen cm^{-1})	Total dissolved solid (mg L^{-1})
Bilaspur	Uslapur	7.2 ± 0.95	28.0 ± 0.20	131.1±3.48	132.0±4.36	25.7±1.15	427.7±10.02
	Ratanpur	7.9 ±0.23	29.6±0.58	81.7±2.83	65.0±1.0	45.4±0.45	83.0±1.76
	Chilhati	8.3±0.28	31.3±0.35	66.0±1.04	130.0± 3.17	64.5±2.56	121.1±1.91
	Turkadih	9.30±0.1	32.10±1.3	142.83±2.0	67.83±1.1	140.33±2.5	568.67±8.3
	Koni	7.4±0.35	28.9±0.91	91.1±1.32	30.8±1.57	78.8±1.44	155.7±1.87
	Mopka	8.5±0.45	28.5±0.43	91.6±1.17	67.4±1.04	99.0±1.38	212.4±3.92
Raigarh	Sarangarh	8.2±0.2	28.7±1.0	41.5±1.0	57.7±0.8	152.0±3.5	101.6±2.6
	Transport Nagar	8.17±0.15	30.37±1.38	41.43±0.45	41.40±1.32	33.92±0.93	104.87±1.4
	Baramkela	7.63±0.25	34.23±1.15	39.77±0.75	57.13±1.55	94.47±1.72	101.80±2.69
	Sariya	7.8±0.35	29.3±0.55	81.1±1.07	28.5±0.47	41.8±2.50	98.6±1.42
	Kharsiya	8.4±0.46	30.3±0.53	75.2±1.23	64.3±1.51	68.1±1.57	98.1±1.10
	Sahaspur	8.2±0.21	34.2±1.97	57.0±1.62	77.0±1.35	90.9±1.50	105.9±1.39
Korba	Gevera	8.5±0.26	32.3±0.46	42.7±0.59	67.8±1.44	85.5±0.90	146.6±3.06
	Kenda	8.8±0.23	27.1±0.40	42.4±1.42	87.5±1.34	35.7±0.59	177.6±2.08
	Balco	7.73±0.50	32.27±0.55	60.93±0.907	20.83±1.35	54.68±1.76	188.27±1.95
Janjgir Champa	Sivrinarayan	8.6±0.36	26.5±1.19	45.0±0.95	16.2±0.61	33.9±0.95	177.8±1.92
	Paamgarh	8.57±0.42	33.47±0.45	41.70±0.62	46.27±0.55	88.50±1.42	141.63±2.05
	Jhangir Champa	7.3±0.10	28.6±0.55	53.2±6.41	69.8±0.75	92.0±1.93	136.1±2.34
	Mulmula	7.13±0.40	30.63±1.09	31.97±0.95	35.63±0.55	52.39±0.60	155.77±1.93
	Rahoud	7.33±0.12	32.30±0.44	56.63±0.60	31.03±1.62	92.23±1.56	166.07±1.79
Mahasmund	Mahasmund	8.1±0.44	30.3±1.47	42.2±1.07	70.9±1.33	96.6±2.07	135.2±3.21

	Basana	8.67±0.32	30.57±0.90	81.67±2.72	40.93±0.95	43.23±1.06	128.00±2.23
	Sankra	8.57±0.49	34.83±0.76	75.77±1.00	66.20±0.70	61.89±0.82	123.70±4.20
	Khallari	8.3±0.10	37.2±0.67	130.5±2.30	33.3±1.48	75.9±0.90	104.5±1.31
Durg	Ganjpara	8.6±0.52	31.9±1.00	91.2±2.36	44.3±1.48	55.4±1.27	200.9±2.85
	Bhilai	7.50±0.52	31.80±0.55	40.93±0.90	67.33±0.92	38.86±1.33	142.97±2.82
	Bhilaii Kala	8.33±0.51	28.07±1.40	39.43±0.51	31.30±1.44	55.93±0.90	130.87±1.58
	Potiya	7.20±0.52	31.80±0.14	40.63±0.40	66.67±0.25	38.90±1.30	166.70±1.11
	Rasmada	8.20±0.30	31.64±1.40	40.93±1.95	30.40±0.42	56.45±0.63	106.83±1.89
Raipur	Birganve	7.93±0.4	29.17±1.12	42.80±1.57	56.53±1.76	151.40±2.08	149.97±1.84
	Aarang	7.87±0.46	30.73±1.66	120.33±1.76	28.73±0.40	109.73±1.16	192.10±3.41
	Simga	7.83±0.45	31.77±1.18	79.50±1.23	27.97±0.95	41.43±2.16	122.23±1.92
	Tilda	7.13±0.65	32.50±1.30	42.43±1.29	66.87±1.46	39.06±1.27	159.70±1.99
	Lalpur	8.40±0.20	31.87±2.48	40.50±1.80	31.87±1.53	56.50±1.14	162.53±3.52
	Navagaon	6.83±0.12	32.17±0.64	38.23±3.69	67.23±1.19	39.00±0.96	174.30±3.06
	Siliyari	8.33±0.12	34.57±0.58	40.30±1.47	29.70±0.78	56.87±1.03	192.63±3.70
Dhamtari	Dhamtari	6.83±0.12	31.67±1.37	40.27±2.05	88.83±1.80	40.30±1.57	172.37±2.80
	Banjari	7.45±0.50	32.50±0.69	46.60±1.47	70.00±3.11	39.20±0.99	146.23±2.30
	Sarangpuri	7.60±0.34	37.43±1.55	39.43±1.31	31.13±1.54	56.97±1.67	151.60±2.53
Kabirdhaam	Kawrdha	8.27±0.40	30.10±1.82	44.67±5.51	66.37±0.84	77.10±0.91	155.10±3.84
	Pandariya	7.90±0.10	34.80±0.98	82.37±3.15	56.97±1.00	95.03±2.57	204.10±5.89
	Pipiriya	7.30±0.265	30.23±0.987	70.27±0.709	57.07±0.862	37.23±1.550	149.83±2.060
	Dongargarh	8.33±0.29	31.33±0.35	66.17±1.61	64.53±3.17	129.93±1.97	121.10±1.91
Rajnandgaon	Somni	7.57±0.38	33.73±1.70	31.27±1.50	31.13±1.55	90.53±1.27	102.90±2.23
	Tilaii	8.47±0.38	31.87±0.91	43.43±1.99	67.77±1.00	83.17±2.41	190.90±3.28

Table 2. Physico-chemical characterization of soil and water samples of different paddy fields of North Hill Regions of Chhattisgarh

District	Area	pH	Temperature (˚C)	Salinity (g L^{-1})	Dissolved Oxygen (ppm)	Conductivity (µ Seimen cm^{-1})	Total Dissolved Solid (mg L^{-1})
Jashpur Nagar	Jashpur Nagar	8.30 ± 0.17	30.27 ± 1.52	39.47 ± 1.29	32.17±1.44	65.77 ±1.12	121.53 ± 2.16
	Jashpur	7.5 ± 0.5	29.7 ± 0.6	86.7 ± 1.1	45.4± 0.5	95.9 ±1.8	101.7 ± 2.0
	Deepatoli	8.33 ± 0.2	31.33 ± 0.29	40.33 ± 0.58	67.47±1.24	77.33 ±1.38	157.83 ± 1.70
	Gholeng	8.6 ± 0.5	30.9 ± 0.9	42.8 ± 1.0	87.8±1.1	110.9 ±2.1	146.6 ± 1.9
	Ranidah	7.9 ± 0.1	34.8 ±1.0	81.4 ± 1.4	57.0 ±1.0	94.4 ±1.4	204.1 ± 5.9
Sarguja	Ambikapur	8.7 ± 0.4	28.7 ±1.1	41.5 ± 0.4	58.2 ±1.3	152.1 ±2.5	106.1 ± 2.4
	Mainpaath	7.40 ± 0.35	29.73 ± 0.47	122.03 ± 2.57	30.43 ±1.36	113.90 ±5.54	122.63 ± 3.52
	Mainpaath	7.57 ± 0.29	27.43 ± 0.60	81.93 ± 2.57	68.40 ±1.32	108.57 ±1.24	142.27 ± 2.97
	Ramgarh	7.3 ± 0.3	30.2 ± 1.0	70.3 ± 0.7	57.1 ±0.9	36.9 ±1.0	150.5 ±3.1
	Sitapur	8.4 ± 0.45	30.633 ± 0.4	73.64±1.132	64.733 ±1.3	77.223 ±1.101	122.1 ± 2.386
Surajpur	Surajpur	8.43 ± 0.50	28.50 ± 0.46	61.57±2.11	36.57 ±1.59	56.83 ±1.03	110.20 ±1.59
	Surajpur	7.2 ± 0.15	32.6 ± 0.56	61.7±1.916	21.2 ±1.021	54.3 ±0.621	152.9 ±2.052
	Premnagar	8.6 ± 0.265	28.5 ±1.380	42.3±1.270	46.1 ±0.757	35.2 ±0.493	102.7 ±2.722
	Ramanujnagar	7.5 ± 0.231	32.3 ± 0.513	110.3±1.539	68.0 ±0.896	129.1±1.300	135.2 ±1.513
	Pratapur	7.77 ± 0.23	31.27 ± 0.35	57.00±0.89	68.20 ±1.47	96.47±2.20	121.93 ±2.32

4.2 MORPHOLOGICAL CHARACTERIZATION OF CYANOBACTERIA

4.2.1 *Microscopic Analysis*

The morphological characters of the cyanobacterial strains were identified microscopically based on the keys provided by (Desikachary, 1959; Komarek *et al*., 2014). All the forty cyanobacterial strains whether isolated from Plain regions or from North hills of Chhattisgarh were morphologically diverse (Table 4 & 5). Based on the phenotypic characters such as shape and size of the vegetative cells, heterocytes and akinetes, position and frequency of heterocytes were compared among the different strains of cyanobacteria collected from different sites of plains region as well as north hills region of Chhattisgarh.

(A) Taxonomic description of cyanobacterial strains isolated from plains regions

1. *Anabaena doliolum* 1a

Pl. no 1; Fig. 1

Thallus mucilaginous; pale blue-green; trichome straight and 3.8 ± 0.2 µm broad; cell barrel shaped, 3.5 ± 0.11 µm length and 4.0 ± 0.13 µm width; heterocyte barrel shaped, intercalary, 5.75 ± 0.25 µm length and 5.5 ± 0.30 µm width; heterocyte frequency was 9.09%; akinetes barrel or ellipsoidal shaped, 10.5 ± 0.25 µm length 5.2 ± 0.35 µm width, apex cell conical shaped.

Class- Cyanophyceae

Order- Nostocales

Family- Nostocaceae

Genus - *Anabaena*

Species - *doliolum* 1a

2. *Nostoc* sp. 1c

Pl.no. 1; Fig. 2

Thallus tuberculate, gelatinous; filament entangled, coiled; sheath colorless, trichomes 3.9 ± 0.10 µm broad; cells oval shaped 4.5 ±1.13 µm length, 4.0 ± 0.95 µm width; heterocyte subspherical, intercalary, 7.0 ± 0.20 µm length, 6.5 ± 0.20 µm width and heterocyte frequency was 11.77%.

Class- Cyanophyceae

Order- Nostocales

Family- Nostocaceae

Genus - *Anabaena*

Species – sp.1f

3. *Nostoc* sp.1d

Pl.no.1; Fig. 3

Mucilaginous thallus, olive color, filamentous loosely entangled; indistinct sheath; trichome3.0 ± 0.1 µm broad; cells ellipsoidal shaped, rarely longer than broad; heterocyte intercalary, spherical, 6.0 ± 0.21µm length and 6.0 ± 0.21 µm width and there frequency was 9.52%.

Class- Cyanophyceae

Order- Nostocales

Family- Nostocaceae

Genus - *Anabaena*

Species – *sp. 1d*

4. *Anabaena sphaerica* 1e

Pl.no. 1; Fig. 4

Trichome straight; blue-green, arranged in parallel, 5-6 µm broad, mucilaginous; cells spherical, 7.5 ± 0.23 µm length and 5.8 ± 0.18 µm width; heterocyte, oval or sub spherical, intercalary, 13.0 ± 0.21 µm length and 10.5 ± 0.20 µm width; akinetes, spherical shaped, 12.0 ± 0.25 µm length, 11.0 ± 0.25 µm width and heterocyte frequency was reported to be 13.04 %.

Class- Cyanophyceae

Order- Nostocales

Family- Nostocaceae

Genus - *Anabaena*

Species – *sphaerica* 1e

5. *Scytonema* sp. *1f*

Pl.no. 2; Fig. 5

Free floating; thallus radially expanded, dark blue-green; filament 18.0 ± 0.5 µm broad, long false branching; firm sheath; cells cylindrical; 4.0 ±1.15 µm length, 3.5 ± 0.12 µm width, heterocyte intercalary, Barrel in shape, 6.0 ± 0.21 µm length, 5.5 ± 0.21 µm width and heterocyte frequency was found 10.0%

Class- Cyanophyceae

Order- Nostocales

Family- Scytonemataceae

Genus - *Scytonema*

Species- sp. 1f

6. *Nostoc* sp. 1h

Pl.no. 2; Fig. 6

Thallus tuberculate, irregularly expanded, gelatinous, filament flexuous; sheath colorless; trichome 3.5 ± 0.12 µm broad; cells barrel, 6.5 ± 0.15µm length and 23.5 ± 0.11 µm width; heterocyte intercalary, Oblong 7.4 ± 0.20 µm length and 8.0 ± 0.30 µm width and there frequency was observed 11.76%

Class- Cyanophyceae

Order- Nostocales

Family- Nostocaceae

Genus – *Nostoc*

Species – sp. 1h

7. *Anabaena* sp. 1n

Pl.no. 2; Fig. 7

Thallus mucilaginous, free floating, bluish in color; trichome 5.4 ± 0.19 µm broad; apical cell conical shaped; cells were cylindrical in shape, 7.0 ± 0.11 µm length, 5.5 ± 0.09 µm width; heterocyte was sub spherical in shape, intercalary, 9.0 ± 0.12 µm length, 8.0 ±0.10 µm width and there frequency was observed to be 15.0%

Class- Cyanophyceae

Order- Nostocales

Family- Nostocaceae

Genus - *Anabaena*

Species – sp.1n

8. *Anabaena* sp. 2a

Pl.no. 2; Fig. 8

Mucilaginous, trichomes single, straight, 4.5 µm broad; pale blue green; apical cell broad conical shaped, vegetative cells barrel shaped, 3.5 ± 0.11µm length and 4.0 ± 0.09 µm width; heterocyte barrel shaped, intercalary, 8.5 ± 0.12 µm length and 5.6 ± 0.10 µm width; akinetes ellipsoidal shaped, 7.5 ± 0.25 µm width, 9.0 ± 0.20 µm length, where as heterocyte frequency was 4.76%

Class- Cyanophyceae

Order- Nostocales

Family- Nostocaceae

Genus - *Anabaena*

Species- sp. 2a

9. *Anabaena* sp. 2b

Pl.no. 3; Fig. 9

Thallus green, gelatinous; trichome short, straight, parallel, 2.6 ± 0.30 µm broad; cells 2-3 µm broad, Ellipsoidal shaped, 5.8 ± 0.09 µm in length, 5.0 ± 0.08 µm in width; heterocyte ellipsoidal and intercalary, 8.0 ± 0.12 µm length and 6.0 ± 0.12 µm width and their frequency was 14.81%

Class- Cyanophyceae

Order- Nostocales

Family- Nostocaceae

Genus - *Anabaena*

Species- sp.2b

***10. Nostoc* sp. 4**

Pl.no. 3; Fig. 10

Thallus bullose, filament flexuous; trichome 4.5 µm broad; cells barrel shaped, 6.5 ± 0.15 µm length and 3.5 ± 0.11 µm width; heterocyte intercalary, oblong, 7.4 ± 0.20 µm length and 8.0 ± 0.30 µm width; akinetes intercalary, oblong, 10 ± 0.32 µm length, 7.0 ± 0.22 µm width and their frequency was 14.29%

Class- Cyanophyceae

Order- Nostocales

Family- Nostocaceae

Genus- *Nostoc*

Species- sp.4

11. ***Desmonostoc muscorum* 9a**

Pl.no. 3; Fig.11

Thallus gelatinous, tuberculate, brownish in colour; filaments were densely entangled, trichome 3-5 µm broad, cells were ellipsoidal shaped, 4.0 ±1.15 µm length and 4.5 ±1.4 µm width; heterocyte intercalary, spherical, 6 ± 0.20 µm length and 6.0 ±0.20 µm width and their frequency was 21.05%

Class- Cyanophyceae

Order- Nostocales

Family- Nostocaceae

Genus – *Desmonostoc*

Species – *muscorum* 9a

12. ***Nostoc* sp. 9c**

Pl.no. 3; Fig. 12

Thallus discoid, yellowish in colour, hyaline inside; trichome 4.0 µm broad, irregularly curved; cells spherical, 4.0 ± 0.15 µm length and 4.0 ± 0.11 µm width; heterocyte intercalary, spherical, 7.4 ± 0.20 µm length and 8.0 ± 0.30 µm width and heterocyte frequency was observed to be 4.0%

Class- Cyanophyceae

Order- Nostocales

Family- Nostocaceae

Genus- *Nostoc*

Species - sp.9c

13. *Nostoc* sp. 9f

Pl.no. 4; Fig. 13

Thallus varying in size; tuberculate, greenish in colour; trichomes 3.5-4 µm broad, irregularly curved; cells barrel shaped, 5.0 ±1.15 µm length and 5.0 ± 1.15µm width; heterocyte intercalary, Oblong, 8.4 ± 0.30 µm length and 8.0 ± 0.30 µm width and heterocyte frequency was observed 5.36%

Class- Cyanophyceae

Order- Nostocales

Family- Nostocaceae

Genus - *Nostoc*

Species-– sp. 9f

14. *Nostoc* sp. 9i

Pl.no. 4; Fig. 14

Thallus gelatinous, expanded, yellowish brown to blue green; trichome 4.0-5.0 μm broad, filamentous numerous, flexuous; cells barrel in shape, 6.5±0.15 μm length and 3.5±0.11 μm width; heterocyte intercalary, Oblong, 7.0 ± 0.20 μm length and 7.5 ± 0.30μm width and heterocyte frequency was 5.26%.

Class- Cyanophyceae

Order- Nostocales

Family- Nostocaceae

Genus - *Nostoc*

Species- sp. 9i

15. *Nostoc* sp.9j

Pl.no. 4; Fig. 15

Thallus gelatinous, irregularly expanded, light blue green; filaments flexuous, trichomes 3.2-3.5 μm broad, irregularly curved; cells spherical in shape, 6.5 ± 0.15 μm length and 6.5 ± 0.15 μm width; heterocyte intercalary, spherical, 7.4 ± 0.20 μm length and 8.0 ± 0.30 μm width and heterocyte frequency was to be 14.81%.

Class- Cyanophyceae

Order- Nostocales

Family- Nostocaceae

Genus - *Nostoc*

Species - sp. 9j

16. *Tolypothrix* sp. 9k

Pl.no. 4; Fig.16

Filaments long, irregularly curved, thin or thick sheath with a single trichome, false branching; trichome 11 ± 0.25 µm broad; cells sub-spherical, 3.5 ± 0.12 µm, 2.5 ± 0.10 µm, heterocyte intercalary, cylindrical in shape, 8.0 ± 0.35 µm length, 6.0 ± 0.35 µm width and heterocyte frequency was 16.67%.

Class- Cyanophyceae

Order - Nostocales

Family- Tolypothrichaceae

Genus - *Tolypothrix*

Species - sp.9k

17. *Nostoc commune* 9n

Pl.no.5; Fig. 17

Thallus mucilaginous; first cell globose to oblong, later globose, foliose, hollow free or attached, filament flexuous curved or entangled, 4.8 ± 0.3 µm broad; cells barrel, 6.5 ± 0.15 µm length and 3.5 ± 0.11 µm width; heterocyte intercalary, barrel, 7.4 ± 0.20 µm length and 8.0 ± 0.30 µm width, heterocyte frequency was 3.23%.

Class- Cyanophyceae

Order- Nostocales

Family- Nostocaceae

Genus – *Nostoc*

Species – *commune* 9n

18. Nostoc* sp. *9ma1

Pl.no. 5; Fig.18

Thallus mucilaginous, foliose, hollow free or attached; filament flexuous, curved cells, Cells were spherical 4.0 ±1.15µm length and 4.0 ± 1.15µm width; heterocyte intercalary, oblong 8.4 ± 0.30 µm length and 8.0 ± 0.30 µm width; heterocyte frequency was observed 9.09%

Class- Cyanophyceae

Order- Nostocales

Family- Nostocaceae

Genus - *Nostoc*

Species- sp. 9ma1

19. *Nostoc* sp. 9ma2

Pl.no. 5; Fig.19

Thallus mucilaginous, foliose, hollow free or attached; filament flexuous curved or entangled; cells spherical, 5.5 ±1.15 µm length and 3.5 ± 0.11 µm width; heterocyte intercalary, oblong, 7.4 ± 0.20 µm length and 8.0 ± 0.30µm width, heterocyte frequency was 5.41%

Class- Cyanophyceae

Order- Nostocales

Family- Nostocaceae

Genus - *Nostoc*

Species - sp. 9ma2

20. *Scytonema* sp. 12c

Pl.no. 5; Fig.20

Free floating; thallus radially expanded, dark blue-green; filament 18.0 ± 0.5 µm broad, long, false branching; firm sheath; cells subspherical; 5.5 ± 0.15 µm length, 4.5 ± 0.11 µm width; heterocyte intercalary, cylindrical or sub quadrate in shape, 8.0 ± 0.35 µm length, 6.0 ± 0.35 µm width and heterocyte frequency was observed 3.33%.

Class- Cyanophyceae

Order- Nostocales

Family- Scytonemataceae

Genus - *Scytonema*

Species- sp.12c

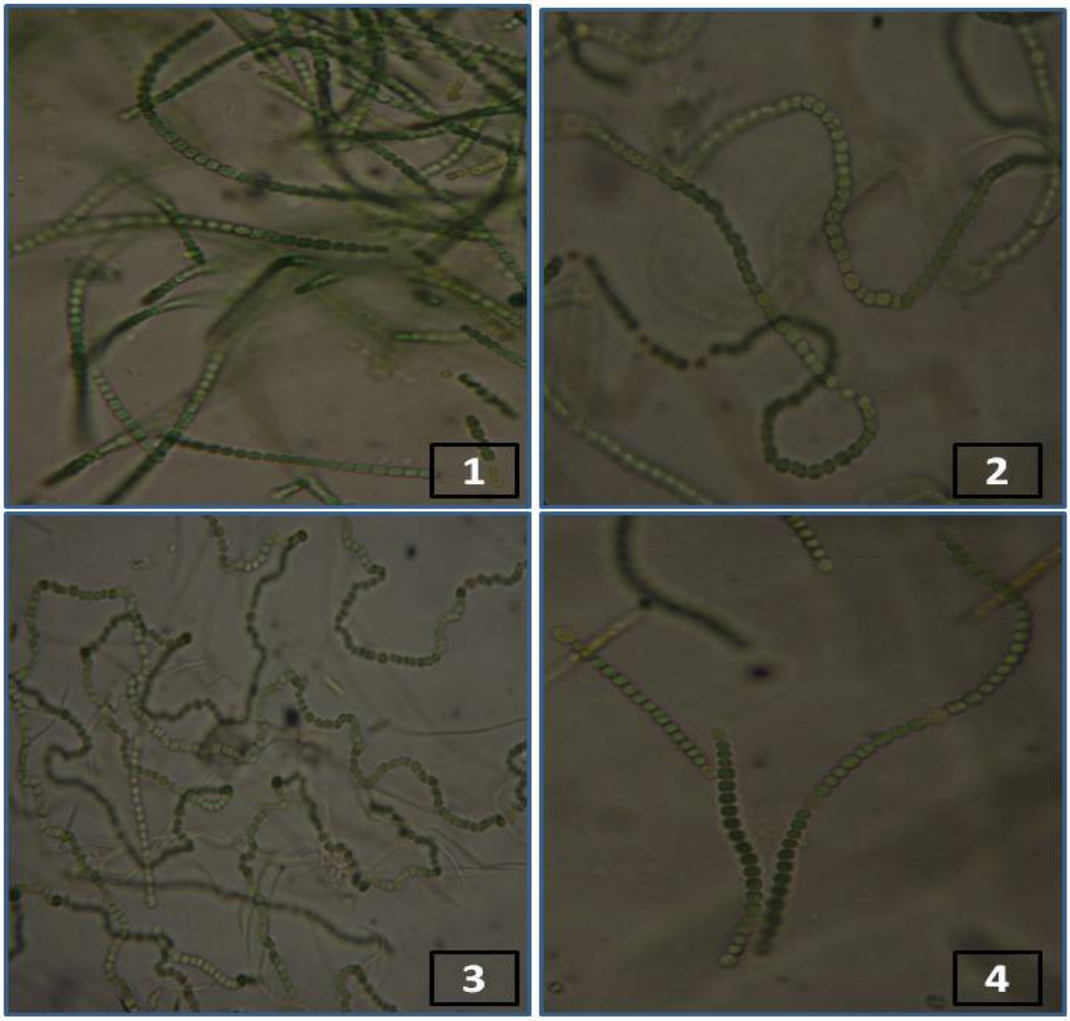

Photo plate 1. (Fig. 1) ***Anabaena doliolum*** **1a, (Fig. 2)** ***Nostoc*** **sp. 1c, (Fig. 3)** ***Nostoc*** **sp. 1d, (Fig. 4)** ***Anabaena sphaerica*** **1e**

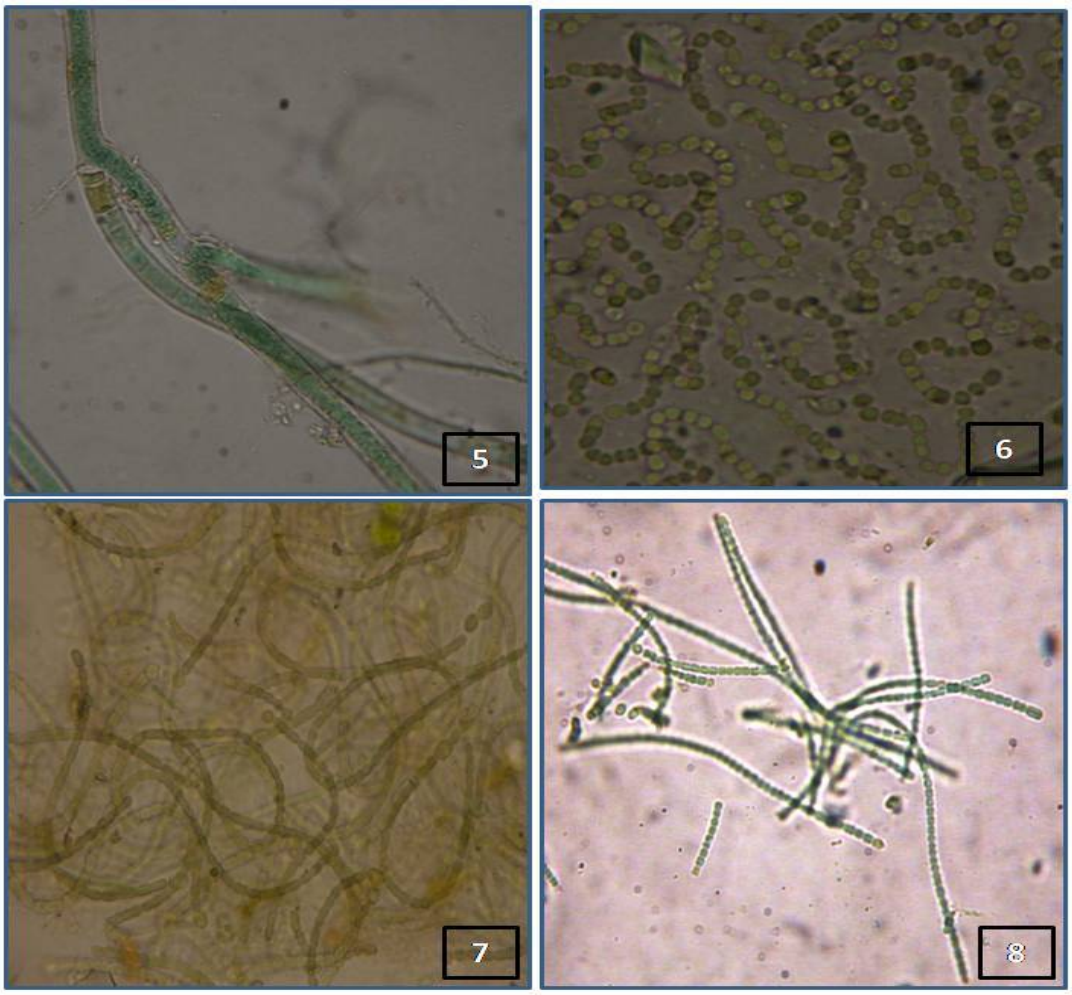

Photo plate 2. (Fig. 5) *Scytonema* sp. 1f, (Fig. 6) *Nostoc* sp. 1h, (Fig. 7) *Anabaena* sp. 1n, (Fig. 8) *Anabaena* sp. 2a

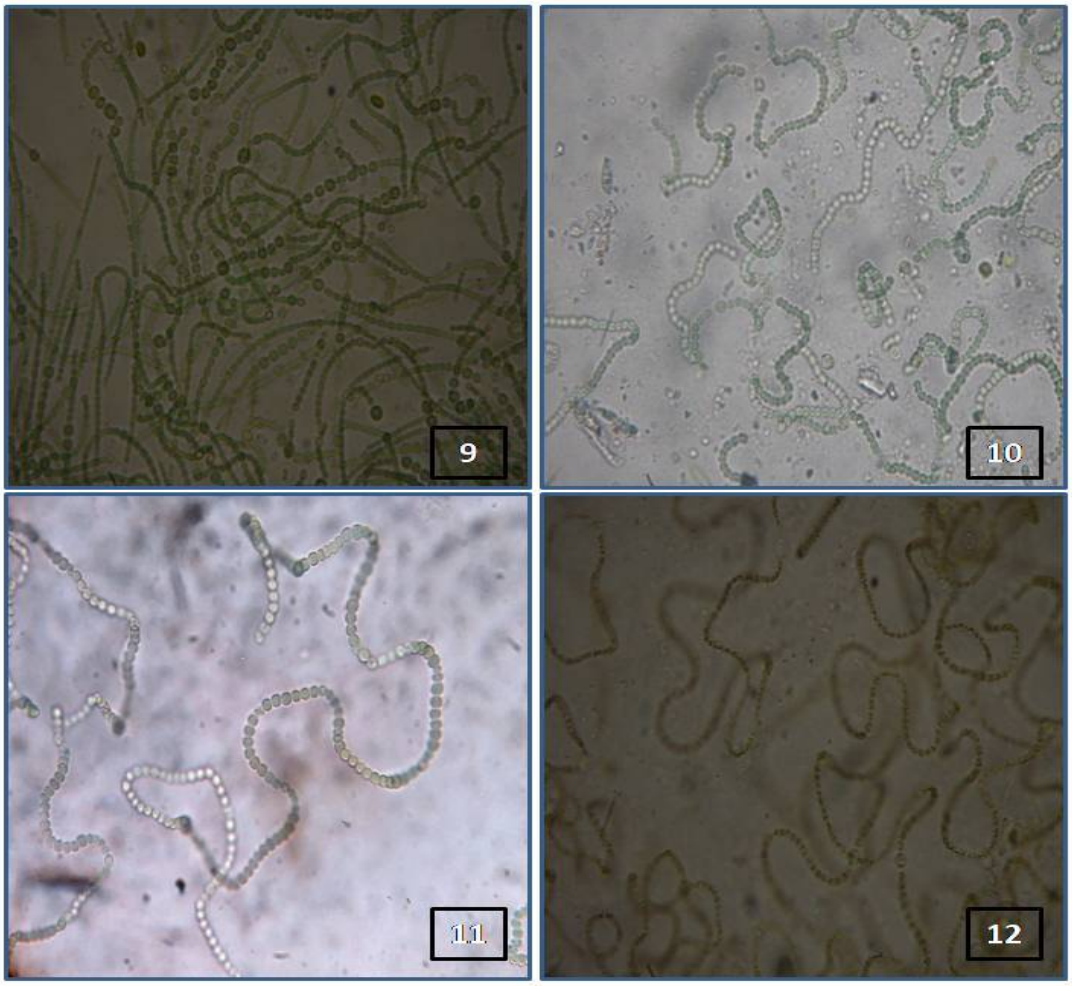

Photo plate 3. (Fig. 9) *Anabaena* sp. 2b, (Fig. 10) *Nostoc* sp. 4, (Fig. 11) *Desmonostoc muscorum* 9a, (Fig. 12) *Nostoc* sp. 9c

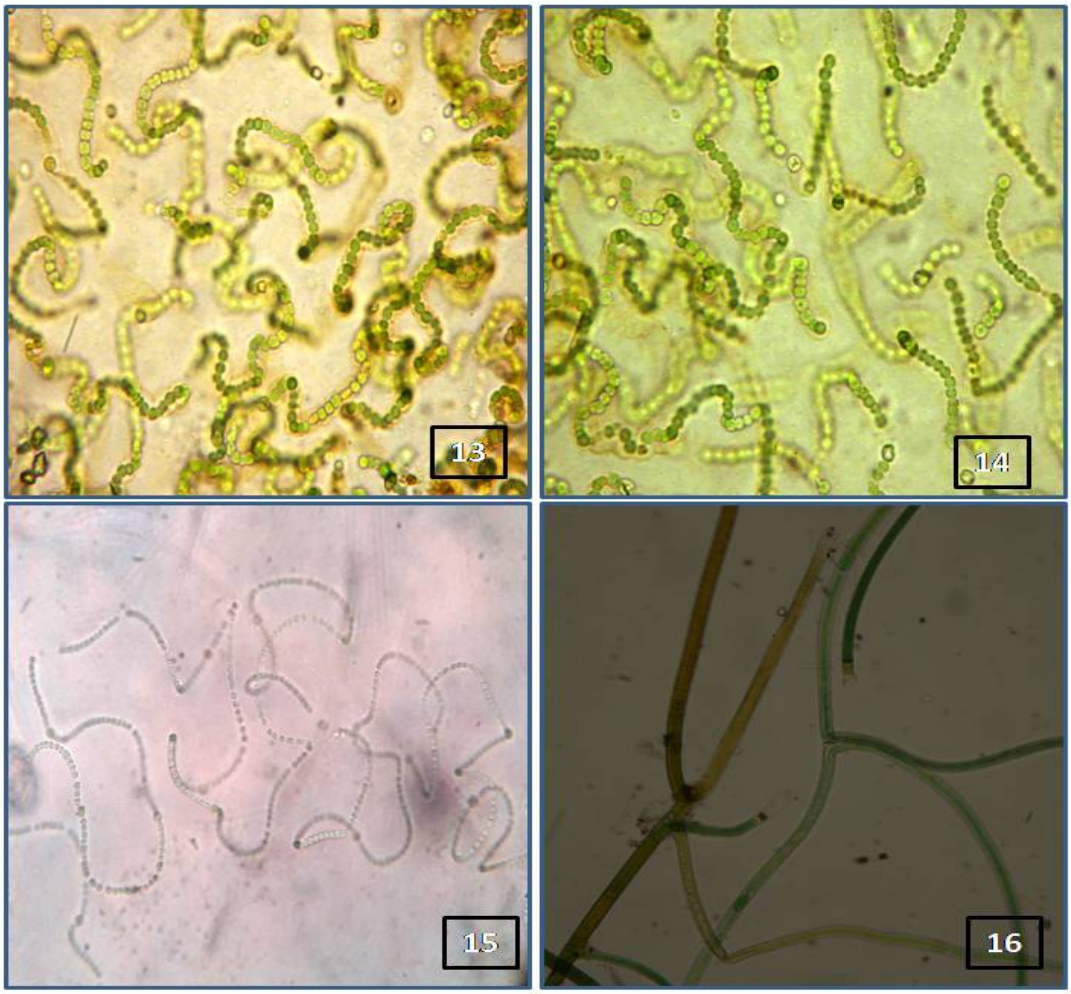

Photo plate 4. (Fig. 13) ***Nostoc*** **sp. 9f, (Fig. 14)** ***Nostoc*** **sp. 9i, (Fig. 15)** ***Nostoc*** **sp. 9j, (Fig. 16)** ***Tolypothrix*** **sp. 9k**

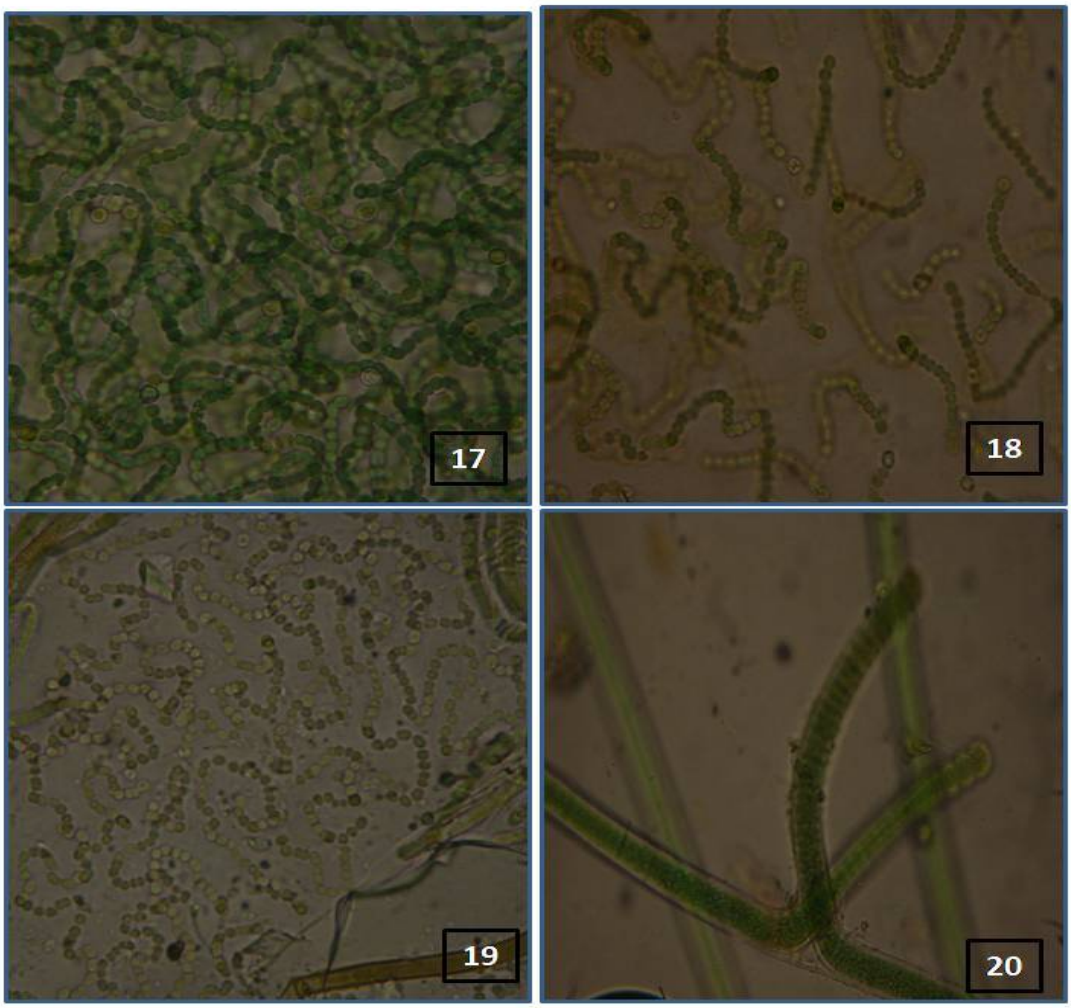

Photo plate 5. (Fig. 17) *Nostoc commune* 9n, (Fig.18) *Nostoc* sp. 9ma1, (Fig. 19) *Nostoc* sp. 9ma2, (Fig. 20) *Scytonema* sp. 12c

Table 4. Morphological study of cyanobacterial strains collected from Plain regions of Chhattisgarh

S.N.	Cyanobacterial strains	Average length filament	Vegetative Cells			Heterocyte				Akinete			
		Cell per Filaments	Shape	Dimension (µm)		Shape	Dimension (µm)		Position	Frequency (%)	Shape	Dimension (µm)		Position
				L	W		L	W				L	W	
1	*Anabaena doliolum* 1a	30	Barrel	3.5±0.11	4.0±0.13	Barrel	5.75±0.25	5.0±0.25	Intercalary	9.09	Ellipsoidal	10.5 ±0.2	5.2 ±0.3	Intercalary
2	*Nostoc* sp. 1c	15	Oval	4.5±1.13	4.0±0.95	Subspherical	7.0±0.20	6.5±0.20	Intercalary	11.76	-	-	-	-
3	*Nostoc* sp. 1d	28.5	Ellipsoidal	3.5±0.11	3.0±0.09	Spherical	6.0± 0.21	6.0±0.21	Intercalary	9.52	-	-	-	-
4	*Anabaena sphaerica* 1e	20	Spherical	7.5±0.23	5.8±0.18	Subspherical	13.0±0.21	10.5±0.20	Intercalary	13.04	Spherical	12.0 ±0.2	12.0 ±0.2	Intercalary
5	*Scytonema* sp. 1f	18	Cylindrical	4.0±1.15	3.5±0.12	Barrel	6.0± 0.21	5.5± 0.21	Intercalary	10.00	-	-	-	-
6	*Nostoc* sp. 1h	15	Barrel	6.5±0.15	3.5±0.11	Oblong	7.4 ± 0.20	8.0 ± 0.30	Intercalary	11.76	-	-	-	-
7	*Anabaena* sp. 1n	17	Cylindrical	7.0±0.11	5.5±0.09	Subspherical	9.0 ± 0.12	8.0 ±0.10	Intercalary	15.00	-	-	-	-
8	*Anabaena* sp. 2a	20	Barrel	3.5±0.11	4.0±0.09	Barrel shaped	8.5 ± 0.12	5.6 ± 0.10	Intercalary	4.76	Ellipsoidal	7.5 ±0.25	9.0 ±0.2	Intercalary
9	*Anabaena* sp. 2b	23	Ellipsoidal	5.8±0.09	5.0±0.08	Ellipsoidal	8.0 ± 0.12	6.0± 0.12	Intercalary	14.81	-	-	-	-
10	*Nostoc* sp. 4	18	Barrel	6.5±0.15	3.5±0.11	Oblong	7.4 ± 0.20	8.0 ± 0.30	Intercalary	14.29	Spherical	8.0 ±0.30	8.0± 0.30	Intercalary
11	*Desmonostoc muscorum* 9a	15	Ellipsoidal	4.0±1.15	4.5 ±1.4	Spherical	6 ± 0.20	6.0 ± 0.20	Intercalary	21.05	-	-	-	-
12	*Nostoc* sp. 9c	48	Spherical	4.0±1.15	4.0±0.11	Spherical	7.4 ± 0.20	8.0 ± 0.30	Intercalary	4.00	-	-	-	-
13	*Nostoc* sp. 9f	53	Barrel	5.0±1.15	5.0±1.11	Oblong	8.4 ± 0.30	8.0 ± 0.30	Intercalary	5.36	-	-	-	-
14	*Nostoc* sp. 9i	54	Barrel	6.5±0.15	3.5±0.11	Oblong	7.0 ± 0.20	7.5 ± 0.30	Intercalary	5.26	-	-	-	-
15	*Nostoc* sp. 9j	23	Round	6.5±0.15	3.5±0.11	Spherical	7.4 ± 0.20	8.0 ± 0.30	Intercalary	14.81	-	-	-	-
16	*Tolypothrix* sp. 9k	10	Sub-Spherical	3.5±0.12	2.5±0.10	Cylindrical	8.0 ± 0.35	6.0 ± 0.35	Intercalary	16.67	-	-	-	-
17	*Nostoc commune* 9n		Barrel	6.5±0.15	3.5±0.11	Barrel	7.4 ± 0.20	8.0 ± 0.30	Intercalary	3.23	-	-	-	-
18	*Nostoc* sp. 9ma1	30	Spherical	4.0±1.15	4.0±0.11	Oblong	8.4 ± 0.30	8.0 ± 0.30	Intercalary	9.09	-	-	-	-
19	*Nostoc* sp. 9ma2	35	Barrel	5.5±1.15	3.5±0.11	Oblong	7.4 ± 0.20	8.0 ± 0.30	Intercalary	5.41	-	-	-	-
20	*Scytonema* sp.12c	58	Subspherical	5.5±0.15	4.5±0.11	Cylindrical	8.0 ± 0.35	6.0 ± 0.35	Intercalary	3.33	-	-	-	-

(B) Taxonomic description of cyanobacterial strains isolated from North hill regions

1. *Scytonema* sp. 1ss

Pl.no. 6; Fig. 1

Free floating; thallus radially expanded, dark blue-green; filament 10-12 μm broad, long false branching, branches single; firm sheath; cells sub quadrate, 4.5 ± 0.15 μm length, 3.5 ± 0.11 μm width; heterocyte intercalary, sub quadrate in shape. 6.5 ± 1.12 μm length, 5.0 ± 0.1 μm width and heterocyte frequency was 9.09%.

Class- Cyanophyceae

Order- Nostocales

Family- Scytonemataceae

Genus - *Scytonema*

Species- sp. 1ss

2. *Anabaena* sp. 2ss

Pl.no. 6; Fig. 2

Thallus green, gelatinous; trichome long, straight, 2.6 ± 0.30 μm broad; cells barrel in shape, 5.5 ± 0.09 μm in length, 5.5 ± 0.09 μm in width; heterocyte barrel and intercalary, 8.0 ± 0.12 μm length and 6.0 ± 0.12 μm width and the observed heterocyte frequency was 11.76%.

Class- Cyanophyceae

Order- Nostocales

Family- Nostocaceae

Genus - *Anabaena*

Species - sp.2ss

3. *Calothrix* sp. 3ss

Pl.no. 6; Fig. 3

Solitary filament, unbranched; colorless sheath, constricted at the cross-walls, filament 5.5 ± 0.2 µm broad at the base, cells spherical or quadrate in shape, 3.0 ± 0.10 µm length and 3.0 ± 0.10 µm width; heterocyte terminal, sub-spherical, 4.0 ± 0.10 length, 4.0 ± 0.10 width, heterocyte frequency was 3.17%

Class - Cyanophyceae

Order- Nostocales

Family - Rivulariaceae

Genus - *Calothrix*

Species - sp.3ss

4. *Aphanothece* sp. 4ss

Pl.no. 6; Fig. 4

Free floating; thallus small, gelatinous; cells spherical, 3.0 ± 0.10 in size; colonies mucilaginous, dull brown or brownish, straight.

Class - Cyanophyceae

Order - Chroococcales

Family- Aphanothecaceae

Genus - *Aphanothece*

Species- sp.4ss

5. *Aphanothece* sp. 5ss

Pl.no. 7; Fig. 5

Free floating; colonies hemispherical or expanded; thallus mucilaginous, dull brown or brownish; cell cylindrical, straight, 3.5 ± 0.13 µm in cell dimension.

Class - Cyanophyceae

Order - Chroococcales

Family- Aphanothecaceae

Genus - *Aphanothece*

Species - sp.5ss

6. *Scytonema* sp. 6ss

Pl.no. 7; Fig. 6

Filamentous false branching, trichome single in each sheath, straight, 10.0 ± 0.15 µm broad; cells hemispherical in shape, 3 ± 0.10 µm length, 2.5 ± 0.10 µm in width, heterocyte intercalary. Cylindrical, 8.0 ± 0.35 µm length, 6.0 ± 0.35 µm width and heterocyte frequency was 11.76%.

Class- Cyanophyceae

Order- Nostocales

Family- Scytonemataceae

Genus - *Scytonema*

Species- sp.6ss

7. *Anabaena* sp. 7ss

Pl.no. 7; Fig. 7

Free floating; thallus soft dark green, gelatinous, trichome single, straight; cells cylindrical in shape, 9.0 ± 0.11 µm length and 4.5 ± 0.09 µm width; heterocyte barrel, intercalary, 9.0 ± 0.10 µm length and 5.0 ± 0.10 µm width and heterocyte frequency 15.00%; akinetes spherical 20 ± 4.0 in size.

Class- Cyanophyceae

Order- Nostocales

Family- Nostocaceae

Genus - *Anabaena*

Species- sp.7ss

8. *Tolypothrix* sp. 8ss

Pl.no. 7; Fig. 8

Prostrate or erect system; filaments with firm, thin sheath, single false branching, Filaments long, thin sheath with a single trichome, cells quadrate, 4.0 ± 1.15 µm length, 4.5 ±1.4 µm width; heterocyte intercalary, cylindrical, 8.0 ± 0.35 µm length, 6.0 ± 0.35 µm width and heterocyte frequency was 4.76%.

Class- Cyanophyceae

Order - Nostocales

Family- Tolypothrichaceae

Genus - *Tolypothrix*

Species - sp. 8ss

9. *Nostoc* sp. 9ss

Pl.no. 8; Fig. 9

Mucilaginous, trichomes single, straight, pale blue green; apical cell broad conical shaped, cells spherical shaped, 3.0 ± 0.10 µm length and 3.0 ± 0.10 µm width; heterocyte round shaped, intercalary, 4.0 ± 0.10 µm length and 4.0 ± 0.10 µm width and heterocyte frequency was 14.81%.

Class- Cyanophyceae

Order- Nostocales

Family- Nostocaceae

Genus - *Anabaena*

Species - sp. 9ss

10. *Aphanothece* sp. 10ss

Pl.no. 8; Fig. 10

Thallus gelatinous, yellow-brown; cells oval or spherical, 3.7 ± 0.10 µm broad, blue green in color.

Class - Cyanophyceae

Order - Chroococcales

Family- Aphanothecaceae

Genus - *Aphanothece*

Species- sp. 10ss

11. *Anabaena* sp. 11ss

Pl.no. 8; Fig. 11

Thallus mucilaginous; cell globose , hollow, free or attached, filament flexuous curved, 4.8 ± 0.3 µm broad; cells hemispherical 3 ± 0.10 µm length and 2.5 ± 0.10 µm width; heterocyte intercalary, spherical, 6.5 ± 0.10µm length and 6.5 ± 0.10 µm width and heterocyte frequency 21.05%.

Class- Cyanophyceae

Order- Nostocales

Family- Nostocaceae

Genus - *Nostoc*

Species- sp. 11ss

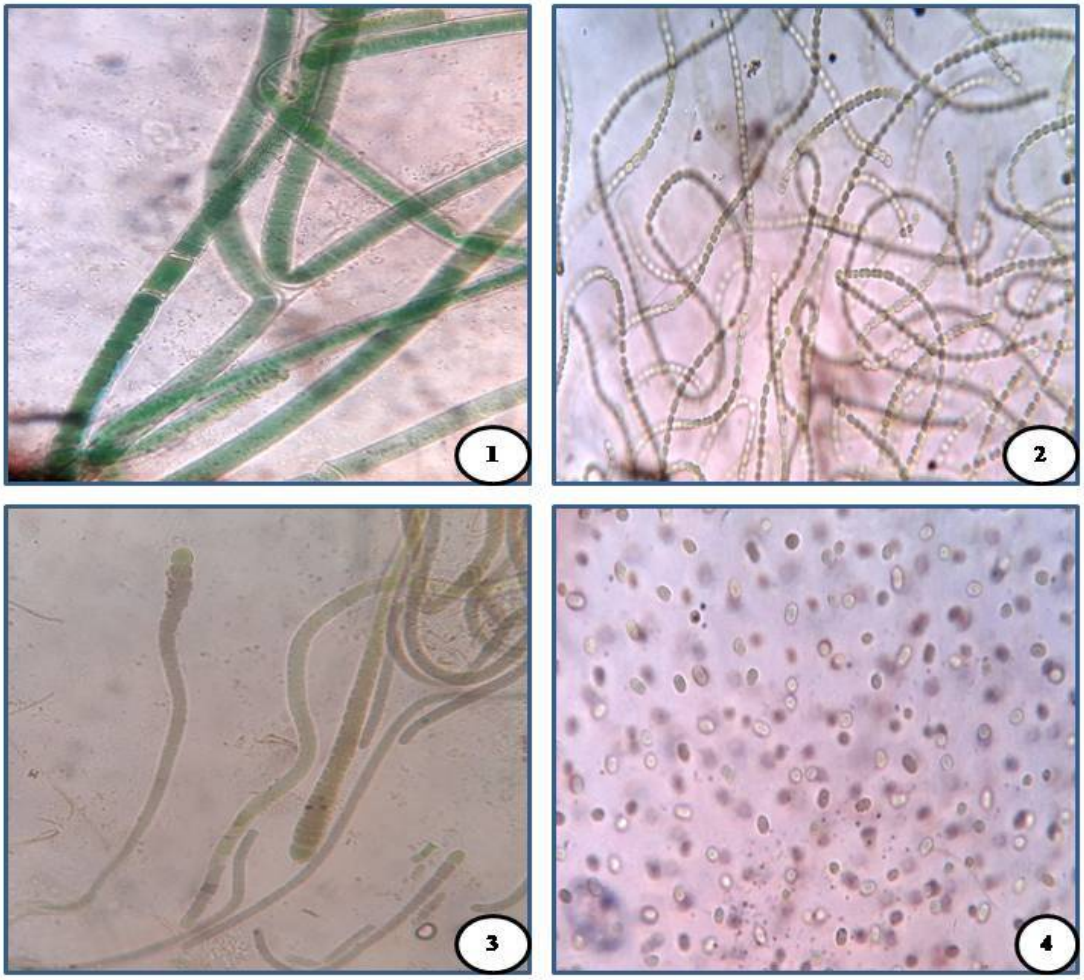

Photo plate 6. (Fig. 1) *Scytonema* sp.1ss, (Fig. 2) *Anabaena* sp. 2ss, (Fig. 3) *Calothrix* sp. 3ss, (Fig. 4) *Aphanothece* sp. 4ss

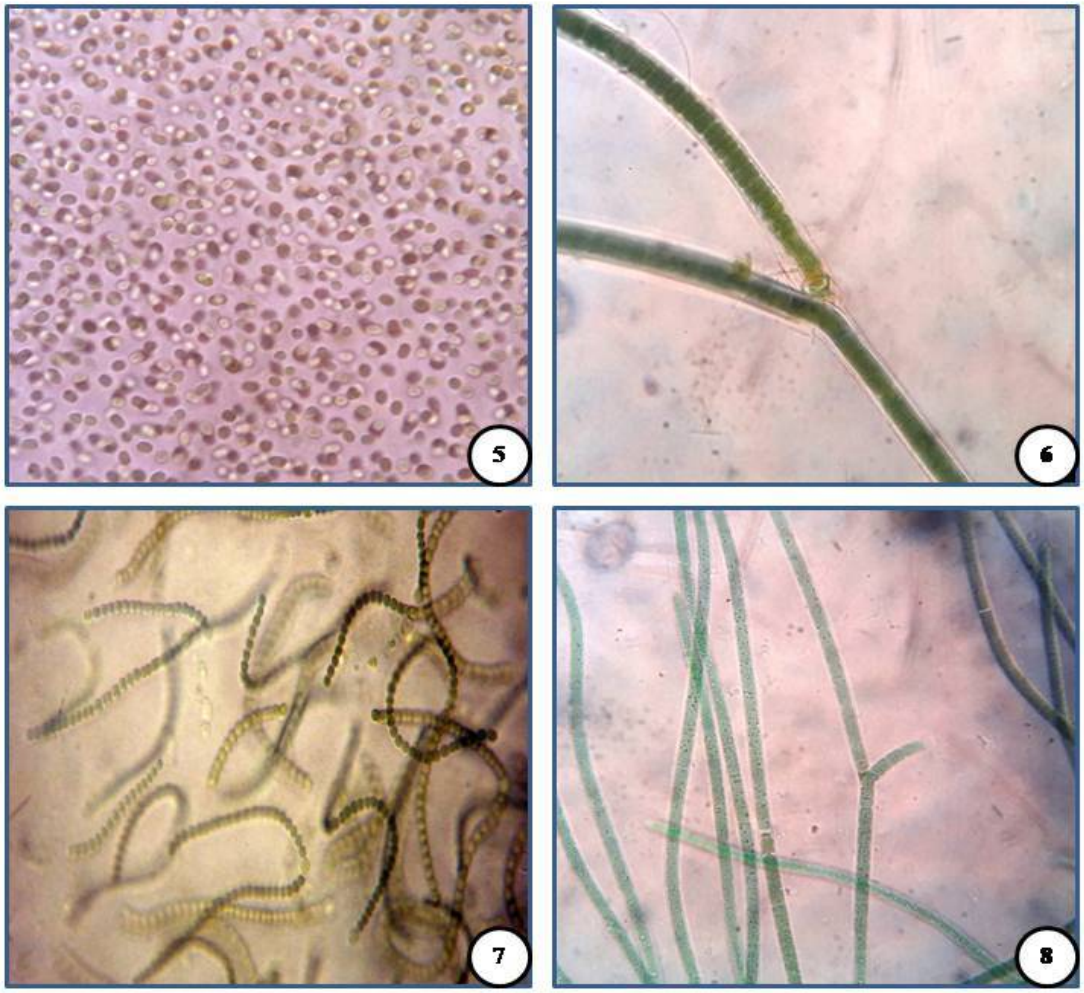

Photo plate 7. (Fig. 5) ***Aphanothece*** **sp. 5ss, (Fig. 6)** ***Scytonema*** **sp. 6ss, (Fig. 7*)* *Anabaena*** **sp. 7ss, (Fig. 8)** ***Tolypothrix*** **sp. 8ss**

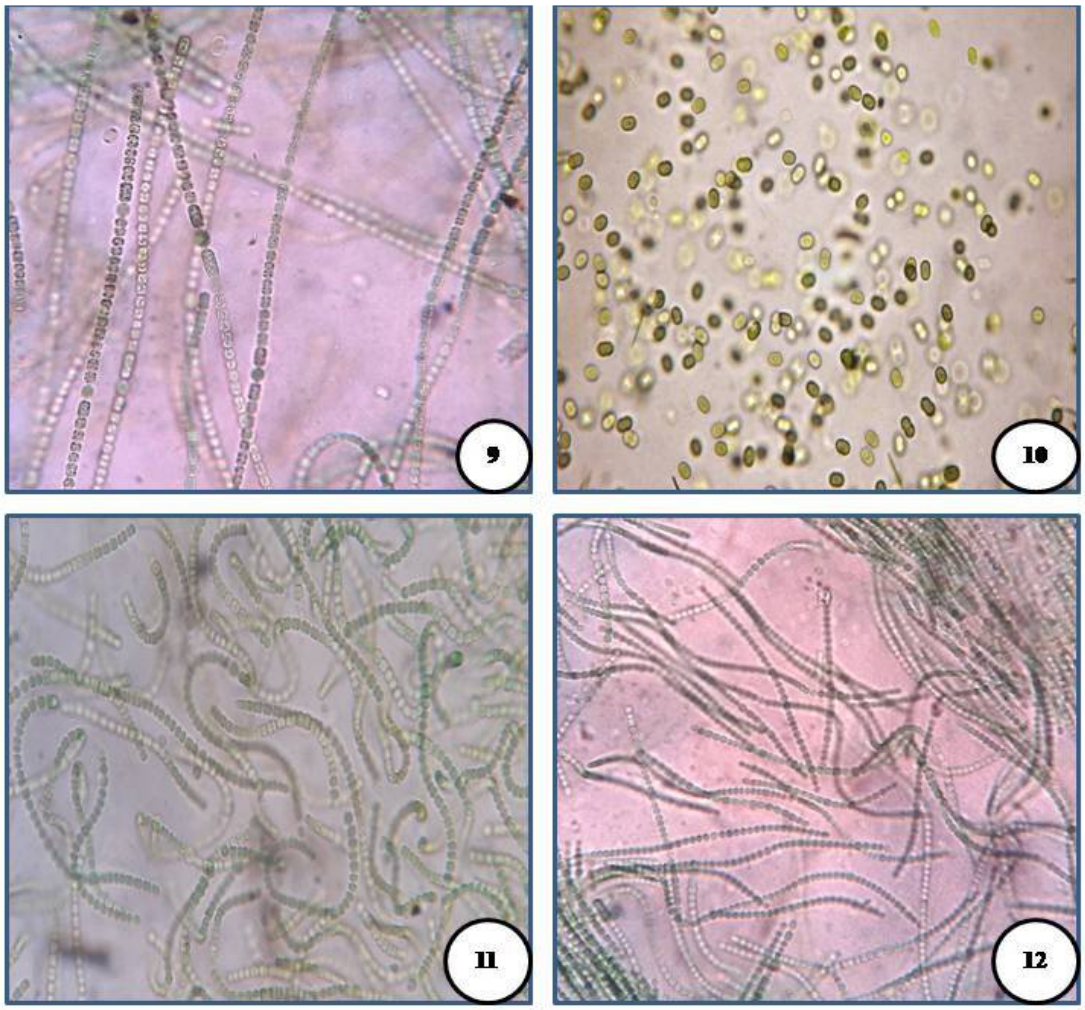

Photo plate 8. (Fig. 9) *Nostoc* sp. 9ss, (Fig. 10) *Aphanothece* sp. 10ss, (Fig. 11) *Anabaena* sp. 11ss, (Fig. 12) *Anabaena* sp. 12ss

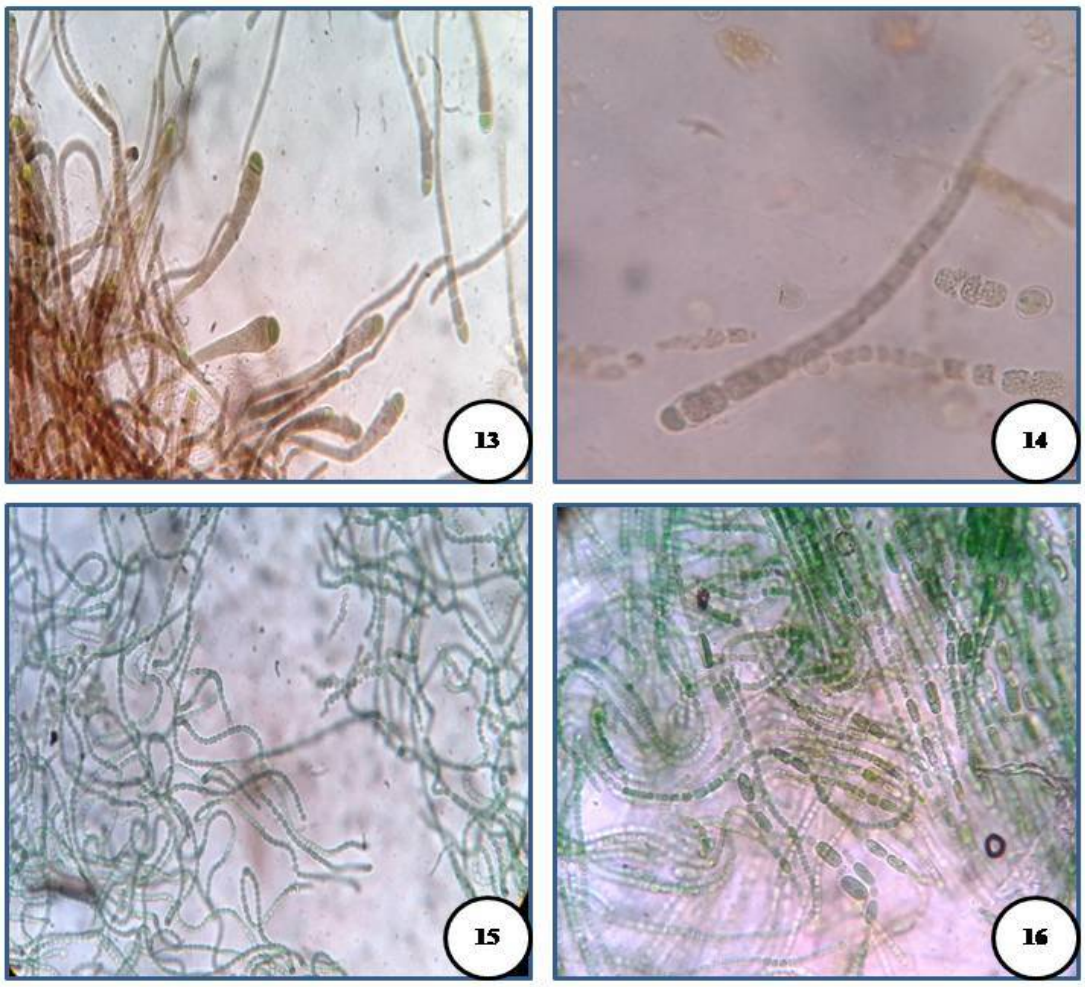

Photo plate 9. (Fig. 13) *Calothrix* sp. 13ss, (Fig. 14) *Calothrix* sp. 14ss, (Fig. 15) *Anabaena* sp. 15ss, (Fig. 16) *Nostoc* sp. 16ss

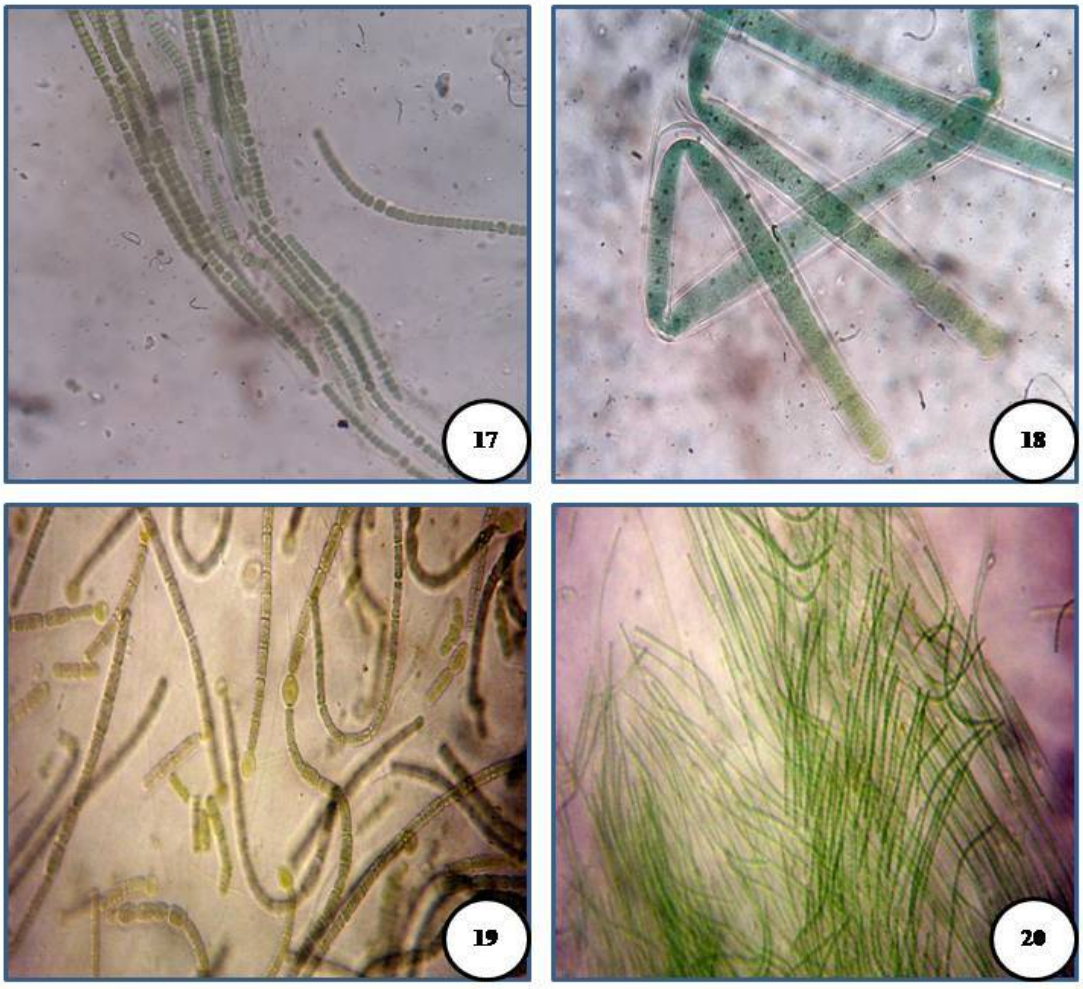

Photo plate 10. (Fig. 17*) Nostoc* sp. 17ss, (Fig. 18) *Scytonema* sp.18ss, (Fig. 19) *Anabaena* sp. 19ss, (Fig. 20) *Anabaena* sp. 20ss

Table 5. Morphological observation of cyanobacterial strains collected from North Hills region of Chhattisgarh

S.N.	Cyanobacterial strains	Average length filament	Vegetative Cell			Heterocyte				Akinites			
		Cell per Fillament	Shape	Dimension (µm)		Shape	Dimension (µm)		Position	Frequency (%)	Shape	Dimension (µM)		Position
				L	W		L	W				L	W	
1	*Scytonema sp.* 1ss	30	Subquadrate	4.5±0.15	3.5±0.11	Subquadrate	6.5±1.125	5.0±0.1	Intercalary	9.09	-	-	-	-
2	*Anabaena sp.*2SS	15	Barrel	5.5±0.09	5.5±0.09	Barrel	-	-	Intercalary	11.76	-	-	-	-
3	*Calothrix sp.* 3ss	28.5	Spherical	3.0±0.10	3.0±0.10	Round	4.0±0.10	4.0±0.10	Terminal	3.17	-	-	-	-
4	*Aphanothece sp.* 4ss	0	Spherical	3.0±0.10	-	--	-	-	-	-	-	-	-	-
5	*Aphanothece sp.* 5ss	0	Cylindrical	3.5±0.13	-	-	-	-	-	-	-	-	-	-
6	*Scytonema sp.* 6ss	15	Hemispherical	3±0.10	2.5±0.10	Cylindrical	8.0 ± 0.35	6.0 ± 0.35	Intercalary	11.76	-	-	-	-
7	*Anabaena sp.* 7ss	17	Cylindrical	9.0 ±0.1	4.5 ±0.09	Barrel	9.0±0.10	5.0±0.10	Intercalary	15.00	Spherical	20±4.0	20±4.0	Intercalary
8	*Tolypothrix sp.*8ss	20	Quadrate	4.0±1.15	4.5 ±1.4	Cylindrical	8.0 ± 0.35	6.0 ± 0.3	Intercalary	4.76	-	-	-	-
9	*Nostoc sp.* 9ss	23	Spherical	3.0±0.10	3.0±0.10	Round	4.0±0.10	4.0±0.10	Intercalary	14.81				
10	*Aphanothece sp.* 10ss	0	Oval and Spherical	3.7±0.10	-	-	-	-	-	-	-	-	-	-
11	*Anabaena sp.*11ss	15	Hemispherical	3±0.10	2.5±0.10	Spherical	6.5±0.10	6.5±0.10	Intercalary	21.05	-	-	-	-
12	*Anabaena sp.* 12ss	36	Barrel	3.0±0.10	4.5±0.10	Barrel	5.5±0.10	5.0±0.10	Intercalary	4.00	Barrel	10.5±0.10	5.0±0.10	Intercalary
13	*Calothrix sp..*13ss	53	Quadrate	5.0±1.15	5.0±1.11	Round	7.0±1.0	5.0±1.0	Terminal	1.79	-	-	-	-
14	*Calothrix sp.*14ss	28	Spherical	3.0±0.10	3.0±0.10	Obelong	5.5±0.10	5.0±0.10	Terminal	1.75	-	-	-	-
15	*Anabaena sp.*15ss	23	Barrel	2.0±0.11	3.0±0.10	Elipsoidal	5.0±0.10	5.5±0.10	Intercalary	14.81	-	-	-	-
16	*Nostoc sp.* 16ss	22	Spherical	4.0±0.10	4.0±0.10	Round	7.0±0.10	7.0±0.10	Intercalary	16.67	-	-	-	-
17	*Nostoc sp.* 17ss	30	Spherical	3.0±0.10	3.0±0.10	Hemispherical	5.5±0.10	5.5±0.10	Intercalary	3.23	-	-	-	-
18	*Scytonema sp.* 18ss	30	Cylindrical	5±0.10	4.5±0.10	Cylindrical	8.0±1.89	7.5±1.0	Intercalary	6.06	-	-	-	-
19	*Anabaena sp.*19ss	35	Spherical	5.0±0.10	5.0±0.10	Hemispherical	6.5±0.10	5.8±0.10	Intercalary	8.11	-	-	-	-
20	*Anabaena sp.* 20ss	58	Oblong	4±0.10	3±0.10	Spherical	5±0.10	5±0.10	Intercalary	3.33	Barrel	7.5	9.0	Intercalary

L-Length, W-Width

12. *Anabaena* sp. 12ss

Pl.no. 8; Fig. 12

Filaments are attached, elongated, straight, solitary having mucilaginous sheath, colorless filaments, trichomes 2.3 ± 0.1 µm broad; cells barrel shaped, 3.0 ± 0.10 length, 4.5 ± 0.10, heterocyte barrel, intercalary, 5.5 ± 0.10 µm length and 5.0 ± 0.10 µm width and heterocyte frequency was 4.11%.

Class- Cyanophyceae

Order- Nostocales

Family- Nostocaceae

Genus - *Anabaena*

Species - sp. 12ss

13. *Calothrix* sp. 13ss

Pl.no. 9; Fig. 13

Filaments in group, grow in small dense tufts, parallel, heterocyte terminal; akinete formed in single. Single filament, unbranched; colorless sheath, constricted at the cross-walls, filament 5.5 ± 0.2 µm broad at the base, cells quadrate shaped, 5.0 ± 1.15 µm length and 5.0 ±1.11µm width; heterocyte terminal sub-spherical, 7.0 ± 1.0 µm length, 5.0 ±1.0 11µm width and their frequency was observed 1.79%.

Class - Cyanophyceae

Order- Nostocales

Family - Rivulariaceae

Genus - *Calothrix*

Species - sp.13ss

14. *Anabaena* sp. 14ss

Pl.no. 9; Fig. 14

Trichomes single, straight, cells spherical shaped, 3.0±0.10 µm length and 3.0±0.10 µm width; heterocyte oblong shaped, intercalary, 5.5±0.10 µm length and 5.0±0.10 µm width and heterocyte frequency was 1.75%.

Class- Cyanophyceae

Order- Nostocales

Family- Nostocaceae

Genus - *Anabaena*

Species - sp. 14ss

15. *Anabaena* sp. 15ss

Pl.no. 9; Fig. 15

Thallus mucilaginous, free floating, bluish in color; trichome 5.4 ± 0.19 µm broad; apical cell conical shaped; cells barrel shaped, 2.0 ± 0.11 µm length, 3.0 ± 0.10 µm width, gas vacuoles present; heterocyte ellipsoidal, intercalary, 5.0 ± 0.10 µm length, 5.5 ± 0.10 µm width and their frequency was 14.81%

Class- Cyanophyceae

Order- Nostocales

Family- Nostocaceae

Genus - *Anabaena*

Species - sp. 15ss

16. *Nostoc* sp. 16ss

Pl.no. 9; Fig. 16

Free floating, single, curved or coiled; cells spherical, 4.0 ± 0.10 µm broad, 4.0 ± 0.10 µm width; heterocyte spherical, intercalary, 7.0 ± 0.10 µm broad and heterocyte frequency was 16.07%.

Class- Cyanophyceae

Order- Nostocales

Family- Nostocaceae

Genus - *Anabaena*

Species - sp. 16ss

17. *Nostoc* sp. 17ss

Pl.no. 10; Fig. 17

Thallus tuberculate, gelatinous; filament entangled, coiled; sheath colorless, trichomes 3.9 ± 0.10 µm broad; cells spherical 3.0 ± 0.10 µm length, 3.0 ± 0.10µm width; heterocyte hemispherical, intercalary, 5.5 ± 0.10 µm length, 5.5 ± 0.10 µm width and heterocyte frequency was observed 3.27%.

Class- Cyanophyceae

Order- Nostocales

Family- Nostocaceae

Genus - *Nostoc*

Species- sp. 17ss

18. *Scytonema* sp.18ss

Pl.no. 10; Fig. 18

Filamentous false branching, trichome single in each sheath, straight, cells cylindrical 5 ± 0.10 µm length, 4.5 ± 0.10 µm width; heterocyte intercalary, cylindrical 8.0 ± 1.89 7.5 ± 1.0 and heterocyte frequency was 6.06%.

Class- Cyanophyceae

Order- Nostocales

Family- Scytonemataceae

Genus - *Scytonema*

Species- sp.18ss

19. *Anabaena* sp. 19ss

Pl.no. 10; Fig. 19

Thallus green, gelatinous; trichome short, straight, parallel, 2.6 ± 0.30 µm broad; cells, spherical shaped, 5.0 ± 0.10 µm length, 5.0 ± 0.10 µm width; heterocyte hemispherical and intercalary, 6.5 ± 0.10 µm length and 5.8 ± 0.10 µm width and heterocyte was observed 8.11%.

Class- Cyanophyceae

Order- Nostocales

Family- Nostocaceae

Genus - *Anabaena*

Species - sp. 19ss

20. *Anabaena* sp. 20ss

Pl.no. 10; Fig. 20

Thallus gelatinous; trichome flexuous, constricted at the cross-walls; end cell conical, 4-5 µm broad; cells oblong shaped, 4 ± 0.10 µm length and 3 ± 0.10 µm width; heterocyte spherical 5 ± 0.10 µm broad and 5 ± 0.10 µm length; akinetes barrel shaped, 7.5 ± 0.25 µm length, 9.0 ± 0.35 µm width and heterocyte frequency was observed 3.33%.

Class- Cyanophyceae

Order- Nostocales

Family- Nostocaceae

Genus - *Anabaena*

Species - sp. 20ss

4.2.2 *Cluster Analysis based on morphological characters*

On the basis of morphological attributes, the constructed dendogram showed that cluster I and II (Figure. 2). Cluster I was divided into two sub cluster IA and IB, IA contained single cyanobacterial strain *Nostoc* sp. 9c, whereas sub-cluster IB was further divided into two sub clusters which comprise three cyanobacterial genera *Nostoc* sp. 9f, Nostoc sp. 9i and *Scytonema* sp. 12c.

Cluster II was further divided into two sub cluster IIA and IIB. Both sub-cluster was again divided into two sub sub clusters. Cluster IIA consisted of two sub cluster *i.e.* IIA1 enclosed the genera *Anabaena doliolum* 1a and IIA2 comprised of the genera *Nostoc* sp. 1d, *Nostoc* sp. 9ma1 and *Nostoc* sp. 9ma2. While cluster IIB further grouped into two sub clusters IIB1 and IIB2. Cluster IIB 1 comprised of the genera *Anabaena sphaerica* 1e, *Nostoc* sp. 4 and *Anabaena* sp. 2a.

Cluster IIB2 was further grouped into two and I group contained *Desmonostoc muscorum* 9a and *Tolypothrix* sp. 9k whereas the second group contained *Scytonema* sp.1f, *Anabaena* sp. 1n, *Nostoc* sp. 1h, *Nostoc* sp. 1c, *Nostoc* sp. 9j and *Anabaena* sp. 2a.

Similarly, the constructed dendrogram of the twenty cyanobacterial strains from the North hill regions of Chhattisgarh showed two major Clusters I and II. Cluster I contained two genera *Calothrix* sp. 13ss and *Anabaena* sp. 20ss**.** (Figure 3).

While cluster II was divided into two group IIA and IIB. Sub cluster IIB contained three cyanobacterial species *Aphanothece* sp. 4ss, *Aphanothece* sp. 5ss and *Aphanothece* sp.10ss while sub cluster IIA was further divided into two groups. Group IIA1 contained *Anabaena* sp. 7ss whereas the sub cluster IIA2 was again divided into groups and its comprised of the different cyanobacterial species *Scytonema*sp.1ss, *Anabaena* sp. 2ss, *Calothrix* sp. 3ss, *Scytonema* sp. 6ss, *Tolypothrix* sp. 8ss, *Anabaena* sp. 9ss, *Nostoc* sp. 11ss, *Anabaena* sp. 12ss, *Anabaena* sp. 14ss, *Anabaena* sp. 15ss, *Anabaena* sp. 16ss, *Nostoc* sp. 17ss, *Scytonema*sp.18ss, *Anabaena* sp. 19ss.

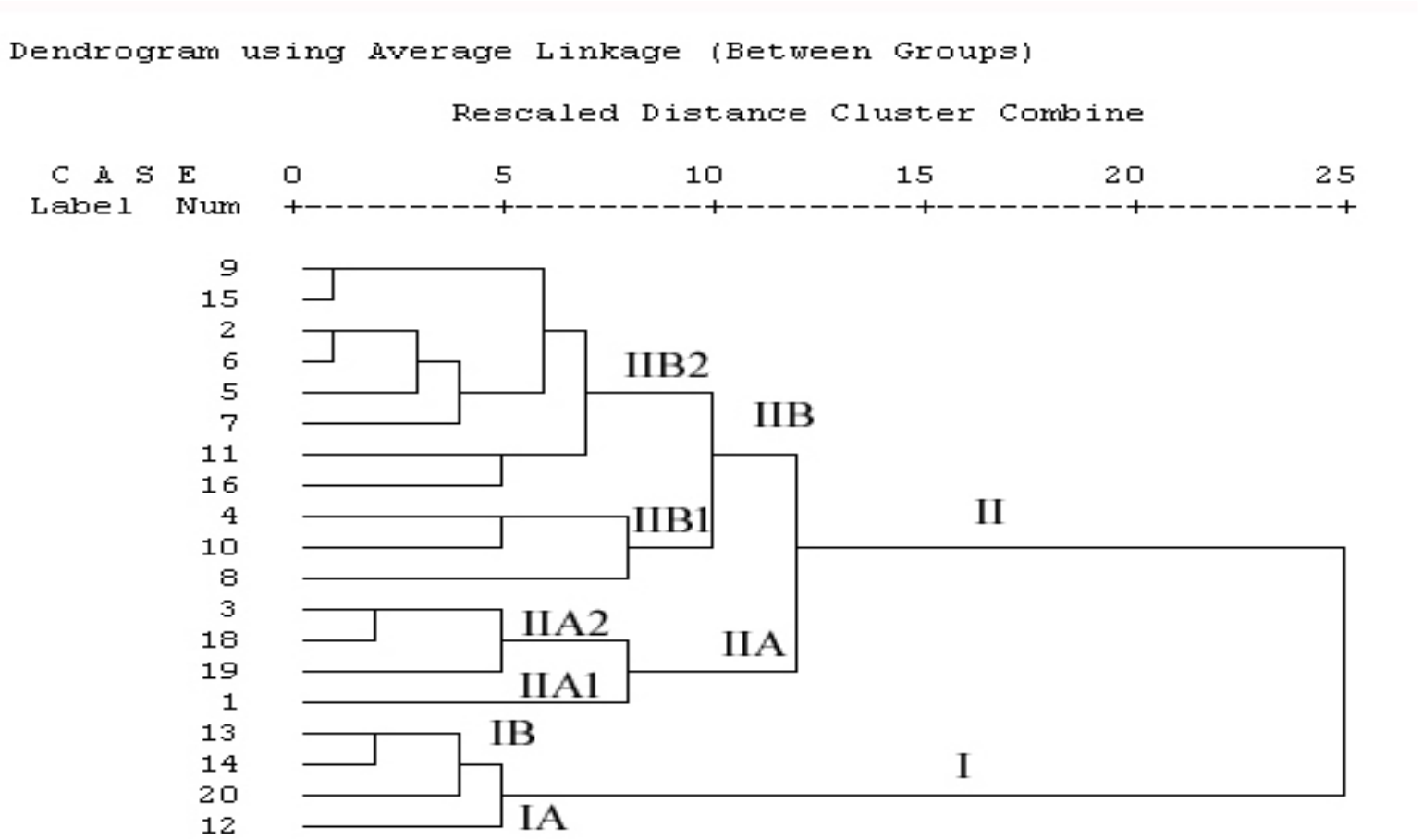

Figure 2. Cluster analysis of the twenty cyanobacterial strains isolated from Plains region based on the morphological attributes (1) *Anabaena doliolum* 1a (2) *Nostoc* sp. 1c (3) *Nostoc* sp. 1d (4) *Anabaena sphaerica* 1e (5) *Scytonema* sp. 1f (6) *Nostoc* sp. 1h (7*) Anabaena* sp. 1n (8) *Anabaena* sp. 2a (9) *Anabaena* sp. 2b (10) *Nostoc* sp. 4 (11) *Desmonostoc muscorum* 9a (12) *Nostoc* sp. 9c (13) *Nostoc* sp. 9f (14) *Nostoc* sp. 9i (15) *Nostoc* sp. 9j (16) *Tolypothrix* sp. 9k (17*) Nostoc* commune 9n (18) *Nostoc* sp. 9ma1 (19) *Nostoc* sp. 9ma2 (20) *Scytonema* sp. 12c

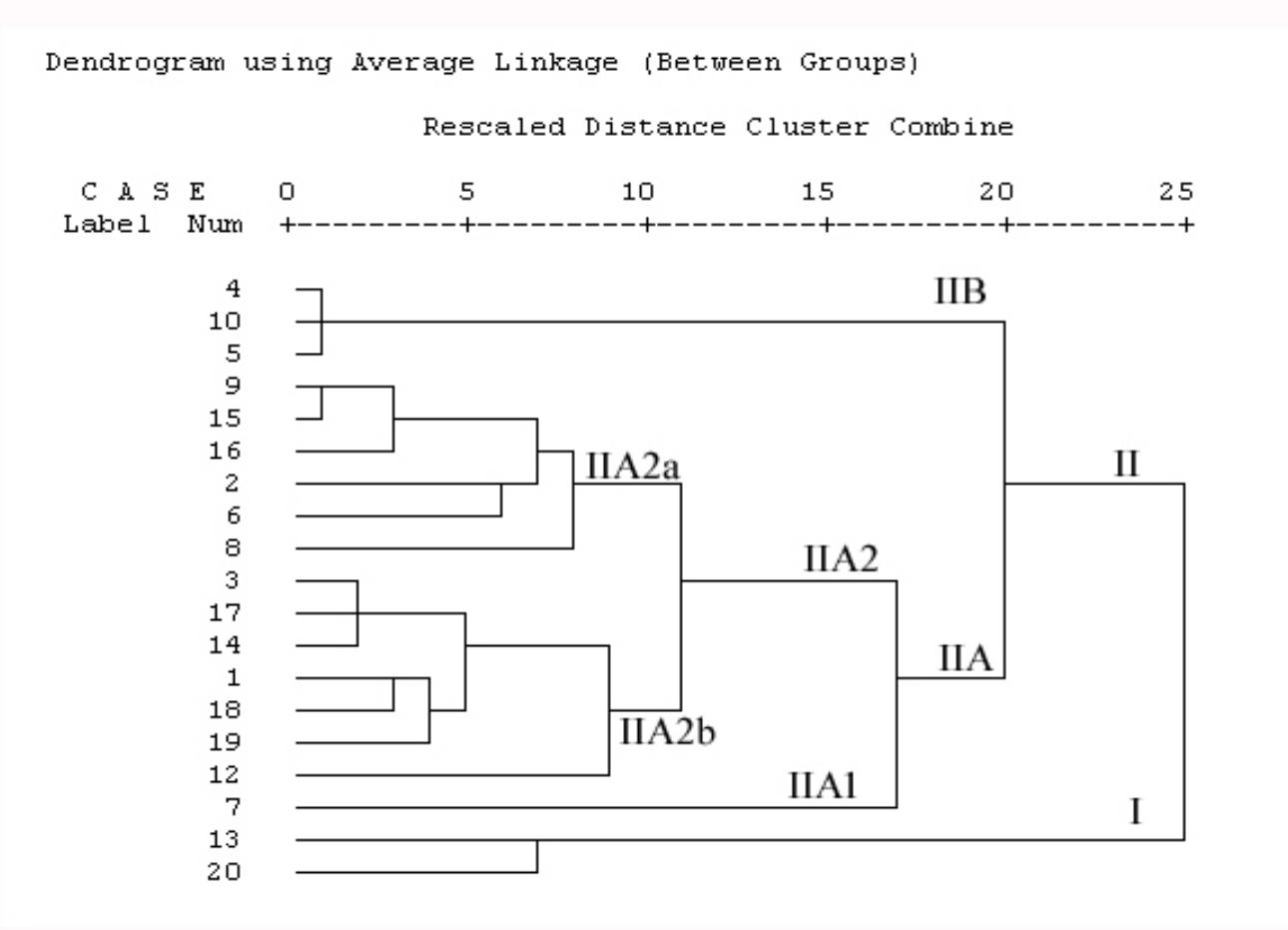

Figure 3. Cluster analysis of the twenty cyanobacterial species isolated from North hills region based on the morphological attributes (1) *Scytonema* sp. 1ss (2)*Anabaena* sp. 2ss (3) *Calothrix* sp. 3ss (4) *Aphanothece* sp. 4ss (5) *Aphanothece* sp. 5ss (6) *Scytonema* sp. 6ss (7*) Anabaena* sp. 7ss (8)*Tolypothrix* sp. 8ss (9) *Nostoc* sp. 9ss (10) *Aphanothece* sp. 10ss (11) *Anabaena* sp. 11ss (12) *Anabaena* sp. 12ss (13) *Calothrix* sp. 13ss (14) *Calothrix* sp. 14ss (15) *Anabaena* sp. 15ss (16) *Nostoc* sp. 16ss (17*) Nostoc* sp. 17ss (18) *Scytonema* sp.18ss (19) *Anabaena* sp. 19ss (20) *Anabaena* sp. 20ss

4.3 PHYSIOLOGICAL CHARACTERIZATION OF CYANOBACTERIA

4.3.1 G*rowth Pattern*

When the growth (in terms of Absorbance 663nm) was compared among different cyanobacterial strains isolated from plain region as well as North hills region at the different time interval (0, 2, 4, 6, 8, 10 and 12 days). It was clear from the data that the growth was rapidly increased from 2nd day and remained in the exponential phase upto 8th day to 12th day of incubation (Figure 4). When the growth was compared among the cyanobcaterial strains of plains region maximum growth was found in *Nostoc* sp. 9n while minimum was observed in *Nostoc* sp. 9ma2.

After observing maximum growth in *Nostoc* sp. 9n, *Tolypothrix* sp. 9k followed growth then *Desmonostoc muscorum* 9a, *Anabaena doliolum* 1a, *Anabaena* sp. 2a, *Anabaena* sp.1n, *Nostoc* sp. 4, *Scytonema* sp. 12c, *Anabaena sphaerica* 1e, *Scytonema* sp.1f, *Nostoc* sp. 9f, *Nostoc* sp. 9c, *Nostoc* sp. 1c, *Nostoc* sp. 9i, *Nostoc* sp. 1h and *Nostoc* sp. 9ma1.

Similarly, the maximum growth was found in *Anabaena* sp. 19ss while minimum growth was *Nostoc* sp. 17ss observed among the cyanobacterial strain isolated from North Hill regions of Chhattisgarh (Figure 5).

Further, growth pattern was compared in other cyanobacterial strains isolated from North hills. *Scytonema* sp. 1ss showed the maximum growth pattern followed by *Anabaena* sp. 16ss, *Anabaena* sp. 2ss, *Anabaena* sp. 7ss, *Aphanothece* sp. 5ss, *Tolypothrix* sp. 8ss, *Aphanothece* sp. 4ss, *Anabaena* sp. 11ss, *Anabaena* sp. 20ss, *Calothrix* sp. 1ss, *Calothrix* sp. 3ss, *Calothrix* sp. 14ss, *Nostoc* sp. 9ss, *Nostoc* sp. 17ss, *Aphanothece* sp. 10ss, *Anabaena* sp. 12ss, *Scytonema* sp. 6ss and *Anabaena* sp. 15ss.

4.3.2 *Chlorophyll a content*

Chlorophyll a content was varied among different cyanobacterial strains isolated from plains region of Chhattisgarh at different time interval (Figure 6). On

the second day of incubation the maximum was found in *Tolypothrix* sp. 9k (3.15 µg ml^{-1}) followed by *Nostoc* sp. 4 (2.26 µg ml^{-1}), *Nostoc* sp. 9c (2.23 µg ml^{-1}), *Nostoc* sp. 1h (2.11 µg ml^{-1}), *Anabaena* sp. 2b (1.00 µg ml^{-1}), *Nostoc commune* 9n (1.37µg ml^{-1}), *Nostoc* sp. 9j (1.03 µg ml^{-1}), *Anabaena* sp. 2a (0.995 µg ml^{-1}), *Anabaena sphaerica* 1e (0.82 µg ml^{-1}), *Nostoc* sp. 9ma2 (0.66 µg ml^{-1}), *Scytonema* sp. 12c (0.68 µg ml^{-1}), *Nostoc* sp. 9i (0.66 µg ml^{-1}), *Anabaena* sp. 1n (0.63µg ml^{-1}), *Anabaena doliolum* 1a (0.66 µg ml^{-1}), *Desmonostoc muscorum* 9a (0.50µg ml^{-1}), *Nostoc* sp. 1d (0.48 µg ml^{-1}), *Nostoc* sp. 1c (0.44 µg ml^{-1}), *Nostoc* sp. 9f (0.36 µg ml^{-1}) and *Nostoc* sp. 9ma1 (0.30 µg ml^{-1}) while minimum was observed in *Scytonema* sp. 1f (0.22 µg ml^{-1}).

Maximum chlorophyll a content was found in *Nostoc* sp. 9i (3.98 µg ml^{-1}) followed by *Nostoc* sp.1h (3.2 µg ml^{-1}) and followed by *Anabaena* sp. 2b (2.08 µg ml^{-1}), *Nostoc* sp. 4 (2.20 µg ml^{-1}), *Tolypothrix* sp. 9k (2.02 µg ml^{-1}), *Nostoc* sp. 9ma2 (1.95 µg ml^{-1}), *Nostoc* sp. 9c (1.85µg ml^{-1}), *Anabaena sphaerica* 1e (1.28 µg ml^{-1}), *Scytonema* sp. 12c (1.03µg ml^{-1}), *Nostoc commune* 9n (1.50 µg ml^{-1}), *Anabaena* sp. 2a (0.99µg ml^{-1}), *Anabaena* sp. 1n (0.71µg ml^{-1}), *Desmonostoc muscorum* 9a (0.78 µg ml^{-1}), *Nostoc* sp. 9f (0.76µg ml^{-1}), *Nostoc* sp. 9j (0.75 µg ml^{-1}), *Nostoc* sp. 1d (0.70 µg ml^{-1}), *Nostoc* sp. 9ma1 (0.64 µg ml^{-1}), *Anabaena doliolum* 1a (0.69 µg ml^{-1}), *Nostoc* sp.1c (0.68 µg ml^{-1}) and *Scytonema* sp. 1f (0.475 µg ml^{-1}) on the 4^{th} day of incubation.

A chlorophyll content was reported to be maximum in *Nostoc* sp. 9i (4.22 µg ml^{-1}) and followed by *Nostoc* sp. 9ma2 (3.30 µg ml^{-1}), *Nostoc* sp. 1h (3.05µg ml^{-1}), *Tolypothrix* sp. 9k (2.79 µg ml^{-1}), *Nostoc commune* 9n (2.82 µg ml^{-1}), *Anabaena* sp. 2b (2.30 µg ml^{-1}), *Anabaena sphaerica* 1e (1.85 µg ml^{-1}), *Nostoc* sp. 4 (1.72 µg ml^{-1}), *Desmonostoc muscorum* 9a (1.29 µg ml^{-1}), *Anabaena* sp. 2a (1.49 µg ml^{-1}), *Nostoc* sp. 9c (1.03 µg ml^{-1}), *Nostoc* sp. 9f (1.2 µg ml^{-1}), *Anabaena doliolum* 1a (1.79 µg ml^{-1}), *Nostoc* sp. 9ma1(1.71 µg ml^{-1}), *Scytonema* sp. 12c (1.718 µg ml^{-1}), *Nostoc* sp. 1c (1.027 µg ml^{-1}), *Nostoc* sp. 1d (1.41 µg ml^{-1}), *Scytonema* sp. 1f (0.82 µg ml^{-1}) and *Anabaena* sp. 1n (0.84 µg ml^{-1}) where as the minimum was found in *Nostoc* sp. 9j (0.76) on the 6^{th} day of incubation.

The pattern of increment in chlorophyll a content was varied on 8th day among the tested cyanobacterial strains. The maximum was found in *Nostoc* sp. 9i (6.18 µg ml^{-1}) followed by *Anabaena doliolum* 1a (3.08 µg ml^{-1}), *Nostoc* sp. 1h (3.0 µg ml^{-1}), *Tolypothrix* sp. 9k (2.73 µg ml^{-1}), *Scytonema* sp. 12c (2.48 µg ml^{-1}), *Nostoc* sp. 9ma2 (2.14 µg ml^{-1}), *Nostoc* sp. 9c (2.18 µg ml^{-1}), *Anabaena* sp. 2a (2.10 µg ml^{-1}), *Anabaena sphaerica* 1e (2.18 µg ml^{-1}), *Nostoc* sp. 4 (1.84 µg ml^{-1}), *Nostoc* sp. 9f (1.18 µg ml^{-1}), *Nostoc commune* 9n (1.62µg ml^{-1}), *Nostoc* sp. 9ma1(1.50 µg ml^{-1}), *Anabaena* sp. 2b (1.32 µg ml^{-1}), *Nostoc* sp. 1d (1.30 µg ml^{-1}), *Scytonema* sp. 1f (1.11 µg ml^{-1}), *Anabaena* sp. 1n (1.095 µg ml^{-1}), *Desmonostoc muscorum* 9a (1.120 µg ml^{-1}), *Nostoc* sp. 9j (0.824 µg ml^{-1}) and *Nostoc* sp. 1c (0.650 µg ml^{-1}).

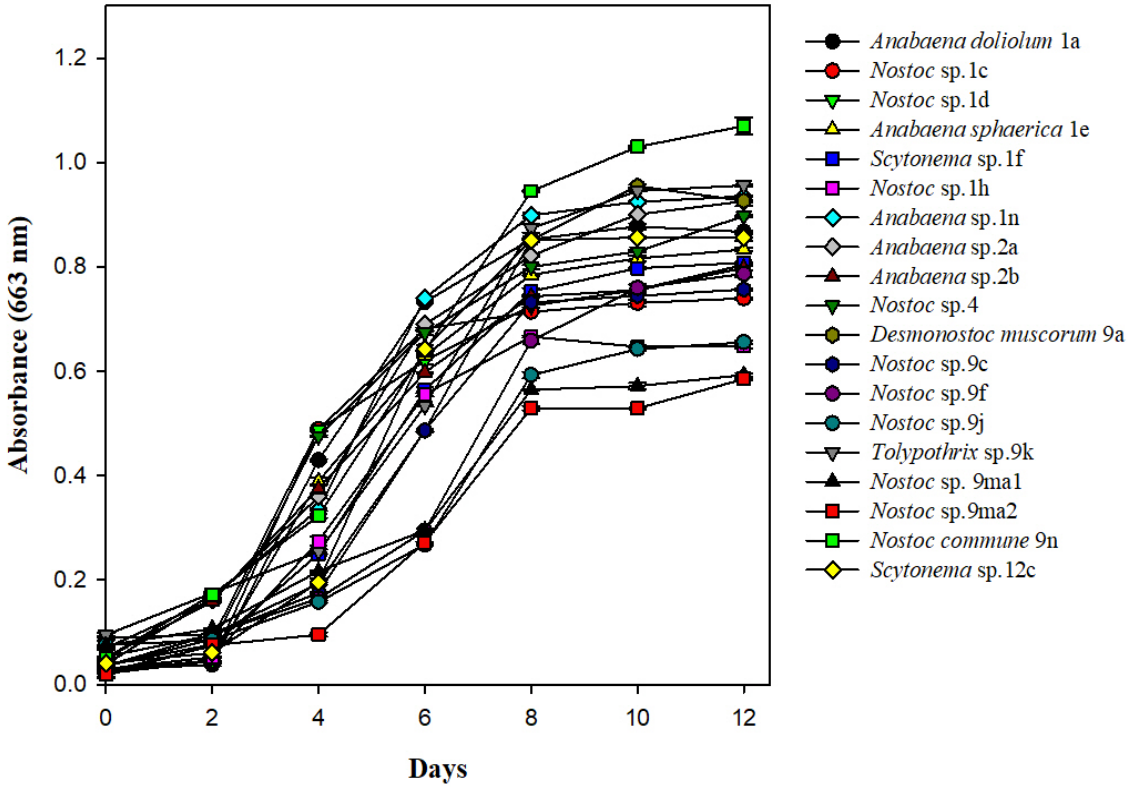

Figure 4. Growth curve (In terms of OD) of different cyanobacterial strains isolated from plain regions of Chhattisgarh.

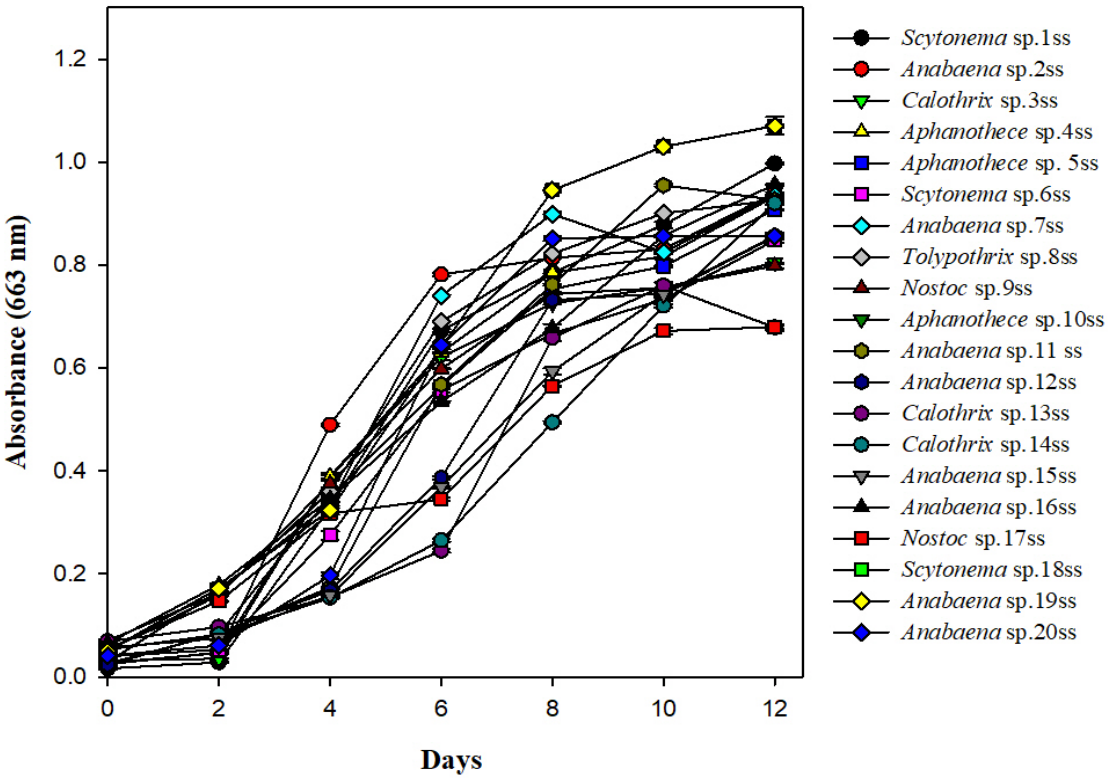

Figure 5. Growth curve (in terms of OD) of different cyanobacterial strains isolated from North Hill regions of Chhattisgarh.

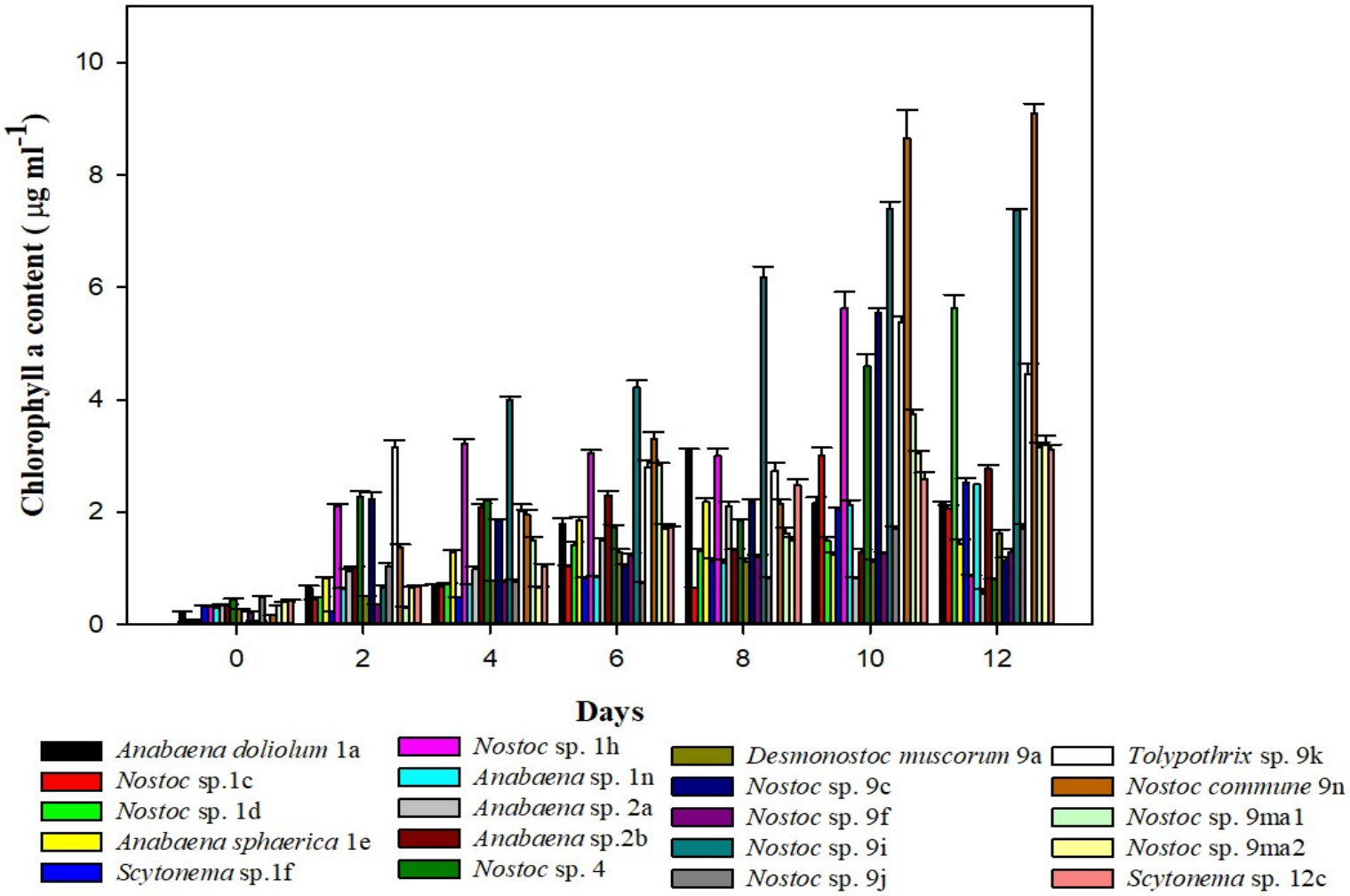

Figure 6. Chlorophyll a content of twenty cyanobacterial strains isolated from plain regions of Chhattisgarh

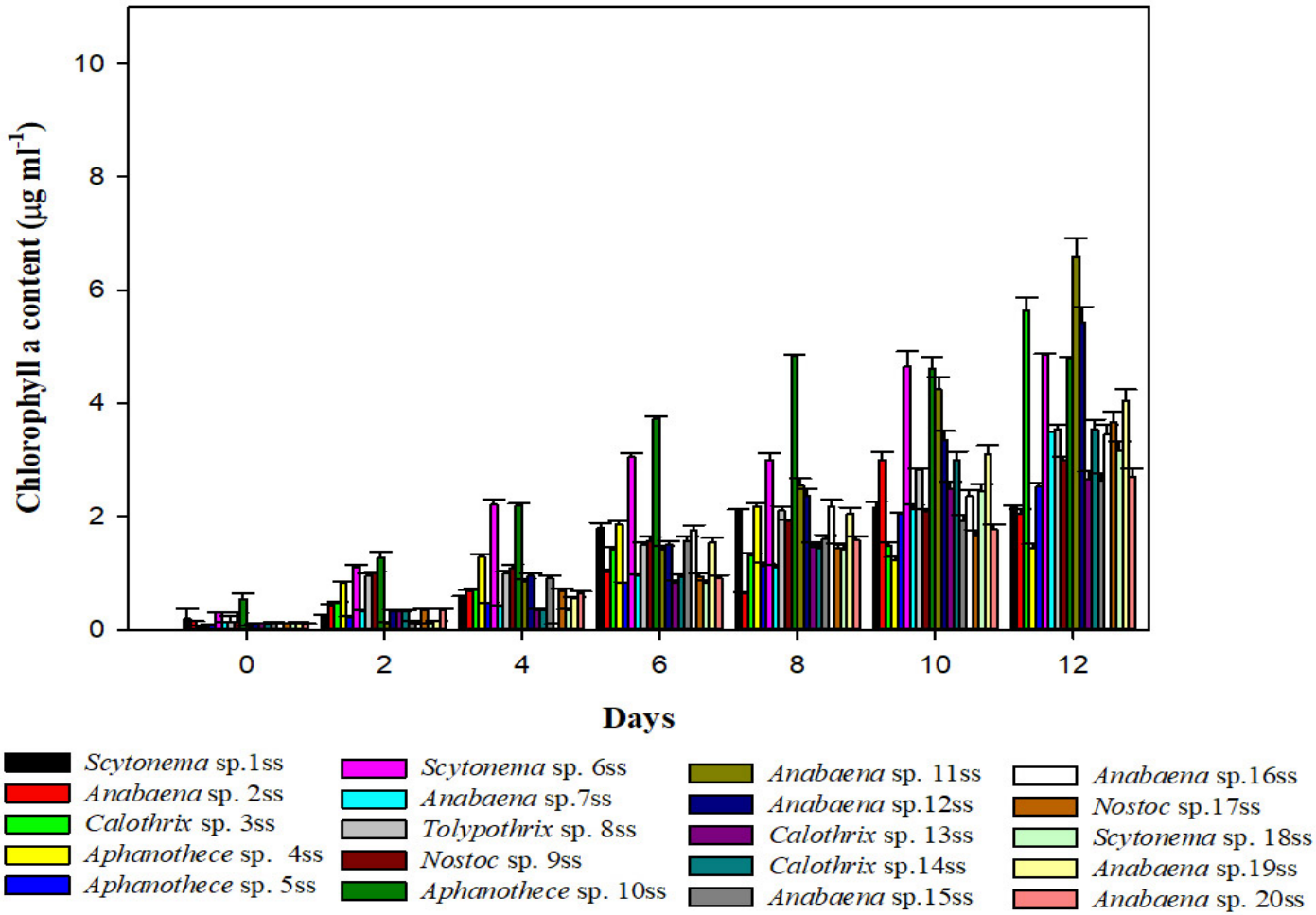

Figure 7. Chlorophyll a content of twenty cyanobacterial strains isolated from North Hills region of Chhattisgarh

While on 10th day, the maximum chlorophyll a content was found in *Nostoc commune* 9n (8.66 µg ml^{-1}) followed *Nostoc* sp. 9i (7.39 µg ml^{-1}) followed by *Nostoc* sp.1h (5.640 µg ml^{-1}), *Nostoc* sp.1c (5.55 µg ml^{-1}), *Tolypothrix* sp. *9k* (4.63 µg ml^{-1}), *Nostoc* sp. 9ma1 (3.75 µg ml^{-1}), *Nostoc* sp. 9ma2 (3.06 µg ml^{-1}), *Nostoc* sp. 4 (2.60 µg ml^{-1}), S*cytonema* sp. 12c (2.59 µg ml^{-1}), *Anabaena* sp. 1n (2.4 µg ml^{-1}), *Nostoc* sp. 9j (1.70 µg ml^{-1}), *Desmonostoc muscorum* 9a (1.62 µg ml^{-1}), *Nostoc* sp. 1d (1.49 µg ml^{-1}), *Anabaena* sp. 2b (1.285 µg ml^{-1}), *Anabaena sphaerica 1e* (1.234 µg ml^{-1}), *Nostoc* sp. 9c (1.14µg ml^{-1}), *Scytonema* sp. 1f (2.05µg ml^{-1}), *Nostoc* sp. 9f (1.2 µg ml^{-1}), *Anabaena doliolum* 1a (2.10 µg ml^{-1}) and *Anabaena* sp. 2a (0.538 µg ml^{-1}).

On the final day the maximum chlorophyll a content was observed in *Nostoc commune* 9n (9.09 µg ml^{-1}) followed by *Nostoc* sp. 9i (7.38 µg ml^{-1}), *Nostoc* sp. 1d (5.63µg ml^{-1}), *Tolypothrix* sp. 9k (4.46 µg ml^{-1}), *Nostoc* sp. 9ma2 (3.19 µg ml^{-1}), *Nostoc* sp. 9ma1 (3.15 µg ml^{-1}), *Nostoc* sp. 4 (3.11 µg ml^{-1}), *Scytonema* sp. 12c (3.11 µg ml^{-1}), *Anabaena* sp. 2b (2.78µg ml^{-1}), *Scytonema* sp. 1f (2.52 µg ml^{-1}) *Anabaena* sp. 1n (2.499 µg ml^{-1}), *Anabaena doliolum* 1a (2.16 µg ml^{-1}), *Nostoc* sp. 1c (2.05 µg ml^{-1}), *Anabaena sphaerica* 1e (1.43 µg ml^{-1}), *Desmonostoc muscorum* 9a (1.62 µg ml^{-1}), *Nostoc* sp. 9c (1.14 µg ml^{-1}), *Nostoc* sp. 9f (1.28 µg ml^{-1}), *Nostoc* sp. 9j (1.71 µg ml^{-1}), *Nostoc* sp. 1h (0.856 µg ml^{-1}) and *Anabaena* sp. 2a (0.538 µg ml^{-1}).

Similarly, all cyanobacteria strains isolated from North hill regions showed a similar pattern and results revealed that the maximum chlorophyll content was found in *Aphanothece* sp. 10ss (1.26 µg ml^{-1}) and followed by *Scytonema* sp. 6ss (1.110 µg ml^{-1}), *Tolypothrix* sp. 8ss (0.958 µg ml^{-1}), *Aphanothece* sp. 4ss (0.824 µg ml^{-1}), *Anabaena* sp. 2ss (0.442 µg ml^{-1}), *Calothrix* sp. 3ss (0.482 µg ml^{-1}), *Nostoc* sp. 17ss (0.354 µg ml^{-1}), *Anabaena* sp. 20ss (0.350 µg ml^{-1}), *Anabaena* sp. 7ss (0.338 µg ml^{-1}), *Calothrix* sp. 14ss (0.338 µg ml^{-1}), *Anabaena* sp. 12ss (0.338 µg ml^{-1}), *Calothrix* sp. 13ss (0.318 µg ml^{-1}), *Scytonema* sp. 1ss (0.239 µg ml^{-1}), *Aphanothece* sp. 5ss (0.22 µg ml^{-1}), *Anabaena* sp. 15ss (0.15 µg ml^{-1}), *Anabaena* sp. 19ss (0.149 µg ml^{-1}), *Nostoc* sp. 9ss (1.00 µg ml^{-1}), *Anabaena* sp. 11ss (0.127 µg ml^{-1}), *Nostoc* sp. 16ss

(0.105 µg ml^{-1}) and *Scytonema* sp.18ss (0.120 µg ml^{-1}) on 2nd day of incubation (Figure 7).

On the 4th day incubation, maximum chlorophyll a content was found in *Scytonema* sp. 6ss (2.21 µg ml^{-1}) and followed by *Aphanothece* sp. 10ss (2.20 µg ml^{-1}), *Nostoc* sp. 9ss (1.08 µg ml^{-1}), *Aphanothece* sp. 4ss (1.28 µg ml^{-1}), *Tolypothrix* sp. 8ss (0.99 µg ml^{-1}), *Nostoc* sp. 17ss (0.945 µg ml^{-1}), *Anabaena* sp.12ss (0.949 µg ml^{-1}), *Anabaena* sp. 15ss (0.906 µg ml^{-1}), *Anabaena* sp. 11ss (0.860 µg ml^{-1}), *Calothrix* sp. 3ss (0.708 µg ml^{-1}), *Anabaena* sp. 2ss (0.68 µg ml^{-1}), *Anabaena* sp. 20ss (0.644 µg ml^{-1}), *Scytonema* sp.1ss (0.578 µg ml^{-1}), *Anabaena* sp. 19ss (0.540 µg ml^{-1}), *Aphanothece* sp. 5ss (0.47 µg ml^{-1}), *Anabaena* sp. 7ss (0.412 µg ml^{-1}), *Calothrix* sp. 13ss (0.349 µg ml^{-1}), *Calothrix* sp. 14ss (0.349 µg ml^{-1}), *Scytonema* sp. 18ss (0.34 µg ml^{-1}) and *Nostoc* sp. 16ss (0.108 µg ml^{-1}).

As the culture was incubated for 6th day, maximum chlorophyll a content was found in *Aphanothece* sp. 10ss (3.72 µg ml^{-1}) followed by *Scytonema* sp. 6ss (3.053 µg ml^{-1}), *Aphanothece* sp. 4ss (1.8543 µg ml^{-1}), *Scytonema* sp.1ss (1.79µg ml^{-1}), *Nostoc* sp. 16ss (1.75 µg ml^{-1}), *Nostoc* sp. 9ss (1.56 µg ml^{-1}), *Anabaena* sp. 15ss (1.564 µg ml^{-1}), *Tolypothrix* sp. 8ss (1.49 µg ml^{-1}), *Calothrix* sp.3ss (1.41 µg ml^{-1}), *Anabaena* sp. 2ss (1.027 µg ml^{-1}), *Anabaena* sp.19ss (1.544 µg ml^{-1}), *Anabaena* sp. 12ss (1.49 µg ml^{-1}), *Anabaena* sp. 11ss (1.410 µg ml^{-1}), *Nostoc* sp.17ss (0.945 µg ml^{-1}), *Calothrix* sp. 14ss (0.939 µg ml^{-1}), *Anabaena* sp. 20ss (0.915 µg ml^{-1}) *Anabaena* sp. 7ss (0.949 µg ml^{-1}), *Calothrix* sp. 13ss (0.839 µg ml^{-1}), *Scytonema* sp.18ss (0.839 µg ml^{-1}) and *Aphanothece* sp. 5ss (0.829 µg ml^{-1}).

On the 8th day of incubation, almost equal increment was observed in chlorophyll a content as observed in 6th day incubated cultures. The chlorophyll content was maximum in *Aphanothece* sp.10ss (4.84 µg ml^{-1}), *Scytonema* sp. 6ss (3.00 µg ml^{-1}), *Anabaena* sp. 11ss (2.540 µg ml^{-1}), *Anabaena* sp.12ss (2.37 µg ml^{-1}), *Aphanothece* sp. 4ss (2.180 µg ml^{-1}), *Nostoc* sp. 16ss (2.181 µg ml^{-1}), *Tolypothrix* sp. 8ss (2.101 µg ml^{-1}), *Scytonema* sp.1ss (2.08 µg ml^{-1}), *Anabaena* sp. 19ss (2.05 µg ml^{-1}), *Nostoc* sp. 9ss (1.92 µg ml^{-1}), *Anabaena* sp.20ss (1.5762 µg ml^{-1}), *Calothrix* sp.

13ss (1.465 µg ml^{-1}), *Nostoc* sp. 17ss (1.443 µg ml^{-1}) *Calothrix* sp. 14ss (1.432 µg ml^{-1}), *Scytonema* sp.18ss (1.42 µg ml^{-1}), *Anabaena* sp. 15ss (1.601 µg ml^{-1}), *Calothrix* sp. 3ss (1.30 µg ml^{-1}), *Aphanothece* sp.5ss (1.119 µg ml^{-1}), *Anabaena* sp. 7ss (1.095 µg ml^{-1}) and *Anabaena* sp. 2ss (0.650 µg ml^{-1}).

When chlorophyll a content was compared among the cyanobacterial strains on 10^{th} day, the maximum was found in *Aphanothece* sp. 10ss (4.601 µg ml^{-1}) followed by *Scytonema* sp. 6ss (4.649 µg ml^{-1}), *Anabaena* sp. 11ss (4.246 µg ml^{-1}), *Anabaena* sp. 12ss (3.99 µg ml^{-1}), *Anabaena* sp. 19ss (3.10 µg ml^{-1}), *Anabaena* sp. 2ss (3.00 µg ml^{-1}), *Tolypothrix* sp. 8ss (2.82 µg ml^{-1}), *Nostoc* sp. 16ss (2.35 µg ml^{-1}), *Nostoc* sp. 9ss (2.08 µg ml^{-1}), *Scytonema* sp. 18ss (2.44 µg ml^{-1}), *Calothrix* sp. 14ss (2.49 µg ml^{-1}), *Calothrix* sp. 13ss (2.49µg ml^{-1}), *Aphanothece* sp. 5ss (2.05 µg ml^{-1}), *Anabaena* sp. 7ss (2.12 µg ml^{-1}), *Scytonema* sp. 1ss (2.16 µg ml^{-1}), *Anabaena* sp. 15ss (1.93 µg ml^{-1}), *Calothrix* sp. 3ss (1.49 µg ml^{-1}), *Anabaena* sp. 20ss (1.77 µg ml^{-1}), *Nostoc* sp. 17ss (1.67 µg ml^{-1}) and *Aphanothece* sp. 4ss (1.23 µg ml^{-1}).

Finally maximum chlorophyll a was found in *Calothrix* sp. 3ss (5.632 µg ml^{-1}) and followed by *Anabaena* sp. 11ss (5.43 µg ml^{-1}), *Anabaena* sp. 12ss (5.432 µg ml^{-1}), *Scytonema* sp. 6ss (4.85 µg ml^{-1}), *Aphanothece* sp. 10ss (4.79 µg ml^{-1}), *Anabaena* sp. 19ss (4.043 µg ml^{-1}), *Nostoc* sp. 17ss (3.665 µg ml^{-1}), *Tolypothrix* sp. 8ss (3.538 µg ml^{-1}) *Calothrix* sp. 14ss (3.537 µg ml^{-1}), *Anabaena* sp. 7ss (3.49 µg ml^{-1}), *Nostoc* sp. 16ss (3.446 µg ml^{-1}), *Scytonema* sp.18ss (3.169 µg ml^{-1}), *Nostoc* sp. 9ss (2.98 µg ml^{-1}), *Anabaena* sp. 20ss (2.70 µg ml^{-1}), *Anabaena* sp. 15ss (2.629 µg ml^{-1}) *Calothrix* sp. 13ss (2.661µg ml^{-1}), *Aphanothece* sp.5ss (2.52 µg ml^{-1}), *Scytonema* sp.1ss (2.160 µg ml^{-1}) and *Anabaena* sp.2ss (2.058 µg ml^{-1}). Minimum was found in *Aphanothece* sp. 4ss (1.43 µg ml^{-1}) as compared to the other cyanobacterial strains.

4.3.3 *Carotenoid content*

Figure 8 showed that the Carotenoid content was going on increased at the different time interval of tested cyanobacterial strains. Initially, the Carotenoid content was almost similar in all the cyanobacteria strains isolated from plains region of Chhattisgarh.

On the 2nd day of incubation the maximum carotenoid content was found in *Anabaena* sp. 2b (40.4 µg ml^{-1}) followed by *Anabaena doliolum* 1a (30.3 µg ml^{-1}), *Nostoc* sp. 9i (33.3 µg ml^{-1}), *Anabaena sphaerica* 1e (29.84 µg ml^{-1}), *Anabaena* sp. 2a (28.1 µg ml^{-1}), *Nostoc* commune 9n (25.7 µg ml^{-1}), *Anabaena* sp. 1n (18.6 µg ml^{-1}), *Nostoc* sp. 9ma1(16.1 µg ml^{-1}), *Nostoc* sp. 9c (15.7 µg ml^{-1}), *Tolypothrix* sp. 9k (12.5 µg ml^{-1}), *Nostoc* sp. 9j (11.6 µg ml^{-1}), *Desmonostoc muscorum* sp. 9a (11.3 µg ml^{-1}), *Nostoc* sp. 4 (11.2 µg ml^{-1}), *Nostoc* sp. 1d (10.75 µg ml^{-1}), *Nostoc* sp. 9f (9.4 µg ml^{-1}), *Nostoc* sp. 1c (8.97 µg ml^{-1}), *Scytonema* sp. 12c(7.31 µg ml^{-1}), *Nostoc* sp. 9ma2(7.2 µg ml^{-1}), *Nostoc* sp. 1h (6.6 µg ml^{-1}) and *Scytonema* sp. 1f (4.36 µg ml^{-1}).

On the 4th day of incubation the Carotenoid content was also increased as compared to the 2nd day incubated cyanobacterial strains. Maximum carotenoid content was observed in *Anabaena* sp. 2b (52.16 µg ml^{-1}), *Anabaena* sp. 1n (37.9 µg ml^{-1}), *Anabaena* sp. 2a (34.6 µg ml^{-1}), *Nostoc* sp. 4 (32.0 µg ml^{-1}), *Nostoc commune* 9n (30.1 µg ml^{-1}), *Scytonema* sp. 1f (30.6 µg ml^{-1}), *Anabaena sphaerica* 1e (29.36 µg ml^{-1}), *Nostoc* sp. 9c (27.9 µg ml^{-1}), *Nostoc* sp. 9ma1 (26.7 µg ml^{-1}), *Desmonostoc muscorum* sp. 9a (20.2 µg ml^{-1}), *Nostoc* sp. 9f (19.2 µg ml^{-1}), *Nostoc* sp. 1h (17.30 µg ml^{-1}), *Tolypothrix* sp. 9k (16.4 µg ml^{-1}), *Nostoc* sp. 9i (15.6 µg ml^{-1}), *Nostoc* sp. 9ma2 (13.3 µg ml^{-1}), *Anabaena doliolum* 1a (11.3 µg ml^{-1}), *Nostoc* sp. 9j (12.5 µg ml^{-1}), *Scytonema* sp. 12c (12.5 µg ml^{-1}), *Nostoc* sp. 1d (9.22 µg ml^{-1}) and *Nostoc* sp. 1c (3.31 µg ml^{-1}).

The maximum carotenoid content was observed in *Nostoc* sp. 9j (89.1 µg ml^{-1}) followed by *Nostoc* commune 9n (56.3 µg ml^{-1}), *Anabaena* sp. 1n (39.1 µg ml^{-1}), *Nostoc* sp. 9c (39.1 µg ml^{-1}), *Nostoc* sp. 4 (39.7 µg ml^{-1}), *Anabaena* sp. 2a (38.2 µg ml^{-1}), *Anabaena* sp. 2b (38.1 µg ml^{-1}), *Nostoc* sp. 1c (37.4 µg ml^{-1}), *Nostoc* sp. 9ma1 (30.3 µg ml^{-1}), *Nostoc* sp. 1d (33.4 µg ml^{-1}), *Anabaena sphaerica* 1e (33.1 µg ml^{-1}), *Scytonema* sp. 1f (31.3 µg ml^{-1}), *Desmonostoc muscorum* sp. 9a (24.7 µg ml^{-1}), *Nostoc* sp. 9f (23.6 µg ml^{-1}), *Nostoc* sp. 9i (19.5 µg ml^{-1}), *Tolypothrix* sp. 9k (19.6 µg ml^{-1}), *Anabaena doliolum* 1a (18.1 µg ml^{-1}), *Nostoc* sp. 1h (18.8 µg ml^{-1}), *Nostoc* sp.

9ma2 (16.0 µg ml^{-1}) an 4^{th} day of incubation. Minimum was found in *Scytonema* sp. 12c (14.6 µg ml^{-1}).

on the 8^{th} day the maximum carotenoid content was observed in *Nostoc* sp. 9j (98.6 µg ml^{-1}) followed by *Desmonostoc muscorum* sp. 9a (48.3 µg ml^{-1}), *Anabaena* sp. 2a (45.03 µg ml^{-1}), *Scytonema* sp. 1f (44.7 µg ml^{-1}), *Nostoc* commune 9n (43.4 µg ml^{-1}), *Nostoc* sp. 4 (40.93 µg ml^{-1}), *Nostoc* sp. 1c (39.9 µg ml^{-1}), *Nostoc* sp. 9ma1 (39.8 µg ml^{-1}), *Anabaena* sp. 2b (39.2 µg ml^{-1}), *Nostoc* sp. 9c (39.4 µg ml^{-1}), *Nostoc* sp. 9ma2 (37.51 µg ml^{-1}), *Anabaena sphaerica* 1e (37.4 µg ml^{-1}), *Nostoc* sp. 9i (35.14 µg ml^{-1}), *Anabaena* sp. 1n (31.0), *Nostoc* sp. 9f (29.9 µg ml^{-1}), *Nostoc* sp. 1h (29.8 µg ml^{-1}), *Nostoc* sp. 1d (26.6 µg ml^{-1}), *Anabaena doliolum* 1a (24.8 µg ml^{-1}), *Tolypothrix* sp. 9k (19.4 µg ml^{-1}) and *Scytonema* sp. 12c (17.2 µg ml^{-1}).

From the 10^{th} to 12^{th} both day, Maximum carotenoid content was observed in *Anabaena* sp. 2b (78.07 µg ml^{-1}) followed by *Nostoc* sp. 9j (68.6 µg ml^{-1}), *Nostoc* commune 9n (76.3 µg ml^{-1}), *Anabaena doliolum* 1a (65.13 µg ml^{-1}), *Scytonema* sp. 12c (53.46 µg ml^{-1}), *Desmonostoc muscorum* sp. 9a (51.6 µg ml^{-1}), *Scytonema* sp. 1f (47.8 µg ml^{-1}), *Nostoc* sp. 4 (46.6 µg ml^{-1}), *Anabaena sphaerica* 1e (46.40 µg ml^{-1}), *Nostoc* sp. 1c (46.3 µg ml^{-1}), *Nostoc* sp. 9c (43.88 µg ml^{-1}), *Nostoc* sp. 9i (41.43 µg ml^{-1}), *Anabaena* sp. 1n (39.57 µg ml^{-1}), *Anabaena* sp. 2a (39.3 µg ml^{-1}), *Tolypothrix* sp. 9k (35.3 µg ml^{-1}), *Nostoc* sp. 1h (32.70 µg ml^{-1}), *Nostoc* sp. 9ma2 (31.52 µg ml^{-1}), *Nostoc* sp. 1d (30.5 µg ml^{-1}), *Nostoc* sp. 9ma1(30.2 µg ml^{-1}) and minimum was found in *Nostoc* sp. 9f (23.85 µg ml^{-1}).

Similarly on 12^{th} day of incubation, maximum carotenoid content was found in *Anabaena* sp. 2b (115.83 µg ml^{-1}) followed by *Nostoc commune* 9n (114.26 µg ml^{-1}), *Nostoc* sp. 1d (107.32 µg ml^{-1}), *Anabaena* sp. 2a (103.6 µg ml^{-1}), *Nostoc* sp. 4 (102.95 µg ml^{-1}), *Nostoc* sp. 9c (100.24 µg ml^{-1}), *Scytonema* sp.1f (83.06 µg ml^{-1}), *Anabaena sphaerica* 1e (82.96 µg ml^{-1}), *Desmonostoc muscorum* sp. 9a (81.80 µg ml^{-1}), *Scytonema* sp. 12c (68.5 µg ml^{-1}), *Nostoc* sp. 9j (67.0 µg ml^{-1}), *Nostoc* sp. 9i (59.6 µg ml^{-1}), *Nostoc* sp. 9ma2 (58.07 µg ml^{-1}), *Nostoc* sp. 1h (45.74 µg ml^{-1}), *Anabaena* sp. 1n (41.37 µg ml^{-1}), *Anabaena doliolum* 1a (40.3 µg ml^{-1}), *Tolypothrix* sp. 9k

(40.89 µg ml^{-1}), *Nostoc* sp. 1c (39.69 µg ml^{-1}), *Nostoc* sp. 9f (33.9 µg ml^{-1}). Minimum carotenoid was observed in *Nostoc* sp. 9ma1 (20.42 µg ml^{-1}).

Similar trend was also observed in the isolated cyanobacterial strains from north hills of Chhattisgarh**.** (Figure 9). At Initial day, all the cyanobacterial strains confined the same carotenoid content at the different time interval. Maximum carotenoid content was found in *Nostoc* sp. 9ss (40.49 µg ml^{-1}) followed by *Calothrix* sp. 14ss (33.34 µg ml^{-1}), *Scytonema* sp.1ss (30.37 µg ml^{-1}), *Aphanothece* sp. 4ss (29.85 µg ml^{-1}), *Tolypothrix* sp. 8ss (28.1 µg ml^{-1}), *Nostoc* sp. 17ss (25.72 µg ml^{-1}), *Anabaena* sp. 7ss (18.61 µg ml^{-1}), *Scytonema* sp.18ss (16.14 µg ml^{-1}), *Anabaena* sp. 12ss (15.66 µg ml^{-1}), *Nostoc* sp. 16ss (12.58 µg ml^{-1}), *Anabaena* sp. 11ss (11.30 µg ml^{-1}), *Anabaena* sp. 15ss (11.59 µg ml^{-1}), *Aphanothece* sp. 10ss (11.26 µg ml^{-1}), *Calothrix* sp. 3ss (10.75 µg ml^{-1}), *Calothrix* sp. 13ss (9.45 µg ml^{-1}), *Anabaena* sp. 2ss (8.96 µg ml^{-1}), *Anabaena* sp. 19ss (7.20 µg ml^{-1}), *Anabaena* sp. 20ss (7.310 µg ml^{-1}), *Scytonema* sp. 6ss (6.6 µg ml^{-1}) and *Aphanothece* sp. 5ss (4.36 µg ml^{-1}).

On the 4^{th} day of incubation the maximum carotenoid content was observed in *Nostoc* sp. 9ss (52.17 µg ml^{-1}) followed by *Anabaena* sp. 7ss (37.91 µg ml^{-1}), *Tolypothrix* sp. 8ss (34.61 µg ml^{-1}), *Aphanothece* sp. 10ss (32.03 µg ml^{-1}), *Aphanothece* sp. 5ss (30.65 µg ml^{-1}), *Nostoc* sp. 17ss (30.16 µg ml^{-1}), *Anabaena* sp. 12ss (27.94 µg ml^{-1}), *Aphanothece* sp. 4ss (29.37 µg ml^{-1}), *Scytonema* sp.18ss (26.67 µg ml^{-1}), *Anabaena* sp. 11ss (20.1 µg ml^{-1}), *Calothrix* sp. 13ss (19.2 µg ml^{-1}), *Scytonema* sp. 6ss (17.3 µg ml^{-1}), *Calothrix* sp.14ss (15.61 µg ml^{-1}), *Nostoc* sp. 16ss (16.41 µg ml^{-1}), *Anabaena* sp. 19ss (13.27 µg ml^{-1}), *Anabaena* sp. 20ss (12.49 µg ml^{-1}), *Anabaena* sp.15ss (12.42 µg ml^{-1}), *Scytonema* sp.1ss (11.3 µg ml^{-1}) and *Calothrix* sp. 3ss (9.22 µg ml^{-1}) while minimum was found in *Anabaena* sp. 2ss (3.31 µg ml^{-1}).

On the 6^{th} day of incubation, the maximum carotenoid content was observed in *Nostoc* sp. 17ss (56.67 µg ml^{-1}) followed by *Anabaena* sp. 7ss (39.1 µg ml^{-1}) *Anabaena* sp. 7ss (39.18 µg ml^{-1}), *Aphanothece* sp. 10ss (39.78 µg ml^{-1}), *Anabaena* sp. 12ss (39.14 µg ml^{-1}), *Nostoc* sp. 9ss (38.12 µg ml^{-1}), *Tolypothrix* sp. 8ss (38.27 µg ml^{-1}), *Anabaena* sp. 2ss (37.41 µg ml^{-1}), *Aphanothece* sp. 4ss (33.18 µg ml^{-1}),

Calothrix sp. 3ss (33.41 µg ml^{-1}), *Aphanothece* sp. 5ss (31.36 µg ml^{-1}), *Scytonema* sp.18ss (30.39 µg ml^{-1}), *Anabaena* sp. 11ss (24.73 µg ml^{-1}), *Calothrix* sp. 13ss (23.60 µg ml^{-1}), *Nostoc* sp. 16ss (19.62 µg ml^{-1}), *Calothrix* sp. 14ss (19.50 µg ml^{-1}), *Anabaena* sp. 15ss (19.09 µg ml^{-1}), *Scytonema* sp. 6ss (18.89 µg ml^{-1}), *Scytonema* sp.1ss (18.19 µg ml^{-1}), *Anabaena* sp. 19ss (15.98 µg ml^{-1}) while lowest carotenoid content was observed in *Anabaena* sp. 20ss (14.64 µg ml^{-1}).

After 8^{th} day old culture and time interval among the all the cyanobacterial strains showed the significant increment in the carotenoid content on the 8^{th} day the maximum was observed in *Anabaena* sp. 11ss (48.31 µg ml^{-1}) followed by *Tolypothrix* sp. 8ss 45.02 µg ml^{-1}), *Aphanothece* sp. 5ss (44.75 µg ml^{-1}), *Nostoc* sp. 17ss (43.43 µg ml^{-1}), *Aphanothece* sp. 10ss (40.93 µg ml^{-1}), *Anabaena* sp. 2ss (39.99 µg ml^{-1}), *Scytonema* sp.18ss (39.78 µg ml^{-1}), *Anabaena* sp. 12ss (39.39 µg ml^{-1}), *Nostoc* sp. 9ss (39.28 µg ml^{-1}), *Anabaena* sp. 15ss (38.73 µg ml^{-1}), *Anabaena* sp. 19ss (37.52 µg ml^{-1}), *Aphanothece* sp. 4ss (37.41 µg ml^{-1}), *Anabaena* sp. 20ss (37.25 µg ml^{-1}), *Calothrix* sp. 14ss (35.14 µg ml^{-1}), *Anabaena* sp. 7ss (31.13 µg ml^{-1}), *Calothrix* sp. 13ss (29.88 µg ml^{-1}), *Scytonema* sp. 6ss (29.82 µg ml^{-1}), *Calothrix* sp. 3ss (26.62 µg ml^{-1}), *Scytonema* sp.1ss (24.89 µg ml^{-1}) and *Nostoc* sp. 16ss (19.35 µg ml^{-1}).

On the 10^{th} day the maximum carotenoid was observed in *Nostoc* sp. 9ss (78.07 µg ml^{-1}), *Nostoc* sp. 17ss (76.52 µg ml^{-1}), *Anabaena* sp. 15ss (54.6 µg ml^{-1}), *Anabaena* sp. 20ss (53.46 µg ml^{-1}), *Anabaena* sp. 11ss (51.64 µg ml^{-1}), *Scytonema* sp.1ss (65.13 µg ml^{-1}), *Aphanothece* sp. 5ss (47.88 µg ml^{-1}), *Aphanothece* sp. 4ss (46.40 µg ml^{-1}), *Anabaena* sp. 2ss (46.37 µg ml^{-1}), *Aphanothece* sp. 10ss (46.66 µg ml^{-1}), *Anabaena* sp. 12ss (43.78 µg ml^{-1}), *Calothrix* sp. 14ss (41.43 µg ml^{-1}), *Anabaena* sp. 7ss (39.56 µg ml^{-1}), *Tolypothrix* sp. 8ss (39.39 µg ml^{-1}), *Nostoc* sp. 16ss (35.26 µg ml^{-1}), *Scytonema* sp. 6ss (32.71 µg ml^{-1}), *Anabaena* sp. 19ss (31.52 µg ml^{-1}), *Calothrix* sp. 3ss (30.54 µg ml^{-1}), *Scytonema* sp.18ss (30.23 µg ml^{-1}) and minimum was found in *Calothrix* sp. 13ss (23.85 µg ml^{-1}).

On the final day of incubation the maximum carotenoid content was observed *Nostoc* sp. 9ss (78.90 µg ml^{-1}) followed by *Anabaena* sp. 20ss (68.45 µg ml^{-1}),

Scytonema sp.1ss (66.60 µg ml^{-1}), *Anabaena* sp. 15ss (66.96 µg ml^{-1}), *Nostoc* sp. 17ss (64.27 µg ml^{-1}), *Anabaena* sp. 11ss (61.81 µg ml^{-1}), *Anabaena* sp. 12ss (60.24 µg ml^{-1}), *Calothrix* sp. 14ss (59.65 µg ml^{-1}), *Anabaena* sp. 19ss (58.08 µg ml^{-1}), *Scytonema* sp.18ss (50.43 µg ml^{-1}), *Nostoc* sp. 16ss (50.89 µg ml^{-1}), *Aphanothece* sp. 5ss (49.09 µg ml^{-1}), *Aphanothece* sp. 10ss (48.67 µg ml^{-1}), *Tolypothrix* sp. 8ss (47.20 µg ml^{-1}), *Anabaena* sp. 2ss (47.78 µg ml^{-1}), *Aphanothece* sp. 4ss(46.67 µg ml^{-1}), *Scytonema* sp. 6ss (45.90 µg ml^{-1}), *Anabaena* sp. 7ss (40.34 µg ml^{-1}) and *Calothrix* sp. 3ss (35.89 µg ml^{-1}) while lowest was observed in *Calothrix* sp. 13ss (33.85 µg ml^{-1}).

4.3.4. *Phycobillin protein content*

From the initial day to 2nd day of incubation, the maximum phycobillin protein content **(**Figure 10) was found in S*cytonema* sp. 1f (1.46 µg ml^{-1}) followed by *Nostoc* sp. 1h (1.912 µg ml^{-1}), *Nostoc* sp. 1c (0.916 µg ml^{-1}), *Nostoc* sp. 9c (0.338 µg ml^{-1}), *Nostoc* sp. 9f (0.318 µg ml^{-1}), *Nostoc* sp. 9i (0.338 µg ml^{-1}), *Nostoc* sp. 1d (0.186 µg ml^{-1}), *Anabaena* sp. 1n (0.157 µg ml^{-1}), *Anabaena* sp. 2a (0.154 µg ml^{-1}), *Nostoc* sp. 9j (0.153 µg ml^{-1}), *Scytonema* sp. 12c (0.150 µg ml^{-1}), *Anabaena sphaerica* 1e (0.50 µg ml^{-1}), *Nostoc* sp. 9ma2 (0.149 µg ml^{-1}), *Nostoc commune* 9n (0.135 µg ml^{-1}), *Anabaena doliolum* 1a (0.1228 µg ml^{-1}), *Nostoc* sp. 4 (0.1267 µg ml^{-1}), *Desmonostoc muscorum* 9a (0.1267 µg ml^{-1}), *Tolypothrix* sp. 9k (0.1049 µg ml^{-1}), *Nostoc* sp. 9ma1 (0.1004 µg ml^{-1}) and *Anabaena* sp. 2b (0.060 µg ml^{-1}).

On the 4th day of incubation, the maximum phycobilline protein content was found in *Scytonema* sp. 1f (1.91 µg ml^{-1}) followed by *Nostoc* sp. 1c (1.531 µg ml^{-1}), *Nostoc* sp. 9j (0.506 µg ml^{-1}), *Nostoc* sp. 9ma1 (0.343 µg ml^{-1}), *Nostoc* sp. 9c (0.348 µg ml^{-1}), *Nostoc* sp. 9f (0.349µg ml^{-1}), *Nostoc* sp. 9i (0.348 µg ml^{-1}), *Anabaena* sp. 1n (0.355 µg ml^{-1}), *Nostoc* sp. 1h (0.281 µg ml^{-1}), *Nostoc* sp. 1d (0.215 µg ml^{-1}), *Anabaena* sp. 2a (0.284 µg ml^{-1}), *Nostoc* sp. (0.2604 µg ml^{-1}), *Desmonostoc muscorum* 9a (0.260 µg ml^{-1}), *Nostoc* sp. 9ma2 (0.255 µg ml^{-1})**,** *Anabaena doliolum* 1a (0.154 µg ml^{-1}), *Anabaena sphaerica* 1e (0.176 µg ml^{-1}), *Anabaena* sp. 2b (0.154 µg ml^{-1}), *Tolypothrix* sp. 9k (0.107 µg ml^{-1}), *Nostoc* c*ommune* 9n (0.168µg ml^{-1}) and *Scytonema* sp. 12c (0.164 µg ml^{-1}).

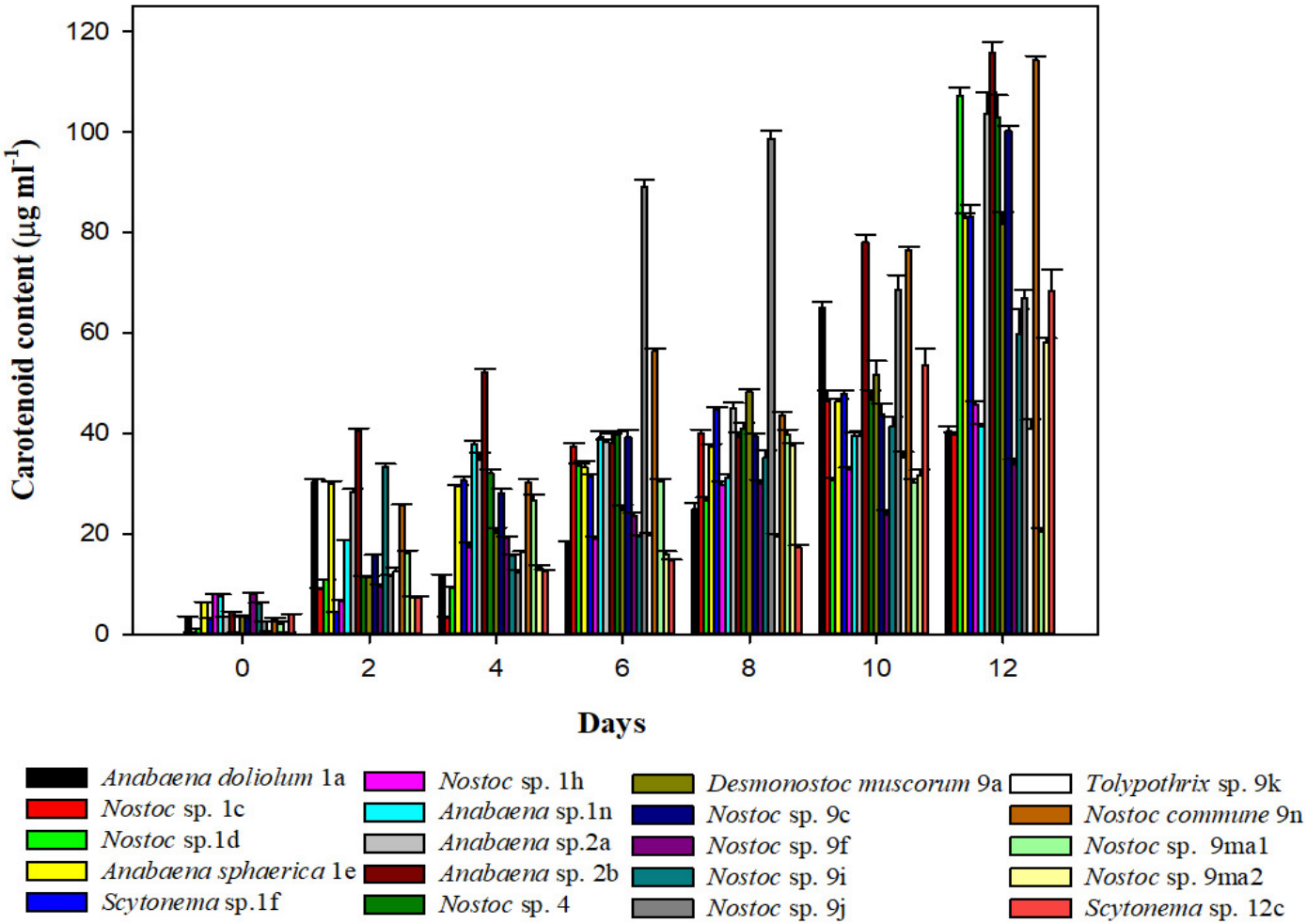

Figure 8. Carotenoid content of twenty cyanobacterial strains isolated from plain regions of Chhattisgarh

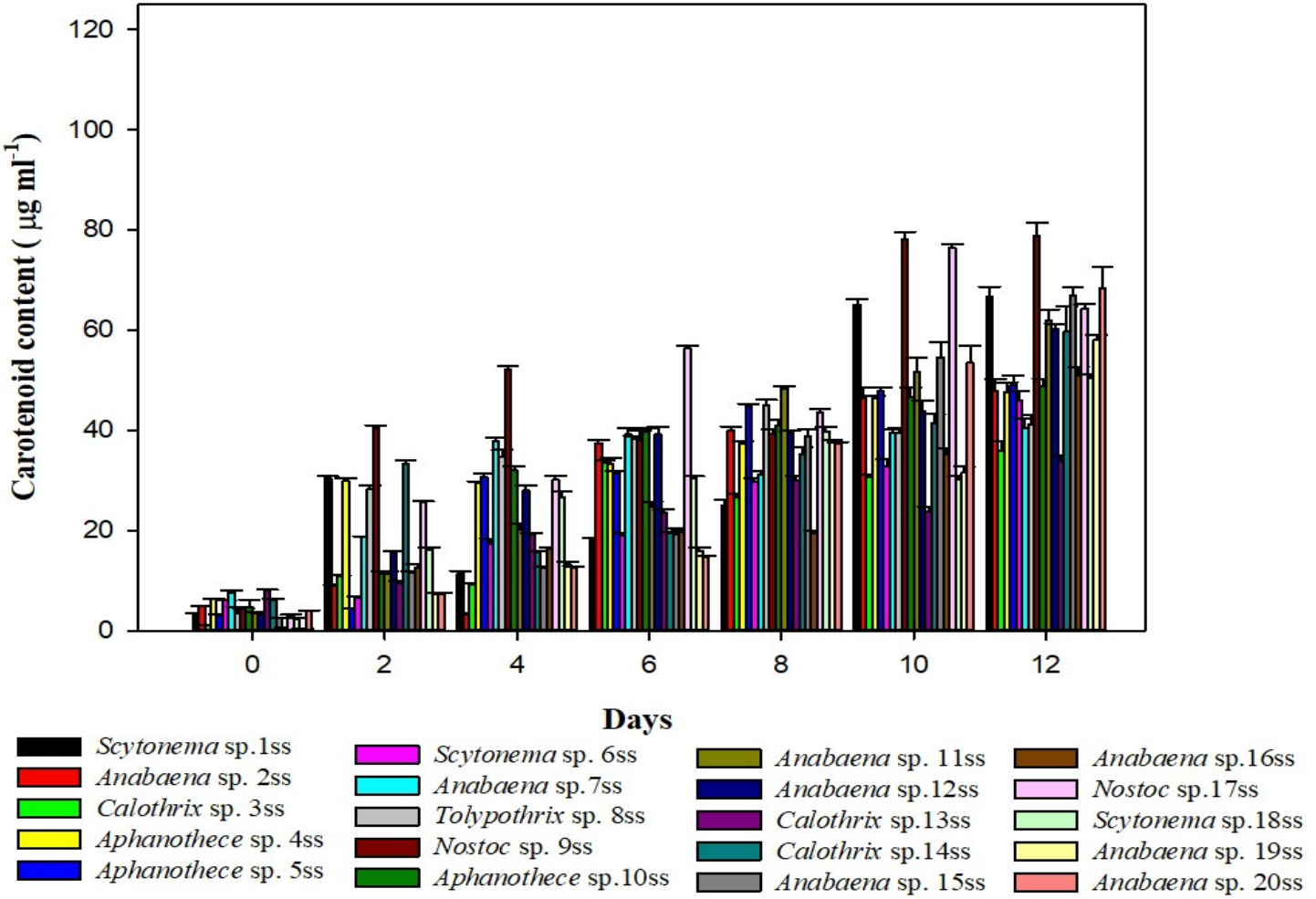

Figure 9. Carotenoid content of twenty cyanobacterial strains isolated from North Hills region of Chhattisgarh

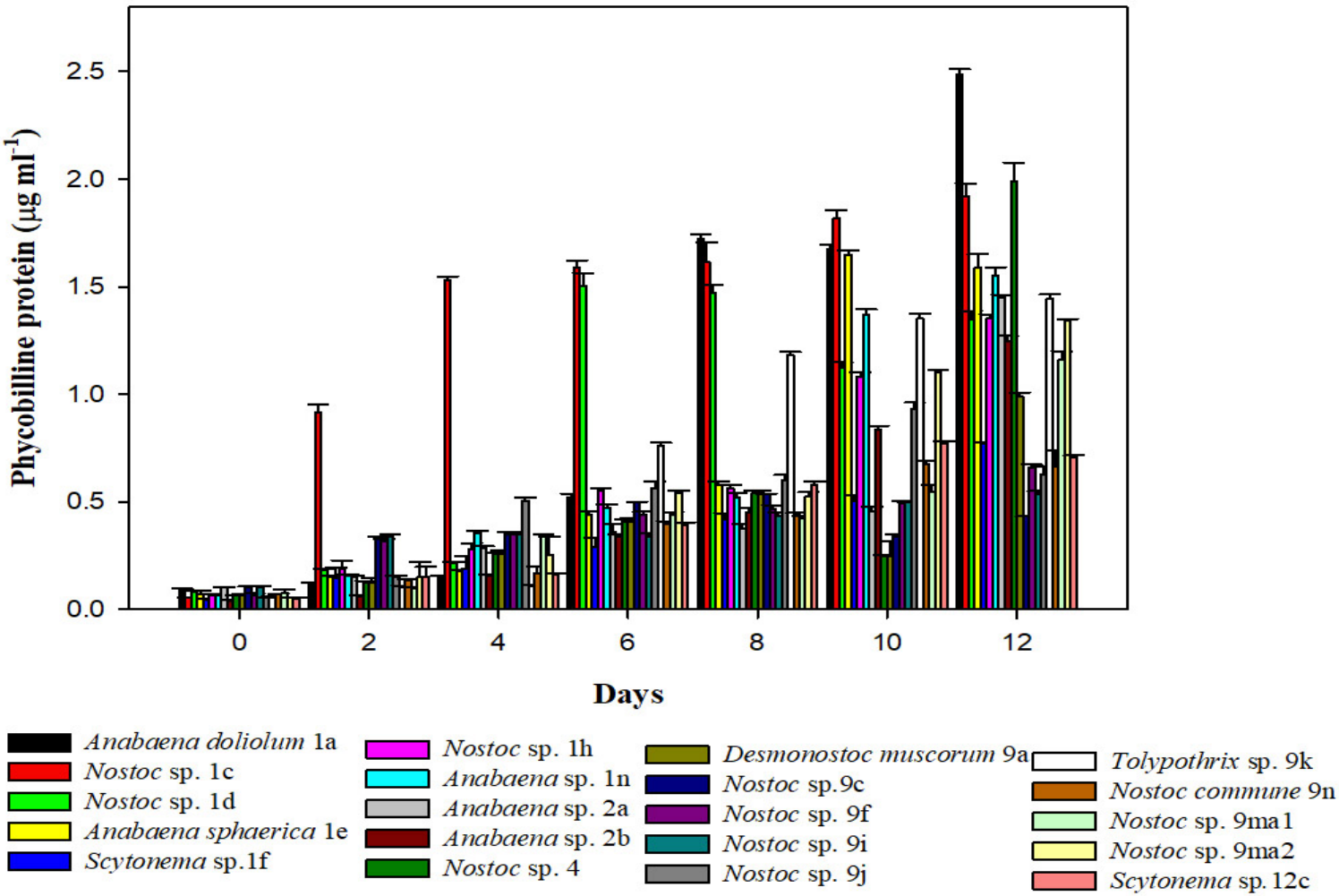

Figure 10. Phycobilin protein of twenty cyanobacterial strains isolated from Plain regions of Chhattisgarh

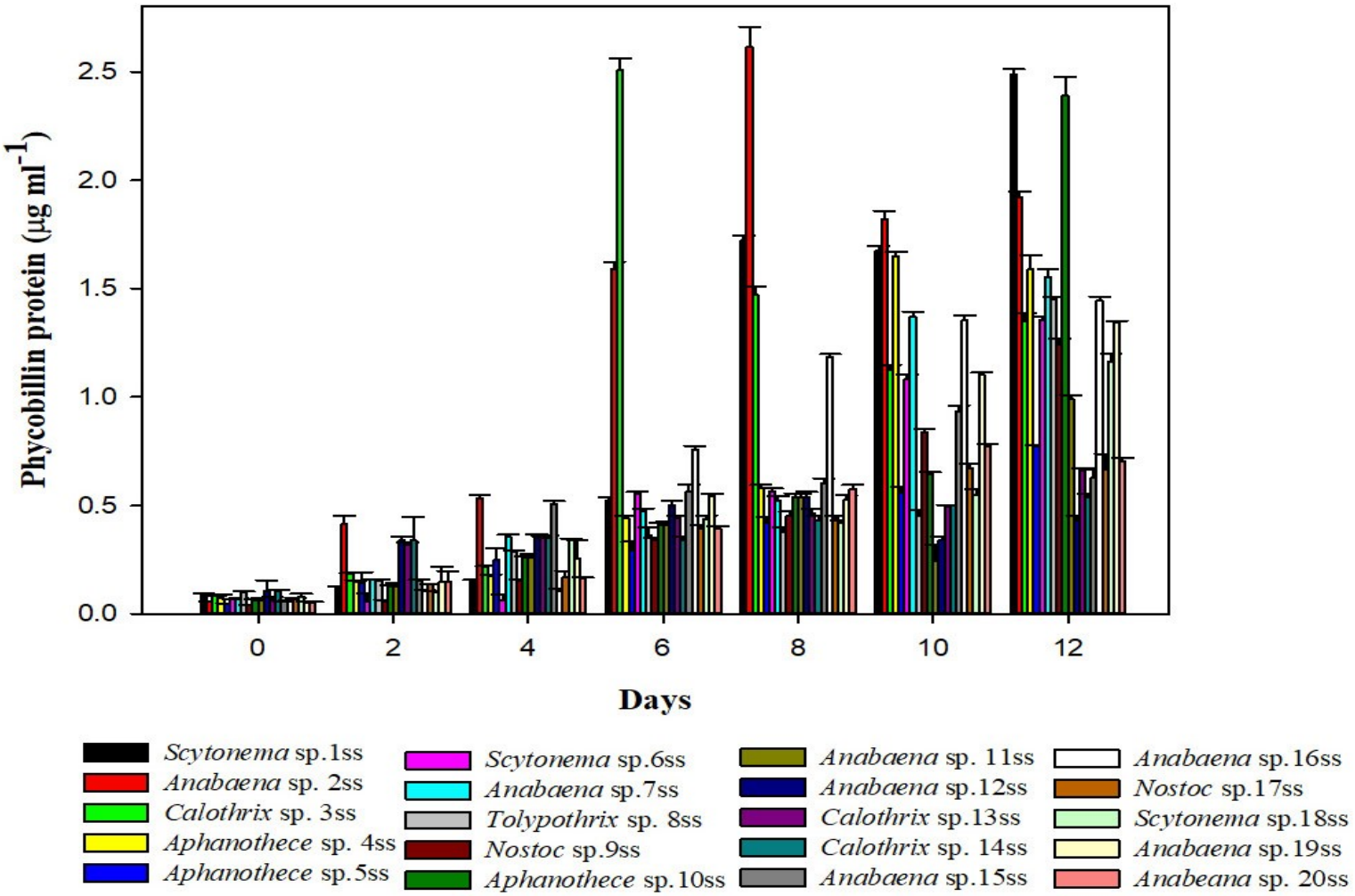

Figure 11. Phycobilin protein of twenty cyanobacterial strains isolated from North hills region of Chhattisgarh

On the 6th day of incubation the higher phycobiline protein was observed in *Nostoc* sp. 1d (1.59 µg ml^{-1}) followed by *Nostoc* sp. 1c (1.591 µg ml^{-1}), *Tolypothrix* sp. 9k (0.758 µg ml^{-1}), *Nostoc* sp. 9j (0.564 µg ml^{-1}), *Nostoc* sp. 1h (0.551 µg ml^{-1}), *Nostoc* sp. 9ma2 (0.543 µg ml^{-1}), *Anabaena doliolum* 1a (0.522 µg ml^{-1}), *Nostoc* sp. 9c (0.499 µg ml^{-1}), *Anabaena* sp. 1n (0.474 µg ml^{-1}), *Nostoc* sp. 9ma1 (0.439 µg ml^{-1}), *Nostoc* sp. 9f (0.439 µg ml^{-1}), *Desmonostoc muscorum* 9a (0.410 µg ml^{-1}), *Anabaena sphaerica* 1e (0.440 µg ml^{-1}), *Nostoc* sp. *4* (0.410 µg ml^{-1}), *Nostoc* commune 9n (0.394 µg ml^{-1}), *Scytonema* sp. 12c (0.391 µg ml^{-1}), *Anabaena* sp. 2a (0.361 µg ml^{-1}), *Anabaena* sp. 2b (0.337 µg ml^{-1}), *Nostoc* sp. 9i (0.339 µg ml^{-1}) and *Scytonema* sp. 1f (0.2904 µg ml^{-1}).

Maximum phycobilin protein was found in *Nostoc* sp. 1c (1.614 µg ml^{-1}) followed by *Anabaena doliolum* 1a (1.72 µg ml^{-1}), *Nostoc* sp. 1d (1.47 µg ml^{-1}), *Tolypothrix* sp. 9k (1.18 µg ml^{-1}), *Nostoc* sp. 9j (0.60 µg ml^{-1}), *Anabaena sphaerica* 1e (0.57 µg ml^{-1}), *Scytonema* sp. 12c (0.576 µg ml^{-1}), *Nostoc* sp. 1h (0.564 µg ml^{-1}), *Nostoc* sp. 4 (0.540 µg ml^{-1}), *Desmonostoc muscorum* 9a (0.540 µg ml^{-1}), *Nostoc* sp. 9c (0.537µg ml^{-1}), *Nostoc* sp. 9ma2 (0.527µg ml^{-1}), *Anabaena* sp.1n (0.522 µg ml^{-1}), *Anabaena* sp. 2b (0.450 µg ml^{-1}), *Nostoc* sp. 9f (0.465 µg ml^{-1}), *Nostoc* sp. 9i (0.432 µg ml^{-1}), *Nostoc* sp. 9ma1 (0.422 µg ml^{-1}), *Scytonema* sp. 1f (0.421 µg ml^{-1}), *Nostoc* sp. 9ma1 (0.444 µg ml^{-1}) and *Anabaena* sp. 2a (0.378 µg ml^{-1}) on 8th day of incubation.

The highest phycobilin protein was found in *Scytonema* sp. 1f (5.102 µg ml^{-1}) 10th day of incubation followed by *Nostoc* sp.1c (1.819 µg ml^{-1}), *Anabaena doliolum* 1a (1.677 µg ml^{-1}), *Anabaena sphaerica* 1e (1.649 µg ml^{-1}), *Anabaena* sp.1n (1.369 µg ml^{-1}), *Tolypothrix* sp. 9k (1.353 µg ml^{-1}), *Nostoc* sp. 1d (1.124 µg ml^{-1}), *Nostoc* sp. 9ma2 (1.1017 µg ml^{-1}), *Nostoc* sp. 1h (1.082 µg ml^{-1}), *Nostoc* sp. 9j (0.932 µg ml^{-1}), *Anabaena* sp. 2b (0.838 µg ml^{-1}), *Scytonema* sp. 12c (0.772 µg ml^{-1}), *Nostoc commune* 9n (0.673 µg ml^{-1}), *Nostoc* sp. 9ma1 (0.547 µg ml^{-1}), *Nostoc* sp. 9f (0.493 µg ml^{-1}), *Nostoc* sp. 9i (0.49 µg ml^{-1}), *Nostoc* sp. 9c (0.33 µg ml^{-1}), *Anabaena* sp. 2a

(0.455 µg ml^{-1}), *Nostoc* sp. 4 (0.245µg ml^{-1}) and *Desmonostoc muscorum* 9a (0.245 µg ml^{-1}).

On last day of incubation the maximum phycobilin protein was found in *Nostoc* sp. 4 (1.989 µg ml^{-1}) followed by *Anabaena doliolum* 1a (2.49 µg ml^{-1}), *Nostoc* sp. 1c (1.922 µg ml^{-1}), *Anabaena sphaerica* 1e (1.588 µg ml^{-1}), *Anabaena* sp. 1n (1.553 µg ml^{-1}), *Anabaena* sp. 2a (1.449 µg ml^{-1}), *Tolypothrix* sp. 9k (1.446 µg ml^{-1}), *Nostoc* sp. 1d (1.351 µg ml^{-1}), *Nostoc* sp. 9ma2 (1.342µg ml^{-1}), *Nostoc* sp. 1h (1.353 µg ml^{-1}), *Anabaena* sp. 2b (1.245 µg ml^{-1}), *Nostoc commune* 9n (1.163 µg ml^{-1}), *Desmonostoc muscorum* 9a (0.989 µg ml^{-1}), *Scytonema* sp.1f (0.770 µg ml^{-1}), *Scytonema* sp.12c (0.705 µg ml^{-1}), *Nostoc* sp. 9f (0.661 µg ml^{-1}), *Nostoc* sp. 9j (0.629 µg ml^{-1}), *Nostoc commune* 9n (0.665µg ml^{-1}), *Nostoc* sp. 9i (0.537 µg ml^{-1}) and *Nostoc* sp. 9c (0.432 µg ml^{-1}).

Similar observation was carried also out in twenty cyanobacterial strains collected from North hill regions of Chhattisgarh (Figure 11).

On the 2^{nd} day of incubation the highest phycobilin protein content was observed in *Anabaena* sp. 2ss (0.415 µg ml^{-1}) followed by *Anabaena* sp. 12ss (0.338 µg ml^{-1}), *Anabaena* sp. 14ss (0.338 µg ml^{-1}), *Calothrix* sp. 13ss (0.318 µg ml^{-1}), *Calothrix* sp. 3ss (0.186 µg ml^{-1}), *Anabaena* sp. 7ss (0.157 µg ml^{-1}), *Anabaena* sp. 15ss (0.1533 µg ml^{-1}), *Tolypothrix* sp. 8ss (0.154 µg ml^{-1}), *Anabaena* sp. 20ss (0.1504 µg ml^{-1}), *Aphanothece* sp. 4ss (0.150 µg ml^{-1}), *Aphanothece* sp. 5ss (0.145 µg ml^{-1}), *Nostoc* sp. 17ss (0.135 µg ml^{-1}), *Scytonema* sp. 1ss (0.123 µg ml^{-1}), (*Aphanothece* sp. 10ss (0.127 µg ml^{-1}), *Nostoc* sp. 11ss (0.127 µg ml^{-1}), *Anabaena* sp. 16ss (0.105 µg ml^{-1}), *Scytonema* sp. 18ss (0.100 µg ml^{-1}), *Anabaena* sp. 19ss (0.149 µg ml^{-1}), *Anabaena* sp. 9ss (0.059 µg ml^{-1}) and *Scytonema* sp. 6ss (0.059 µg ml^{-1}) respectively.

Maximum Phycobilin protein content was found in *Anabaena* sp. 2ss (0.531 µg ml^{-1}) followed by *Anabaena* sp. 15ss (0.506 µg ml^{-1}), *Calothrix* sp. 13ss (0.349 µg ml^{-1}), *Anabaena* sp. 14ss (0.348 µg ml^{-1}), *Anabaena* sp. 7ss (0.355 µg ml^{-1}), *Scytonema* sp.18ss (0.343 µg ml^{-1}), *Anabaena* sp. 12ss (0.349 µg ml^{-1}), *Tolypothrix*

sp. 8ss (0.284 µg ml^{-1}), *Aphanothece* sp. 5ss (0.249 µg ml^{-1}), *Calothrix* sp. 3ss (0.215 µg ml^{-1}), *Aphanothece* sp. 10ss (0.260 µg ml^{-1}), *Anabaena* sp. 19ss (0.255 µg ml^{-1}), *Nostoc* sp. 11ss (0.260 µg ml^{-1}), *Aphanothece* sp. 4ss (0.177 µg ml^{-1}), *Anabaena* sp. 20ss (0.1644 µg ml^{-1}) *Nostoc* sp. 17ss (0.169 µg ml^{-1}), *Anabaena* sp. 9ss (0.155 µg ml^{-1}), *Scytonema* sp.1ss (0.154 µg ml^{-1}) and *Anabaena* sp. 16ss (0.108 µg ml^{-1}) where as minimum was found in *Scytonema* sp. 6ss (0.063 µg ml^{-1}) on the 4^{th} days of incubation.

On the 6^{th} day of incubation, the highest phycobilin protein was found in *Calothrix* sp. 3ss (2.506 µg ml^{-1}) and followed by *Anabaena* sp. 2ss (1.5911 µg ml^{-1}), *Anabaena* sp. 16ss (0.757 µg ml^{-1}), *Anabaena* sp. 15ss (0.564 µg ml^{-1}), *Scytonema* sp. 6ss (0.551 µg ml^{-1}), *Anabaena* sp. 19ss (0.544 µg ml^{-1}), *Scytonema* sp.1ss (0.522 µg ml^{-1}), *Anabaena* sp. 12ss (0.499 µg ml^{-1}), *Anabaena* sp. 7ss (0.474 µg ml^{-1}), *Aphanothece* sp. 4ss (0.440 µg ml^{-1}), *Calothrix* sp. 13ss (0.439 µg ml^{-1}), *Scytonema* sp. 18ss (0.439 µg ml^{-1}), *Aphanothece* sp. 10ss (0.410 µg ml^{-1}), *Nostoc* sp. 11ss (0.410 µg ml^{-1}), *Nostoc* sp. 17ss (0.394 µg ml^{-1}), *Anabaena* sp. 20ss (0.391 µg ml^{-1}). *Tolypothrix* sp. 8ss (0.361 µg ml^{-1}), *Anabaena* sp. 9ss (0.33 µg ml^{-1}) and *Anabaena* sp. 14ss (0.33 µg ml^{-1}) whereas minimum was observed in *Aphanothece* sp. 5ss (0.290 µg ml^{-1}).

Maximum phycobilin protein was observed in *Anabaena* sp. 2ss (2.614 µg ml^{-1}) followed by *Scytonema* sp.1ss (1.72 µg ml^{-1}), *Calothrix* sp. 3ss (1.47 µg ml^{-1}), *Anabaena* sp. 16ss (1.18 µg ml^{-1}), *Anabaena* sp. 15ss (0.601 µg ml^{-1}), *Aphanothece* sp. 4ss (0.578 µg ml^{-1}), *Anabaena* sp. 20ss (0.576 µg ml^{-1}), *Scytonema* sp. 6ss (0.564 µg ml^{-1}), *Nostoc* sp. 11ss (0.5400 µg ml^{-1}), *Aphanothece* sp. 10ss (0.5400 µg ml^{-1}), *Anabaena* sp. 12ss (0.5371 µg ml^{-1}), *Anabaena* sp. 19ss (0.527 µg ml^{-1}), *Anabaena* sp. 7ss (0.523 µg ml^{-1}), *Calothrix* sp. 13ss (0.465 µg ml^{-1}), *Anabaena* sp. 9ss (0.450µg ml^{-1}), *Anabaena* sp. 14ss (0.433 µg ml^{-1}), *Nostoc* sp. 17ss (0.44 µg ml^{-1}), *Aphanothece* sp. 5ss (0.4210 µg ml^{-1}), *Scytonema* sp. 18ss (0.422 µg ml^{-1}) and *Tolypothrix* sp. 8ss (0.378 µg ml^{-1}) on the 8^{th} days of incubation.

On 10th day of incubation, the maximum phycobilin protein was found in *Anabaena* sp. 2ss (1.82 µg ml^{-1}) followed by *Scytonema* sp.1ss (1.678 µg ml^{-1}), *Aphanothece* sp. 4ss (1.649 µg ml^{-1}), *Anabaena* sp. 16ss (1.353 µg ml^{-1}), *Anabaena* sp. 7ss (1.369 µg ml^{-1}), *Calothrix* sp. 3ss (1.123 µg ml^{-1}), *Scytonema* sp. 6ss (1.082 µg ml^{-1}), *Anabaena* sp. 19ss (1.101 µg ml^{-1}), *Anabaena* sp. 15ss (0.932 µg ml^{-1}), *Anabaena* sp. 9ss (0.83 µg ml^{-1}), *Anabaena* sp. 20ss (0.77µg ml^{-1}), *Aphanothece* sp. 10ss (0.64 µg ml^{-1}), *Nostoc* sp. 17ss (0.673 µg ml^{-1}), *Aphanothece* sp. 5ss (0.56 µg ml^{-1}), *Scytonema* sp. 18ss (0.54 µg ml^{-1}), *Calothrix* sp. 13ss (0.49 µg ml^{-1}), *Anabaena* sp. 14ss (0.49 µg ml^{-1}), *Tolypothrix* sp. 8ss (0.45 µg ml^{-1}), *Anabaena* sp. 12ss (0.33 µg ml^{-1}) and *Nostoc* sp. 11ss (0.24 µg ml^{-1}) respectively.

On the 12th day maximum phycobilin protein was observed in *Aphanothece* sp. 10ss (2.38 µg ml^{-1}) followed by *Scytonema* sp.1ss (2.490 µg ml^{-1}), *Anabaena* sp. 2ss (1.922 µg ml^{-1}), *Aphanothece* sp. 4ss (1.58 µg ml^{-1}), *Anabaena* sp. 7ss (1.552 µg ml^{-1}), *Tolypothrix* sp. 8ss (1.449 µg ml^{-1}), *Anabaena* sp. 16ss (1.44 µg ml^{-1}), *Calothrix* sp. 3ss (1.35 µg ml^{-1}), *Scytonema* sp. 6ss (1.35 µg ml^{-1}), *Anabaena* sp. 19ss (1.34 µg ml^{-1}), *Anabaena* sp. 9ss (1.24 µg ml^{-1}), *Scytonema* sp.18ss (1.16 µg ml^{-1}), *Nostoc* sp. 11ss (0.98 µg ml^{-1}), *Aphanothece* sp. 5ss (0.77 µg ml^{-1}), *Anabaena* sp. 20ss (0.70 µg ml^{-1}), *Calothrix* sp. 13ss (0.66 µg ml^{-1}), *Nostoc* sp. 17ss (0.66 µg ml^{-1}), *Anabaena* sp. 15ss (0.62 µg ml^{-1}), *Anabaena* sp. 14ss (0.53 µg ml^{-1}) while minimum was observed in *Anabaena* sp. 12ss (0.43 µg ml^{-1}).

4.3.5 *Protein content*

When the total protein was observerd and compared among the twenty cyanobacterial strains isolated from plain regions of Chhattisgarh (Figure 12).

On the second day of incubation the maximum protein content was found in *Desmonostoc muscorum* 9a (37.02 µg ml^{-1}) followed by *Nostoc* sp. 9c (36.97 µg ml^{-1}), *Nostoc* sp. 9ma2 (35.91 µg ml^{-1}), *Scytonema* sp. 12c (35.29 µg ml^{-1}), *Anabaena* sp. 1n (35.97 µg ml^{-1}), *Nostoc* sp. 1h (34.21 µg ml^{-1}), *Anabaena* sp. 2a (33.61 µg ml^{-1}), *Nostoc* sp. 4 (33.26 µg ml^{-1}), *Anabaena* sp. 2b (32.07 µg ml^{-1}), *Nostoc* sp. 1d (31.14

µg ml^{-1}), *Tolypothrix* sp. 9k (30.20 µg ml^{-1}), *Nostoc* sp. 1c (29.78 µg ml^{-1}), *Nostoc* sp. 9j (27.56 µg ml^{-1}), *Nostoc* sp. 9f (24.16 µg ml^{-1}), *Anabaena sphaerica* 1e (23.07 µg ml^{-1}), *Scytonema* sp. 1f (22.64 µg ml^{-1}), *Nostoc* sp. 9i (22.96 µg ml^{-1}), *Nostoc* commune 9n (25.85 µg ml^{-1}), *Nostoc* sp. 9ma1 (26.97 µg ml^{-1}) and *Anabaena doliolum* 1a (20.00 µg ml^{-1}).

Maximum protein was found in *Anabaena* sp. 1n (58.30 µg ml^{-1}) followed by *Nostoc* sp. 4 (57.13 µg ml^{-1}), *Nostoc* sp. 1h (57.03 µg ml^{-1}), *Nostoc* sp. 1d (56.11 µg ml^{-1}), *Nostoc* sp. 9ma2 (56.47 µg ml^{-1}), *Anabaena* sp. 2b (50.22 µg ml^{-1}), *Desmonostoc muscorum* sp. 9a (49.97 µg ml^{-1}), *Nostoc* sp. 9ma1 (50.37 µg ml^{-1}), *Scytonema* sp. 12c (46.30 µg ml^{-1}), *Nostoc* sp. 9f (45.14 µg ml^{-1}), *Nostoc* sp. 1c (44.38 µg ml^{-1}), *Anabaena* sp. 2a (43.01 µg ml^{-1}), *Nostoc* sp. 9c (39.37 µg ml^{-1}), *Nostoc* sp. 9i (39.36 µg ml^{-1}), *Nostoc commune* 9n (39.85 µg ml^{-1}), *Anabaena sphaerica* 1e (38.34 µg ml^{-1}), *Scytonema* sp. 1f (38.42 µg ml^{-1}), *Tolypothrix* sp. 9k (38.11 µg ml^{-1}), *Nostoc* sp. 9j (36.96 µg ml^{-1}) and *Anabaena doliolum* 1a (31.54 µg ml^{-1}) on the 4^{th} day of incubation.

On the 6^{th} day of incubation the maximum protein was found in *Nostoc* sp. 4 (77.70 µg ml^{-1}) followed by *Nostoc* sp. 9ma2 (74.64 µg ml^{-1}), *Anabaena* sp. 1n (74.46 µg ml^{-1}), *Anabaena* sp. 2b (71.61 µg ml^{-1}), *Nostoc* sp. 1h (68.69 µg ml^{-1}), *Anabaena* sp. 2a (66.17 µg ml^{-1}), *Nostoc* sp. 1d (60. 97 µg ml^{-1}), *Nostoc* sp. 1c (58.83 µg ml^{-1}), *Anabaena doliolum* 1a (58.00 µg ml^{-1}), *Desmonostoc muscorum* 9a (57.72 µg ml^{-1}), *Nostoc* sp. 9ma1(57.37 µg ml^{-1}), *Nostoc* sp. 9i (51.36 µg ml^{-1}), *Nostoc* sp. 9f (50.48 µg ml^{-1}), *Nostoc* sp. 9j (49.92 µg ml^{-1}), *Tolypothrix* sp. 9k (48.85 µg ml^{-1}), *Anabaena sphaerica* 1e (45.91 µg ml^{-1}), *Scytonema* sp. 1f (42.50 µg ml^{-1}), *Scytonema* sp. 12c (41.63 µg ml^{-1}), *Nostoc* sp. 9c (41.00 µg ml^{-1}) while minimum was observed in *Nostoc commune* 9n (40.85 µg ml^{-1}).

On the 8^{th} day of incubation, the protein content was varied and maximum was found in *Nostoc* sp. 4 (89.34 µg ml^{-1}) followed by *Anabaena* sp. 1n (89.56 µg ml^{-1}), *Anabaena* sp. 2b (87.80 µg ml^{-1}), *Anabaena doliolum* 1a (80.00 µg ml^{-1}), *Nostoc* sp. 9i (79.97 µg ml^{-1}), *Nostoc* sp. 1d (78.98 µg ml^{-1}), *Anabaena* sp. 2a (78.90 µg ml^{-1}),

Nostoc sp. 1c (76.00 µg ml^{-1}), *Desmonostoc muscorum* 9a (73.54 µg ml^{-1}), *Nostoc* sp. 1h (69.00 µg ml^{-1}), *Anabaena sphaerica* 1e (69.00 µg ml^{-1}), *Nostoc* sp. 9ma2 (67.27 µg ml^{-1}), *Nostoc* sp. 9ma1(61.00 µg ml^{-1}), *Nostoc* sp. 9f (59.78 µg ml^{-1}), *Scytonema* sp. 1f (59.05 µg ml^{-1}), *Nostoc* sp. 9j (54.44 µg ml^{-1}), *Nostoc commune* 9n (55.85 µg ml^{-1}) and minimum was found in *Scytonema* sp. 12c (52.61 µg ml^{-1}) followed by *Nostoc* sp. 9c (52.39 µg ml^{-1}) *and Tolypothrix* sp. 9k (52.85 µg ml^{-1}).

On the 10^{th} day of incubation the maximum protein was found in *Nostoc* sp. 4 (98.67 µg ml^{-1}) followed by *Anabaena* sp. 1n (95.50 µg ml^{-1}), *Anabaena* sp. 2b (94.95 µg ml^{-1}), *Anabaena* sp. 2a (90.45 µg ml^{-1}), *Desmonostoc* muscorum 9a (89.30 µg ml^{-1}), *Nostoc* sp.1c (85.00 µg ml^{-1}), *Nostoc* sp. 9f (81.09 µg ml^{-1}), *Scytonema* sp. 1f (80.50 µg ml^{-1}), *Anabaena doliolum* 1a (79.80 µg ml^{-1}), *Nostoc* sp. 1d (79.34 µg ml^{-1}), *Nostoc* sp. 1h (79.67 µg ml^{-1}), *Nostoc* sp. 9j (77.40 µg ml^{-1}), *Nostoc* sp. 9c (79.68 µg ml^{-1}), *Anabaena sphaerica* 1e (75.71 µg ml^{-1}), *Nostoc* sp. 9ma1 (72.39 µg ml^{-1}), *Tolypothrix* sp. 9k (70.85 µg ml^{-1}), *Nostoc commune* 9n (67.85 µg ml^{-1}), *Scytonema* sp. 12c (67.69 µg ml^{-1}). Minimum was found in *Nostoc* sp. 9ma2 (66.47 µg ml^{-1}) followed by *Nostoc* sp. 9i (63.61 µg ml^{-1}).

On the final day of incubation, the maximum protein was found in *Anabaena* sp. 2a (109.23 µg ml^{-1}) followed *Anabaena doliolum* 1a (105.00 µg ml^{-1}), *Anabaena* sp. 1n (101.40 µg ml^{-1}), *Nostoc* sp. 4 (103.11 µg ml^{-1}), *Anabaena* sp. 2b (99.50 µg ml^{-1}), *Desmonostoc muscorum* 9a (99.49 µg ml^{-1}), *Anabaena sphaerica* 1e (99.40 µg ml^{-1}), *Nostoc* sp. 9ma1 (99.68 µg ml^{-1}), *Nostoc* sp. 9c (98.75 µg ml^{-1}), *Nostoc* sp. 9i (95.77 µg ml^{-1}), *Scytonema* sp.1f (95.40 µg ml^{-1}), *Nostoc* sp. 1c (90.00 µg ml^{-1}), *Nostoc* sp. 1d (88.70 µg ml^{-1}), *Nostoc* sp. 1h (88.80 µg ml^{-1}), *Nostoc* sp. 9ma2 (85.54 µg ml^{-1}), *Nostoc* sp. 9j (84.36 µg ml^{-1}), *Nostoc* sp. 9f (83.16 µg ml^{-1}), *Tolypothrix* sp. 9k (82.85 µg ml^{-1}) and *Scytonema* sp. 12c (81.90 µg ml^{-1}). The lowest was found in *Nostoc* commune 9n (79.85 µg ml^{-1}).

Similarly, when the protein content was investigated among cyanobacterial strains isolated from north hill regions of Chhattisgarh (Figure 13). Results showed the variation in different time interval. Whereas, on the 2^{nd} day of incubation the

maximum protein was found in *Calothrix* sp. 3ss (47.35 µg ml^{-1}) followed by *Anabaena* sp. 19ss (43.83 µg ml^{-1}), *Tolypothrix* sp. 8ss (37.021 µg ml^{-1}), *Aphanothece* sp. 5ss (36.65 µg ml^{-1}), *Aphanothece* sp. 4ss (36.65 µg ml^{-1}), *Scytonema* sp. 18ss (35.40 µg ml^{-1}), *Scytonema* sp. 6ss (33.066 µg ml^{-1}), *Nostoc* sp. 17ss (33.34 µg ml^{-1}), *Nostoc* sp. 16ss (31.35 µg ml^{-1}), *Anabaena* sp. 7ss (29.31 µg ml^{-1}), *Anabaena* sp. 15ss (29.45 µg ml^{-1}), *Aphanothece* sp. 10ss (27.56 µg ml^{-1}), *Anabaena* sp. 12ss (27.70 µg ml^{-1}), *Nostoc* sp. 9ss (26.09 µg ml^{-1}), *Scytonema* sp. 1ss (20.85 µg ml^{-1}), *Anabaena* sp. 11ss (24.70 µg ml^{-1}), *Anabaena* sp. 20ss (20.48 µg ml^{-1}), *Anabaena* sp. 2ss (19.70 µg ml^{-1}), *Calothrix* sp. 14ss (19.14 µg ml^{-1}) and *Calothrix* sp. 13ss (13.55 µg ml^{-1}).

On the 4^{th} day of incubation, the highest protein was found in *Scytonema* sp. 18ss (55.62 µg ml^{-1}) followed by *Calothrix* sp. 3ss (47.91 µg ml^{-1}), *Nostoc* sp. 9ss, (47.63 µg ml^{-1}), *Nostoc* sp. 16ss (45.77 µg ml^{-1}), *Nostoc* sp. 17ss (43.31 µg ml^{-1}), *Anabaena* sp. 19ss (43.84 µg ml^{-1}), *Aphanothece* sp. 5ss (39.69 µg ml^{-1}), *Scytonema* sp. 6ss (38.34 µg ml^{-1}), *Tolypothrix* sp. 8ss (38.68 µg ml^{-1}), *Aphanothece* sp. 4ss (37.0 µg ml^{-1}), *Anabaena* sp. 15ss (36.30 µg ml^{-1}), *Anabaena* sp. 11ss (34.17 µg ml^{-1}), *Aphanothece* sp. 10ss (33.58 µg ml^{-1}), *Anabaena* sp. 12ss (32.74 µg ml^{-1}), *Anabaena* sp. 20ss (30.74 µg ml^{-1}), *Anabaena* sp. 7ss (29.83 µg ml^{-1}), *Calothrix* sp. 14ss (24.37 µg ml^{-1}), *Calothrix* sp. 13ss (23.54 µg ml^{-1}), *Anabaena* sp. 2ss (23.06 µg ml^{-1}). Minimum was found in *Scytonema* sp.1ss (22.95 µg ml^{-1}).

Maximum protein was observed in *Anabaena* sp. 19ss (66.96 µg ml^{-1}) followed by *Nostoc* sp. 9ss (59.98 µg ml^{-1}), *Anabaena* sp. 11ss (59.76 µg ml^{-1}), *Aphanothece* sp. 4ss (59.62 µg ml^{-1}), *Calothrix* sp. 3ss (58.8 µg ml^{-1}), *Nostoc* sp. 16ss (57.12 µg ml^{-1}), *Scytonema* sp.18ss (56.14 µg ml^{-1}), *Nostoc* sp. 17ss (55.47 µg ml^{-1}), *Scytonema* sp. 6ss (55.91 µg ml^{-1}), *Anabaena* sp. 12ss (50.65 µg ml^{-1}), *Aphanothece* sp. 10ss (46.13 µg ml^{-1}), *Aphanothece* sp. 5ss (43.06 µg ml^{-1}), *Tolypothrix* sp. 8ss (41.83 µg ml^{-1}), *Anabaena* sp. 15ss (41.63 µg ml^{-1}), *Anabaena* sp. 7ss (40.43 µg ml^{-1}), *Calothrix* sp. 13ss (39.95 µg ml^{-1}), *Anabaena* sp. 2ss (38.34 µg ml^{-1}), *Anabaena* sp. 20ss (37.79 µg ml^{-1}) and *Scytonema* sp. 1ss (33.65 µg ml^{-1}). The lowest was found in *Calothrix* sp. 14ss (30.00 µg ml^{-1}) on the 6^{th} day of incubation.

On the 8th day of incubation the maximum was found in *Anabaena* sp. 19ss (79.92 µg ml^{-1}) followed by *Scytonema* sp. 18ss (69.18 µg ml^{-1}), *Aphanothece* sp. 5ss (68.2 µg ml^{-1}), *Nostoc* sp. 16ss (68.39 µg ml^{-1}), *Anabaena* sp. 12ss (67.64 µg ml^{-1}), *Anabaena* sp. 11ss (64.23 µg ml^{-1}), *Tolypothrix* sp. 8ss (62.53 µg ml^{-1}), *Anabaena* sp. 15ss (62.60 µg ml^{-1}), *Scytonema* sp. 6ss (61.24 µg ml^{-1}), *Calothrix* sp. 3ss (59.7 µg ml^{-1}), *Aphanothece* sp. 4ss (58.13 µg ml^{-1}), *Anabaena* sp. 2ss (55.2 µg ml^{-1}), *Nostoc* sp. 9ss (54.45 µg ml^{-1}), *Aphanothece* sp. 10ss (52.82 µg ml^{-1}), *Nostoc* sp. 17ss (51.72 µg ml^{-1}), *Calothrix* sp. 13ss (45.29 µg ml^{-1}), *Anabaena* sp. 7ss (42.49 µg ml^{-1}), *Calothrix* sp. 14ss (42.39 µg ml^{-1}) and *Anabaena* sp. 20ss (42.80 µg ml^{-1}). Minimum was found in *Scytonema* sp.1ss (34.7 µg ml^{-1}).

On the 10th day of incubation the maximum was found in *Anabaena* sp. 15ss (87.61 µg ml^{-1}) followed by *Anabaena* sp. 11ss (87.13 µg ml^{-1}), *Anabaena* sp. 19ss (87.40 µg ml^{-1}), *Nostoc* sp. 16ss (83.63 µg ml^{-1}), *Nostoc* sp. 17ss (78.90 µg ml^{-1}), *Aphanothece* sp. 5ss (75.2 µg ml^{-1}), *Scytonema* sp. 6ss (75.2 µg ml^{-1}), *Nostoc* sp. 9ss (75.96 µg ml^{-1}), *Tolypothrix* sp. 8ss (74.21 µg ml^{-1}), *Scytonema* sp.18ss (72.30 µg ml^{-1}), *Calothrix* sp. 3ss (69.8 µg ml^{-1}), *Anabaena* sp. 12ss (64.22 µg ml^{-1}), *Aphanothece* sp. 4ss (62.32 µg ml^{-1}), *Anabaena* sp. 2ss (60.05 µg ml^{-1}), *Calothrix* sp. 14ss (59.67 µg ml^{-1}), *Aphanothece* sp. 10ss (59.36 µg ml^{-1}), *Anabaena* sp. 20ss (58.09 µg ml^{-1}) *Anabaena* sp. 7ss (52.63 µg ml^{-1}), *Scytonema* sp. 1ss (50.65 µg ml^{-1}) and *Calothrix* sp. 13ss (49.49 µg ml^{-1}).

On the final day of incubation the maximum protein was found in *Anabaena* sp. 15ss (99.90 µg ml^{-1}) followed by *Anabaena* sp. 19ss (98.36 µg ml^{-1}), *Anabaena* sp. 11ss (92.15 µg ml^{-1}), *Nostoc* sp. 16ss (91.61 µg ml^{-1}), *Tolypothrix* sp. 8ss (89.51 µg ml^{-1}), *Nostoc* sp. 17ss (88.00 µg ml^{-1}), *Calothrix* sp. 3ss (80.8 µg ml^{-1}), *Aphanothece* sp. 5ss (80.03 µg ml^{-1}), *Aphanothece* sp. 4ss (79.6 µg ml^{-1}), *Nostoc* sp. 9ss (78.301 µg ml^{-1}), *Anabaena* sp. 12ss (77.13 µg ml^{-1}), *Anabaena* sp. 20ss (76.27 µg ml^{-1}), *Anabaena* sp. 2ss (73.91 µg ml^{-1}), *Aphanothece* sp. 10ss (69.99 µg ml^{-1}), *Scytonema* sp.18ss (68.56 µg ml^{-1}), *Anabaena* sp. 7ss (60.3 µg ml^{-1}), *Calothrix* sp. 13ss (57.72 µg ml^{-1}), *Calothrix* sp. 14ss (53.34 µg ml^{-1}), *Scytonema* sp.1ss (51.5 µg ml^{-1}) and *Scytonema* sp. 6ss (30.05 µg ml^{-1}).

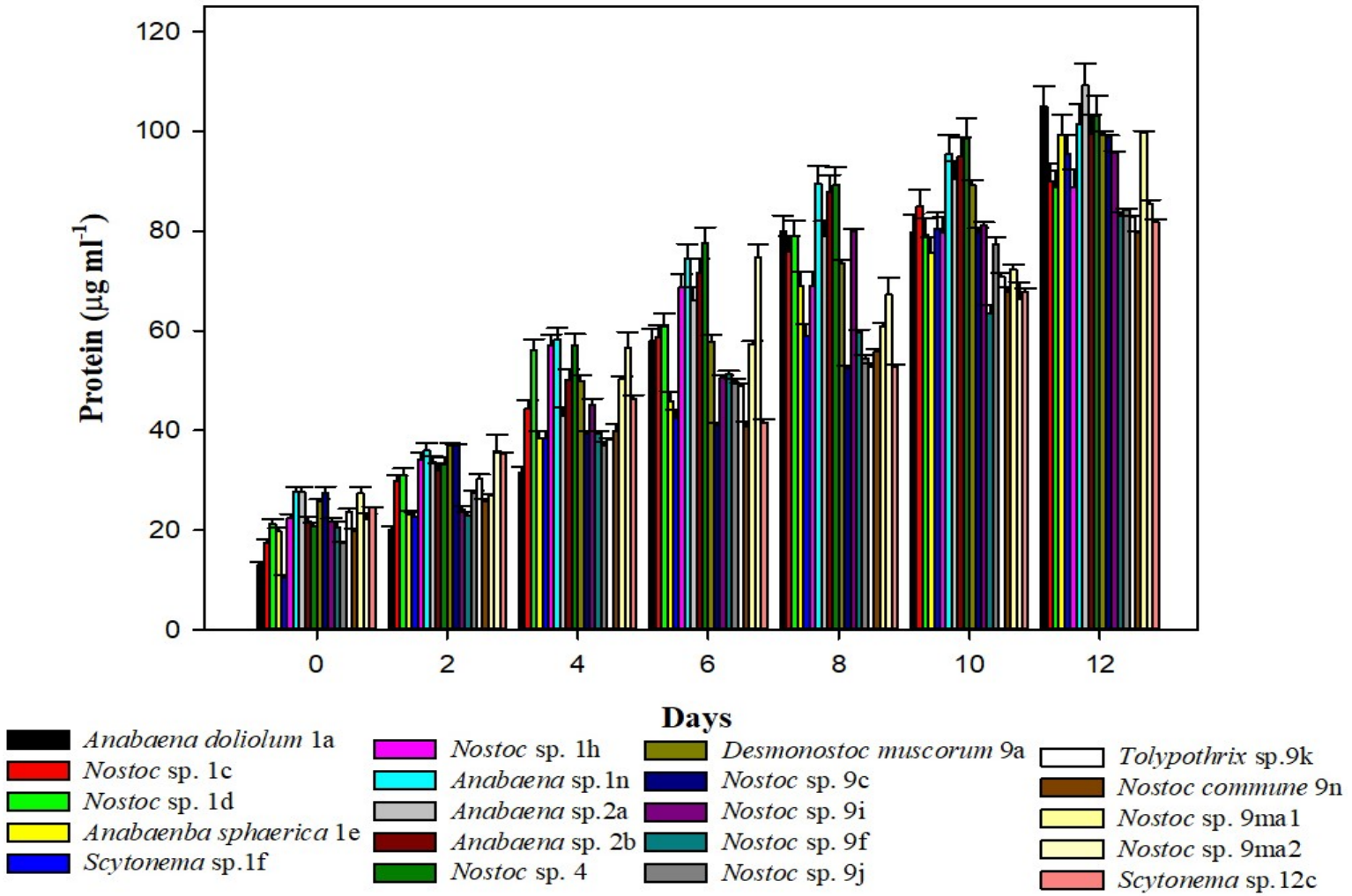

Figure 12. Protein content of twenty cyanobacterial strains isolated from Plain regions of Chhattisgarh

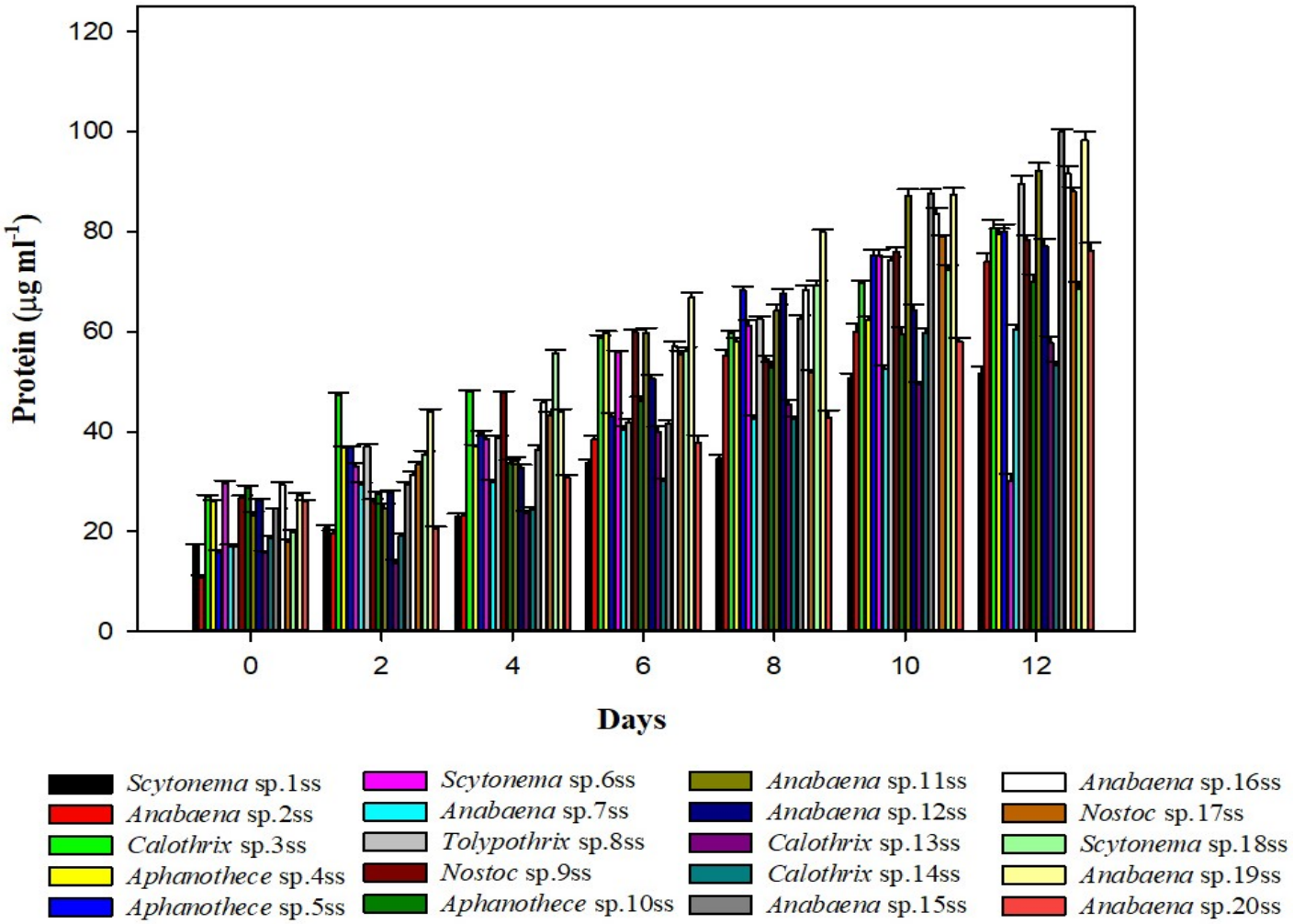

Figure 13. Protein content of twenty cyanobacterial strains isolated from North hills of Chhattisgarh

4.3.6. *Carbohydrate content*

Carbohydrate content was observed among the twenty cyanobacteria strains isolated from plain regions of Chhattisgarh suggested that the carbohydrate content was increased from initial to last of incubation. All the cyanobacteria showed the remarkable increase in the carbohydrate content on second day. (Figure 14) Maximum carbohydrate was found in *Anabaena* sp. 1n (7.38 µg ml^{-1}) followed by *Nostoc sp.* 9f (6.012 µg ml^{-1}), *Anabaena* sp. 2b (6.65 µg ml^{-1}), *Anabaena sp.* 2a (6.93 µg ml^{-1}), *Anabaena spherical* 1e (6.34 µg ml^{-1}), *Desmonostoc muscorum* sp. 9a (5.77 µg ml^{-1}), *Scytonema* sp. 12c (4.99 µg ml^{-1}), *Nostoc sp.* 1h (4.732µg ml^{-1}), *Tolypothrix sp.*9k (4.20 µg ml^{-1}), *Scytonema* sp. 1f (4.42 µg ml^{-1}), *Nostoc* sp. 9ma2 (3.23 µg ml^{-1})), *Nostoc sp.* 9ma2 (3.23 µg ml^{-1}), *Nostoc commune* 9n (3.09 µg ml^{-1}), *Nostoc* sp. 9c (2.86 µg ml^{-1}), *Nostoc* sp. 9i (3.12 µg ml^{-1}), *Nostoc* sp. 9j (2.14 µg ml^{-1}), *Anabaena doliolum* 1a (1.97 µg ml^{-1}), *Nostoc* sp. 1d (1.55 µg ml^{-1}), *Nostoc* sp. 1c (1.26 µg ml^{-1}) and the minimum was found in *Nostoc* sp. 4 (0.921µg ml^{-1}) respectively.

On the 4th day of incubation the maximum carbohydrate was found in *Scytonema* sp. 12c (25.43 µg ml^{-1}) followed by *Nostoc* sp. 9ma2 (13.40 µg ml^{-1}), *Nostoc* sp. 9ma1 (12.28 µg ml^{-1}), *Anabaena* sp. 2a (10.34 µg ml^{-1}), *Anabaena* sp. 1n (10.17 µg ml^{-1}), *Nostoc* sp. 1d (10.0 µg ml^{-1}), *Nostoc* sp. 9j (8.21 µg ml^{-1}), *Nostoc* sp. 9f (7.93 µg ml^{-1}), *Anabaena sphaerica* 1e (7.70 µg ml^{-1}), *Tolypothrix* sp. 9k (7.81 µg ml^{-1}), *Nostoc commune* 9n (7.48 µg ml^{-1}), *Nostoc* sp. 9i (5.20 µg ml^{-1}), *Desmonostoc muscorum* 9a (6.98 µg ml^{-1}), *Nostoc* sp. 9c (4.14 µg ml^{-1}), *Nostoc* sp. 1h (5.164 µg ml^{-1}), *Scytonema sp.* 1f (4.927 µg ml^{-1}), *Nostoc sp.* 1c (2.56 µg ml^{-1}), *Anabaena doliolum* 1a (2.69 µg ml^{-1}), *Nostoc* sp. 4 (1.04 µg ml^{-1}) and *Anabaena* sp. 2b (1.72 µg ml^{-1}).

Similarly, On 6th day of incubation the maximum carbohydrate was found in *Scytonema sp.* 12c (40.82 µg ml^{-1}) followed by *Scytonema* sp. 1f (37.04 µg ml^{-1}), *Anabaena doliolum* 1a (25.4 µg ml^{-1}), *Nostoc* sp. 1c (24.4 µg ml^{-1}), *Nostoc* sp. 9ma1 (12.28 µg ml^{-1}), *Anabaena* sp. 2a (10.31 µg ml^{-1}), *Anabaena* sp. 1n (10.10 µg ml^{-1}), *Nostoc* sp. 9ma2 (10.60 µg ml^{-1}), *Anabaena sphaerica* 1e (9.95 µg ml^{-1}), *Desmonostoc muscorum* 9a (8.94 µg ml^{-1}), *Nostoc* sp. 9n (8.77 µg ml^{-1}), *Nostoc* sp. 9f

(8.29 µg ml^{-1}), *Nostoc* sp. 1h (8.73 µg ml^{-1}), *Nostoc* sp. 9i (7.99 µg ml^{-1}), *Nostoc* sp. 9c (7.815 µg ml^{-1}), *Tolypothrix* sp. 9k (7.650 µg ml^{-1}), *Nostoc* sp. 9j (7.178 µg ml^{-1}), *Anabaena* sp. 2b (7.87 µg ml^{-1}), *Nostoc* sp. 1d (1.52 µg ml^{-1}) and *Nostoc* sp. 4 (2.31 µg ml^{-1}).

On the 8^{th} day of incubation, the highest carbohydrate was found in *Scytonema* sp. 12c (42.34 µg ml^{-1}) followed by *Anabaena doliolum* 1a (37.25 µg ml^{-1}), *Anabaena sphaerica* 1e (36.70 µg ml^{-1}), *Scytonema* sp. 1f (37.52 µg ml^{-1}), *Nostoc* sp. 1c (36.7 µg ml^{-1}), *Nostoc* sp. 9j (18.71 µg ml^{-1}), *Nostoc* sp. 9ma1 (14.75 µg ml^{-1}), *Nostoc* sp. 9ma1 (14.75 µg ml^{-1}), *Nostoc* sp. 9f (12.50 µg ml^{-1}), *Desmonostoc muscorum* 9a (12.20 µg ml^{-1}), *Anabaena* sp. 2a (12.77 µg ml^{-1}), *Nostoc* sp. 9ma2 (11.36 µg ml^{-1}), *Anabaena* sp. 1n (11.34µg ml^{-1}), *Nostoc* sp. 9i (11.84 µg ml^{-1}), *Nostoc* sp. 1h (10.71 µg ml^{-1}), *Nostoc* sp. 9c (9.109 µg ml^{-1}), *Tolypothrix* sp. 9k (8.30 µg ml^{-1}), *Nostoc* sp. 4 (3.62 µg ml^{-1}) and *Anabaena* sp. 2b (1.322 µg ml^{-1}). Minimum carbohydrate was reported in *Nostoc* sp. 1d (1.23 µg ml^{-1}).

Whereas the on 10^{th} day of incubation the carbohydrate content was maximum in *Scytonema* sp. 12c (50.670) followed by *Anabaena doliolum* 1a (45.23 µg ml^{-1}), *Scytonema* sp. 1f (40.098 µg ml^{-1}), *Nostoc* sp. 9ma1 (39.50 µg ml^{-1}), *Tolypothrix* sp. 9k (33.11 µg ml^{-1}), *Nostoc* sp. 9j (32.33 µg ml^{-1}), *Nostoc* sp. 9i (30.47 µg ml^{-1}), *Desmonostoc muscorum* sp. 9a (29.40 µg ml^{-1}), *Nostoc* sp. 9f (26.98 µg ml^{-1}), *Anabaena* sp. 2b (22.84 µg ml^{-1}), *Nostoc* sp. 1c (22.50 µg ml^{-1}), *Anabaena* sp. 1n (18.45µg ml^{-1}), *Anabaena* sp. 2a (14.3µg ml^{-1}), *Nostoc* sp. 9ma2 (13.85 µg ml^{-1}), *Anabaena sphaerica* 1e (13.17 µg ml^{-1}), *Nostoc commune* 9n (12.22 µg ml^{-1}), *Nostoc* sp. 1h (11.98 µg ml^{-1}), *Nostoc* sp. 9c (10.92 µg ml^{-1}), *Nostoc* sp. 4 (5.12 µg ml^{-1}) and *Nostoc* sp.1d (4.15 µg ml^{-1}).

On the final day of incubation, cyanobacterial strains showed the maximum carbohydrate content in *Scytonema* sp. 12c (55.82 µg ml^{-1}) followed by *Anabaena doliolum* 1a (47.2 µg ml^{-1}), *Scytonema* sp. 1f (44.09 µg ml^{-1}), *Nostoc* sp. 9ma1 (40.28 µg ml^{-1}), *Tolypothrix* sp. 9k (33.65 µg ml^{-1}), *Nostoc* sp. 9j (33.18 µg ml^{-1}), *Nostoc* sp. 9i (32.99 µg ml^{-1}), *Nostoc* sp. 9f (28.29 µg ml^{-1}), *Desmonostoc muscorum* 9a (28.94

µg ml^{-1}), *Nostoc* sp. 1c (21.5 µg ml^{-1}), *Anabaena* sp. 2b (21.84 µg ml^{-1}), *Anabaena* sp. 2a (17.39 µg ml^{-1}), *Anabaena* sp. 1n (16.45µg ml^{-1}), *Anabaena sphaerica* 1e (15.17 µg ml^{-1}), *Nostoc commune* 9n (13.77 µg ml^{-1}), *Nostoc* sp. 9ma2 (12.60 µg ml^{-1}), *Nostoc* sp. 9c (11.81 µg ml^{-1}), *Nostoc* sp. 1d (5.63 µg ml^{-1}), *Nostoc* sp. 1h (10.98 µg ml^{-1}) and *Nostoc* sp. 4 (6.12 µg ml^{-1}).

Carbohydrate content were also investigated of the cyanobacteria strains isolated from North hills. All the respective cyanobacterial strains showed significant changes in the carbohydrate content **(**Figure15).

On the initial day, all the respective cyanobacterial strains showed an equal carbohydrate content but on the 2nd day of incubation, maximum carbohydrate was observed in *Anabaena* sp. 7ss (7.39 µg ml^{-1}) followed by *Nostoc* sp. 9ss (6.65 µg ml^{-1}), *Calothrix* sp. 13ss (6.012 µg ml^{-1}), *Tolypothrix* sp. 8ss (6.930 µg ml^{-1}), *Aphanothece* sp. 4ss (6.39 µg ml^{-1}), *Anabaena* sp. 20ss (5.99 µg ml^{-1}), *Anabaena* sp. 11ss (5.77 µg ml^{-1}), *Scytonema* sp. 6ss (4.73 µg ml^{-1}), *Aphanothece* sp. 5ss (4.42 µg ml^{-1}), *Calothrix* sp. 14ss (4.12 µg ml^{-1}), *Anabaena* sp. 19ss (3.85 µg ml^{-1}), *Nostoc* sp. 17ss (3.12 µg ml^{-1}), *Anabaena* sp. 12ss (2.86 µg ml^{-1}), *Scytonema* sp.1ss (1.98 µg ml^{-1}), *Nostoc* sp. 16ss (1.195 µg ml^{-1}), *Anabaena* sp. 15ss (1.43 µg ml^{-1}), *Aphanothece* sp. 10ss (1.311 µg ml^{-1}), *Scytonema* sp.18ss (1.23 µg ml^{-1}), *Anabaena* sp. 2ss (1.269 µg ml^{-1}) and *Calothrix* sp. 3ss (1.01 µg ml^{-1}).

Similarly on the 4th day of incubation the maximum carbohydrate content was showed in *Anabaena* sp. 7ss (9.77 µg ml^{-1}) followed by *Tolypothrix* sp. 8ss (8.69 µg ml^{-1}), *Anabaena* sp. 15ss (8.21 µg ml^{-1}), *Calothrix* sp. 13ss (7.93 µg ml^{-1}), *Nostoc* sp. 16ss (7.82 µg ml^{-1}), *Nostoc* sp. 17ss (7.47 µg ml^{-1}), *Aphanothece* sp. 4ss (7.70 µg ml^{-1}), *Anabaena* sp. 20ss (7.43 µg ml^{-1}), *Scytonema* sp. 18ss (5.40 µg ml^{-1}), *Scytonema* sp. 1ss (5.7 µg ml^{-1}), *Anabaena* sp. 11ss (5.98 µg ml^{-1}), *Calothrix* sp. 14ss (5.20 µg ml^{-1}), *Scytonema* sp. 6ss (5.16 µg ml^{-1}), *Aphanothece* sp. 5ss (4.93 µg ml^{-1}), *Anabaena* sp. 19ss (4.38 µg ml^{-1}), *Anabaena* sp. 12ss (4.14 µg ml^{-1}), *Aphanothece* sp. 10ss (3.623 µg ml^{-1}) and *Anabaena* sp. 2ss (2.56 µg ml^{-1}). while the minimum

carbohydrate was found in *Nostoc* sp. 9ss (1.72 µg ml^{-1}) and *Calothrix* sp. 3ss (1.234 µg ml^{-1}).

On the 6th day of incubation the maximum carbohydrate conetnt was found in *Nostoc* sp. 17ss (12.28 µg ml^{-1}) followed by *Scytonema* sp.18ss (10.60 µg ml^{-1}), *Anabaena* sp. 7ss (10.11 µg ml^{-1}), *Aphanothece* sp. 4ss (9.95 µg ml^{-1}), *Anabaena* sp. 20ss (9.82 µg ml^{-1}), *Tolypothrix* sp. 8ss (10.31 µg ml^{-1}), *Scytonema* sp.1ss (9.44 µg ml^{-1}), *Scytonema* sp. 6ss (8.73 µg ml^{-1}), *Anabaena* sp. 11ss (8.94 µg ml^{-1}), *Anabaena* sp. 19ss (8.77 µg ml^{-1}), *Calothrix* sp. 13ss (8.29 µg ml^{-1}), *Calothrix* sp. 14ss (7.99 µg ml^{-1}), *Nostoc* sp. 9ss (7.87 µg ml^{-1}), *Anabaena* sp. 12ss (7.81 µg ml^{-1}), *Nostoc* sp. 16ss (7.65 µg ml^{-1}), *Anabaena* sp. 15ss (7.17 µg ml^{-1}), *Aphanothece* sp. 5ss (7.04 µg ml^{-1}), *Calothrix* sp. 3ss (5.52 µg ml^{-1}) and *Aphanothece* sp. 10ss (5.12 µg ml^{-1}). Minimum was found in *Anabaena* sp. 2ss (3.41 µg ml^{-1}).

On the 8th of incubation, Maximum carbohydrate content was found in *Anabaena* sp. 15ss (18.715 µg ml^{-1}) followed by *Nostoc* sp. 17ss (14.75 µg ml^{-1}), *Nostoc* sp. 9ss (13.86 µg ml^{-1}), *Tolypothrix* sp. 8ss (12.77 µg ml^{-1}), *Calothrix* sp. 13ss (12.50 µg ml^{-1}), *Anabaena* sp. 20ss (12.33 µg ml^{-1}), *Anabaena* sp. 11ss (12.20 µg ml^{-1}), *Calothrix* sp. 14ss (11.84 µg ml^{-1}), *Anabaena* sp. 7ss (11.35 µg ml^{-1}), *Scytonema* sp.18ss (11.36 µg ml^{-1}), *Scytonema* sp. 6ss (10.72 µg ml^{-1}), *Scytonema* sp.1ss (10.25 µg ml^{-1}), *Anabaena* sp. 12ss (9.109 µg ml^{-1}), *Aphanothece* sp. 5ss (9.53µg ml^{-1}), *Aphanothece* sp. 10ss (8.92 µg ml^{-1}), *Aphanothece* sp. 4ss (8.87 µg ml^{-1}), *Nostoc* sp. 16ss (8.30 µg ml^{-1}), *Anabaena* sp. 19ss (7.02 µg ml^{-1}), *Anabaena* sp. 2ss (6.71 µg ml^{-1}) and minimum was found in *Calothrix* sp. 3ss (6.557).

On the 10th day of incubation the maximum carbohydrate was found in *Anabaena* sp. 15ss (32.33 µg ml^{-1}), *Calothrix* sp. 14ss (30.46 µg ml^{-1}), *Calothrix* sp. 13ss (26.99 µg ml^{-1}), *Nostoc* sp. 9ss (22.85 µg ml^{-1}), *Anabaena* sp. 7ss (18.46 µg ml^{-1}), *Aphanothece* sp. 10ss (16.33 µg ml^{-1}), *Scytonema* sp. 1ss (15.24 µg ml^{-1}), *Anabaena* sp. 11ss (14.40 µg ml^{-1}), *Tolypothrix* sp. 8ss (14.38 µg ml^{-1}), *Aphanothece* sp. 5ss (14.1 µg ml^{-1}), *Scytonema* sp. 18ss (13.86 µg ml^{-1}), *Anabaena* sp. 20ss (13.670 µg ml^{-1}), *Nostoc* sp. 16ss (13.11 µg ml^{-1}), *Aphanothece* sp. 4ss (13.18 µg ml^{-1}),

Anabaena sp. 19ss (12.22 µg ml^{-1}), *Anabaena* sp. 2ss (12.50 µg ml^{-1}), *Nostoc* sp. 17ss (11.50 µg ml^{-1}) *Scytonema* sp. 6ss (11.98 µg ml^{-1}), *Anabaena* sp. 12ss (10.93 µg ml^{-1}) and minimum was showed in *Calothrix* sp. 3ss (9.15 µg ml^{-1}).

Whereas the final day of incubation the maximum carbohydrate was found in *Anabaena* sp.15ss (30.211 µg ml^{-1}) followed by *Calothrix* sp. 14ss (29.201 µg ml^{-1}), *Calothrix* sp. 13ss (27.93 µg ml^{-1}), *Anabaena* sp. 7ss (20.10 µg ml^{-1}), *Nostoc* sp. 9ss 20.87 µg ml^{-1}), *Anabaena* sp. 20ss (17.43 µg ml^{-1}), *Scytonema* sp.1ss (16.4 µg ml^{-1}), *Aphanothece* sp. 5ss (16.04 µg ml^{-1}), *Anabaena* sp. 11ss (15.98 µg ml^{-1}), *Anabaena* sp. 19ss (15.38 µg ml^{-1}), *Aphanothece* sp. 10ss (15.13 µg ml^{-1}), *Scytonema* sp.18ss (14.40 µg ml^{-1}), *Scytonema* sp. 6ss (13.74 µg ml^{-1}), *Anabaena* sp. 2ss (13.41 µg ml^{-1}), *Tolypothrix* sp. 8ss (13.31 µg ml^{-1}), *Aphanothece* sp. 4ss (12.9 µg ml^{-1}), *Nostoc* sp. 16ss 12.82 µg ml^{-1}), *Anabaena* sp. 12ss (12.14 µg ml^{-1}), *Nostoc* sp. 17ss (12.47 µg ml^{-1}) and minimum was found in *Calothrix* sp. 3ss (10.526 µg ml^{-1}).

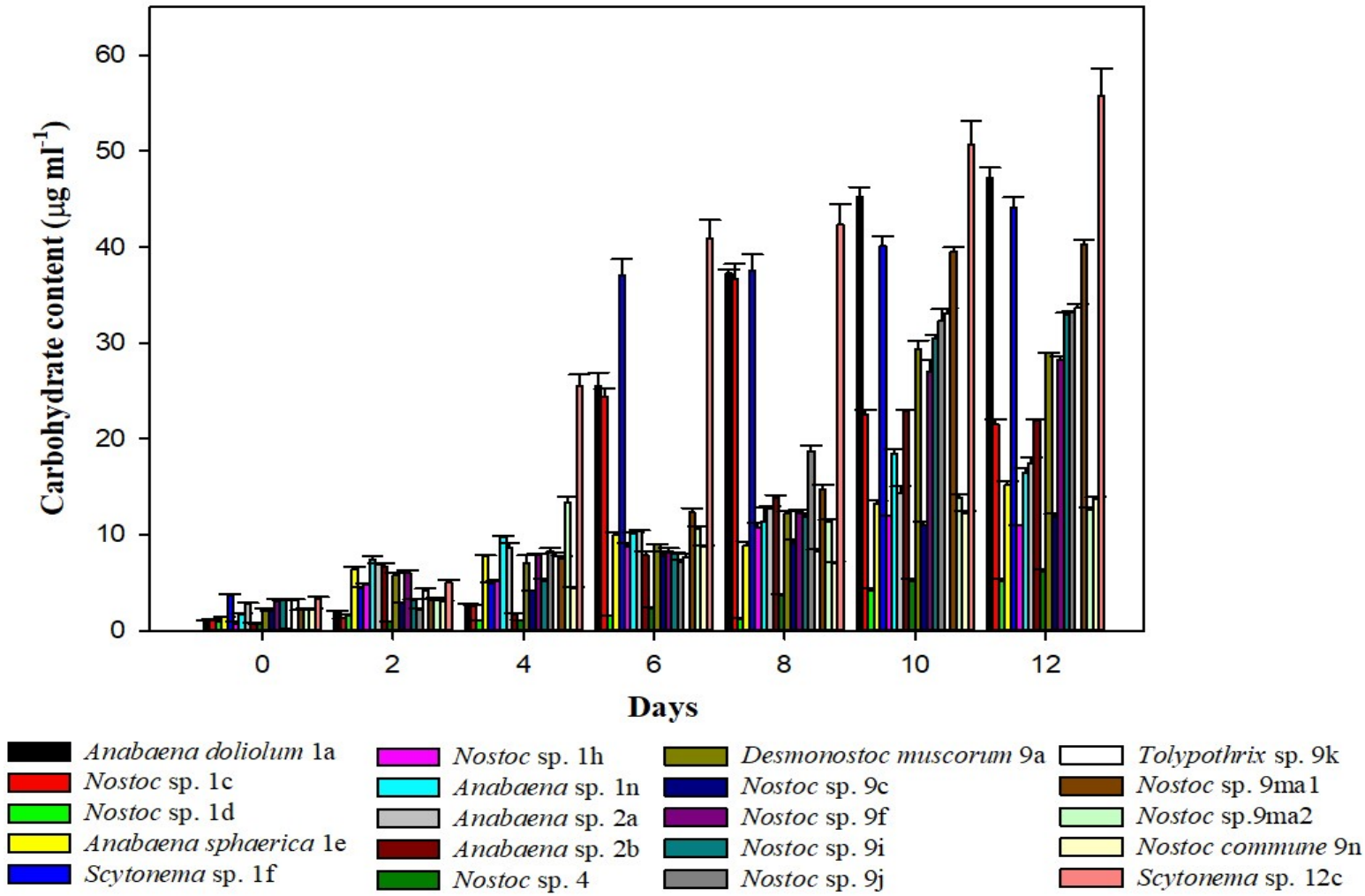

Figure 14. Carbohydrate content of twenty cyanobacterial strains isolated from plain regions of chhattisgarh

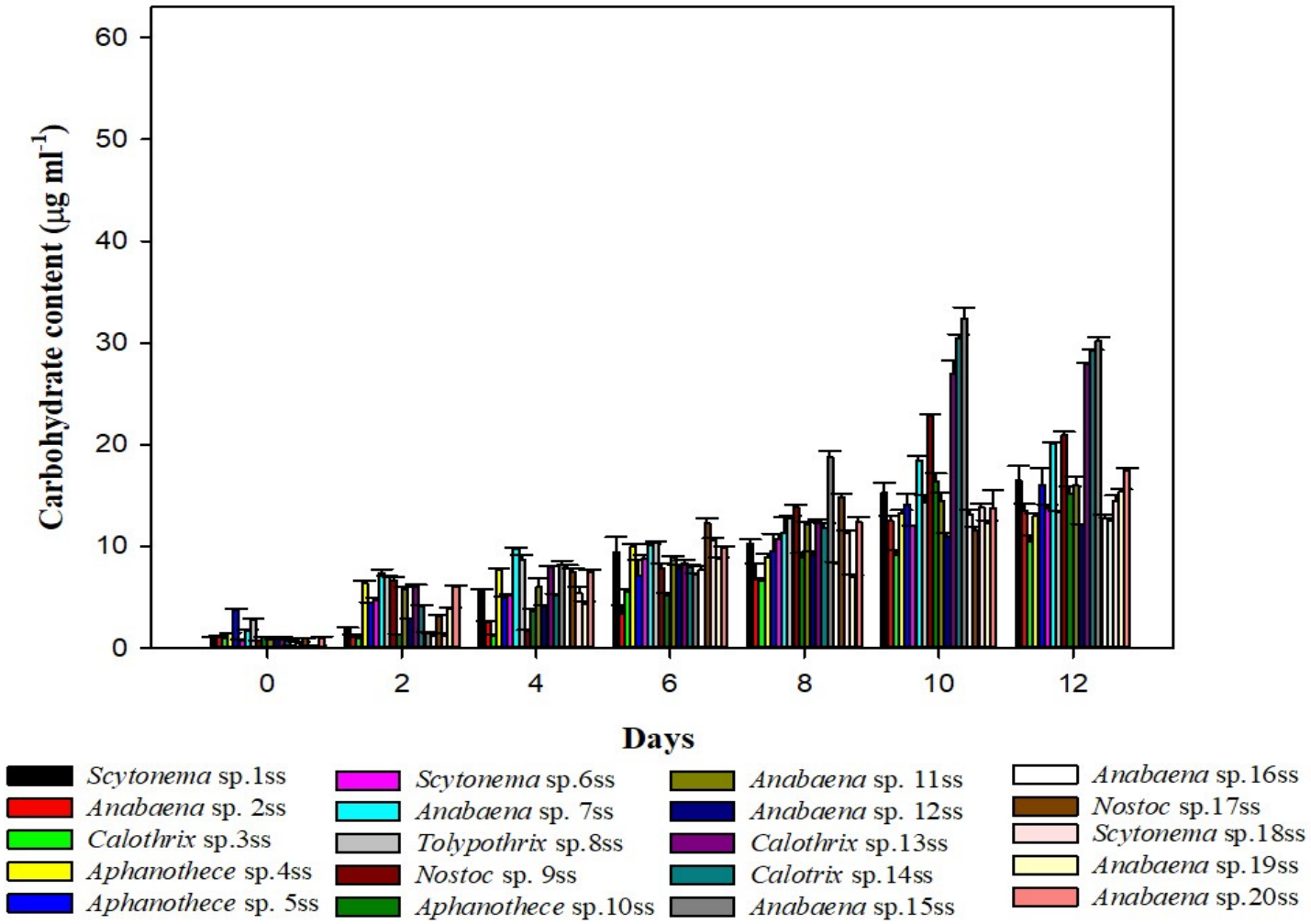

Figure 15. Carbohydrate content of twenty cyanobacterial strains isolated from North hills region of Chhattisgarh

4.4 ENZYMATIC ACTIVITY

4.4.1 *Nitrate reductase activity*

The activity of Nitrate reductase (NR) was observed in forms of µmol No_2^- $µg^{-1}$ protein among different cyanobacterial strains isolated from Plains regions of Chhattisgarh (Figure 17). Nitrate reductase activity was maximum in *Nostoc* sp. 9f (70.96) followed by *Anabaena sphaerica* 1e (66.00), *Nostoc* sp. 1c (66.0), *Anabaena* sp. 1n (66.0), *Scytonema* sp. 12c (56.48), *Desmonostoc muscorum* 9a (43.14), *Anabaena* sp. 2a (42.19), *Scytonema* sp. 1f (39.81), *Anabaena* sp. 2b (36.95), *Nostoc* sp. 9j (34.57), *Anabaena doliolum* 1a (32.67), *Nostoc* sp. 4 (32.19), *Nostoc* sp. 9c (32.67), *Nostoc* sp. 9i (31.24), *Nostoc commune* 9n (26.00), *Nostoc* sp. 9ma2 (25.14), *Nostoc* sp. 1h (21.71), *Nostoc* sp. 9ma1 (21.71) and *Nostoc* sp. 1d (18.38) Minimum was recorded in *Tolypothrix* sp. 9k (16.48).

NR activity was observed in selected cyanobacterial strains isolated in North hills region of Chhattisgarh (Figure 18). Maximum NR activity was found in *Scytonema* sp. 1ss (85.0) followed by *Nostoc* sp. 17ss (79.8), *Anabaena* sp.19ss (79.8) *Nostoc* sp. 9ss (74.6), *Nostoc* sp. 16ss (69.3), *Anabaena* sp.15ss (61.2), *Scytonema* sp. 18ss (58.9), *Anabaena* sp.2ss (51.7), *Anabaena* sp. 12ss (46.5), *Aphanothece* sp. 5ss (35.0), *Anabaena* sp. 7ss (35.0), *Anabaena* sp. 11ss (32.7), *Scytonema* sp. 6ss (30.8), *Anabaena* sp. 20ss (32.1), *Calothrix* sp.13ss (23.1), *Calothrix* sp. 14ss (27.9), *Calothrix* sp. 3ss (22.7), *Aphanothece* sp. 10ss (23.1). Minimum NR activity was reported in *Tolypothrix* sp.8ss (21.7) followed by *Aphanothece* sp. 4ss (21.0).

4.4.2 *Glutamine synthetase (GS) activity*

The level of glutamine synthetase activity among different cyanobacterial species was observed in terms of (nmol γ-glutamyl hydroxamate $µg^{-1}$ protein min^{-1}) (Figure 19). Maximum Glutamine synthetase activity was found in *Scytonema* sp.12c (96.4) followed by *Tolypothrix* sp. 9k (89.8), *Nostoc* sp. 9ma1 (83.1), *Anabaena sphaerica* 1e (83.1), *Nostoc* sp. 9i (79.8), *Nostoc commune* 9n (79.8), *Anabaena doliolum* 1a (76.4), *Desmonostoc muscorum* 9a (73.1), *Nostoc* sp. 9ma2 (73.1),

Anabaena sp. 1n (68.4), *Nostoc* sp. 9c (59.8), *Nostoc* sp. 9j (66.8), *Nostoc* sp. 4 (5.4), *Nostoc* sp. 1c (49.8), *Scytonema* sp. 1f (39.8), *Nostoc* sp. 9f (29.4), *Nostoc* sp. 1d (29.8), *Nostoc* sp. 1h (23.1) and *Anabaena* sp. 2b (22.1). Minimum GS activity was found in *Anabaena sp.* 2a (11.1).

Similarly, *Scytonema* sp. 1ss (99.77) showed maximum GS activity followed by *Nostoc* sp. 9ss (83.10), *Scytonema* sp. 18ss (83.10), *Calothrix* sp.14ss (79.77), *Anabaena* sp. 12ss (73.10), *Anabaena* sp.7ss (66.43), *Anabaena* sp.11ss (55.43), *Anabaena* sp.2ss (49.77), *Scytonema* sp. 6ss (43.10), *Calothrix* sp.13ss (32.43), *Anabaena* sp. 20ss (32.43), *Calothrix* sp. 3ss (32.43), *Tolypothrix* sp. 8ss (29.77), *Nostoc* sp. 17ss (25.77), *Anabaena* sp.19ss (25.77), *Nostoc* sp. 16ss (26.43), *Anabaena* sp. 15ss (23.10). Minimum GS activity was observed in *Aphanothece* sp. 5ss (13.43) followed by *Aphanothece* sp. 4ss (14.6) and *Aphanothece* sp. 10ss (16.43) (Figure.20).

4.4.3 *Nitrogenase activity*

Nitrogen fixing ability was observed among the different cyanobacterial strains isolated from plain regions of Chhattisgarh (Figure 21). Maximum activity was observed in *Desmonostoc muscorum* 9a (198.01) followed by *Nostoc* sp. 9c (106.49), *Anabaena* sp. 1n (117.39), *Nostoc* sp. 1h (145.83), *Nostoc* sp. 9i (73.98), *Anabaena doliolum* 1a (66.02), *Scytonema* sp. 12c (48.70), *Nostoc* sp. 4 (46.51), *Nostoc* sp. 9ma1(28.73), *Anabaena* sp. 2b (21.75), *Anabaena sphaerica* 1e (21.75), *Anabaena* sp. 2a (18.05), *Scytonema* sp. 1f (18.45), *Nostoc* sp. 1c (14.31), *Nostoc* sp. 9ma2 (13.61), *Nostoc* sp. 1d (9.93), *Nostoc* sp. 9j (8.79), *Nostoc* sp. 9f (4.83), *Tolypothrix* sp. 9k (2.55) and *Nostoc commune* 9n (2.00).

There was a great variation in nitrogenase activity of the cyanobacterial species isolated from hills region of Chhattisgarh (Figure 22). Result also suggested that the maximum nitrogenase activity was found in *Anabaena* sp. 7ss (211.85) followed by *Calothrix* sp.13ss (211.28), *Scytonema* sp. 6ss (207.55), *Scytonema* sp. 18ss (177.64), *Calothrix* sp.3ss (174.99), *Anabaena* sp.15ss (123.30), *Nostoc* sp.17ss

(105.53), *Anabaena* sp.11ss (75.61), *Anabaena* sp. 20ss (73.31), *Tolypothrix* sp.8ss(68.58), *Nostoc* sp. 16ss (62.08), *Nostoc* sp. 9ss (57.67), *Anabaena* sp.2ss (44.72), *Aphanothece* sp. 10ss (37.54), *Calothrix* sp.14ss (37.43), *Scytonema* sp. 1ss (37.11), *Anabaena* sp.19ss (31.19), *Anabaena* sp.12ss (25.54), *Aphanothece* sp.5ss (19.07) and the minimum was reported in *Aphanothece* sp. 4ss (16.73).

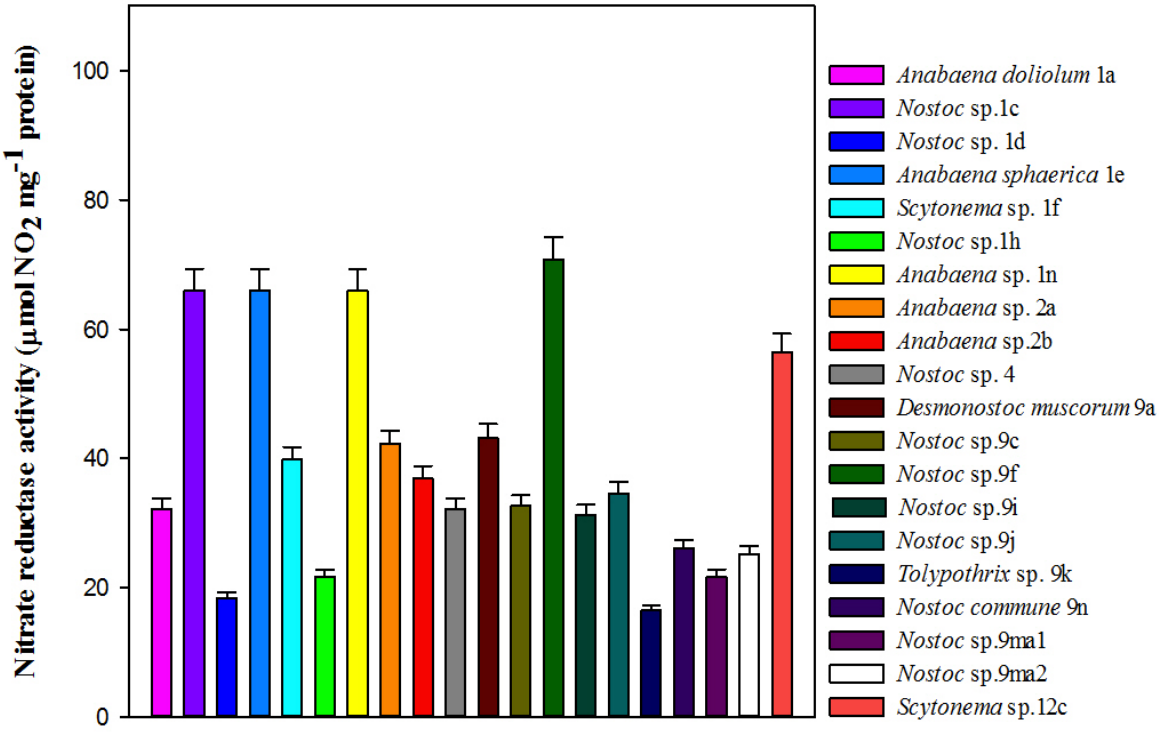

Figure 16. Nitrate reductase activity in cyanobacterial strains isolated from Plain regions of Chhattisgarh

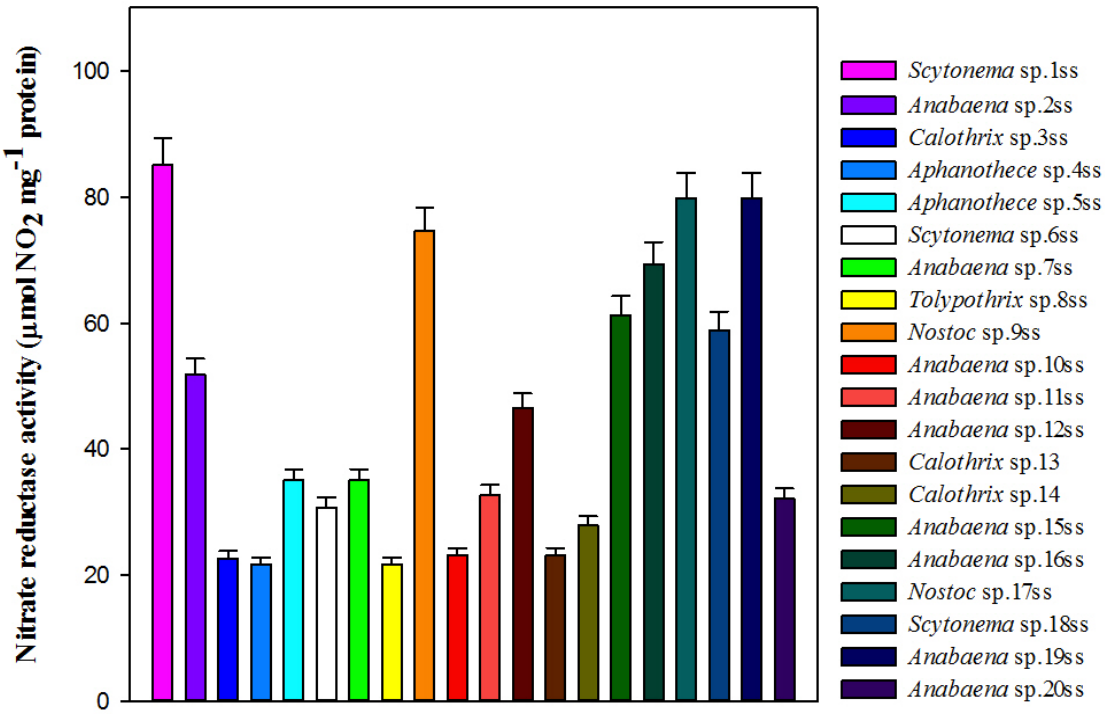

Figure 17. Nitrate reductase activity in cyanobacterial strains isolated from North hills region of Chhattisgarh

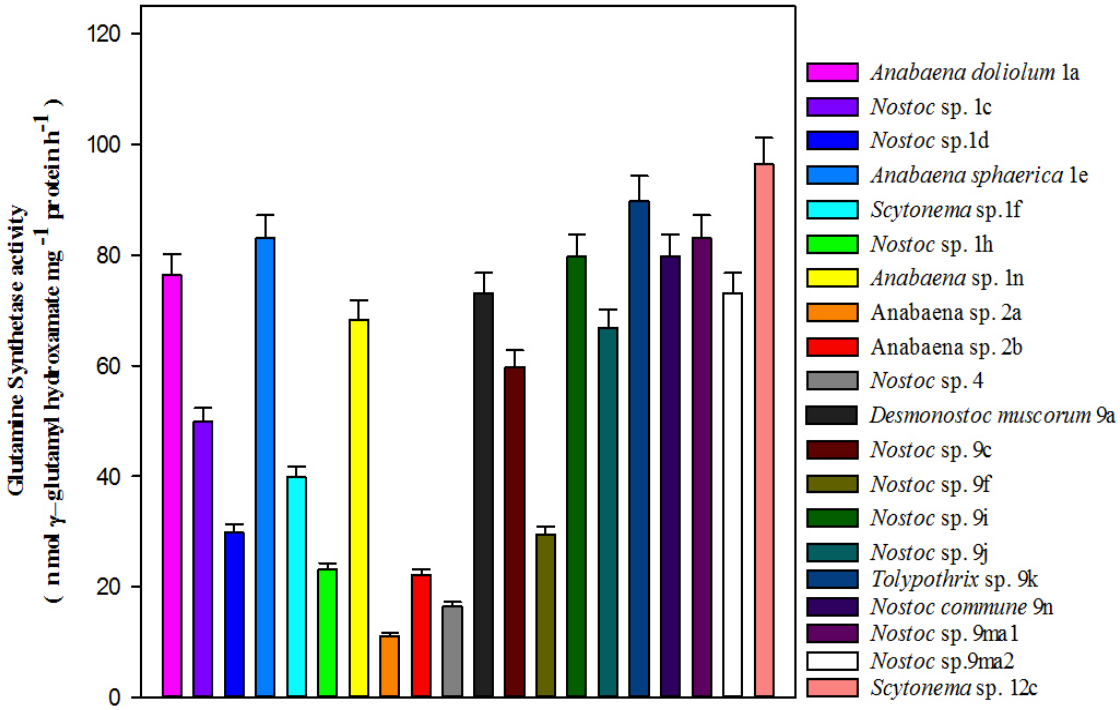

Figure 18. Glutamine synthetase activity in cyanobacterial strains isolated from Plain regions of Chhattisgarh

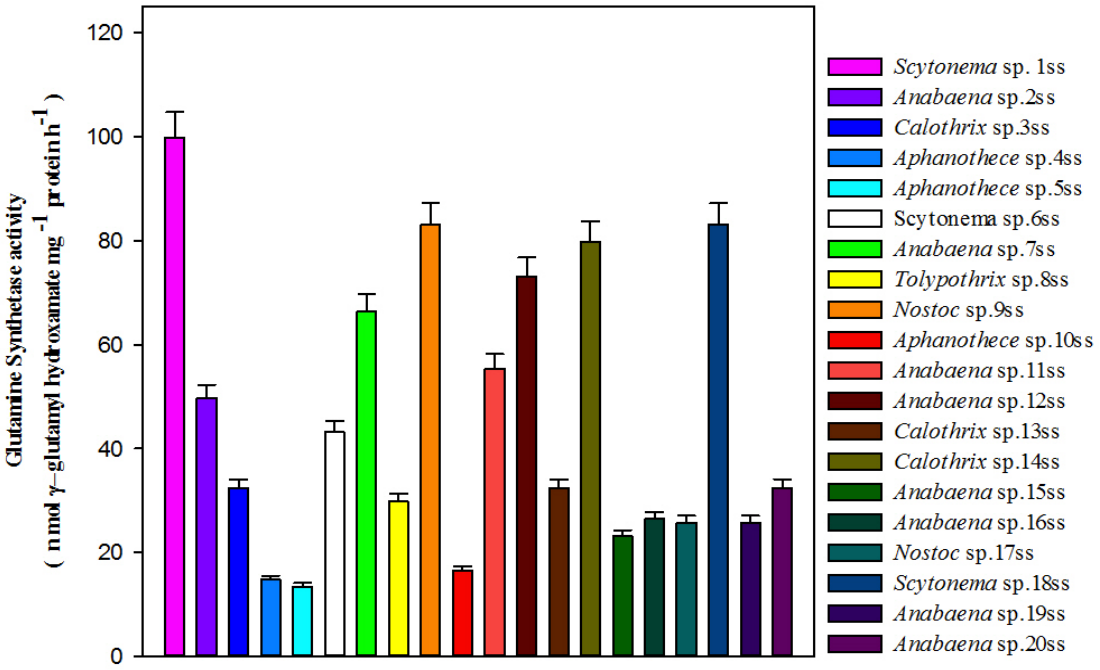

Figure 19. Glutamine synthetase activity in cyanobacterial strains isolated from North hills regions of Chhattisgarh

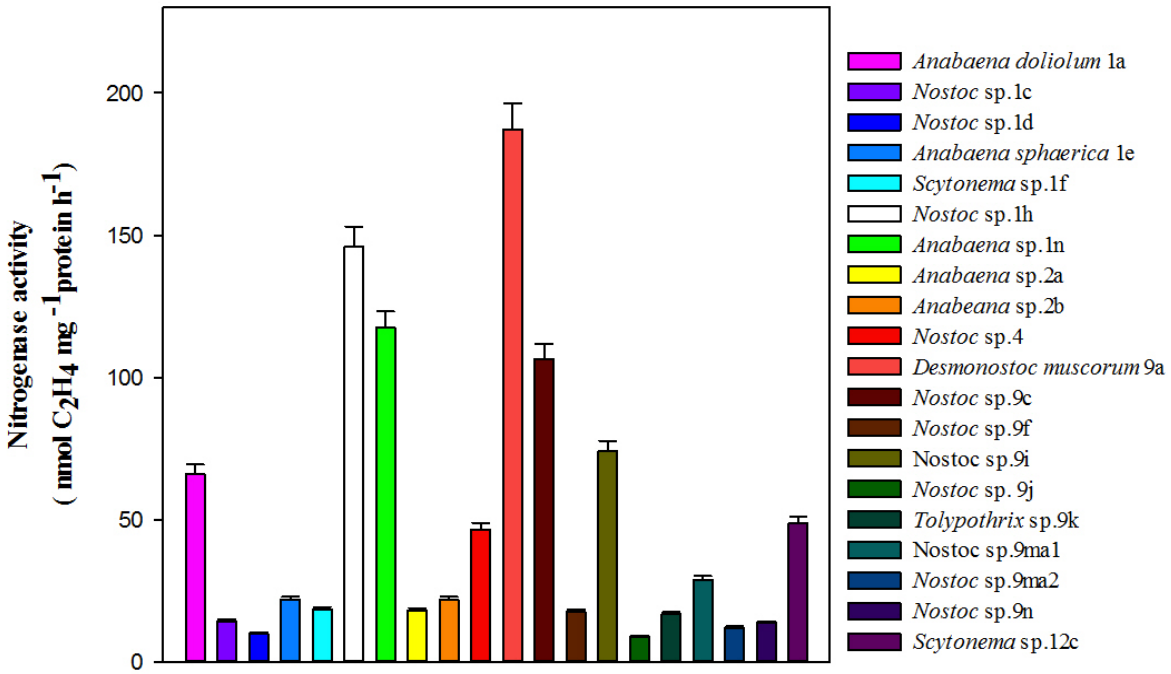

Figure 20. Nitrogenase enzyme activity in cyanobacterial strains isolated from plain regions of Chhattisgarh

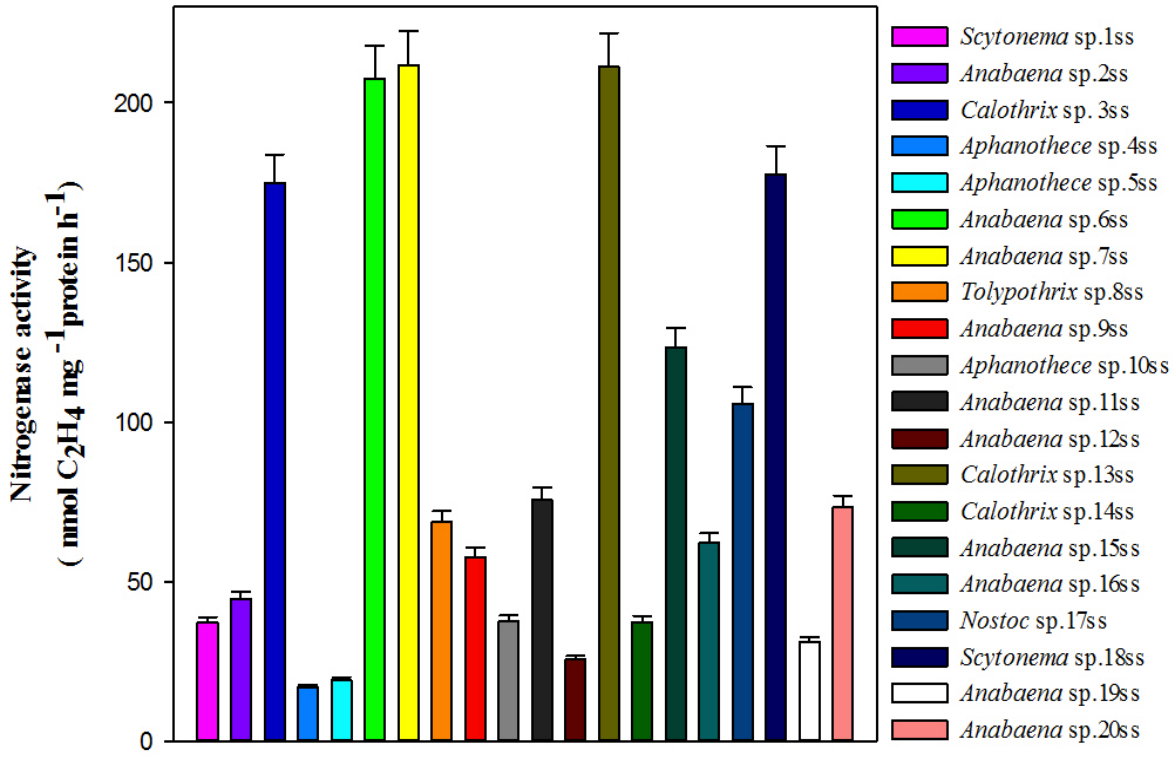

Figure 21. Nitrogenase enzyme activity in cyanobacterial strains isolated from North hills region of Chhattisgarh

4.5 BIOCHEMICAL CHARACTERIZATION

4.5.1 *FAME (Fatty Acid Methyl Ester) Analysis*

Biochemical characterization has been done in terms of FAME profiling of diffferent cyanobacterial strains isolated from plains of Chhattisgarh by using Gas Chromatograph (Figure 22-26) and results suggested that the total twenty eight different types of fatty acids were observed in cyanobacteria (Table 5).

In the present study, Three fatty acid were found in all the cyanobacterial strains *i.e.* 9-hexadecenoic fatty acid (C:16:1) and 9,12 octadecadienoic acid (C18:2).

Undecanoic acid (C11:0) was found only in *Scytonema* sp. 1f and *Scytonema* sp. 12c.

Almost equal amount of Dodecanoic acid or Lauric acid (C12:0) was found in almost equal percent in *Anabaena doliolum* 1a, *Nostoc* sp. 9ma1 and *Nostoc* sp. 9ma2 whereas Tetradecanoic acid or Myristic acid (C14:0) was found only in *Nostoc* sp. 9j and the rest of ninteen cyanobacterial strains were devoid of Tetradecanoic acid content.

The amount of Pentadecanoic acid (C15:0) was highest in *Anabaena* sp. 2b (1.7%) followed by *Anabaena doliolum* 1a (1.036%), *Nostoc* sp. 9n (0.89%), *Nostoc* sp. 9j (0.5%), *Anabaena* sp. 2a (0.55%), *Scytonema* sp. 1f (0.2%) and *Nostoc* sp. 4 (0.22%). Minimum was observed in *Anabaena* sp. 1n (0.13%) whereas rest of twenty cyanobacterial strains did not have pentadecanoic acid.

Hexadecanoic acid (C16:0) was detected among the cyanobacterial strains except *Anabaena* sp. 2a. Maximum percent of Hexadecanoic acid was found in *Nostoc* sp. 9j (51.7%) followed by Nostoc sp. 9ma1 (49.07%), *Tolypothrix* sp. 9k (46.0%), *Nostoc* sp. 1c (46.7%), *Nostoc* sp. 9i (41.8%), *Scytonema* sp. 12c (40.3%), *Nostoc* sp. 9c (38.5%), *Nostoc* sp. 9f (38.3%), *Nostoc* sp. 1d (37.9%), *Nostoc* sp. 1h (36.6%), *Nostoc* sp. 9a (34.9%), *Desmonostoc muscorum* 9a (33.6%), *Anabaena* sp. 1n (32.3%), *Anabaena doliolum* 1a (31.22%), *Nostoc* sp. 4(30.5%) and *Scytonema* sp.1f (28.0%). Minimum was observed in *Anabaena* sp. 2b (14.0%).

Maximum 9-Hexadecenoic acid (16:1) was found in *Anabaena doliolum* 1a (17.4%) followed by *Nostoc* sp. 1c (13.3%), *Anabaena* sp. 1d (14.5%), *Anabaena sphaerica* 1e (20.0%), *Nostoc* sp. 9j (10.0%), *Nostoc* sp. 9n (10.6 %), *Nostoc* sp. 9ma1(5.30%), *Nostoc* sp. 9ma2 (5.30%), *Scytonema* sp. 12c (7.45%), *Nostoc* sp. 9j (0.32%) and *Nostoc* sp. 9i (0.39%).

Amount of Heptadecanoic acid (Margaric acid) (17:0) was found in maximally *Anabaena* sp. 2a (35.36%) followed by *Nostoc* sp. 9c (0.88%), *Anabaena* sp. 2b (0.63%), *Nostoc* sp. 9k (0.49%), *Nostoc* sp. 9n (0.48%), *Nostoc* sp. 9ma1(0.26%), *Nostoc* 9ma2 (0.267%), *Desmonostoc muscorum* sp. 9a (0.289%), *Nostoc* sp. 1h (0.20%), *Scytonema* sp. 1f (0.15%) and *Anabaena sphaerica* 1e (0.123%).

Cis 10- Heptadecenoic (17:1) acid found only in six cyanobacterial strains and it was maximum found *Nostoc* sp. 9n (0.60%) followed by *Nostoc* sp. 9j (0.54%), *Anabaena* sp. 2b (0.49%), *Anabaena* sp. 1n (0.32%), *Nostoc* sp. 4 (0.24%) and *Scytonema* sp. 1f (0.21%).

The amount of Cis-10 Nonadecanoic acid (C17:1) was maximum in found *Nostoc* sp. 9n (1.29%) followed by *Anabaena* sp. 2a (0.77%), *Nostoc* sp. 9f (0.58%), *Nostoc* sp. 9a (0.55%), *Nostoc* sp. 9j (0.39%), *Anabaena* sp. 2b (0.329%), *Nostoc* sp. 1h (0.31%) and *Nostoc* sp.4 (0.19%).

Maximum Octadecanoic acid (Stearic acid) (18:0) was found in maximum in *Anabaena* sp. 1d (8.22%) followed by *Nostoc* sp. 1c (5.22%), *Anabaena doliolum* 1a (0.64%), *Nostoc* sp. 9c (0.64%), *Anabaena* sp. 2a (0.30%), *Nostoc* sp. 9f (0.3%) and minimum stearic acid was found in *Anabaena sphaerica* 1e (0.13%) followed by *Nostoc* sp. 9n (0.19%).

9-Octadecenoic acid (C18:1) was found maximally in *Anabaena* sp. 1n (18.1%) followed by *Nostoc* sp. 1c (4.05%) followed by *Anabaena doliolum* 1a (0.402%), *Anabaena sphaerica* 1e (0.30%) and *Nostoc* sp. 9i (0.30%). Whereas minimum was found in *Nostoc* sp. 1h (0.15%).

Maximum 11-octadecenoic acid (C18:1) was found in *Anabaena doliolum* 1a (20.6%) followed by *Anabaena sphaerica* 1e (20.5%), *Desmonostoc muscorum* 9a (12.4%) and *Nostoc* sp. 9c (12.4%), *Tolypothrix* sp. 9k (9.97%), *Nostoc* sp. 9ma1 (9.66%), *Nostoc* sp. 9ma2 (9.66%), *Nostoc* sp. 9j (8.6%), *Anabaena* sp. 2b (5.8%), *Nostoc* sp. 9i (3.9%) and *Nostoc* sp. 9f (3.53%). The lowest fatty acid content was found in *Scytonema* sp. 12c (2.96%) followed by *Nostoc* sp. 4(11.0%) and *Nostoc commune* 9n (5.80%).

9,12- Octadecadienoic acid (Linolenic acid) (18:2) was found to be highest in *Nostoc* sp. 1c (24.1%) followed by *Nostoc* sp. 9c (23.3%), *Anabaena doliolum* 1a (21.5%), *Nostoc* sp. 9i (20.3%), *Anabaena* sp. 2b (19.9%), *Anabaena* sp. 2a (19.8%), *Scytonema* sp. 1f (19.7%), *Anabaena* sp.1d (19.6%), *Tolypothrix* sp. 9k (19.01%), *Anabaena sphaerica* 1e (19.3%), *Nostoc* sp. 4 (19.2%), *Nostoc* sp. 1h (18.3%), *Scytonema* sp. 12c (18.5%), *Nostoc* sp. 9n (16.0%), *Nostoc* sp. 9f (14.0%), *Nostoc* sp. 9ma1(s)and *Nostoc* sp. 9ma2 (8.43%), *Nostoc* sp. 9j (6.020%) and *Desmonostoc muscorum* 9a (2.0%). The minimum was found in *Anabaena* sp. 1n (0.20%).

9, 12, 15-Octadecatrienoic acid (α-Linolenic acid) (C18:3) was maximumally found in *Nostoc* sp. 9n (25.4%) followed by *Nostoc* sp. 9f (22.7%), *Anabaena* sp. 2b (22.06%), *Nostoc* sp. 1h (21.2%), *Scytonema* sp. 1f (18.1%), *Desmonostoc muscorum* 9a (1.3%). The lowest amount of α-Linolenic acid was found in *Nostoc* sp. 9c (0.91%) and *Nostoc* sp. 9j (0.26%).

Oxiranectonoic acid (C19:0) was observed in maximumally in *Nostoc* sp. 9f (2.57%), *Scytonema* sp. 12c (0.54%) and followed by *Nostoc* sp.9ma1 and *Nostoc* sp. 9ma2 (0.40%), *Desmonostoc muscorum* 9a (0.34%), *Nostoc* sp. 9n (0.30%), *Anabaena* sp. 2a (0.27%) and *Nostoc* sp. 1h (0.16%).

Cis-13 Octadecenoic acid (19:1) was found in the range of 1.5 to 4.9% and mmaximum was reported in *Anabaena doliolum* 1a (4.90%) followed by *Anabaena sphaerica* 1e (4.60%), *Anabaena* sp. 1n (3.41%), *Nostoc* sp. 1h (3.39%), *Nostoc* sp. 9c (3.04%), *Scytonema* sp. 12c (3.28%), *Nostoc* sp. 9i (2.83%), *Desmonostoc muscorum*

9a (2.76%), *Anabaena* sp. 1d (2.53%), *Tolypothrix* sp. 9k (2.06%), *Nostoc* sp. 9ma1 and *Nostoc* sp. 9ma2 (1.50%).

Gamma-linolenic acid (C19:3) was only found in four cyanobacterial species. Maximum was reported in *Nostoc* sp. 9ma1 (8.0%), *Nostoc* sp. 9ma2 (8.0%) and *Nostoc* sp. 9j (6.2%) whereas the minimum was observed in *Nostoc* sp. 9n (1.0%).

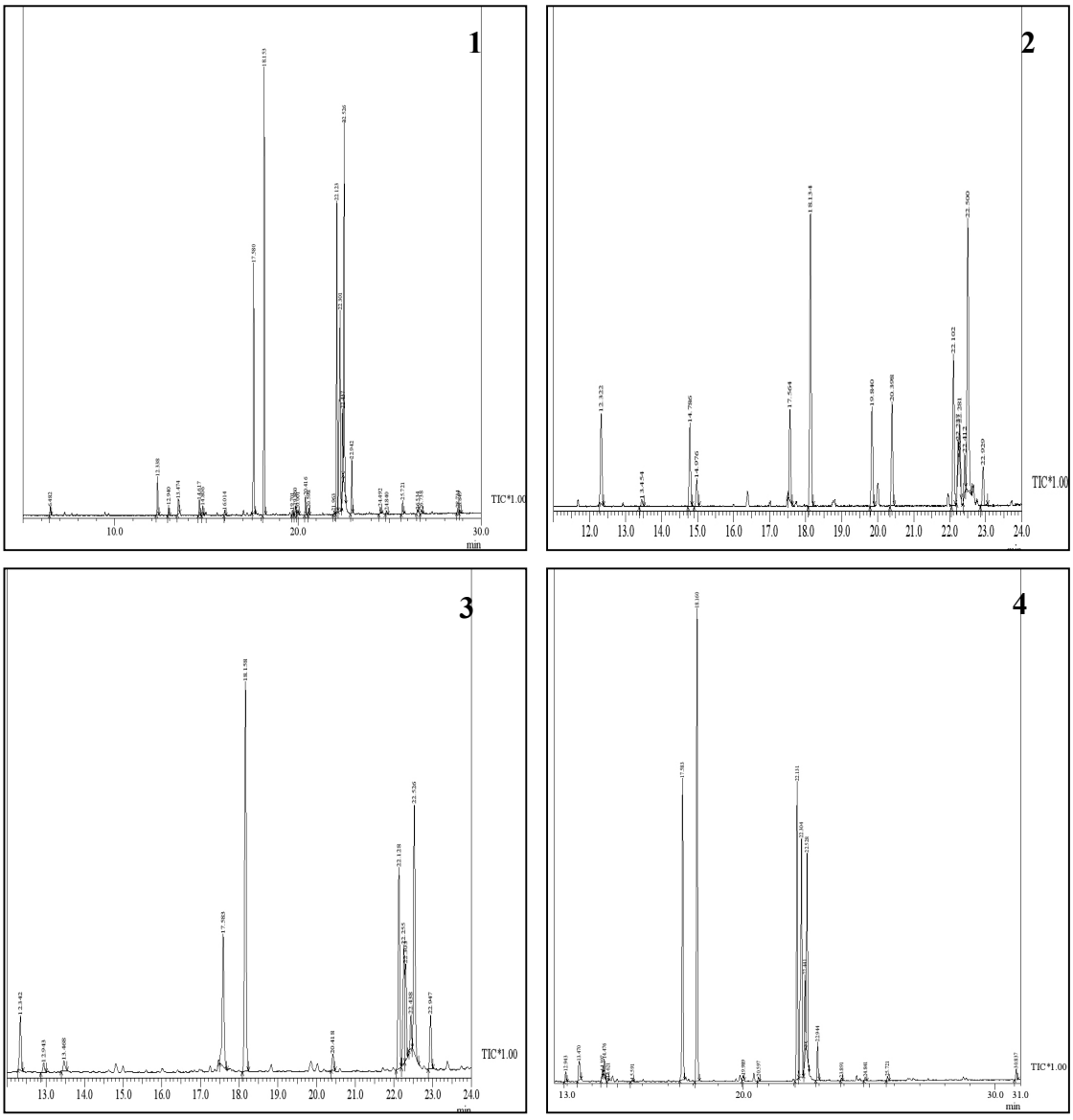

Figure 22. GCFID chromatogram of cyanobacterial species isolated from plain regions of Chhattisgarh (1) *Anabaena doliolum* 1a (2) *Nostoc* sp. 1c (3) *Nostoc* sp. 1d (4) *Anabaena sphaerica* 1e. Each peak depicts presence of fatty acids at specific retention time.

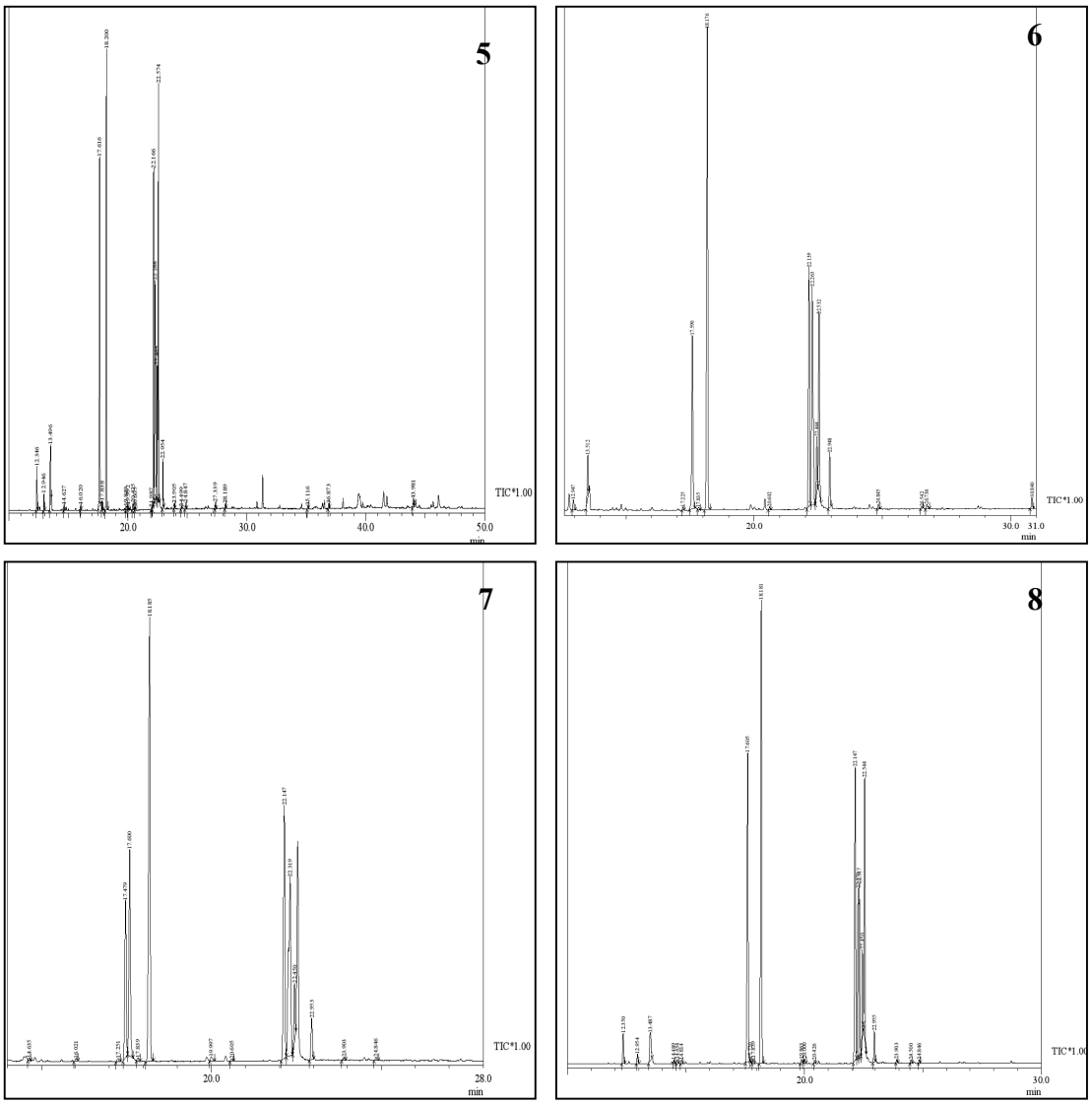

Figure 23. GCFID chromatogram of cyanobacterial species isolated from plain regions of Chhattisgarh (5) *Scytonema* sp. 1f (6) *Nostoc* sp. 1h (7*) Anabaena* sp. 1n (8) *Anabaena* sp. 2a. Each peak depicts presence of fatty acids at specific retention time.

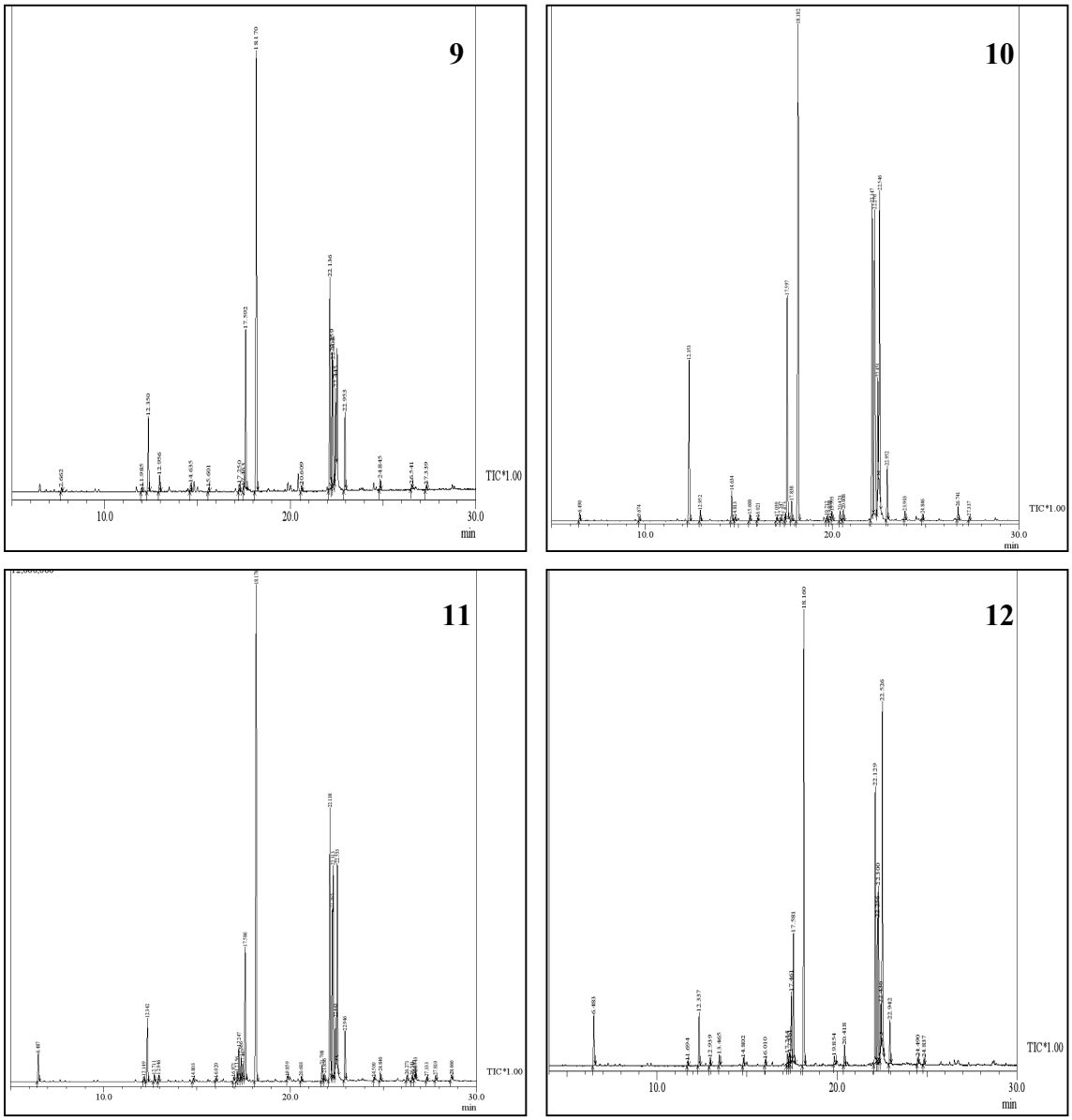

Figure 24. GCFID chromatogram of cyanobacterial species isolated from plain regions of Chhattisgarh. (9) *Anabaena* sp. 2b (10) *Nostoc* sp. 4 (11) *Desmonostoc muscorum* 9a (12) *Nostoc* sp. 9c Each peak depicts presence of fatty acids at specific retention time.

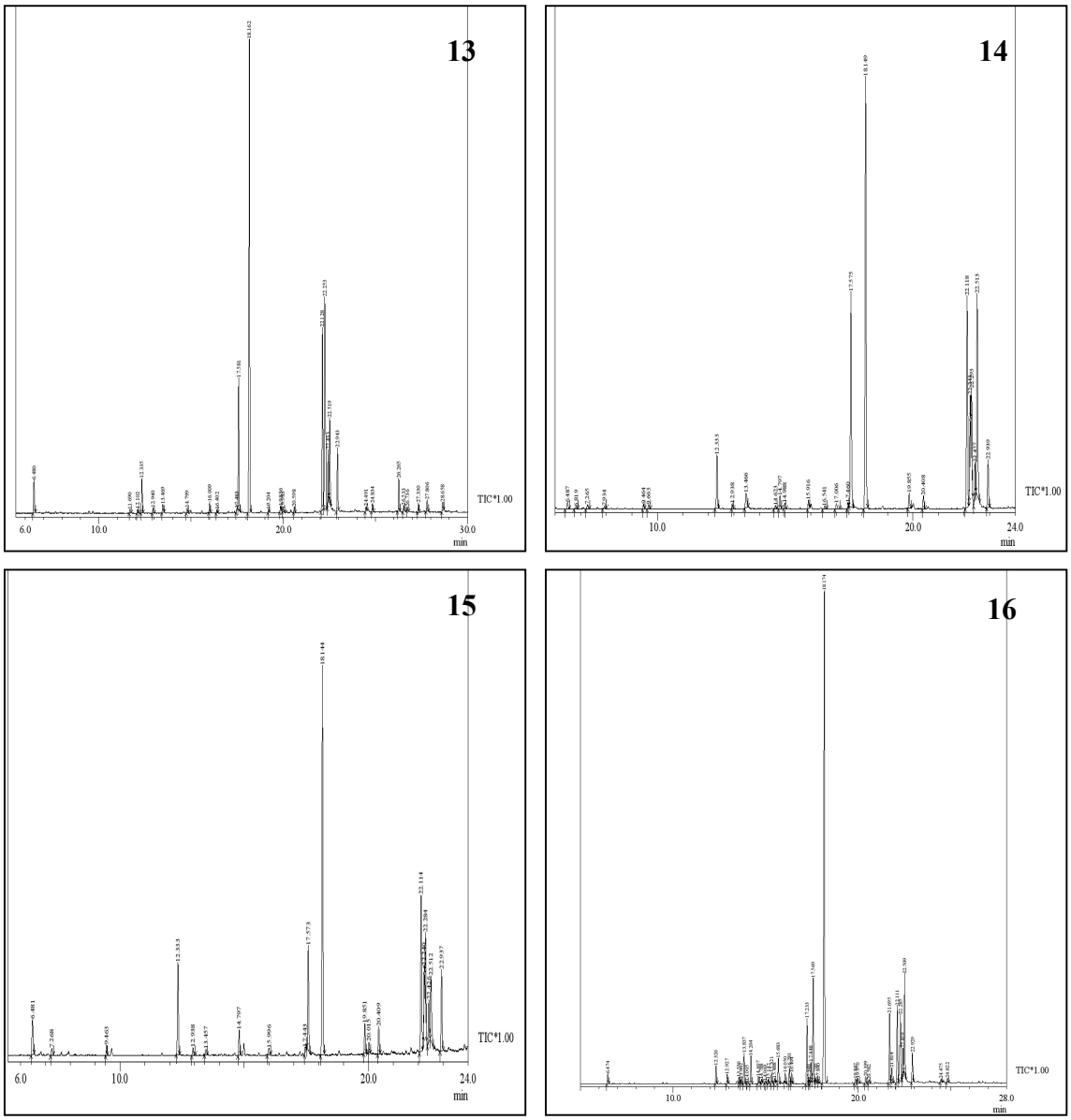

Figure 25. GCFID chromatogram of cyanobacterial species isolated from plain region sof Chhattisgarh (13) *Nostoc* sp. 9f (14) *Nostoc* sp. 9i (15) *Nostoc* sp. 9j (16) *Tolypothrix* sp. 9k. Each peak depicts presence of fatty acids at specific retention time.

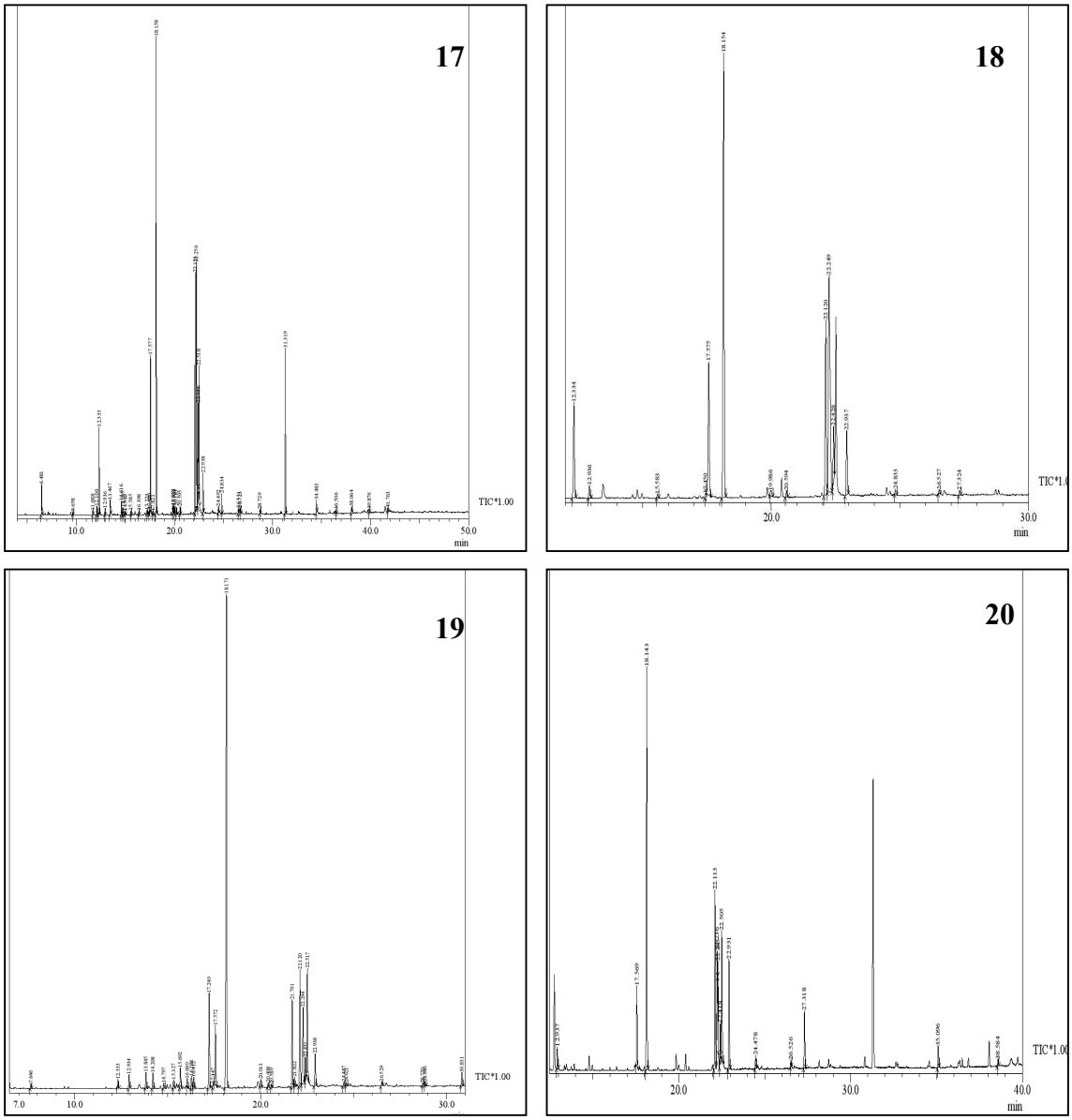

Figure 26. GCFID chromatogram of cyanobacterial species isolated from plain regions of Chhattisgarh (17*) Nostoc commune* 9n (18) *Nostoc* sp. 9ma1 (19) *Nostoc* sp. 9ma2 (20) *Scytonema* sp. 12c. Each peak depicts presence of fatty acids at specific retention time.

Table 5. Fatty acid composition and percentage of total fatty acid in cyanobacteria isolated from Plain regions of Chhattisgarh

	Fatty acid	Cyanobacterial strains																		
		1a	**1c**	**1d**	**1e**	**1f**	**1h**	**1n**	**4**	**2a**	**2b**	**9a**	**9c**	**9f**	**9i**	**9k**	**9j**	**9n**	**9ma1**	**9ma2**	**12c**
1	Undecanoic acid **(C11:0)**	-	-	-	-	0.605	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.196
2	Dodecanoic acid **(C12:0)**	0.41	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.43	0.43	-
3	Tetradecanoic acid **(C14:0)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.79	-	-	-	-
4	13-Tetradecynoic acid **(C14:1)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.33	-	-	-
5	Pentadecanoic acid **(C15:0)**	1.03	-	-	-	0.22	-	0.13	0.2	0.55	1.7	-	-	-	-	-	0.5	0.89	-	-	-
6	Hexadecanoic acid, **(C16:0)**	31.2	46.7	37.9	30.5	28.	36.6	32.3	30.5	-	14.0	34.9	38.5	38.3	41.8	46.0	51.7	33.6	49.07	49.07	40.36
7	9-hexadecenoic acid **(C16:1)**	17.4	13.3	14.5	20.0	20.5	13.5	24.8	21.4	12.2	0.2	8.6	10.5	10.0	21.7	11.7	10.0	10.6	5.30	5.30	7.45
8	Heptadecanoic acid **(C17:0)**	-	-	-	0.123	0.155	0.20	0.15	-	35.3	0.63	0.28	-	0.39	0.49	0.88	0.32	0.48	0.26	0.267	-
9	Cis-10-Heptadecenoic acid **(C17:1)**	-	-	-	-	0.211	-	0.32	0.24	-	0.49	-	-	-	-	-	0.54	0.60	-	-	-
10	Cis-10-Nonadecenoic acid **(C17:1)**	-	-	-	-	-	0.31	-	0.19	0.77	0.32	0.55	-	0.58	-	-	0.39	1.292	-	-	-
11	Octadecanoic acid **(C18:0)**	0.64	5.22	8.22	0.13	-	-	-	-	0.30	-	-	0.64	0.3	-	-	-	0.19	-	-	-
12	9-octadecenoic acid **(C18:1)**	0.40	4.05	-	0.30	-	0.15	18.1	-	-	-	-	-	-	0.3	-	-	-	-	-	
13	11-Octadecenoic acid **(C18:1)**	20.6	-	-	20.5	-	-	-	11.0	-	5.8	12.4	12.4	3.53	3.9	9.97	8.6	5.800	9.66	9.66	2.96
14	3,6-Octadecadienoic acid **(C18:2)**	-	-	-	-	-	0.43	-	-	-	-	-	-	0.3	0.27	-	-	0.34	-	-	-
15	8,11-Octadecadienoic acid **(C18:2)**	-	-	-	-	-	-	-	0.20 -	-	-	-	-	-	-	-	-	-	-	-	-
16	9,12-octadecadienoic acid **(C18:2)**	21.5	24.1	19.6	19.3	19.7	18.3	0.20	19.2	19.8	19.9	2.0	23.3	14.0	20.3	19.0	6.02	16.0	8.43	8.43	18.5

17	9,12,15-Octadecatrienoic acid **(C18:3)**	-	-	-	-	18.101	21.2	-	-	-	22.6	1.3	0.91	22.7		-	0.26	25.4	-	-	-
18	Oxiranectonoic acid, **(C19:0)**	-	-	-	-	-	0.16	-	-	0.272	-	0.34	-	2.57	-	-	-	0.305	0.400	0.400	0.543
19	Cis-13 Octadecenoic acid **(C19:1)**	4.90	-	2.53	4.60	-	3.39	3.41	-	-	-	2.74	3.04	-	2.83	-	2.06	-	1.50	1.505	*3.282*
20	Gamma.-linolenic acid, **(C19:3)**	-	-	-	-	-	-	-	-	-	-	1.0	-	-	-	-	6.2	-	8.00	8.00	-
21	Eicosanoic acid **(C20:0)**	-	-	-	-	-	-	-	-	0.35	0.21	0.26	-	0.19	0.66	-	-	-	-	-	-
22	5,8,11,14-Eicosatetraenoic acid **(C20:5)**	0.912	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	Docosanoic acid **(C22:0)**	-	-	-	-	0.30	-	-	-	-	-	-	-	0.7	-	-	-	-	1.22	1.22	-
24	Cyclopropaneoctanoic acid **(C22:0)**	-	-	-	-	0.30	-	0.23	-	-	-	-	0.51	-	-		-	-	-	-	-
25	13-docosenoic acid, **(C22:1)**	-	-	-	0.704	7.53	0.82	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	11,14-Eicosadienoic acid **(C22:2)**	-	-	-	-	-	-	-	-	-	0.16	-	-	-	-	-	-	-	-	-	-
27	Tetracosanoic acid **(C24:0)**	0.32	6.43	-	-	0.15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.28
28	Hexacosanoic acid **(C26:0)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.690

Legends showed different strain of Cyanobacteria isolated from Plains region of Chhattisgarh. (1) *Anabaena doliolum* 1a (2) *Nostoc* sp. 1c (3) *Nostoc* sp. 1d (4) *Anabaena spherical* 1e (5) *Scytonema* sp. 1f (6) *Nostoc* sp. 1h (7*) Anabaena* sp. 1n (8) *Anabaena* sp. 2a (9) *Anabaena* sp. 2b (10) *Nostoc* sp. 4 (11) *Desmonostoc muscorum* 9a (12) *Nostoc* sp. 9c (13) *Nostoc* sp. 9f (14) *Nostoc* sp. 9i (15) *Nostoc* sp. 9j (16) *Tolypothrix* sp.9k (17*) Nostoc commune* 9n (18) *Nostoc* sp. 9ma1 (19) *Nostoc* sp. 9ma2 (20) *Scytonema* sp. 12c

Table 6. Percent and ratio of MUFAs, PUFAs and SAFAs in twenty different Cyanobacterial strains isolated from plain regions

Cyanobacterial strains	SAFAs (%)	MUFAs (%)	PUFAs (%)	MUFA+PUFA/TOTAL	MUFA/PUFA	SAFA/MUFA
Anabaena doliolum 1a	33.65	43.47	22.50	0.662	1.931	0.77
Nostoc sp. 1c	58.44	17.44	24.11	0.415	0.723	3.35
Nostoc sp. 1d	46.14	17.03	19.6	0.443	0.869	2.354
Anabaena sphaerica 1e	31.2	28.50	19.33	0.605	1.474	1.616
Scytonema sp. 1f	36.97	18.22	39.96	0.611	0.456	0.925
Nostoc sp. 1h	0.5	79.1	17.8	0.995	4.442	0.029
Anabaena sp. 1n	30.54	33.76	19.22	0.634	1.757	1.589
Anabaena sp. 2a	36.84	12.99	19.89	0.472	0.653	1.852
Anabaena sp. 2b	47.9	13.5	42.8	0.540	0.316	1.120
Nostoc sp. 4	35.90	24.43	1.01	0.415	24.13	35.47
Desmonostoc muscorum 9a	39.1	26.5	24.2	0.56	1.09	1.61
Nostoc sp. 9c	41.12	13.60	37.42	0.554	0.363	1.099
Nostoc sp. 9f	43.00	28.94	20.31	0.534	1.424	2.117
Nostoc sp. 9i	47.0	21.7	19.0	0.464	1.141	2.469
Nostoc sp. 9j	53.40	22.52	19.75	0.441	1.140	2.704
Tolypothrix sp.9k	34.85	10.99	16.06	0.437	0.684	2.170
Nostoc commune 9n	0.68	0.740	8.43	0.437	0.684	2.169
Nostoc sp. 9ma1	49.8	16.6	8.0	0.331	2.074	6.222
Nostoc sp. 9ma2	47.9	13.5	42.8	0.540	0.316	1.120
Scytonema sp. 12	42.57	17.22	18.53	0.456	0.930	2.298

SAFAs **-Saturated fatty acid,** ***MUFAs*** **-Monounsaturated fatty acid,** ***PUFAs*** **-Polyunsaturated fatty acid**

Eicosanoic acid or Arachidic acid (C20:0) was found maximumally in Nostoc sp. 9i (0.66%) followed by *Anabaena* sp. 2a (0.35%), *Desmonostoc muscorum* 9a (0.26%), *Anabaena* sp. 2b (0.21%) and *Nostoc* sp. 9f (0.19%).

Some fatty acids were only found in a single cyanobacterial strains *i.e.* 5,8,11,14- Eicostateraenoic acid or Icosapentaenoic acid (C20:5) was reported only in *Anabaena doliolum* 1a (0.912%), 8,11-Octadecadienoic (C18:2) acid was found in *Nostoc* sp. 4 (0.20%), 11,14-Ecosadienoic acid (22:2) was reported in *Anabaena* sp. 2b (0.16%), 13-Tetradecynoic acid (C14:1) was found in *Nostoc* sp. 9n (0.33%), Tetradecanoic acid (C14:0) was found in Nostoc sp. 9j (0.79%), Hexacosanoic acid (C26:0) was found in *Scytonema* sp. 12c (0.69%).

Tetracosanic acid or Lignoceric acid (C24:0) was found in *Scytonema* sp. 12c (2.28 %), *Nostoc* sp. 1c (6.43%), *Anabaena doliolum* 1a (0.32%) and *Scytonema* sp. 1f (0.15%).

The ratio of MUFAs, PUFAs and SAFAs, Total MUFAs + PUFAs, MUFAs/PUFAs and SAFAs/MUFAs were calculated on the basis of fatty acid compositions of different cyanobacterial species isolated from plains region of Chhattisgarh. The percentage of Saturated fatty acid (SAFA) was also calculated and maximum in *Nostoc* sp. 1c (58.44%) followed by *Tolypothrix* sp. 9k (53.40%) and followed by *Nostoc* sp. 9ma1 (49.8%), *Nostoc* sp. 9ma2 (47.9%), *Anabaena* sp. 2b (47.9%), Nostoc sp. 9i (47.0%), *Anabaena* sp. 1d (46.14%), *Nostoc* sp. 9f (43.00%), *Scytonema* sp. 12c (42.57%), *Nostoc* sp. 9c (41.12%), *Desmonostoc muscorum* 9a (39.1%), *Nostoc* sp. 4 (36.84%), *Scytonema* sp. 1f (36.97%), *Anabaena* sp. 2b (35.90%), *Nostoc* sp. 9j (34.85%), *Anabaena doliolum* 1a (33.65%), *Anabaena sphaerica* 1e (31.23%), *Anabaena* sp. 1n (30.54%), *Nostoc* sp. 9n (0.68%) and *Nostoc* sp. 1h (0.5%) (Table 6).

Similarly, the observed percentage of Monounsaturated fatty acid (MUFA) was maximum *Nostoc* sp. 1h (79.1%), *Anabaena doliolum* 1a (43.47%), *Anabaena* sp. 1n (33.76%), *Nostoc* sp. 9f (28.94%), *Anabaena sphaerica* 1e (28.50%), Desmo*nostoc muscorum* 9a (26.5%), *Anabaena* sp. 2b (24.43%), *Tolypothrix* sp. 9k

(13.5%), *Nostoc* sp. 9i (21.7%), *Scytonema* sp. 1f (18.22%), *Nostoc* sp. 1c (17.44%), *Anabaena* sp. 1d (17.03%), *Scytonema* sp. 12c (17.22%), *Nostoc* sp. 9ma2 (16.6%), *Nostoc* sp. 9ma1(13.5%), *Anabaena* sp. 2a (13.5%), *Nostoc* sp. 9c (13.60%), *Nostoc* sp. 4 (12.99%), *Nostoc* sp. 9j (10.99%) whereas the minimum was repoterd in *Nostoc commune* 9n (0.740%).

The total percent of Polyunsaturated fatty acid (PUFA) was maximumally reported in *Anabaena* sp. 2a (42.8%), *Nostoc* sp. 9ma2 (42.2%), *Scytonema* sp. 1f (39.96%), *Nostoc* sp. 9c (37.42%), *Desmonostoc muscorum* 9a (24.2%), *Nostoc* sp. 1c (24.11%), *Anabaena doliolum* 1a (22.5%), *Nostoc* sp. 9f (20.31%), *Nostoc* sp. 9i (19.0%), *Tolypothrix* sp. 9k (19.75%), *Anabaena* sp. 1d (19.6%), *Anabaena sphaerica* 1e (19.3%), *Anabaena* sp. 1n (19.2%), *Nostoc* sp. 4 (19.8%), *Scytonema* sp. 12c (18.53%), *Nostoc* sp. 1h (17.8%), *Nostoc* sp. 9j (16.06%), *Nostoc* sp. 9n (8.43%), *Nostoc* sp. 9ma1 (8.0%) and the minimum was found in *Anabaena* sp. 2b (1.01%).

It was also observed significant variation wqas observed in *i.e.* 0.33 to 0.99 in all respective cyanobacterial species whereas the ratio MUFA/PUFA was varied between 0.31 to 24.13 and *Nostoc* sp. 4 showed the highest rang of the MUFA/PUFA ratio. On the other hand, the ratio of SAFA and MUFA was varied between 0.02 to 35.467 and highest was observed in the Nostoc sp. 4 (Table 6).

Further, fatty acid compostion of other twenty cyanobacterial strains isolated from North Hills region of Chhattisgarh was investigated (Figure. 27-30).

Octanoic acid (C8:0) was found in *Anabaena* sp. 12ss and *Calothrix* sp. 13ss (0.09%) respectively. Decanoic acid (C10:0) was observed in *Anabaena* sp. 14ss (0.087%) and *Anabaena* sp. 20ss (0.070%). Undecanoic acid (C11:0) was found in *Anabaena* sp. 2ss (0.093%); *Anabaena* sp. 15ss (0.286%) and *Anabaena* sp. 16ss (0.222%). (Table 7)

Lauric acid (C12:0) was found to be highest in *Nostoc* sp. 17ss (0.575%) followed by followed by *Aphanothece* sp. 4ss (0.532%), *Aphanothece* sp. 5ss (0.416%), *Anabaena* sp. 12ss (0.368%), *Calothrix* sp. 13ss (0.368%), *Anabaena* sp. 14ss (0.30%), *Nostoc* sp. 11ss (0.247%), *Calothrix* sp. 3ss (0.211%), *Tolypothrix* sp.

8ss (0.181%), *Scytonema* sp. 6ss (0.17%), *Anabaena* sp. 9ss (0.170%), *Scytonema* sp.1ss (0.158%), *Anabaena* sp. 7ss (0.152%), *Anabaena* sp. 19ss (0.150%) and *Scytonema* sp.18ss (0.124%) it is absent in rest of the cyanobacterial strains *i.e.* *Anabaena* sp. 2ss, *Aphanothece* sp. 10ss, *Anabaena* sp. 15ss, *Anabaena* sp. 16ss and *Anabaena* sp. 20ss.

Maximum Tridecanoic acid (C13:0) was found in *Scytonema* sp. 18ss (0.170%) followed by *Nostoc* sp. 17ss (1.140%). Tridecanoic acid was not found in *Scytonema* sp.1ss, *Anabaena* sp. 2ss, *Calothrix* sp. 3ss, *Aphanothece* sp. 4ss, *Aphanothece* sp. 5ss, *Scytonema* sp. 6ss, *Anabaena* sp. 7ss, *Tolypothrix* sp. 8ss, *Anabaena* sp. 9ss, *Aphanothece* sp. 10ss, *Nostoc* sp. 11ss, *Anabaena* sp. 12ss, *Calothrix* sp. 13ss, *Anabaena* sp. 14ss, *Anabaena* sp. 15ss, *Anabaena* sp. 16ss, *Anabaena* sp. 19ss and *Anabaena* sp. 20ss.

Amount of Tetradecanoic acid (C14:0) was maximum in *Aphanothece* sp. 5ss (15.0%) followed by *Anabaena* sp. 15ss (9.722%), *Anabaena* sp. 7ss (2.69%), *Anabaena* sp. 20ss (1.882%), *Calothrix* sp. 3ss (1.882%), *Nostoc* sp. 17ss (1.665%), *Scytonema* sp. 6ss (1.47%), *Calothrix* sp. 13ss (1.407%), *Scytonema* sp.1ss (0.889%), *Anabaena* sp. 19ss (0.84%), *Nostoc* sp. 11ss (0.847%), *Aphanothece* sp. 4ss (0.854%), *Tolypothrix* sp. 8ss (0.785%), *Anabaena* sp. 9ss (0.626%), *Anabaena* sp. 2ss (0.476%) whereas the rest of cyanobacterial strains were devoid of such fatty acid.

Similarly, Pentadecanoic acid (C15:0) content was also varied among the cyanobacterial strains and the maximum percentage was observed in *Nostoc* sp. 17ss (3.121%), *Anabaena* sp. 16ss (2.917%), *Scytonema* sp. 6ss (0.958%), *Nostoc* sp. 11ss (0.812%), *Aphanothece* sp. 5ss (0.845%), *Anabaena* sp. 19ss (0.85%), *Aphanothece* sp. 4ss (0.714%), *Anabaena* sp. 15ss (0.606%), *Scytonema* sp.18ss (0.540%), *Tolypothrix* sp. 8ss (0.483%), *Calothrix* sp. 3ss (0.395%), *Calothrix* sp. 13ss (0.384%), *Anabaena* sp. 9ss (0.352%), *Anabaena* sp. 12ss (0.368%), *Scytonema* sp.1ss (0.100%), *Anabaena* sp. 2ss (0.18%), *Anabaena* sp. 7ss (0.140%) and *Anabaena* sp. 20ss (0.192%). Pentadecanoic acid was not found in *Aphanothece* sp. 10ss and *Anabaena* sp. 14ss.

Hexadecadienoic acid (C16:0) was maximum in *Scytonema* sp.1ss (54.31%) followed by *Anabaena* sp. 15ss (43.717%), *Tolypothrix* sp. 8ss (41.42%), *Anabaena* sp. 20ss (40.296%), *Aphanothece* sp. 10ss (39.10%), *Calothrix* sp. 13ss (39.15%), *Anabaena* sp. 12ss (39.76%), *Anabaena* sp. 16ss (39.64%), *Nostoc* sp. 17ss (39.45%), *Anabaena* sp. 19ss (37.98%), *Scytonema* sp.18ss (37.92%), *Anabaena* sp. 9ss (36.483%), *Calothrix* sp. 3ss (35.25%), *Aphanothece* sp. 4ss (35.81%), *Anabaena* sp. 2ss (32.147%), *Aphanothece* sp. 5ss (28.4%), *Scytonema* sp. 6ss (35.59%), *Anabaena* sp. 14ss (29.18%), *Anabaena* sp. 7ss (24.05%) and *Nostoc* sp. 11ss (30.08%).

Higher content of 9-hexadecenoic acid (C16:1) was found in *Anabaena* sp. 19ss (31.19%) followed by *Nostoc* sp. 11ss (29.96%), *Scytonema* sp. 6ss (27.08%), *Scytonema* sp.18ss (27.33%), *Calothrix* sp. 3ss (23.71%), *Anabaena* sp. 20ss (22.61%), *Anabaena* sp. 9ss (21.842%), *Aphanothece* sp. 4ss (21.63%), *Aphanothece* sp. 5ss (21.6%), *Anabaena* sp. 7ss (21.40%), *Tolypothrix* sp. 8ss (21.34%), *Nostoc* sp. 17ss (19.54%), *Anabaena* sp. 14ss (19.96%), *Scytonema* sp.1ss (7.57%), *Anabaena* sp. 2ss (14.68%), *Anabaena* sp. 12ss (13.30%), *Calothrix* sp. 13ss (13.49%), *Anabaena* sp. 15ss (13.08%), *Anabaena* sp. 16ss (11.46%) and *Aphanothece* sp. 10ss (1.2%) respectively.

Heptadecanoic acid (C17:0) was found in all cyanobacteria of the isolated strains except *Aphanothece* sp. 10ss. The amount of such fatty acid *i.e. Nostoc* sp. 17ss (0.929%) followed by *Anabaena* sp. 16ss (0.696%), *Aphanothece* sp. 5ss (0.651%), *Scytonema* sp. 6ss (0.663%), *Tolypothrix* sp. 8ss (0.529%), *Anabaena* sp. 12ss (0.544%), *Calothrix* sp. 3ss (0.474%), *Anabaena* sp. 9ss (0.404%), *Calothrix* sp. 13ss (0.480%), *Scytonema* sp.1ss (0.330%), *Anabaena* sp. 2ss (0.397%), *Scytonema* sp.18ss (0.340%), *Aphanothece* sp. 4ss (0.378%), *Anabaena* sp. 15ss (0.219%), *Anabaena* sp. 7ss (0.216%), *Nostoc* sp. 11ss (0.106%), *Anabaena* sp. 14ss (0.188%), *Anabaena* sp. 19ss (0.150%) and *Anabaena* sp. 20ss (0.174%).

Cis-10-Heptadecenoic acid (C17:1) was found in *Anabaena* sp. 19ss (1.156%) followed by *Anabaena* sp. 16ss (1.007%), *Nostoc* sp. 17ss (1.069%), *Anabaena* sp. 12ss (0.624%), *Calothrix* sp. 13ss (0.639%), *Anabaena* sp. 2ss (0.635%), *Calothrix* sp. 3ss (0.500%), *Aphanothece* sp. 5ss (0.527%), *Scytonema* sp.18ss (0.525%),

Anabaena sp. 15ss (0.572%), *Tolypothrix* sp. 8ss (0.559%), *Scytonema* sp.1ss (0.488%), *Anabaena* sp. 7ss (0.444%), *Anabaena* sp. 20ss (0.436%), *Anabaena* sp. 9ss (0.470%) and *Nostoc* sp. 11ss (0.494%).

Maximum Cis-10 Nonadecenoic acid (C17:1) was observed in *Aphanothece* sp. 4ss (1.554%) followed by *Nostoc* sp. 17ss (1.017%), *Anabaena* sp. 7ss (0.939%), *Anabaena* sp. 16ss (0.84%), *Aphanothece* sp. 5ss (0.721%), *Scytonema* sp. 6ss (0.75%), *Tolypothrix* sp. 8ss (0.75%), *Nostoc* sp. 11ss (0.74%), *Anabaena* sp. 2ss (0.569%), *Scytonema* sp.18ss (0.571%), *Anabaena* sp. 9ss (0.404%), *Anabaena* sp. 19ss (0.489%) and *Calothrix* sp. 13ss (0.49%).

4,7,10 - Hexadecatrienoic acid (17:3) and 6,9 - Octadecadienoic acid were found only in *Calothrix* sp. 3ss (0.197%).

Octadecanoic acid (C18:0) was maximum *Anabaena* sp. 14ss (0.866%) followed by *Aphanothece* sp. 10ss (0.427%), *Calothrix* sp. 3ss (0.158%), *Anabaena* sp.16ss (0.148%), *Anabaena* sp. 19ss (0.123%), *Anabaena* sp. 20ss (0.139%), *Scytonema* sp.1ss (0.086%), *Anabaena* sp. 9ss (0.039%) and *Scytonema* sp.18ss (0.077%) respectively.

Highest 11-Octadecenoic acid (C18:1) was reported in *Aphanothece* sp. 10ss (7.69%) followed by *Scytonema* sp.18ss (6.455%), *Nostoc* sp. 17ss (5.680%), *Anabaena* sp. 7ss (5.370%), *Anabaena* sp. 9ss (5.311%), *Nostoc* sp. 11ss (5.08%), *Aphanothece* sp. 4ss (5.181%), *Anabaena* sp. 2ss (4.815%), *Aphanothece* sp. 5ss (4.158%), *Tolypothrix* sp. 8ss (4.38%), *Anabaena* sp. 16ss (4.44%), *Anabaena* sp. 19ss (4.568%), *Calothrix* sp. 3ss (1.553%), *Anabaena* sp. 12ss (2.83%), *Calothrix* sp. 13ss (2.78%), *Anabaena* sp. 14ss (3.43%) and *Anabaena* sp. 15ss (2.775%).

6-Octadecenoic acid (C18:1), Cis-11-Eicosenoic acid (C21:1) and Hexadecanoic acid (C16:0) were found only in three genus of *Anabaena* i.e. *Anabaena* sp. 2ss (0.066%), *Anabaena* sp. 12ss (0.160%) and *Anabaena* sp. 2ss (2.394%).

9-octadecenoic acid (C18:1) was found in *Nostoc* sp. 11ss (34.14%), *Anabaena* sp. 7ss (22.8%), *Scytonema* sp. 6ss (19.82%), *Anabaena* sp. 9ss (19.50%),

Tolypothrix sp. 8ss (18.03%), *Aphanothece* sp. 4ss (17.60%), *Scytonema* sp.18ss (17.48%), *Nostoc* sp. 17ss (17.42%), *Anabaena* sp. 20ss (17.64%), *Calothrix* sp. 3ss (16.32%), *Anabaena* sp. 12ss (10.2%), *Anabaena* sp. 19ss (9.23%), *Scytonema* sp.1ss (8.148%), *Anabaena* sp. 2ss (0.242%), *Aphanothece* sp. 5ss (11.17%), *Aphanothece* sp. 10ss (5.76%), *Calothrix* sp. 13ss (10.10%), *Anabaena* sp. 14ss (0.866%) and *Anabaena* sp. 15ss (0.235%).

Among all the tested cyanobacterial strains, the highest content of 9,12-octadecadienoic acid (C18:2) was found in *Aphanothece* sp. 10ss (45.08%) followed by *Anabaena* sp. 14ss (30.65%), *Anabaena* sp. 2ss (21.89%), *Anabaena* sp. 12ss (21.80%), *Calothrix* sp. 13ss (21.39%), *Scytonema* sp.1ss (20.85%), *Anabaena* sp. 7ss (20.73%), *Anabaena* sp. 15ss (17.44%), *Anabaena* sp. 20ss (15.54%), *Nostoc* sp. 17ss (15.656%), *Anabaena* sp. 16ss (13.43%), *Aphanothece* sp. 4ss (14.7%), *Aphanothece* sp. 5ss (14.4%), *Nostoc* sp. 11ss (12.7%), *Calothrix* sp. 3ss (12.384%), *Tolypothrix* sp. 8ss (11.5%), *Scytonema* sp. 6ss (10.82%), *Anabaena* sp. 9ss (9.742%), *Scytonema* sp.18ss (7.93%) and *Anabaena* sp. 19ss (3.30%) respectively.

9,12,15-Octadecatrienoic acid (C18:3) was found maximumally in *Anabaena* sp. 2ss (19.7%) followed by *Anabaena* sp. 14ss (13.6%), *Scytonema* sp.1ss (0.215%) and *Anabaena* sp. 9ss (0.15%). Rest of the cyanobacterial strains were devoid of this fatty acid. Oxiraneoctanoic acid (C19:0) was highest in *Anabaena* sp. 7ss (0.051%) and *Aphanothece* sp. 10ss (0.641%), *Anabaena* sp. 16ss (0.473%).

Gamma.-linolenic acid (C19:3) was maximum observed in *Anabaena* sp. 15ss (10.41%), *Scytonema* sp.1ss (8.894%), *Calothrix* sp. 13ss (8.297%), *Anabaena* sp. 16ss (8.293%), *Aphanothece* sp. 5ss (0.430%), *Anabaena* sp. 12ss (8.391%) and *Anabaena* sp. 19ss (7.016%).

Eicosanoic acid (C20:0) was observed in *Anabaena* sp. 2ss (0.251%) followed by *Anabaena* sp. 15ss (0135%), *Calothrix* sp. 3ss (0.079%), *Anabaena* sp. 7ss (0.114%) and *Anabaena* sp. 9ss (0.052%).

Maximum 8,11,14-Eicosatrienoic acid (C20:3) was found in *Anabaena* sp. 7ss (0.114%) followed by *Anabaena* sp. 15ss (0.286%), *Anabaena* sp. 16ss (0.207%)

and *Nostoc* sp. 17ss (0.193%). 5,8,11,14- Eicosatetraenoic acid (C20:5) was found maximally in *Calothrix* sp. 3ss (0.158%) followed by *Anabaena* sp. 2ss (1.032%), *Aphanothece* sp. 4ss (0.224%) and *Aphanothece* sp. 5ss (0.263%). 5,8,11,14,17-Eicosapentaenoic acid (C20:5) was highest observed in *Anabaena* sp. 2ss (0.384%) and *Aphanothece* sp. 5ss (0.263%)

Cyclopropaneoctanoic acid (C22:0) was maximum in *Calothrix* sp. 13ss (0.52%) followed by *Anabaena* sp. 16ss (0.55%), *Anabaena* sp. 14ss (0.56%), *Nostoc* sp. 17ss (0.386%), *Anabaena* sp. 19ss (0.299%), *Aphanothece* sp. 4ss (0.332%), *Calothrix* sp. 3ss (0.197%) and *Anabaena* sp. 7ss (0.127%). However, the least amount of fatty acid was observed in *Scytonema* sp.1ss (0.072%) followed by *Anabaena* sp. 9ss (0.052%).

Heneicosanoic acid (21:0) was found in *Anabaena* sp. 16ss (0.459%) followed by *Nostoc* sp. 17ss (0.333%), *Anabaena* sp. 14ss (0.245%) and *Scytonema* sp.18ss (0.077%). 13-docosenoic acid, (22:1) was observed in *Scytonema* sp. 6ss (0.324%) followed by *Anabaena* sp. 12ss (0.176%) and *Calothrix* sp. 13ss (0.160%). Maximum Tetracosanoic acid (C24:0) was found in *Scytonema* sp.1ss (0.215%) followed by *Aphanothece* sp. 4ss (0.210%) and *Anabaena* sp. 19ss (0.068%). Cis-5,8,11,14,17-Eicosapentaenoic acid (20:5) and Cis-13,16-Docasadienoic acid was only found in *Anabaena* sp. 7ss (0.102%) and *Aphanothece* sp. 5ss (0.097%) whereas the amount of Octanoic acid (C8:0) was found in *Anabaena* sp. 12ss (0.906%) and *Calothrix* sp. 13ss (0.906%).

7,10,13-eicosatrienoic acid (C21:3) was found in *Anabaena* sp. 19ss (0.245%), *Anabaena* sp. 12ss (0.112%) and *Calothrix* sp. 13ss (0.128%). 17-Octadecynoic acid (C18:0) was observed in *Aphanothece* sp. 5ss (0.055%) followed by *Anabaena* sp. 12ss (0.160%), *Calothrix* sp. 13ss (0.160%) and *Scytonema* sp. 18ss (0.062%). Cis-13 Octadecenoic acid (C19:1) *Scytonema* sp. 6ss (1.960%) and *Anabaena* sp. 20ss (0.714%). 14-Pentadecynoic acid (C16:0) and Eicosenoic acid (C20:1) was found in *Anabaena* sp. 15ss (0.101%) and *Anabaena* sp. 19ss (0.408%) respectively. 9-Hydroxypentadecanoic acid (C16:0) was found in *Anabaena* sp. 15ss (0.067%) and *Anabaena* sp. 16ss (0.888%).

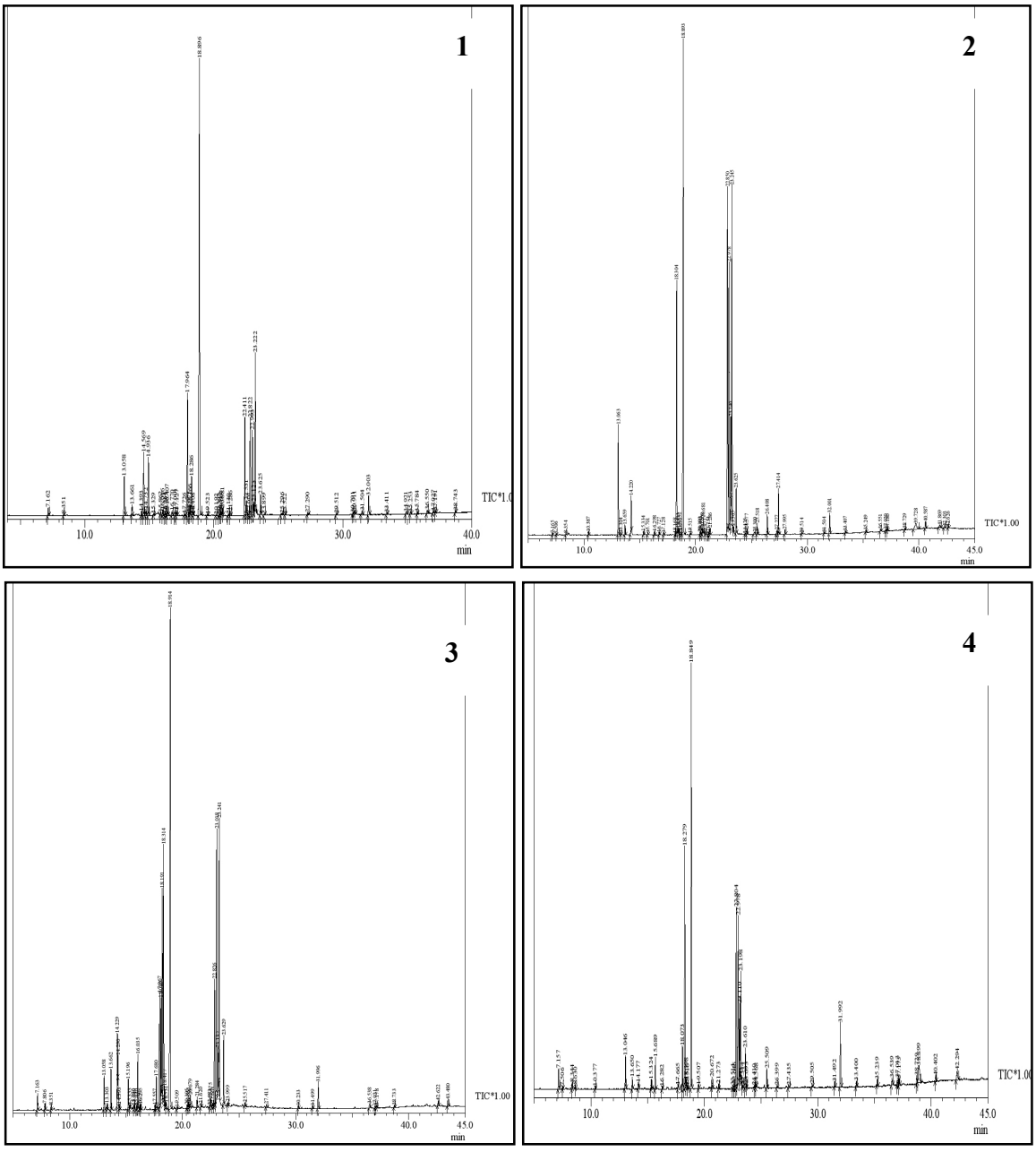

Figure 27. GCFID chromatogram of cyanobacterial species isolated from hill regions of Chhattisgarh (1) *Scytonema* sp.1ss (2)*Anabaena* sp. 2ss (3) *Calothrix* sp. 3ss (4) *Aphanothece* sp. 4ss. Each peak depicts presence of fatty acids at specific retention time.

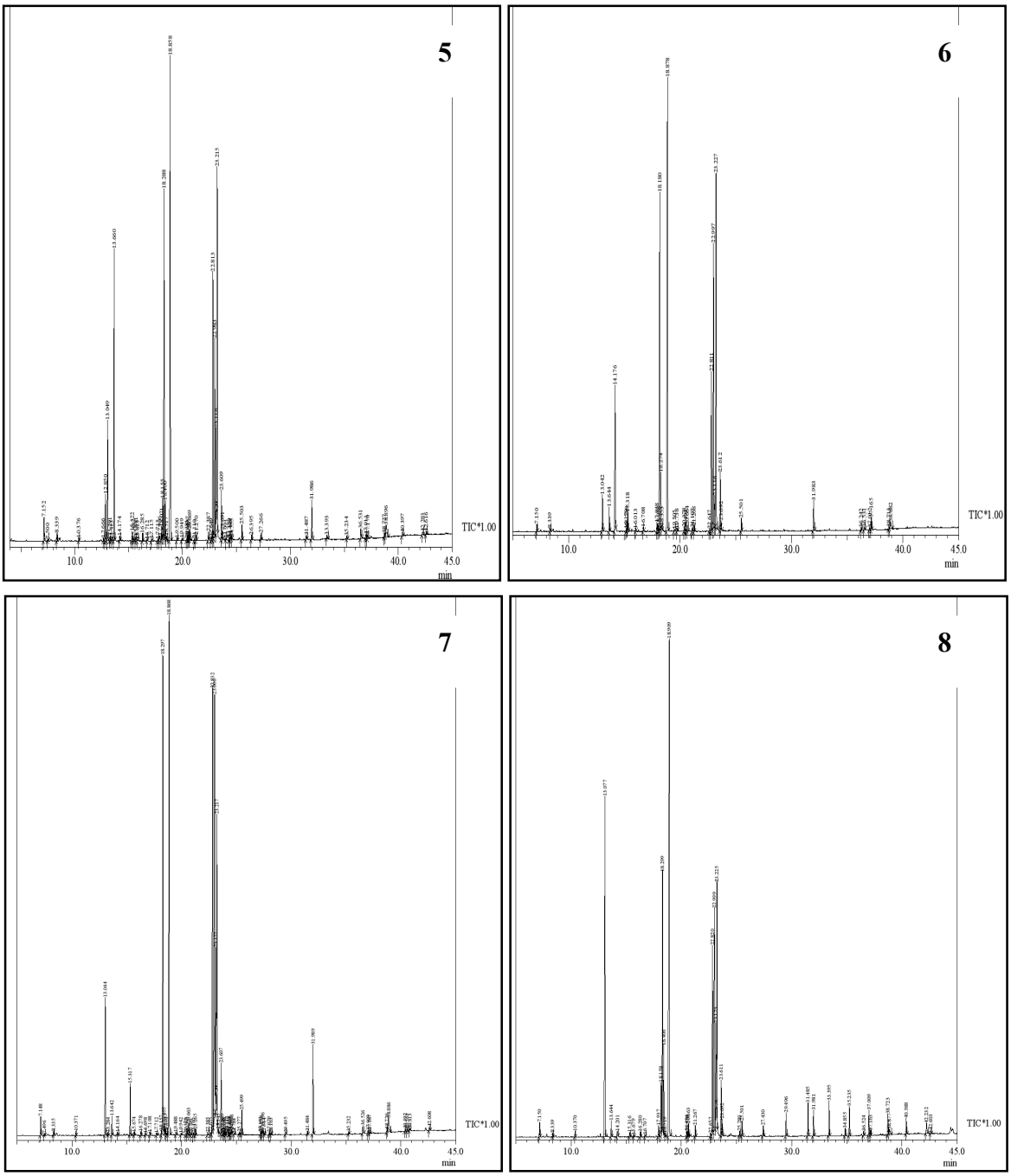

Figure 28. GCFID chromatogram of cyanobacterial species isolated from North hills region of Chhattisgarh. (5) *Aphanothece* sp. 5ss (6) *Scytonema* sp. 6ss (7*) *Anabaena* sp. 7ss (8)*Tolypothrix* sp. 8ss. Each peak depicts presence of atty acids at specific retention times.

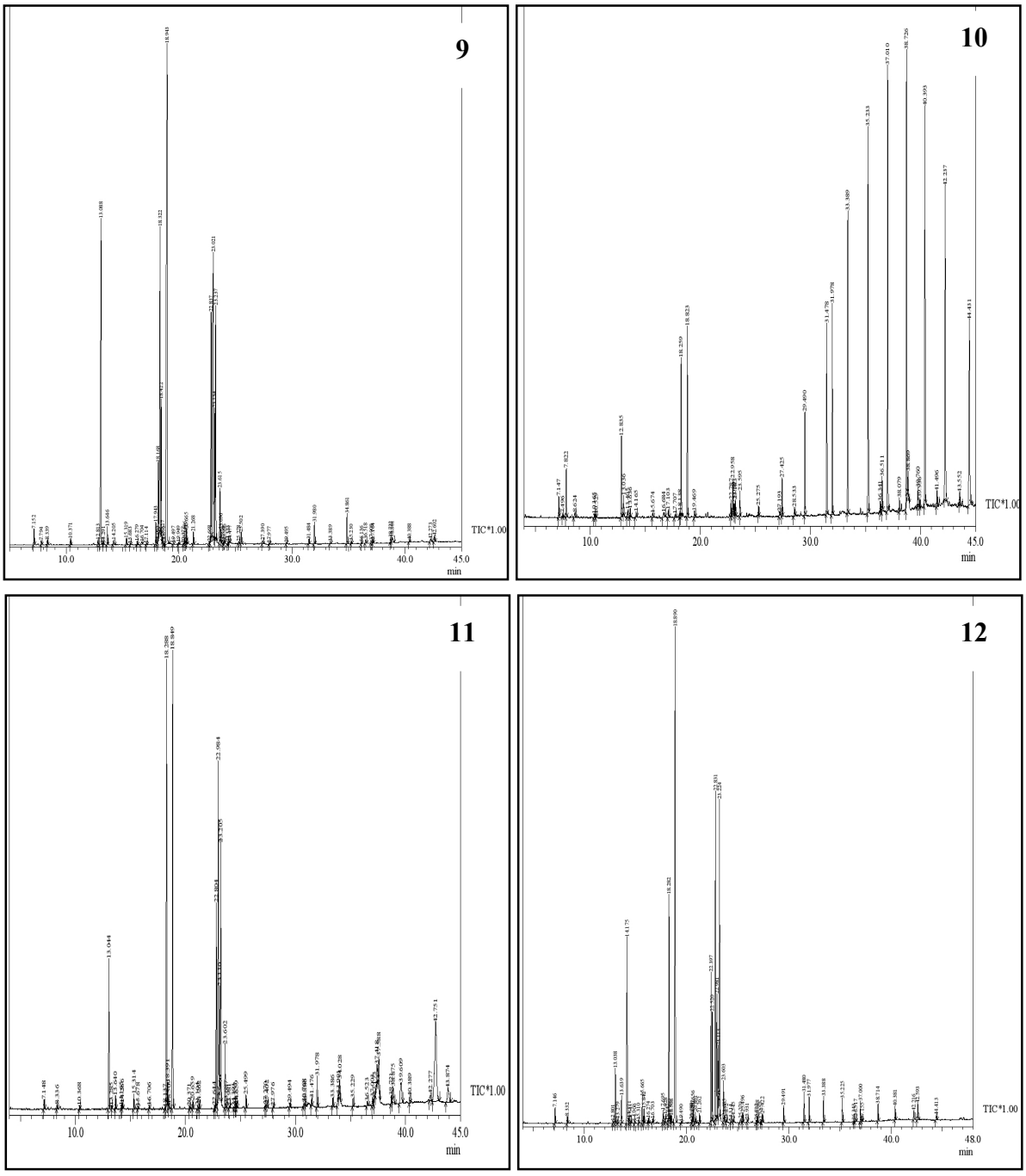

Figure 29. GCFID chromatogram of cyanobacterial species isolated from North hills of Chhattisgarh (9) *Anabaena* sp. 9ss (10) *Aphanothece* sp. 10ss (11) *Nostoc* sp. 11ss (12) *Anabaena* sp. 12ss. Each peak depicts presence of fatty acids at specific retention time.

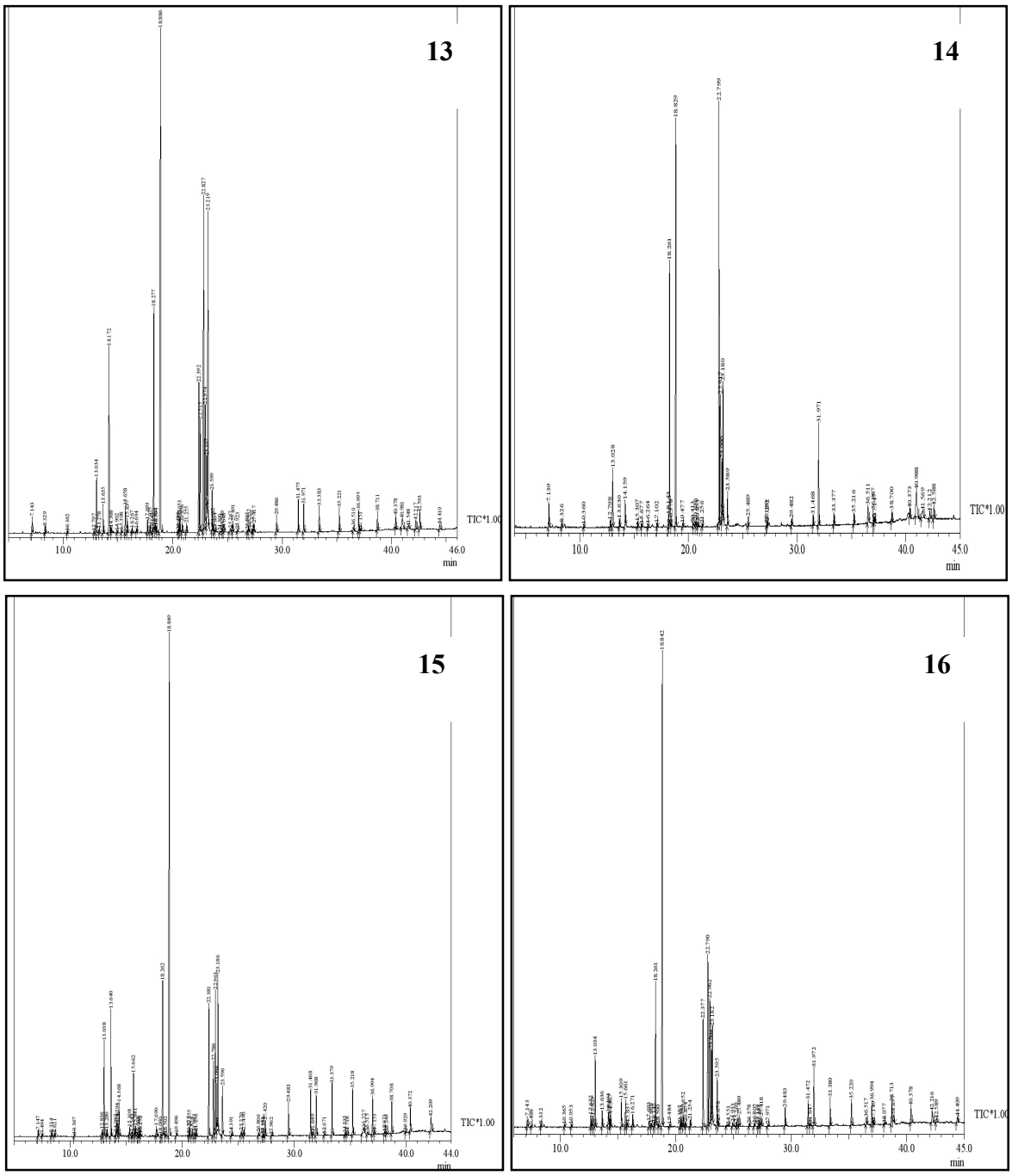

Figure 30. GCFID chromatogram of cyanobacterial species isolated from North hills region of Chhattisgarh. (13) *Calothrix* sp. 13ss (14) *Calothrix sp.* 14ss (15) *Anabaena* sp. 15ss (16) *Anabaena* sp. 16ss Each peak depicts presence of fatty acids at specific retention time.

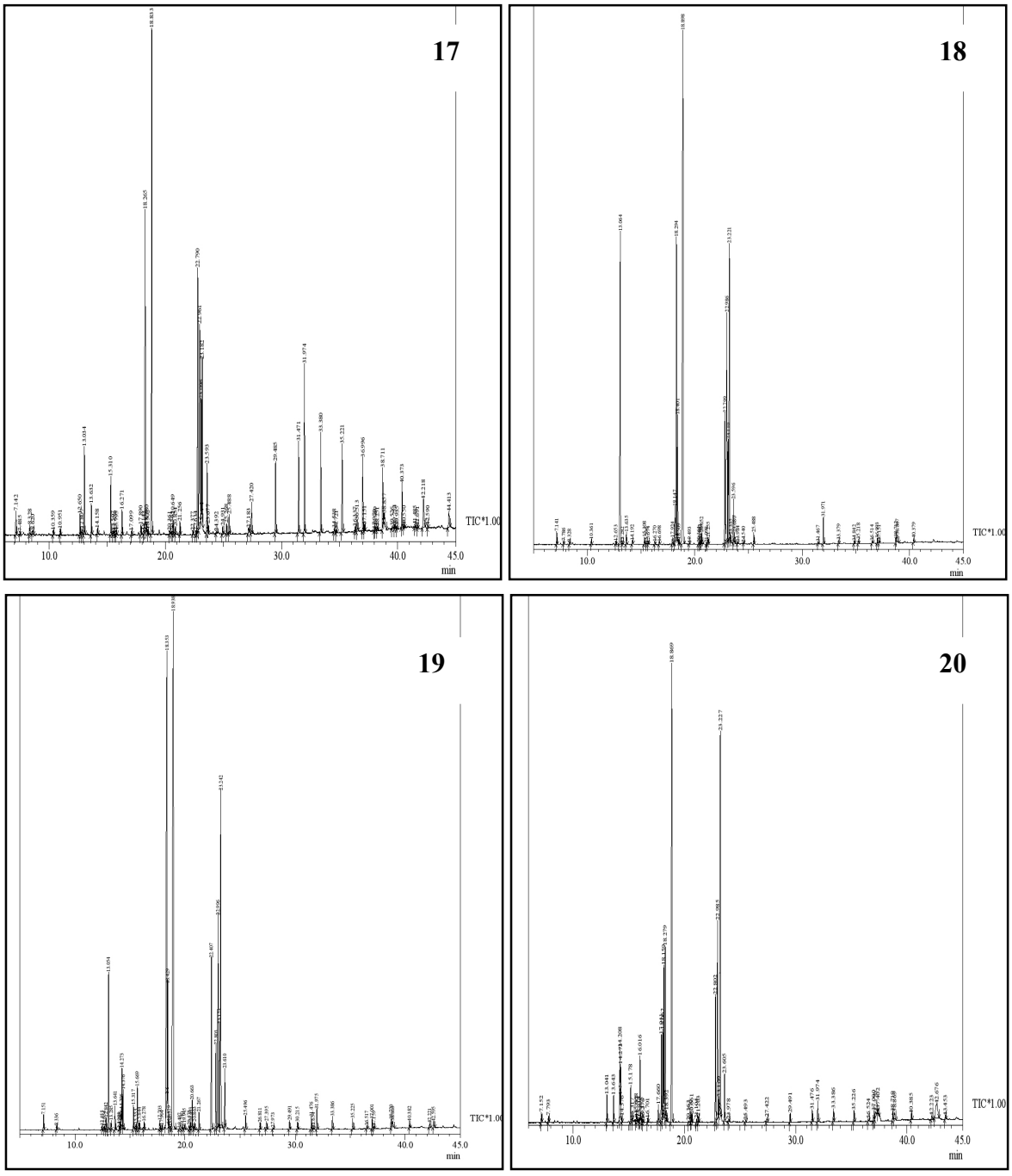

Figure 31. GCFID chromatogram of cyanobacterial species isolated from North hills region of Chhattisgarh. (17) *Nostoc* sp. 17ss (18) *Scytonema* sp.18ss (19) *Anabaena* sp. 19ss (20) *Anabaena* sp. 20ss. Each peak depicts presence of fatty acids at specific retention time.

Table 7. Fatty acid composition and percentage of total fatty acid in cyanobacteria isolated from North Hills region

SN	Fatty acid	Cyanobacterial strains																			
		1ss	**2ss**	**3ss**	**4ss**	**5ss**	**6ss**	**7ss**	**8ss**	**9ss**	**10ss**	**11ss**	**12ss**	**13ss**	**14ss**	**15ss**	**16ss**	**17ss**	**18ss**	**19ss**	**20ss**
1	Octanoic acid, **(C8:0)**	-	-	-	-	-	-	-	-	-	-	-	0.09	0.09	-	-	-	-	-	-	-
2	Decanoic acid, **(C10:0)**	-	-	-	-	-	-	-	-	-	-	-	-	-	0.087	-	-	-	-	-	0.070
3	Undecanoic acid, **(C11:0)**	-	0.093	-	-	-	-	-	-	-	-	-	-	-	-	0.286	0.222	-	-	-	-
4	Lauric acid **(C12:0)**	0.158	-	0.211	0.532	0.416	0.17	0.152	0.18	0.170	-	0.24	0.36	0.36	0.30	-	-	0.57	0.124	0.150	-
5	Tridecanoic acid **(C13:0)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.140	0.170	-	-
6	Tetradecanoic acid **(C14:0)**	0.889	0.476	1.882	0.854	15.0	1.47	2.69	0.78	0.626	--	0.84	-	1.40	-	9.722	-	1.665	-	0.84	1.882
7	Pentadecanoic acid **(C15:0)**	0.100	0.18	0.395	0.714	0.845	0.95	0.140	0.48	0.352	-	0.81	0.36	0.38	-	0.606	2.91	3.121	0.540	0.85	0.192
8	Hexadecanoic acid **(C16:0)**	54.31	32.14	35.25	35.81	28.4	35.5	24.05	41.4	36.48	39.1	30.0	39.7	39.1	29.1	43.71	39.6	39.45	37.92	37.98	40.29
9	Hexadecadienoic acid, **(C16:0)**	-	-	-	-	-	-	-	-	-	-	-	0.16	-	-	-	-	-	-	--	-
10	14-Pentadecynoic acid **(C16:0)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.101	-	-	-	-	-
11	9 -Hydroxypentadecanoic acid **(C16:0)**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.067	0.88	-	-	-	-
12	9-hexadecenoic acid**(C16:1)**	7.57	14.68	23.71	21.63	21.6	27.0	21.40	21.3	21.84	1.2	29.9	13.3	13.4	19.9	13.08	11.4	19.54	27.33	31.19	22.61
13	Heptadecanoic acid **(C17:0)**	0.330	0.397	0.474	0.378	0.651	0.66	0.216	0.52	0.404	-	0.10	0.54	0.48	0.18	0.219	0.69	0.929	0.340	0.150	0.174
14	Cis-10-Nonadecenoic acid **(C17:1)**	-	0.569	-	1.554	0.721	0.75	0.939	0.75	0.404	-	0.74	-	0.49	-	-	0.84	1.017	0.571	0.489	-
15	Cis-10-Heptadecenoic acid **(C17:1)**	0.488	0.635	0.500	-	0.527		0.444	0.55	0.470	-	0.49	0.62	0.63	-	0.572	1.00	1.069	0.525	1.156	0.436
16	4,7,10-Hexadecatrienoic acid **(C17:3)**	-	-	0.197	-	-	-	-	-	-	-	-	-	-	-	-	-	-	--	-	-
17	Octadecanoic acid, **(C18:0)**	0.086	-	0.158	-	-	-	-	-	0.039	0.42	-	-	-	0.86	-	0.14	-	0.077	0.123	0.139
18	17-Octadecynoic acid **(C18:0)**	-	-	-	-	0.055	-	-	-	-	-	-	0.16	0.16	-	-	-	-	0.062	-	-
19	6,9-Octadecadienoic acid, **(C18:0)**	-	-	0.158	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	9-octadecenoic acid **(C18:1)**	8.148	0.242	16.32	17.60	11.17	19.8	22.83	18.0	19.50	5.76	24.1	10.2	10.1	0.86	0.235		17.42	17.48	9.23	17.64

21	6-Octadecenoic acid (**C18:1**)	-	0.066	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	11-Octadecenoic acid (**C18:1**)	-	4.815	1.553	5.181	4.158	-	5.370	4.38	5.311	7.69	5.08	2.83	2.78	3.43	2.775	4.44	5.680	6.455	4.568	-
23	9,12-octadecadienoic acid (**C18:2**)	20.85	21.89	12.38	14.7	14.4	10.8	20.73	11.5	9.742	45.0	12.7	21.8	21.3	30.6	17.44	13.4	15.65	7.93	3.30	15.54
24	9,12,15-Octadecatrienoic acid (**C18:3**)	0.215	19.7	-	-	-	-	-	-	0.15	-	-	-	-	13.6	-		-	-	-	-
25	Oxiraneoctanoic acid (**C19:0**)	-	-	-	-	-	-	0.051	-	-	0.64	-	-	-	-	-	-	0.473	-	-	-
26	Cis-13 Octadecenoic acid (**C19:1**)	-	-	-	-	-	1.96	-	-	-	-	-	-	-	-	-	-	-	-	-	0.714
27	Gamma.-linolenic acid (**C19:3**)	8.894	-	-	-	0.430	-	-	-	-	-	-	8.39	8.29	-	10.41	8.29	-	-	7.016	-
28	Eicosanoic acid (**C20:0**)	-	0.251	0.079	-	-	-	0.114	-	0.052	-	-	-	-	-	0.135	-	-	-	-	-
29	Cis-11-Eicosenoic acid, (**C20:1**)	-	-	-	-	-	-	-	--	-	-	-	-	-	-	-	-	-	-	0.408	-
30	8,11,14-Eicosatrienoic acid, (**C20:3**)	-	-		-			0.114	-	-	-	-	-	-	-	0.286	0.20	0.193	-	-	-
31	Cis-5,8,11,14,17-Eicosapentaenoic acid (**C20:5**)	-	-	-	-	-		0.102	-	-	-	-	-	-	-	--	-	-	-	-	-
32	5,8,11,14-Eicosatetraenoic acid, (**C20:5**)	-	1.032	0.158	0.224	0.263	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
33	5,8,11,14,17-Eicosapentaenoic acid, (**C20:5**)	-	0.384	-	-	0.263	-	-	-	-	-	-	-	-	-	-		-	-	-	-
34	Cis-11-Eicosenoic acid, (**C21:1**)	-	2.394	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	Heneicosanoic acid (**C21:0**)	-	-	-	-	-	-	-	-	-	-	-	-	-	0.245	-	0.45	0.333	0.077	-	-
36	7,10,13-eicosatrienoic acid (**C21:3**)	-	-	-	-	-	-	-	-	-	-	-	0.11	0.12	-	-	-	-	-	0.245	-
37	Cyclopropaneoctanoic acid (C22:0)	0.072	-	0.197	0.332	-	-	0.127		0.052	-	-	-	0.52	0.56	-	0.55	0.386		0.299	-
38	13-docosenoic acid, (**C22:1**)	-	-	-	-	-	0.32	-	-	-	-	-	0.17	0.16	-	-	-	-	-	-	-
39	Cis-13,16-Docasadienoic acid (**C22:2**)	-	-	-		0.097	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
40	Tetracosanoic acid (**C24:0**)	0.215	-	-	0.210	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.068	-

Table 8. Percent and ratio of MUFAs, PUFAs and SAFAs in twenty different cyanobacterial strains isolated from North Hill regions

Cyanobacterial strains	SAFAs (%)	MUFAs (%)	PUFAs (%)	MUFA+PUFA/TOTAL	MUFA/PUFA	SAFA/MUFA
Scytonema sp. 1ss	56.16	16.20	29.95	0.451	0.541	3.465
Anabaena sp.2ss	33.54	23.40	44.03	0.667	0.531	1.433
Calothrix sp. 3ss	38.43	42.08	13.12	0.590	3.207	0.913
Aphanothece sp. 4ss	38.91	45.96	0.22	0.542	205.2	0.846
Aphanothece sp. 5ss	41.36	38.17	15.45	0.565	2.470	1.084
Scytonema sp. 6ss	38.84	49.93	10.82	0.610	4.615	0.778
Anabaena sp. 7ss	27.63	50.43	20.94	0.721	2.408	0.548
Tolypothrix sp. 8ss	43.39	45.06	11.52	0.565	3.911	0.963
Nostoc sp. 9ss	38.17	47.53	9.895	0.601	4.804	0.803
Aphanothece sp.10ss	40.16	14.65	45.08	0.598	0.325	2.741
Anabaena sp.11ss	32.09	60.41	12.77	0.695	4.731	0.531
Anabaena sp. 12ss	41.45	24.96	30.30	0.571	0.824	1.661
Calothrix sp.13ss	42.57	27.66	29.81	0.575	0.928	1.539
Calothrix sp.14ss	31.43	24.26	44.25	0.686	0.548	1.296
Anabaena sp.15ss	55.42	16.09	38.26	0.495	0.420	3.445
Nostoc sp. 16ss	45.52	17.72	21.93	0.466	0.810	2.564
Nostoc sp. 17ss	48.06	44.72	15.84	0.558	2.822	1.075
Scytonema sp. 18ss	39.31	52.93	7.93	0.608	6.675	0.743
Anabaena sp.19ss	40.44	47.75	10.56	0.590	4.521	0.847
Anabaena sp. 20ss	42.75	41.40	15.54	0.57	2.66	1.03

***SAFAs* -Saturated fatty acid, *MUFAs* -Monounsaturated fatty acid, *PUFAs* -Polyunsaturated fatty acid**

The diversity of fatty acids was also observed in terms of saturated fatty acid (SAFA), Monounsaturated fatty acid (MUFA), Polyunsaturated fatty acid (PUFA), MUFAs +PUFAs/Total, MUFAs/PUFAs and SAFAs/MUFAs ratio.

The percentage of SAFA was maximum in *Scytonema* sp.1ss (56.16%) followed by *Anabaena* sp. 15ss (55.42%), *Nostoc* sp. 17ss (48.06%), *Anabaena* sp. 16ss (45.52%), *Tolypothrix* sp. 8ss (43.39%), *Aphanothece* sp. 5ss (41.36%), *Calothrix* sp. 13ss (42.53%), *Anabaena* sp. 20ss (42.75%), *Anabaena* sp. 12ss (41.45%), *Anabaena* sp. 19ss (40.44%), *Aphanothece* sp. 10ss (40.16%), *Scytonema* sp.18ss (39.31%), *Anabaena* sp. 9ss (38.17%), *Calothrix* sp. 3ss (38.43%), *Aphanothece* sp. 4ss (38.91%), *Scytonema* sp. 6ss (38.84%), *Anabaena* sp. 2ss (33.54%), *Nostoc* sp. 11ss (32.09%), *Anabaena* sp. 14ss (31.43%) and *Anabaena* sp. 7ss (27.63%).

The highest percentage of MUFA was found in *Nostoc* sp. 11ss (60.41%) followed by *Scytonema* sp.18ss (52.93%), *Anabaena* sp. 7ss (50.43%), *Scytonema* sp. 6ss (49.93%), *Anabaena* sp. 19ss (47.75%), *Anabaena* sp. 9ss (47.53%), *Aphanothece* sp. 4ss (45.96%), *Nostoc* sp. 17ss (44.72%), *Tolypothrix* sp. 8ss (45.06%), *Calothrix* sp. 3ss (42.08%), *Anabaena* sp. 20ss (41.4%), *Aphanothece* sp. 5ss (38.17%), *Calothrix* sp. 13ss (27.66%), *Anabaena* sp. 12ss (24.96%), *Anabaena* sp. 2ss (23.40%), *Anabaena* sp. 14ss(24.26%), *Scytonema* sp.1ss (16.20%), *Anabaena* sp. 15ss (16.09%), *Anabaena* sp. 16ss (17.75%) and *Aphanothece* sp. 10ss (14.65%).

Similarly, the percent of polyunsaturated fatty acid (PUFA) was also calculated and maximum was found in *Aphanothece* sp. 10ss (45.08%) followed by *Anabaena* sp. 2ss (44.03%), *Anabaena* sp. 14ss (44.25%), *Anabaena* sp. 15ss (38.264%), *Anabaena* sp. 12ss (30.30%), *Scytonema* sp.1ss (29.95%), *Calothrix* sp. 13ss (29.81%), *Anabaena* sp. 16ss (21.93%), *Anabaena* sp. 7ss (20.94%), *Anabaena* sp. 20ss (15.54%), *Nostoc* sp. 17ss (15.84%), *Aphanothece* sp. 5ss (15.45%), *Calothrix* sp. 3ss (13.12%), *Nostoc* sp. 11ss (12.77%), *Anabaena* sp. 19ss (10.56%), *Scytonema* sp.18ss (7.93%), *Anabaena* sp. 9ss (9.89%), *Scytonema* sp. 6ss (10.82%),

Tolypothrix sp. 8ss (11.52%) and minimum was reported in *Aphanothece* sp. 4ss (0.22%).

The ratio of MUFA+PUFA / total fatty acid was calculated and it was found that the all cyanobacterial strains showed a significant changes in fatty acid and it was varied between 0.45 to 0.72. Similarly the ratio of MUFA/PUFA was found to be between 0.32 to 6.6 and the ratio of SAFA/MUFA ranged between 0.53 to 3.4.

4.5.2 *Cluster analysis based on FAME profiling*

Based on the fatty acid composition a dendrogram was constructed and results suggested that the tested twenty cyanobacteria showed cleared two clusters I and II. Cluster II was further subdivided and form IIA and IIB. cluster I contained only one species *Nostoc* sp. 4 whereas Subculture IIA represents only single genus *Anabaena* sp. 2a Subculture IIB was further divided in 2 subculture IIB1 and IIB2 again IIB1 contain single genus *Anabaena* sp. 1n. IIB 2 form two Subculture that contain IIB2a and IIB2b. Subculture of IIB2a was represents the different species of cyanobacteria *i.e. Nostoc* sp. 9f, *Nostoc commune* 9n, *Scytonema* sp. 1f and *Nostoc* sp. 1h, Whereas the second Cluster IIB2b represents almost many groups of cyanobacterial species *i.e. Anabaena doliolum* 1a, *Nostoc* sp. 1c, *Nostoc* sp. 1d, *Anabaena sphaerica* 1e*, Anabaena* sp. 2a, *Desmonostoc muscorum* 9a, *Nostoc* sp. 9c, *Nostoc* sp. 9i, *Nostoc* sp. 9j, *Tolypothrix* sp. 9k, *Nostoc* sp. 9ma1*, Nostoc* sp. 9ma2, *Scytonema* sp. 12c (Figure 32).

Similarly, a dendogram based on the six parameters *i.e.* percentage of saturated fatty acid (SAFA), percentage of monounsaturated fatty acid (MUFA), percentage of polyunsaturated fatty acid (PUFA), sum of MUFA and PUFA with respect to total fatty acid MUFA/PUFA and ratio of SAFA to MUFA, elucidated the degree of relatedness among the cyanobacterial strains (Figure 33).

Among all twenty cyanobacterial strains all were grouped into two major clusters I and II. Cluster I contained single strains of cyanobacteria *Nostoc* sp.1h, similar to the dendogram constructed on the basis of fatty acid percentage

Cluster II was further divided into two sub clusters i.e. IIA and IIB. Sub cluster IIA included single genera *i.e. Nostoc commune* 9n whereas, sub cluster IIB was again divided into two groups IIB1 and IIB2. Sub cluster IIB1 comprise of only one cyanobacterial strains *Anabaena* sp. 4 and Sub cluster IIB2 was again divided into two distinct clusters i.e. IIB2a and IIB2b. Sub cluster IIB2a contained thirteen cyanobacterial strains *Anabaena doliolum* 1a, *Nostoc* sp. 1c, *Nostoc* sp. 1d, *Anabaena Sphaerica* 1e*, Anabaena* sp. 1n, *Anabaena* sp. 2a*, Desmonostoc muscorum* 9a, *Nostoc* sp. 9f, *Nostoc* sp. 9i, *Nostoc* sp. 9j, *Tolypothrix* sp. 9k, *Nostoc* sp. 9ma1 and *Scytonema* sp. 12c. On the other hand sub cluster IIB2b represented four cyanobacterial strains i.e. *Nostoc* sp. 9ma2, *Nostoc* sp. 9c*, Anabaena* sp. 2b, *Scytonema* sp. 1f.

On the basis of total fatty acid composition the constructed dendogram was clearly showed that two distinct clusters were appeared *i.e.* I and II. Cluster I contained single genera *Aphanothece* sp. 10ss while cluster IIB grouped two genus represents *Anabaena* sp. 2ss and *Anabaena* sp. 14ss (Figure 34).

Sub cluster IIA divided into two sub cluster IIA1 and IIA2 which represents *Aphanothece* sp. 5ss and *Anabaena* sp. 7ss. While IIA1 was further grouped in two cluster and contained different cyanobacteria strains i.e. *Scytonema* sp.1ss, *Calothrix* sp. 3ss, *Aphanotheces*p. 4ss, *Scytonema* sp. 6ss, *Tolypothrix* sp. 8ss, *Anabaena* sp. 9ss, *Nostoc* sp. 11ss, *Anabaena* sp. 12ss, *Calothrix* sp. 13ss, *Anabaena* sp. 15ss, *Anabaena* sp.16ss, *Nostoc* sp. 17ss, *Scytonema* sp.18ss, *Anabaena* sp. 19ss and *Anabaena* sp. 20ss.

On the basis of different comprensive parameters of fatty acids the constructed dendogram suggested that there were clearcut distinct three clusters I, II and III. Where as cluster II represents single genus *Aphanothece* sp. 4ss and cluster I divided in two Sub clusters IA and IB (Figure 35).

Cluster III is further divided into two Sub cluster IIIA and IIIB where as IIIA is divided in two sub clusters and represents eleven cyanobacterial species. *Calothrix*

sp. 3ss, *Aphanothece* sp. 5ss, *Scytonema* sp. 6ss, *Anabaena* sp. 7ss, *Tolypothrix* sp. 8ss, *Anabaena* sp. 9ss, *Nostoc* sp. 11ss, *Nostoc* sp. 17ss, *Scytonema*sp.18ss, *Anabaena* sp. 19ss, *Anabaena* sp. 20ss. Sub Cluster IIIB represents five cyanobacterial species *Anabaena* sp. 2ss, *Aphanothece* sp. 10ss, *Anabaena* sp. 12ss, *Calothrix* sp. 13ss and *Anabaena* sp. 14ss.

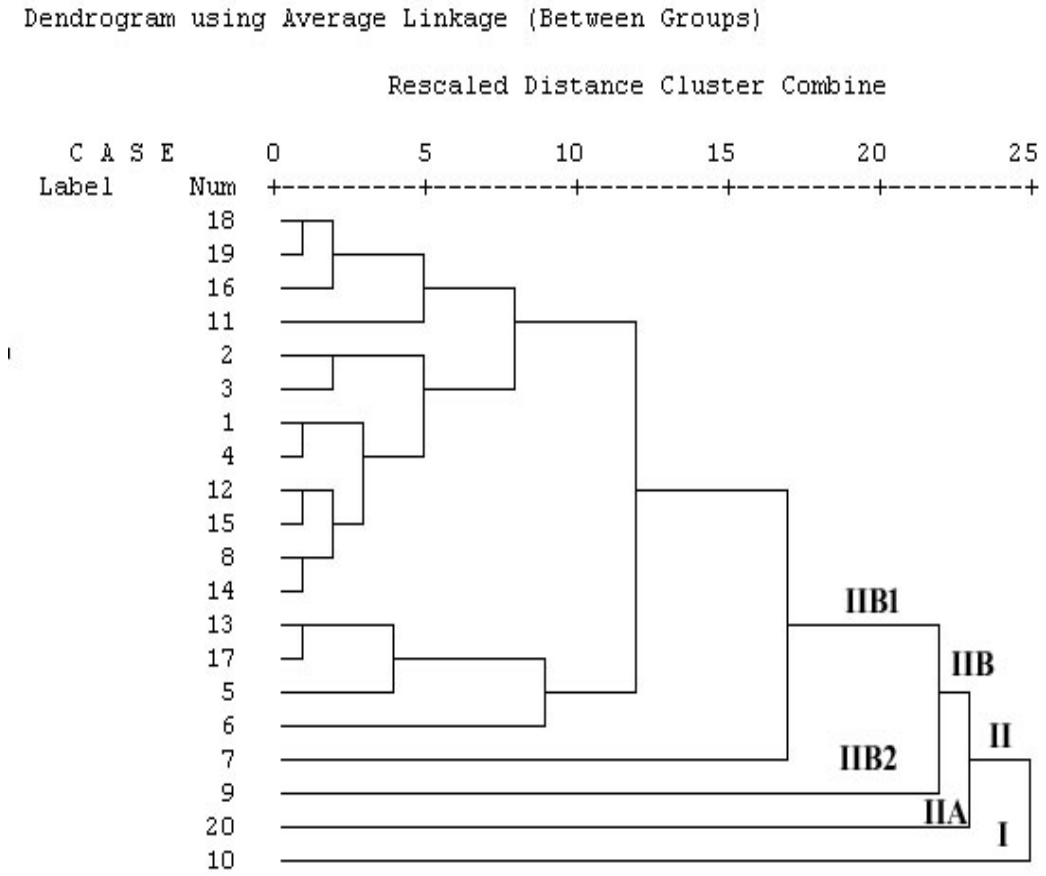

Figure 32. Dendogram depicting the relatedness of twenty cyanobacterial strains using (%) fatty acid profiles (1) *Anabaena doliolum* 1a (2) *Nostoc* sp. 1c (3) *Nostoc* sp. 1d (4) *Anabaena sphaerica* 1e (5) *Scytonema* sp. 1f (6) *Nostoc* sp. 1h (7*) Anabaena* sp. 1n (8) *Anabaena* sp. 2a (9) *Anabaena* sp. 2b (10) *Nostoc* sp. 4 (11) *Desmonostoc muscorum* 9a (12) *Nostoc* sp. 9c (13) *Nostoc* sp. 9f (14) *Nostoc* sp. 9i (15) *Nostoc* sp. 9j (16) *Tolypothrix* sp. 9k (17*) Nostoc commune* 9n (18) *Nostoc* sp. 9ma1 (19) *Nostoc* sp. 9ma2 (20) *Scytonema* sp. 12c

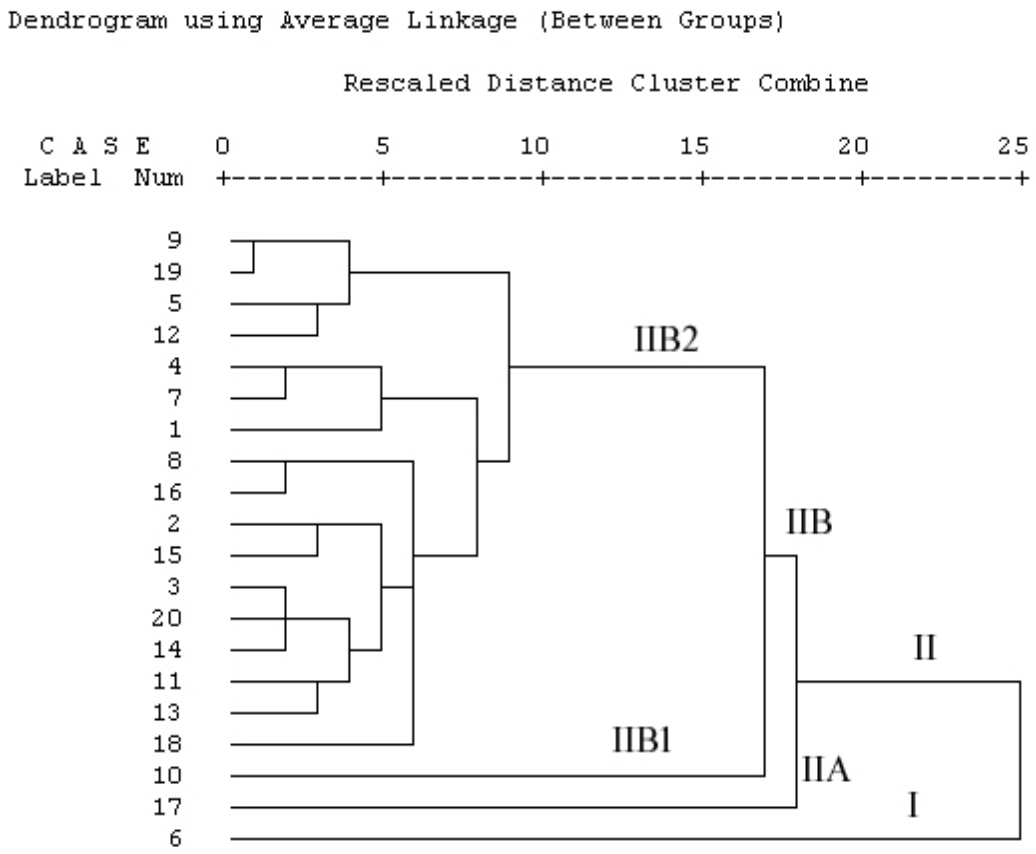

Figure 33. Dendrogram showing the relatedness of twenty cyanobacterial strains using six comprehensive parameters derived from fatty acid content and patterns. (1) *Anabaena doliolum* 1a (2) *Nostoc* sp. 1c (3) *Nostoc* sp. 1d (4) *Anabaena sphaerica* 1e (5) *Scytonema* sp. 1f (6) *Nostoc* sp. 1h (7*) Anabaena* sp. 1n (8) *Anabaena* sp. 2a (9) *Anabaena* sp. 2b (10) *Nostoc sp.* 4 (11) *Desmonostoc muscorum* 9a (12) *Nostoc* sp. 9c (13) *Nostoc* sp. 9f (14) *Nostoc* sp. 9i (15) *Nostoc* sp. 9j (16) *Tolypothrix* sp.9k (17*) Nostoc commune* 9n (18) *Nostoc* sp. 9ma1 (19) *Nostoc* sp. 9ma2 (20) *Scytonema* sp. 12c

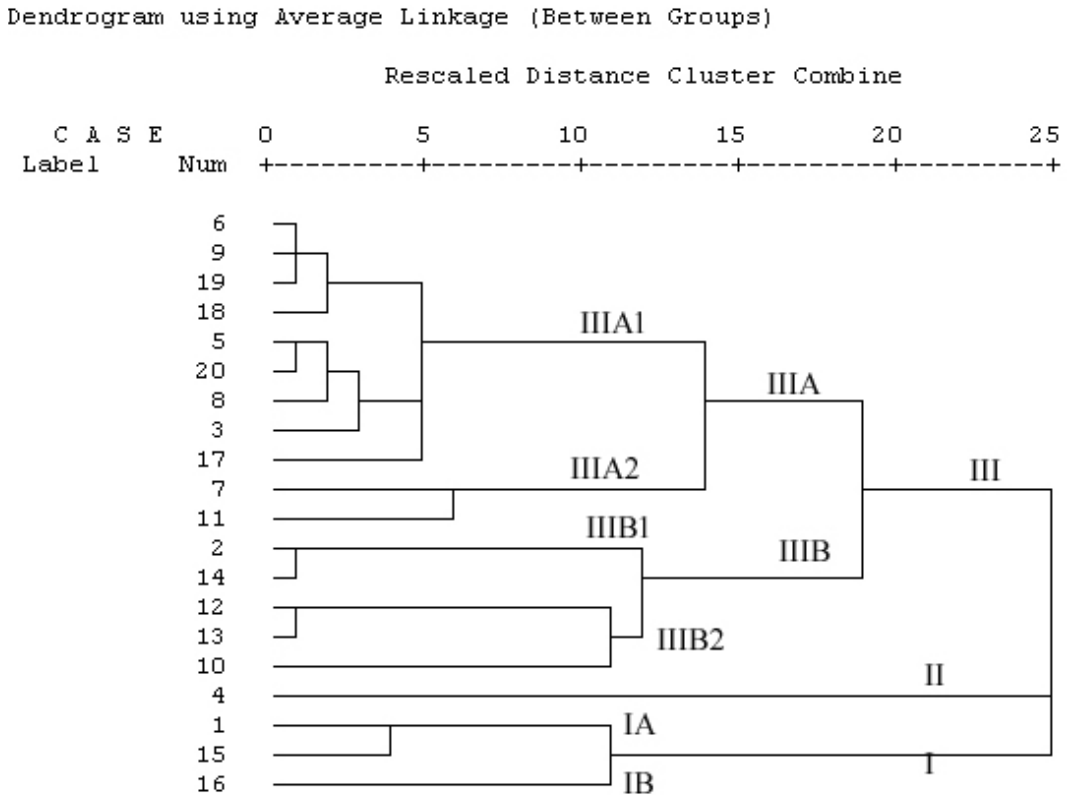

Figure 34. Dendogram depicting the relatedness of twenty cyanobacterial strains using (%) fatty acid profiles (1) *Scytonema* sp. 1ss (2) *Anabaena* sp. 2ss (3) *Calothrix* sp. 3ss (4) *Aphanothece* sp. 4ss (5) *Aphanothece* sp. 5ss (6) *Scytonema* sp. 6ss (7*) Anabaena* sp. 7ss (8)*Tolypothrix* sp. 8ss (9) *Nostoc* sp. 9ss (10) *Aphanothece* sp. 10ss (11) *Anabaena* sp. 11ss (12) *Anabaena* sp. 12ss (13) *Calothrix* sp. 13ss (14) *Calothrix* sp. 14ss (15) *Anabaena* sp. 15ss (16) *Nostoc* sp. 16ss (17*) Nostoc* sp. 17ss (18) *Scytonema* sp.18ss (19) *Anabaena* sp. 19ss (20) *Anabaena* sp. 20ss

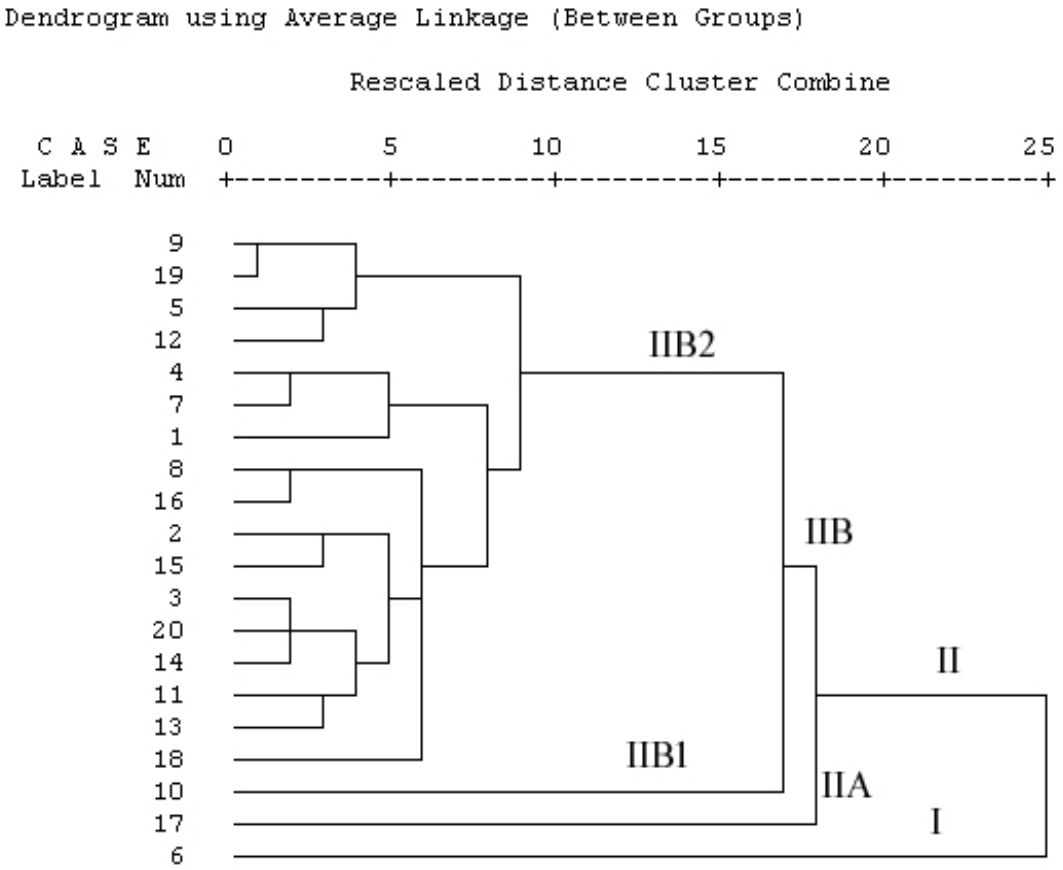

Figure 35. Dendogram showing the relatedness of twenty cyanobacterial strains using six comprehensive parameters derived from fatty acid content and patterns. (1) *Scytonema* sp.1ss (2) *Anabaena* sp. 2ss (3) *Calothrix* sp. 3ss (4) *Aphanothece* sp. 4ss (5) *Aphanothece* sp. 5ss (6) *Scytonema* sp. 6ss (7*) *Anabaena* sp. 7ss (8) *Tolypothrix* sp. 8ss (9) *Nostoc* sp. 9ss (10) *Aphanothece* sp. 10ss (11) *Anabaena* sp. 11ss (12) *Anabaena* sp. 12ss (13) *Calothrix* sp. 13ss (14) *Calothrix* sp. 14ss (15) *Anabaena* sp. 15ss (16) *Nostoc* sp. 16ss (17*) *Nostoc* sp. 17ss (18) *Scytonema* sp.18ss (19) *Anabaena* sp. 19ss (20) *Anabaena* sp. 20ss

4.5.3 *Protein Pattern analysis using* SDS-PAGE

Protein profiling was done among all the forty cyanobacterial strains isolated from North hills as well as plains regions of Chhattisgarh by using one dimensional polyacrylamide gel electrophoresis (SDS-PAGE) for visualizing the different protein banding pattern.Each and every cyanobacterial strains showed variation in its protein banding pattern. Results showed that protein bands of high molecular weight werevery dissimilar while considerable similarity was found among the bands of lower molecular weight (Figure 36 & 37) Some unique protein bands were observed in each cyanobacterial strains *viz*. band of 95.4 kDa was found *in Anabaena sphaerica* 1e, *Anabaena* sp. 2a, *Nostoc sp.* 9f. Similarly, the band of 62.0 kDa was found in *Nostoc* sp.1d, *Anabaena* sp. 2a, *Nostoc* 9f. Again band of 66.1 and 43.4 kDa were found in *Anabaena doliolum* 1a and band of 44.4 kDa was found in *Scytonema* sp.1f. The protein bands of 96.5kDA (high molecular weight) was found in *Nostoc* sp.1h. Similarly, the bands of 66.3 kDa and 46.4 were found in *Anabaena* sp. 1n and *Nostoc sp.* 9i. Likewise, band of 45.6 kDa was found in *Anabaena* sp. 2b and *Nostoc* sp. 4 while band of 45.1 kDa found in *Nostoc* sp. 9f.

29 kDa band was found in *Nostoc sp.*1d, *Anabaena sphaerica* 1e, *Anabaena sp.* 2a, *Desmonostoc muscorum* 9a, *Nostoc* sp. 9c, *Nostoc* sp. 9f whereas 25.4 band was found in *Anabaena sphaerica* 1e and *Anabaena sp. 2a*. Low molecular weight protein band was observed (19.5 kDa) band was found in *Nostoc* sp. 1d, *Anabaena sphaerica* 1e, *Anabaena* sp.2a and *Nostoc sp.* 9c. Similar bands of 46.4 kDa and 27.5kDa were observed in *Nostoc sp.* 1c, *Nostoc sp.*9ma1, *Nostoc sp.* 9ma2, *Nostoc sp.*9j, *Tolypothrix sp.*9k, *Nostoc commune* 9n and *Scytonema* sp. 12c.

Results also suggested that some common protein bands 21.kDa were found in all cyanobacterial strains. Bands of 67.4 kDa was found in Calothrix sp. 3ss while other band (48.0 kDa) in appeared in *Calothrix* sp. 3ss. Similar band of 66.2 kDa and 48.8kDa were observed in *Aphanothece* sp. 4ss and *Aphanothece* sp. 5ss. *Nostoc* sp.8ss showed the unique band 50.0 kDa. The band of 40 kDa was found in *Anabaena* sp. 9ss and the bands of 92.0 kDa, 70.4 kDa, 48.4 kDa were observed in *Anabaena* sp.

11ss and *Anabaena* sp. 12ss.The band of 66.2 kDa found in almost all cyanobacterial strain. Similarly, band of 45.0 kDa was also found in all cyanobacterial strains. *Anabaena* sp. 19ss showed the low molecular weight 38.0 kDa protein band.

4.5.4 *Cluster analysis based on protein profiling*

On the basis of variable protein profile (Fig. 38 & 39) the dendogram was constructed by using Jaccard's similarity coefficient 0.11-1.0. The dendogram showed two major clusters I and II. *Scytonema* sp. 1f was most distinctly apart and formed two separate out-group/outliers. *Anabaena* sp. 1d was found to be present at the top and *Scytonema* sp. 1f at the base of the dendogram. The dendogram clearly separated in two cluster I and II. Whereas cluster I comprise of only one cyanobacterial strains Nostoc sp. 9c. while cluster II was separated in two sub cluster IIA and IIB. Both sub clusters further divided in again two sub cluster IIA divide in IIA 1 and IIB2 whereas IIB was divided in IIB1 and IIB2. *Anabaena doliolum* 1a and *Anabaena sphaerica* 1e were found in same clade or same cluster IIB1 with *Anabaena* sp.1d. whereas sub cluster IIA2 comprised the *Anabaena* sp. 2a, *Desmonostoc muscorum* 9a, *Nostoc* sp. 9i and *Anabaena* sp. 4. Similarly, sub cluster IIB further divided in two sub cluster and *Nostoc* sp. 9f clearly showed the different group in sub cluster IIB1 and rest of the seven cyanobacterial strains comprise viz. *Anabaena* sp. 1h, *Tolypothrix* sp. 9k, *Scytonema* sp. 12c, *Nostoc* sp. 1c, *Nostoc* sp. 9ma1 and *Nostoc* sp. 9ma2 and *Nostoc* sp. 9j. Sub cluster IIB2 comprises *Anabaena* sp. 1n, *Anabaena* sp. 2b, *Nostoc* sp.9n and *Nostoc* sp. 9c.

The dendogram clearly separated into major clusters I and II. Whereas cluster II comprised of the two cyanobacterial strains *viz. Aphanothece* sp. 4ss and *Scytonema* sp. 8ss. Cluster I further separated two sub clusters IA1 and IA2. Whereas sub cluster II A2 comprised of *Anabaena* sp. 9ss and sub cluster IA1 further separate in two sub cluster IA1a and IA2b. Whereas IA2b contains single cyanobacterial species.

Dendogram was clearly showed that the sub cluster IA1a included 14 cyanobacterial species i.e. *Anabaena* sp. 1ss, *Scytonema* sp. 6ss, *Calothrix* sp. 14ss,.

Aphanothece sp. 5ss, *Anabaena* sp. 15ss, *Anabaena* sp. 2ss, *Anabaena* sp. 20ss, *Calothrix* sp. 13ss, *Calothrix* sp. 3ss, *Anabaena* sp. 12ss and *Tolypothrix* sp. 18ss

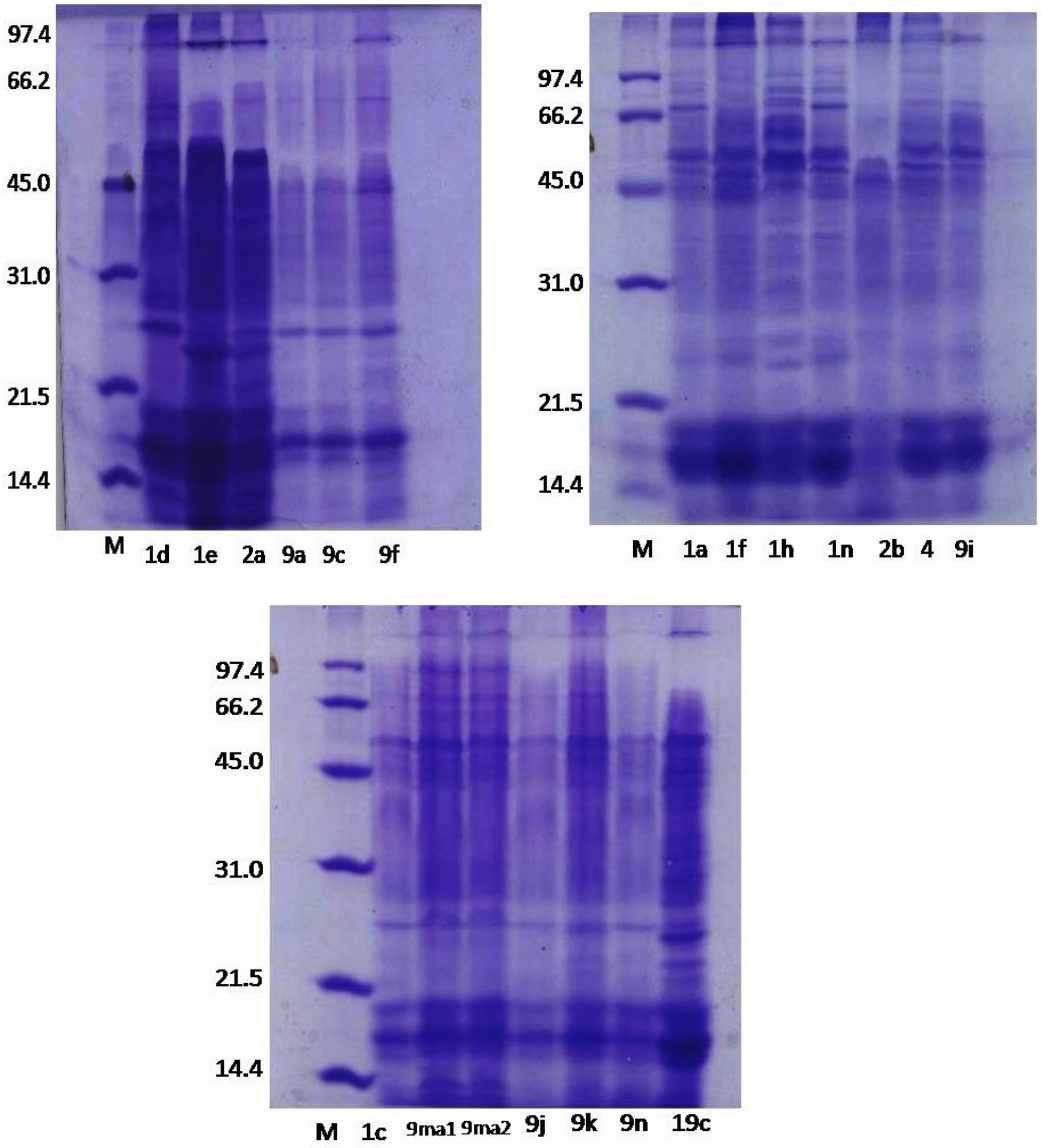

Figure 36. Whole cell protein profiling pattern of the forty cyanobacterial strains obtained by using SDS-PAGE analysis. M represents standard molecular weight marker (kDa). (1) *Anabaena doliolum* 1a (2) *Nostoc* sp. 1c (3) *Nostoc* sp. 1d (4) *Anabaena sphaerica* 1e (5) *Scytonema* sp. 1f (6) *Nostoc* sp. 1h (7) *Anabaena* sp. 1n (8) *Anabaena* sp. 2a (9) *Anabaena* sp. 2b (10) *Nostoc* sp. 4 (11) *Desmonostoc muscorum* 9a (12) *Nostoc* sp. 9c (13) *Nostoc* sp. 9f (14) *Nostoc* sp. 9i (15) *Nostoc* sp. 9j (16) *Tolypothrix* sp. 9k (17) *Nostoc commune* 9n (18) *Nostoc* sp. 9ma1 (19) *Nostoc* sp. 9ma2 (20) *Scytonema* sp. 12c.

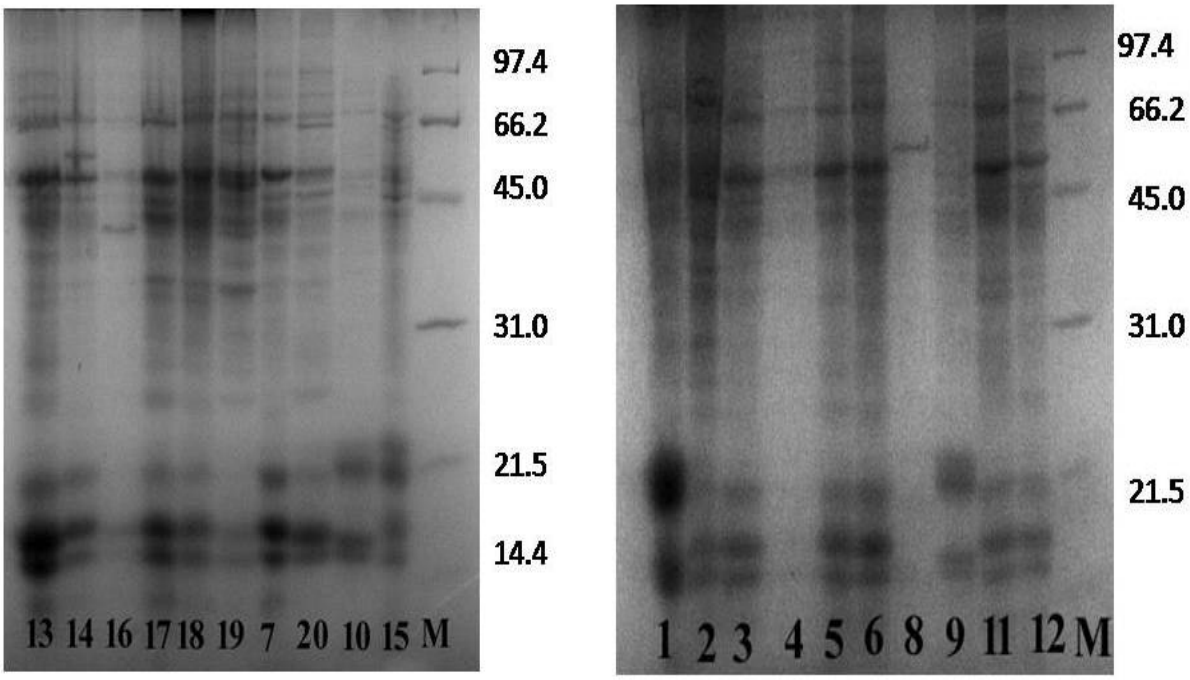

Figure 37. Whole cell protein profiling pattern of the forty cyanobacterial strains obtained by using SDS-PAGE analysis. M represents standard molecular weight marker (kDa).
(1) *Scytonema* sp.1ss (2)*Anabaena* sp. 2ss (3) *Calothrix* sp. 3ss (4) *Aphanothece* sp. 4ss (5) *Aphanothece* sp. 5ss (6) *Scytonema* sp. 6ss (7*) Anabaena* sp. 7ss (8)*Tolypothrix* sp. 8ss (9) *Nostoc* sp. 9ss (10) *Aphanothece* sp. 10ss (11) *Anabaena* sp. 11ss (12) *Anabaena* sp. 12ss (13) *Calothrix* sp. 13ss (14) *Calothrix* sp. 14ss (15) *Anabaena* sp. 15ss (16) *Nostoc* sp. 16ss (17*) Nostoc* sp. 17ss (18) *Scytonema* sp.18ss (19) *Anabaena* sp. 19ss (20) *Anabaena* sp. 20ss

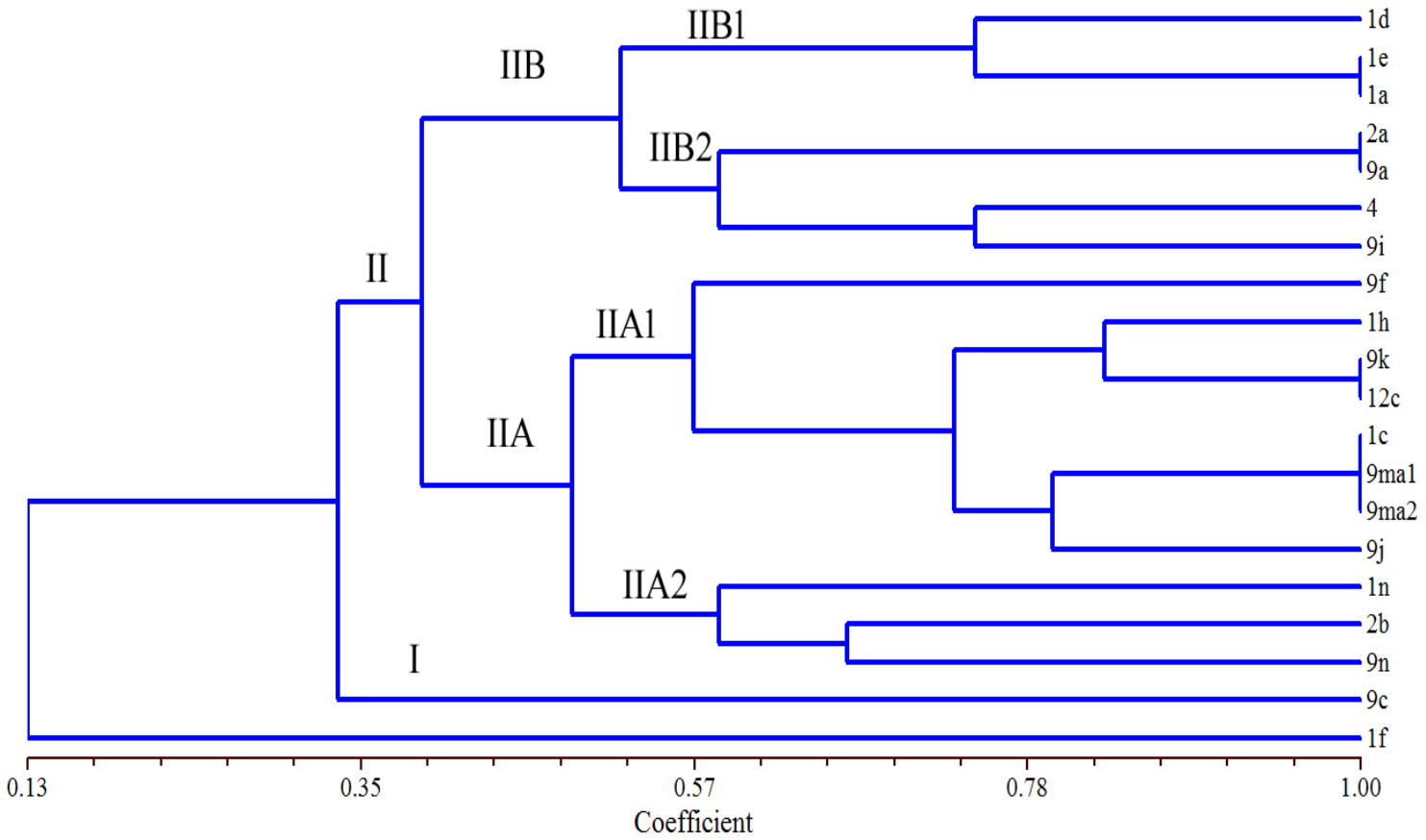

Figure 38. Dendogram generated with UPGMA clustering algorithm by using NTYSYS software (version 2) based on SDS-PAGE of whole cell protein profiling of the forty cyanobacterial strains (1) *Anabaena doliolum* 1a (2) *Nostoc* sp. 1c (3) *Nostoc* sp. 1d (4) *Anabaena sphaerica* 1e (5) *Scytonema* sp. 1f (6) *Nostoc* sp. 1h (7*) Anabaena* sp. 1n (8) *Anabaena* sp. 2a (9) *Anabaena* sp. 2b (10) *Nostoc* sp. 4 (11) *Desmonostoc muscorum* 9a (12) *Nostoc* sp. 9c (13) *Nostoc sp.* 9f (14) *Nostoc* sp. 9i (15) *Nostoc* sp. 9j (16) *Tolypothrix* sp.9k (17) *Nostoc commune* 9n (18) *Nostoc* sp. 9ma1(19) *Nostoc* sp. 9ma2 (20) *Scytonema* sp. 12c

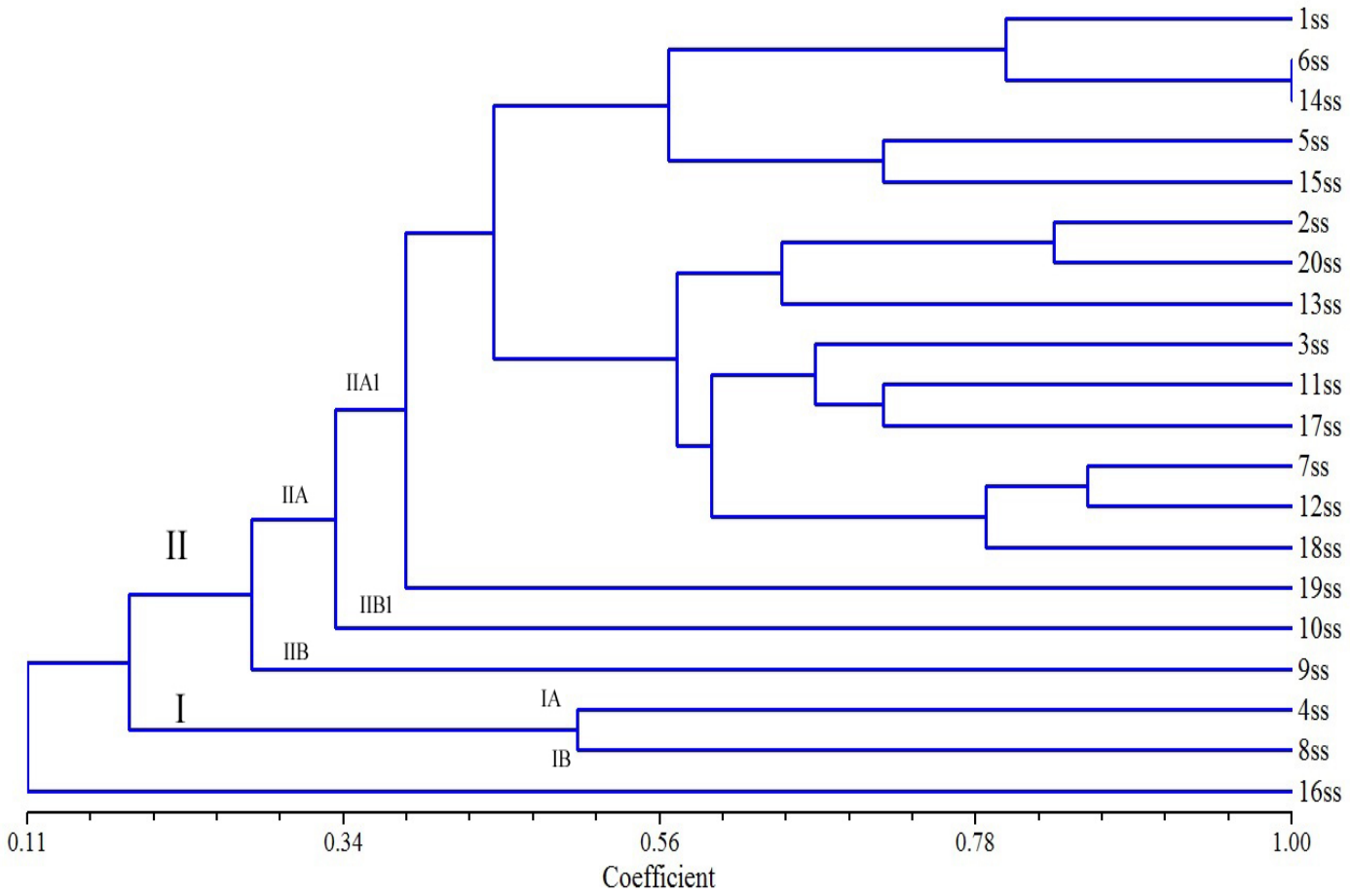

Figure 39. Dendogram generated with UPGMA clustering algorithm by using NTYSYS software (version 2) based on SDS-PAGE of whole cell protein profiling of the 40 cyanobacterial strains. (1) ***Scytonema*** **sp.1ss (2)** ***Anabaena*** **sp. 2ss (3)** ***Calothrix*** **sp. 3ss (4)** ***Aphanothece*** **sp. 4ss (5)** ***Aphanothece*** **sp. 5ss (6)** ***Scytonema*** **sp. 6ss (7)** ***Anabaena*** **sp. 7ss (8)** ***Tolypothrix*** **sp. 8ss (9)** ***Nostoc*** **sp. 9ss (10)** ***Aphanothece*** **sp. 10ss (11)** ***Anabaena*** **sp. 11ss (12)** ***Anabaena*** **sp. 12ss (13)** ***Calothrix*** **sp. 13ss (14)** ***Calothrix*** **sp. 14ss (15)** ***Anabaena*** **sp. 15ss (16)** ***Nostoc*** **sp. 16ss (17)** ***Nostoc*** **sp. 17ss (18)** ***Scytonema*** **sp.18ss (19)** ***Anabaena*** **sp. 19ss (20)** ***Anabaena*** **sp. 20ss**

4.6 MOLECULAR CHARACTERIZATION

4.6.1 *Phylogenetic analysis among the cyanobacterial strains based on 16S rRNA gene*

To investigate the molecular diversity and relatedness among the cyanobacterial strains isolated from different region of Chhattisgarh, 16S rRNA gene was amplified using universal primers and forty cyanobacterial strains have been submitted to the NCBI database and accordingly accession number have been allotted (Table 9 & 10).

In the present study the constructed phylogenetic tree (Figure 40) based on 16S rRNA gene sequences using the Neighbor Joining(NJ), Maximum Likelihood (ML), Maximum Parsimony (MP), algorithm whereas all Phylogenetic tree showed same tree topologies with good bootstrap value at all Nodes were results revealed robust topology. The constructed tree consisted of seven major clusters. The cluster I represented the false branching genus *Scytonema* consisting of *Scytonema* sp. 1f along with the other members of *Scytonema i.e*. *Scytonema bilaspurensis*, *Scytonema singhii* and *Scytonema hofmanni* PCC 7110 found the separate cluster. Similarly the cluster II showed the intermixed clustering between *Nostoc* and *Anabaena* comprising of *Anabaena doliolum* 1a, *Nostoc* sp. 1d, *Anabaena sphaerica* 1e, *Nostoc* sp. 4, *Nostoc* sp. 9ma1, *Scytonema* sp. 12c and *Nostoc* sp. 9i.

The Cluster III also represented the Nostocales members and comprised of *Nostoc* sp. 1h, *Nostoc* sp. 9c, *Nostoc* sp. 9f and *Nostoc* sp. 9j whereas the *Nostoc* sp. 1c and *Nostoc* sp. 9n were clustered in sub cluster. Cluster IV comprised of *Anabaena* sp. 2b and *Anabaena* sp. 1n while Cluster V having *Desmonostoc muscorum* 9a and showed significant similarities with *Anabaena variabilis.* Cluster VI consisted of the *Tolypothrix* sp. 9k while the Cluster VII consisted of *Anabaena* sp. 2a (Figure 41)

Similar observations were also done in the phylogenetic tree constructed based on 16S rRNA gene sequences of twenty cyanobacterial strains isolated from North Hill regions whereas Phylogenetic tree consisted of many small clusters but overall five clusters were consistently formed. Cluster I represented separate and

distinct clusters and comprise the unicellular group *Aphanothece* sp. 4ss, *Aphanothece* sp. 5ss, *Aphanothece* sp. 10ss. Whereas cluster II consist of *Scytonema* sp. 6ss, S*cytonema* sp. 18ss, *Scytonema* sp. 1ss and S*cytonema* sp. 8ss. (Table 10)

Similarly cluster III represented the strains *Anabaena* sp. and *Nostoc* sp.; *Nostoc* sp. 17ss followed by *Anabaena* sp. 19ss, *Anabaena* sp. 15ss, *Anabaena* sp. 12ss, *Anabaena* sp. 12ss, *Anabaena* sp. 11ss, *Anabaena* sp. 20ss, *Anabaena* sp. 7ss, *Anabaena* sp. 2ss, *Nostoc* sp. 16ss. The Cluster IV comprised of the strains *Calothrix* sp. 14ss, *Calothrix* sp. 13ss and *Calothrix* sp. 3ss and Only one strain of *Nostoc* sp. 9ss in cluster V. (Figure 41)

4.6.2 Determination of secondary structure

The 16S rRNA gene sequences of forty cyanobacterial strains isolated from plains region as well as North hills of Chhattisgarh were used for the construction of secondary structure models. (Figure 42-51).

Table 9. List Cyanobacterial strains isolated from Plain regions of Chhattisgarh with Accession number obtained from NCBI

S.N.	Cyanobacterial strains	Accession no.
1.	*Anabaena doliolum* 1a	KJ774520
2.	*Nostoc* sp. 1c	KU668899
3.	*Nostoc* sp. 1d	KU668902
4.	*Anabaena sphaerica* 1e	KJ774521
5.	*Scytonema* sp1f	KU668900
6.	*Nostoc* sp. 1h	KU668904
7.	*Anabaena* sp. 1n	KU668903
8.	*Anabaena* sp. 2a	KU668906
9.	*Anabaena* sp. 2b	KU668921
10.	*Nostoc* sp. 4	KU668920
11.	*Desmonostoc muscorum* 9a	KU668905
12.	*Nostoc* sp. 9c	KU668910
13.	*Nostoc* sp. 9f	KU668912
14.	*Nostoc* sp. 9i	KU668909
15.	*Nostoc* sp. 9j	KU668907
16.	*Tolypothrix* sp. 9k	KU668914
17.	*Nostoc commune* 9n	KU668913
18.	*Nostoc* sp. 9ma1	KU668918
19.	*Nostoc* sp. 9ma2	KU668917
20.	*Scytonema* sp.12c	KU668915

Table 10. List of Cyanobacterial strains isolated from North Hill regions of Chhattisgarh with Accession number obtained from NCBI

S.N.	Cyanobacterial strains	Accession no.
1	*Scytonema* sp. 1ss	MH341427
2	*Anabaena* sp.2ss	MH341434
3	*Calothrix* sp. 3ss	MH341433
4	*Aphanothece* sp. 4ss	MH341432
5	*Aphanothece* sp. 5ss	MH341435
6	*Scytonema* sp. 6ss	MH341438
7	*Anabaena* sp. 7ss	MH341437
8	*Tolypothrix* sp. 8ss	MH341436
9	*Nostoc* sp. 9ss	MH341440
10	*Aphanothece* sp. 10ss	MH341439
11	*Anabaena* sp.11ss	MH341423
12	*Anabaena* sp. 12ss	MH341422
13	*Calothrix* sp.13ss	MH341424
14	*Calothrix* sp.14ss	MH341421
15	*Anabaena* sp.15ss	MH341425
16	*Nostoc* sp. 16ss	MH341426
17	*Nostoc* sp. 17ss	MH341431
18	*Scytonema* sp. 18ss	MH341429
19	*Anabaena* sp.19ss	MH341430
20	*Anabaena* sp. 20ss	MH341428

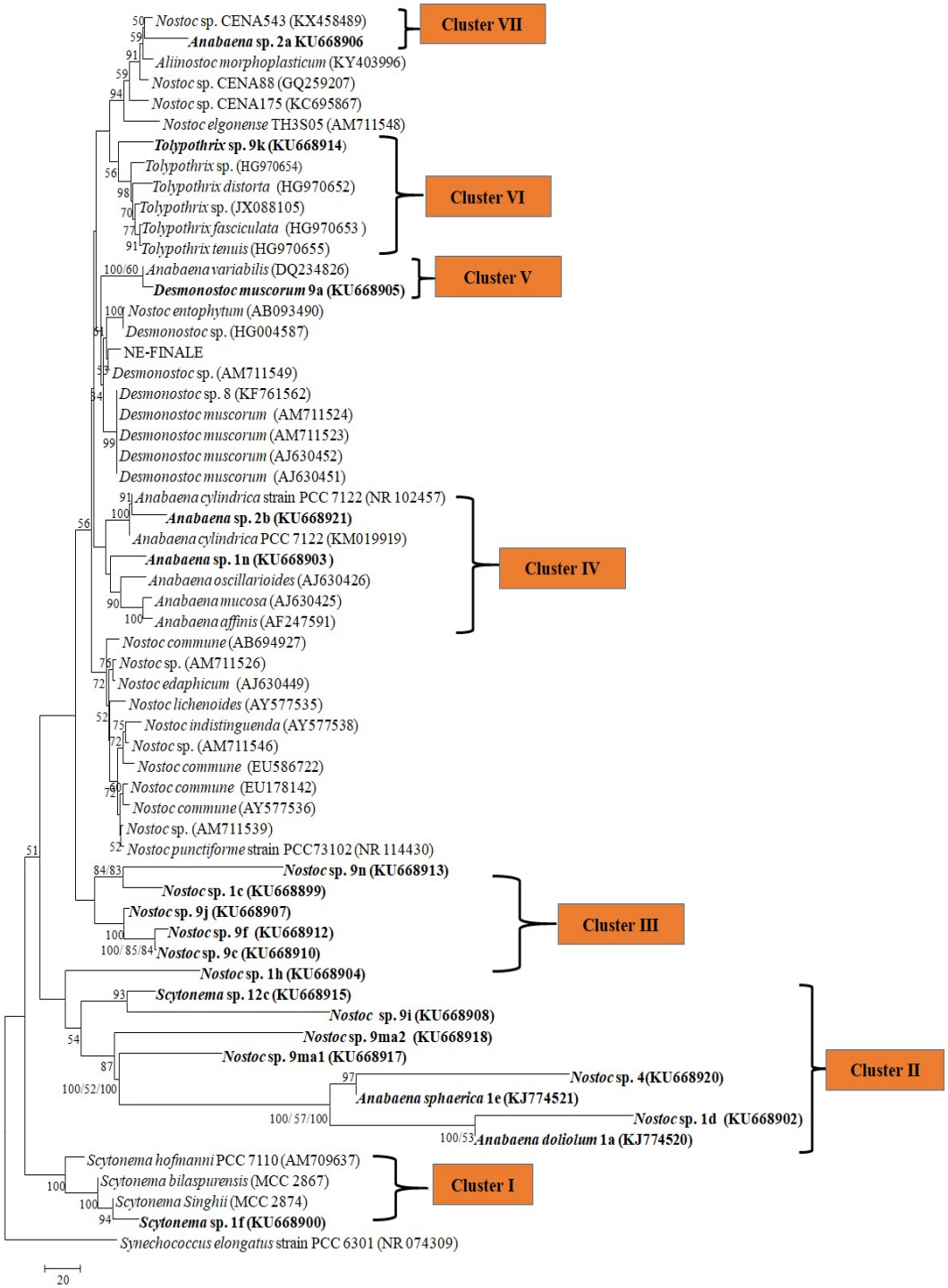

Figure 41. Neighbor joining tree showing phylogenetic relationship of the cyanobacterial strains of the plains regions based 16S rRNA gene sequences. Total 1000 bootstraps were performed and only more than 50% bootstrap support values are mentioned. All the Phylogenetic analysis was performed using MEGA 6.0 software. Numbers designate the clade.

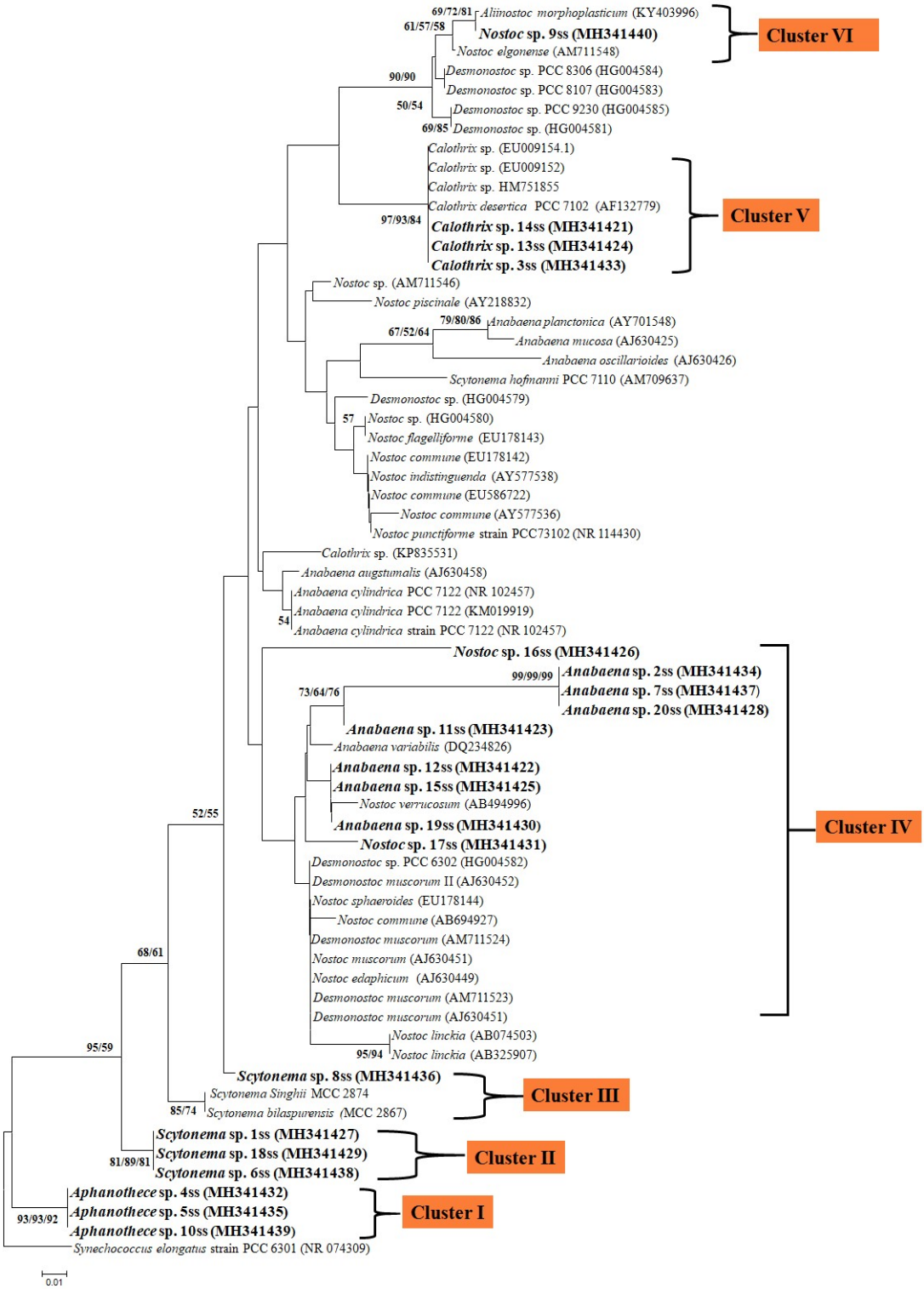

Figure 42. Neighbor joining tree showing phylogenetic relationship of the cyanobacterial strains of the north hills regions based 16S rRNA gene sequences. Total 1000 bootstraps were performed and only more than 50% bootstrap support values are mentioned. All the Phylogenetic analysis was performed using MEGA 6.0 software. Numbers designate the clade.

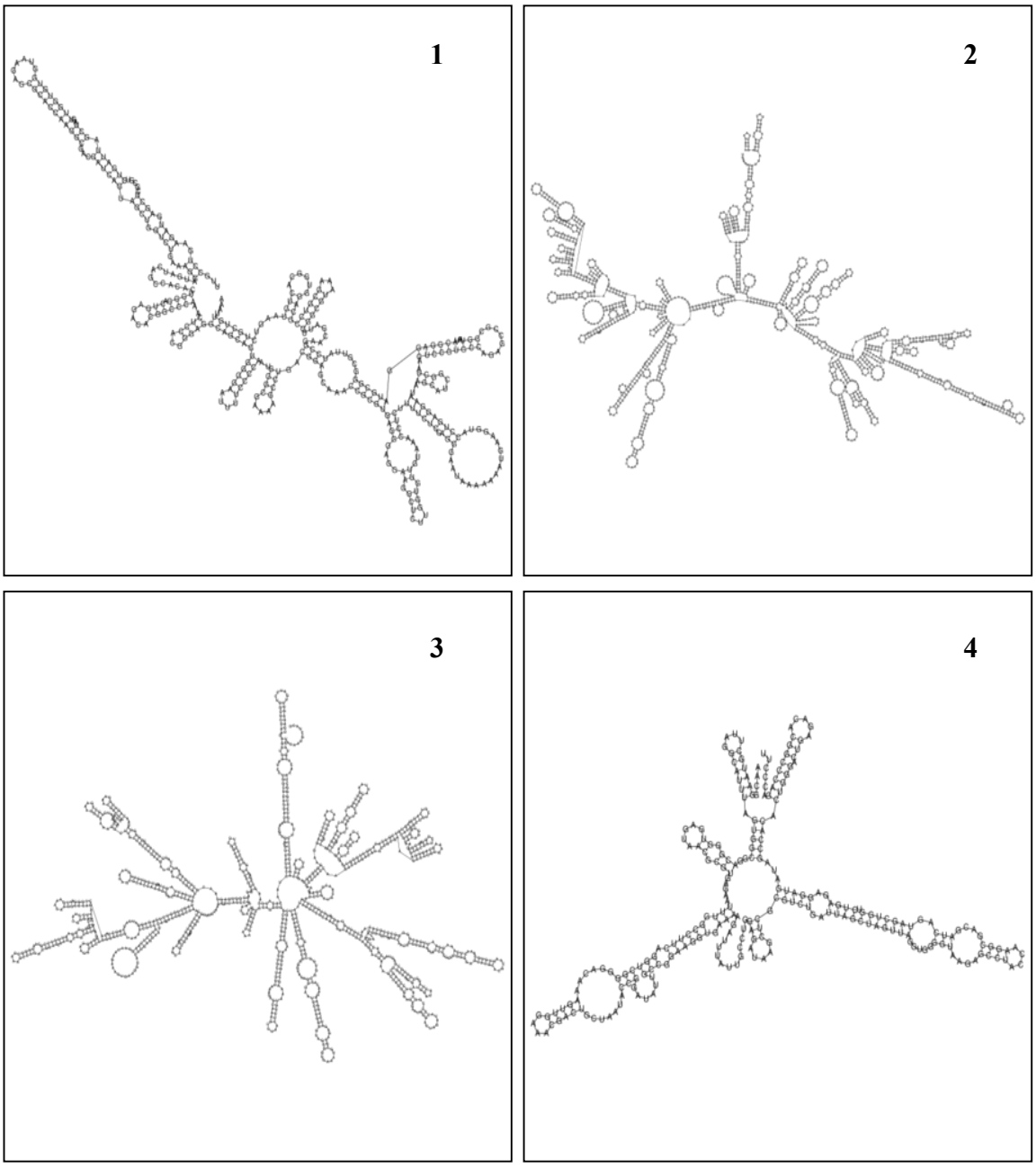

Figure 42. Secondary structure of 16S rRNA gene sequences isolated from plain regions of Chhattisgarh. (1) *Anabaena doliolum* 1a, (2) *Nostoc* sp. 1c, (3) *Nostoc* sp. 1d, (4) *Anabaena sphaerica* 1e

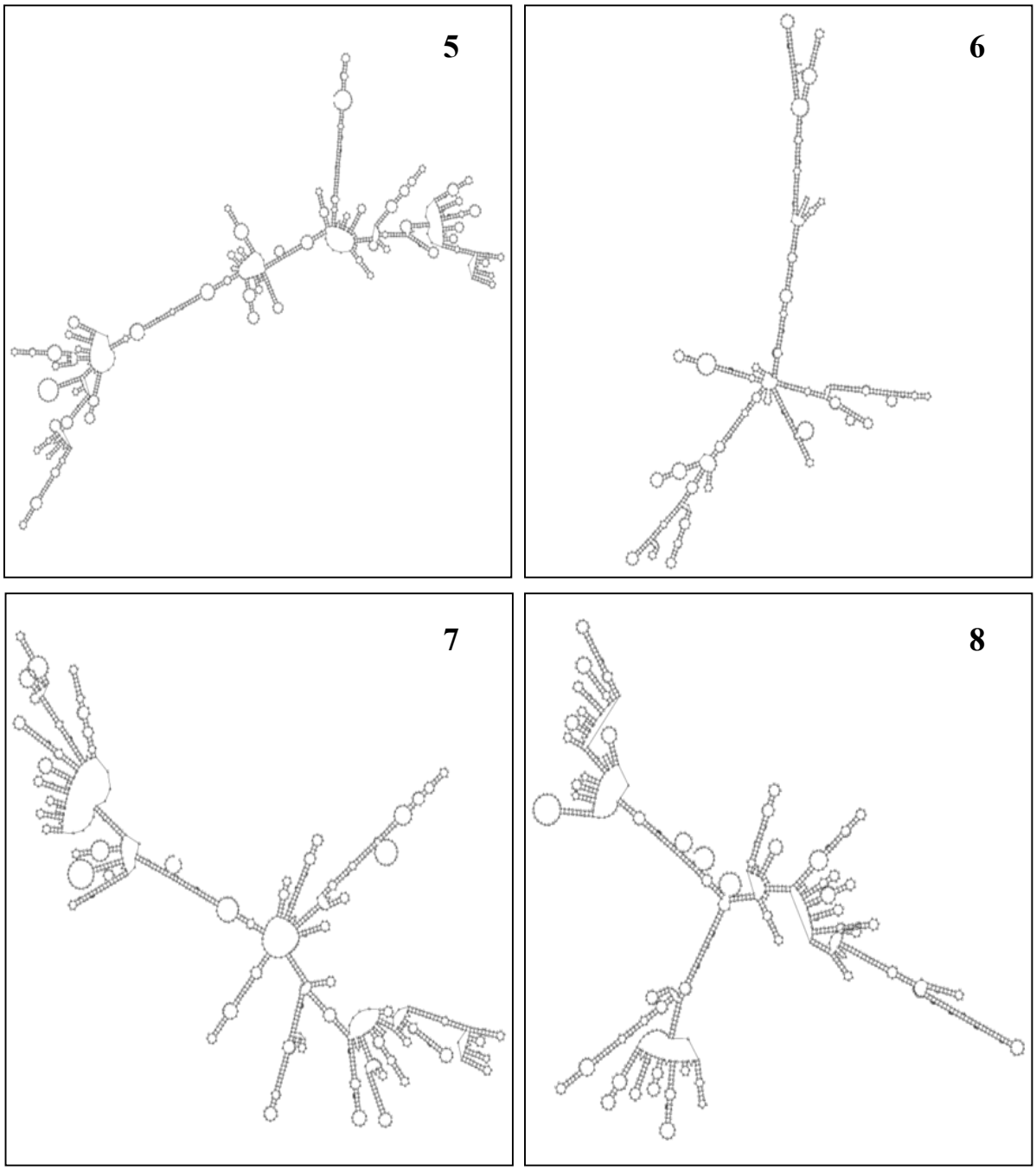

Figure 43. Secondary structure of 16S rRNA gene sequences isolated from plain regions of Chhattisgarh. (5) *Scytonema* sp. 1f, (6) *Nostoc* sp. 1h, (7) *Anabaena* sp. 1n, (8) *Anabaena* sp. 2a

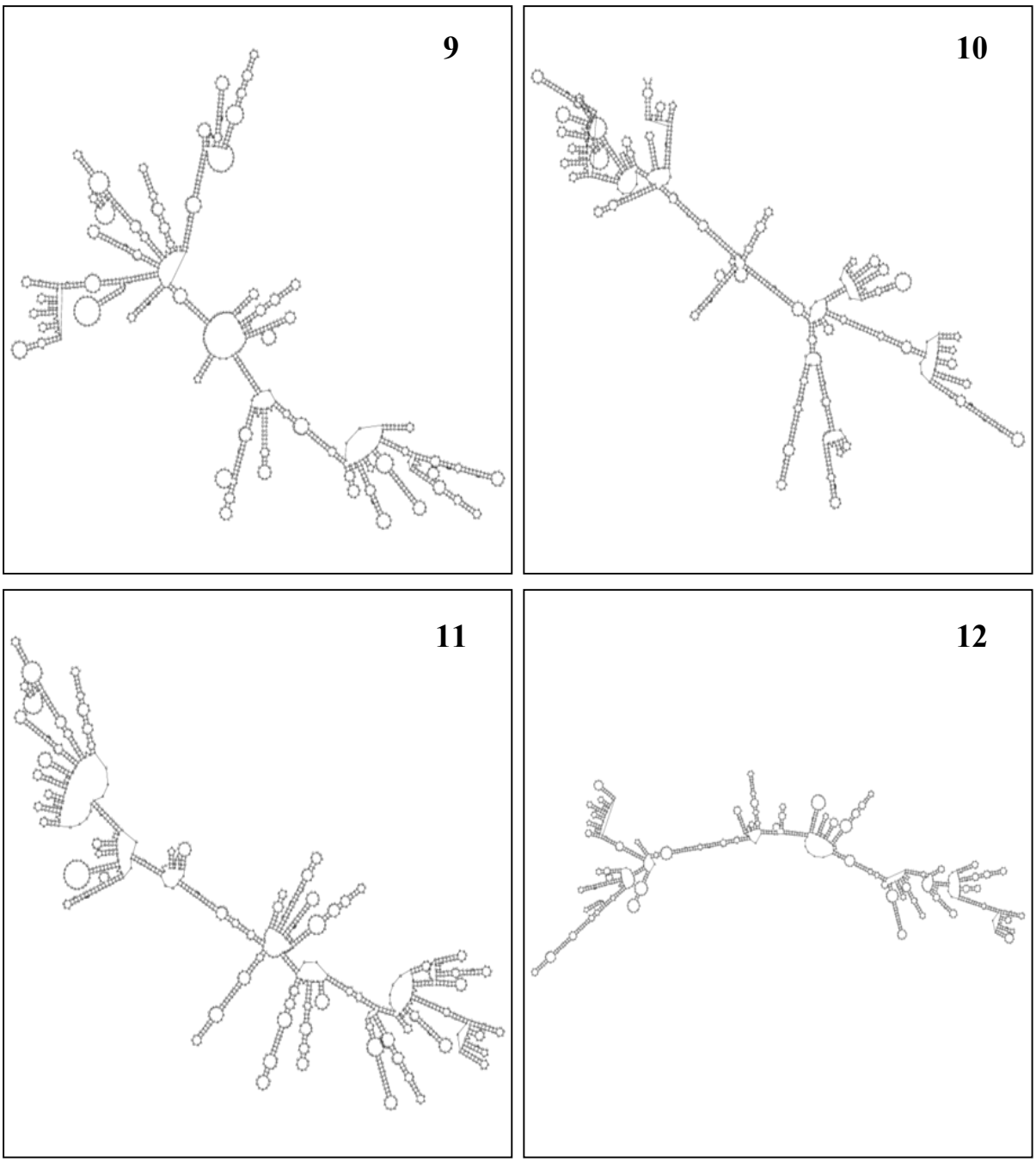

Figure 44. Secondary structures of 16S rRNA gene sequences isolated from plain regions of Chhattisgarh. (9) *Anabaena* sp. 2b, (10) *Nostoc* sp. 4, (11) Desmo*nostoc muscorum* 9a, (12) *Nostoc* sp. 9c

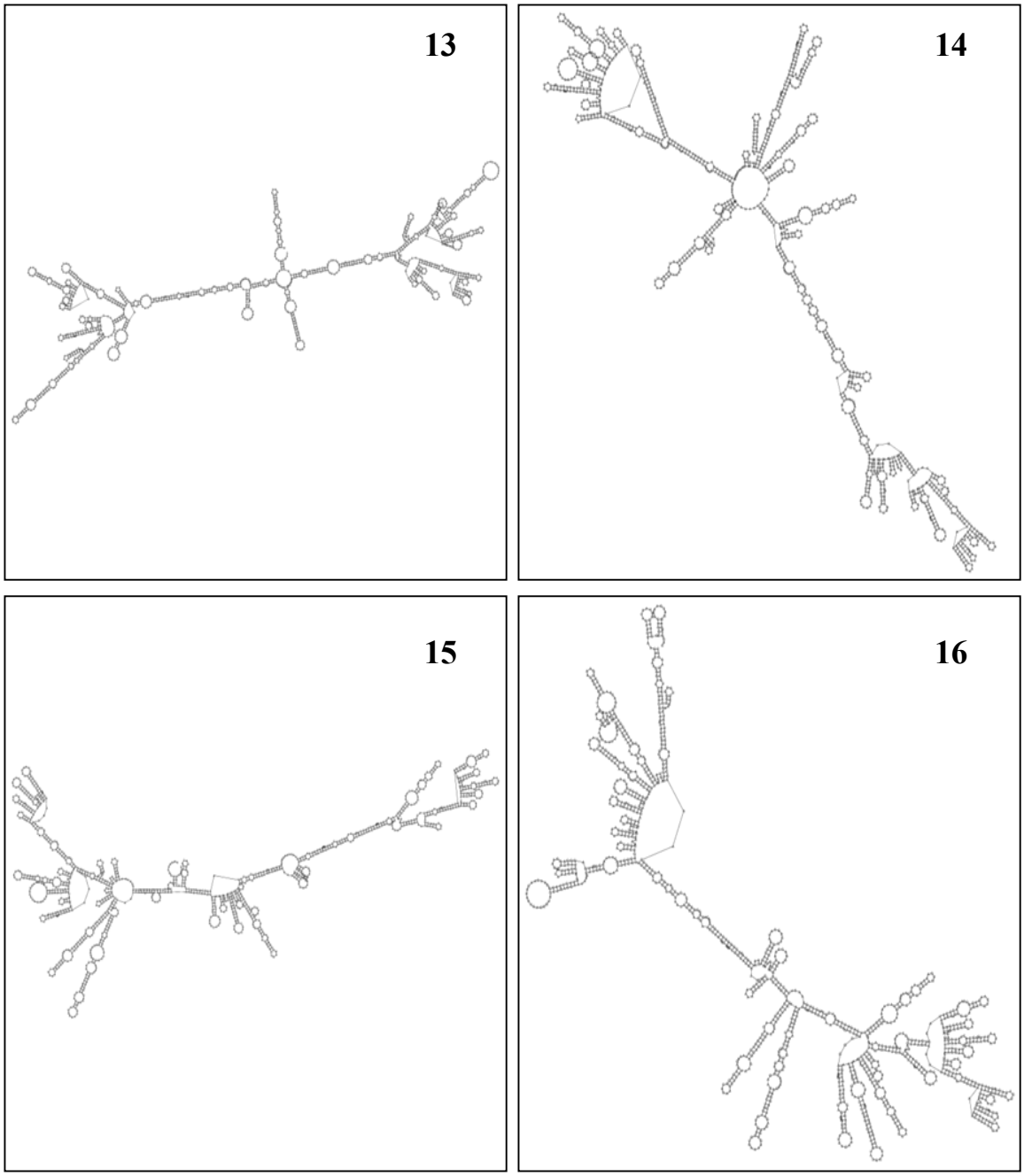

Figure 45. Secondary structure of 16S rRNA gene sequences isolated from plain regions of Chhattisgarh. (13) *Nostoc* sp. 9f, (14) *Nostoc* sp. 9i, (15) *Nostoc* sp. 9j, (16) *Tolypothrix* sp. 9k

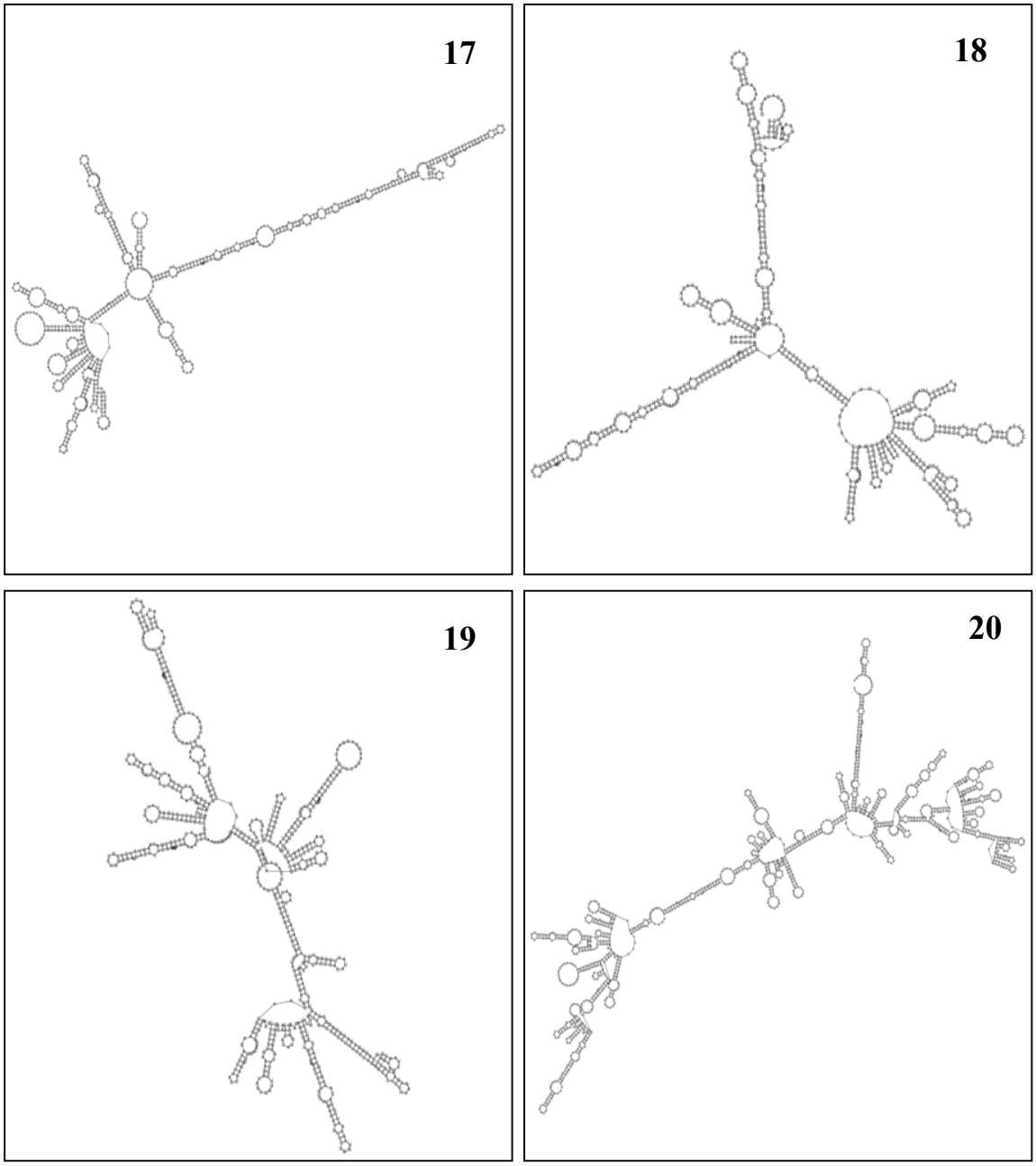

Figure 46. Secondary structure of 16S rRNA gene sequences isolated from plain regions of Chhattisgarh. (17) *Nostoc commune* 9n, (18) *Nostoc* sp. 9ma1, (19) *Nostoc* sp. 9ma2, (20) *Scytonema* sp. 12c

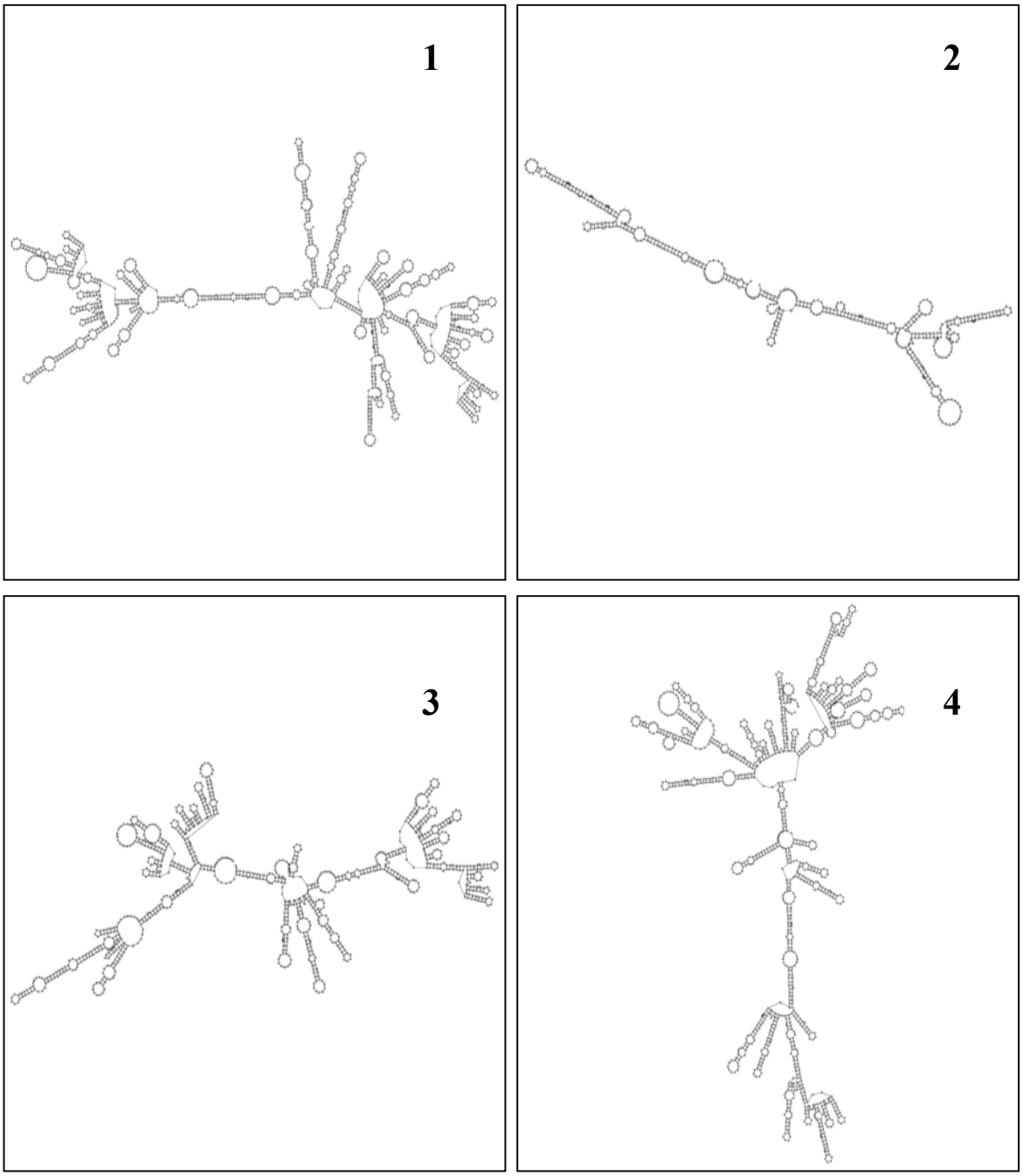

Figure 47. Secondary structures of 16S rRNA gene sequences isolated from north hill regions of Chhattisgarh. (1) *Scytonema* sp.1ss, (2) *Anabaena* sp. 2ss, (3) *Calothrix* sp. 3ss, (4) *Aphanothece* sp. 4ss

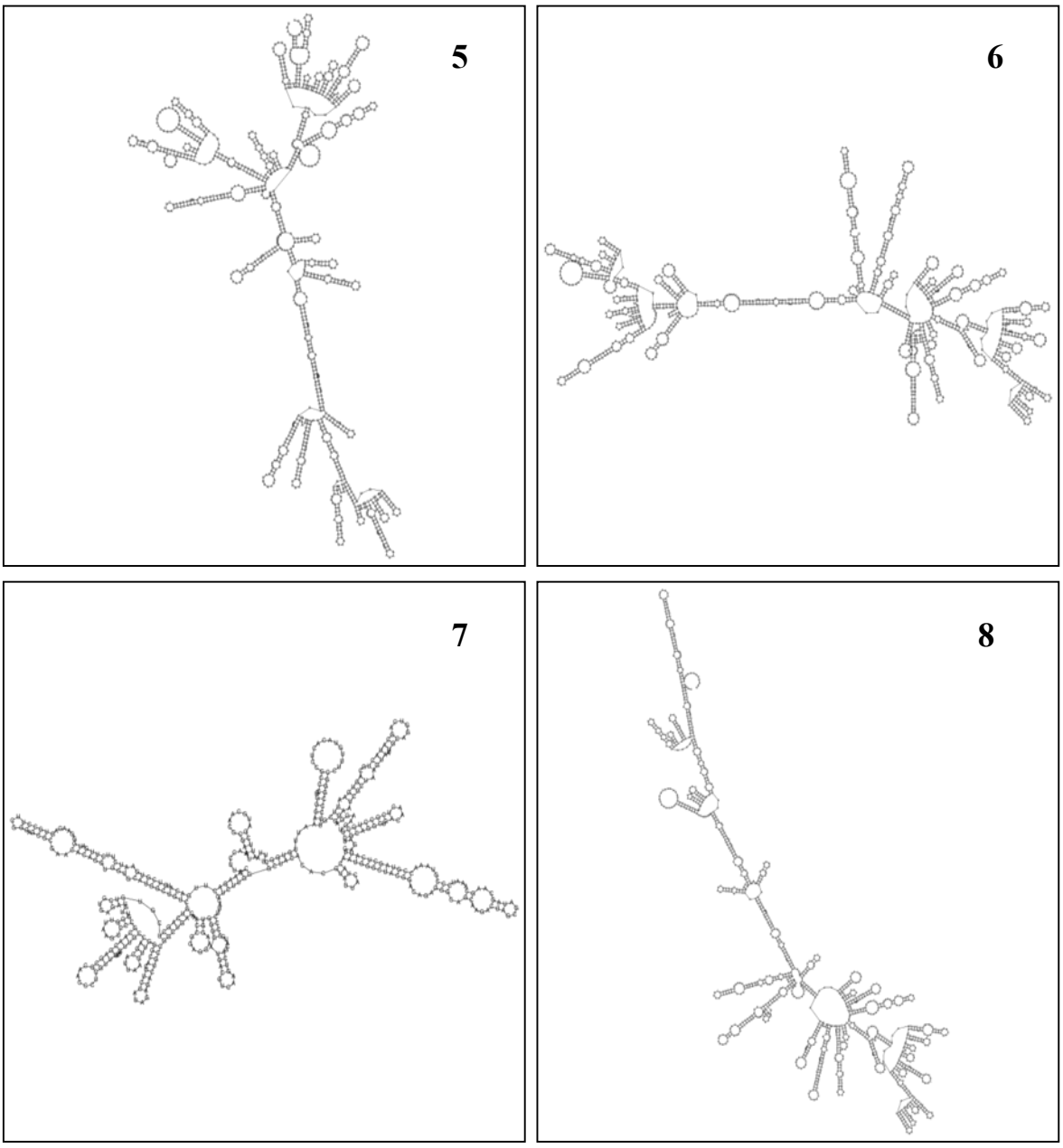

Figure 48. Secondary structures of 16S rRNA gene sequences isolated from north hill regions of Chhattisgarh. (5) *Aphanothece* sp. 5ss, (6) *Scytonema* sp. 6ss, (7*) Anabaena* sp. 7ss, (8)*Tolypothrix* sp. 8ss

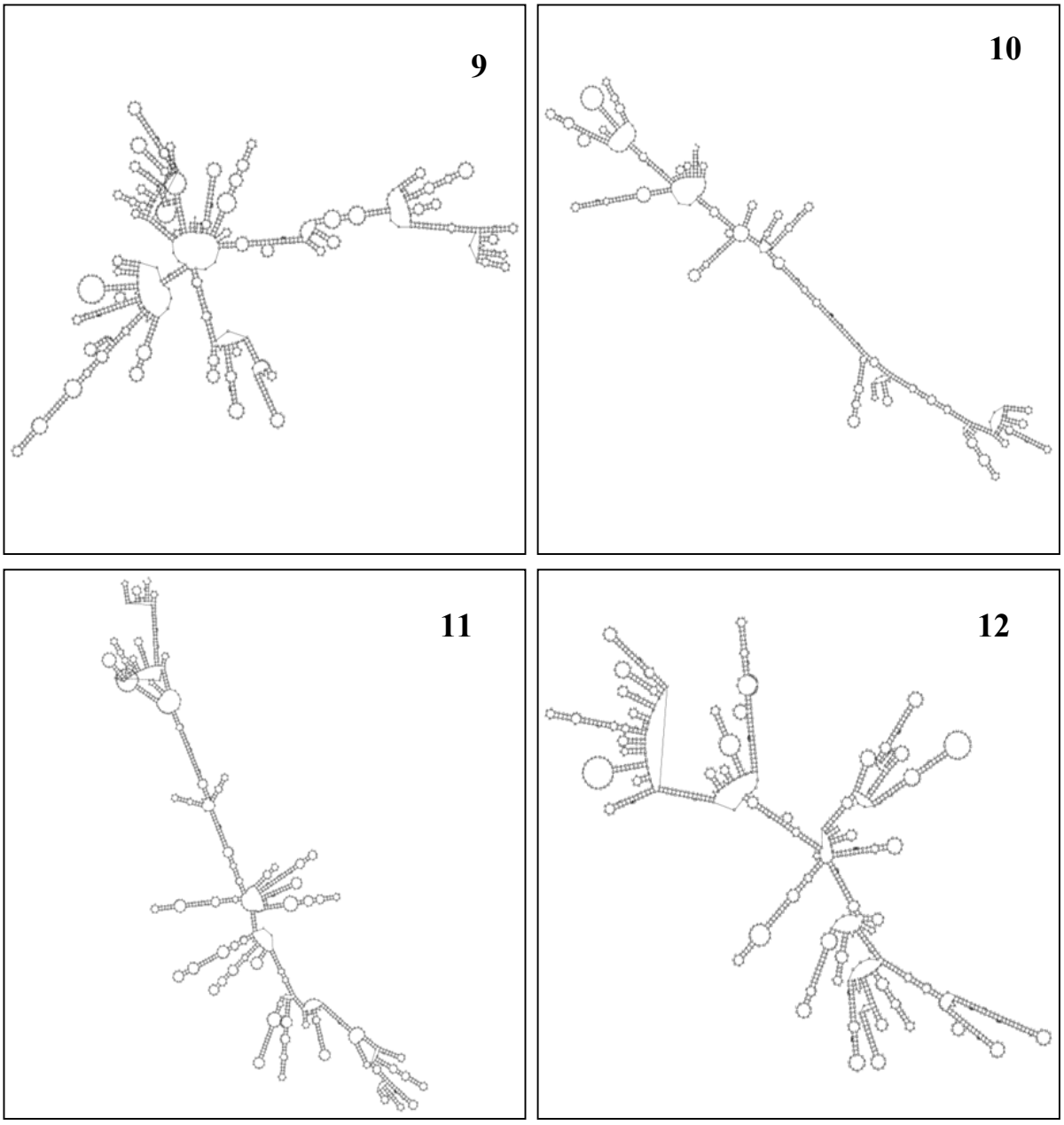

Figure 49. Secondary structures of 16S rRNA gene sequences isolated from north hills regions of Chhattisgarh. (9) *Nostoc* sp. 9ss, (10) *Aphanothece* sp. 10ss, (11) *Anabaena* sp. 11ss, (12) *Anabaena* sp. 12ss

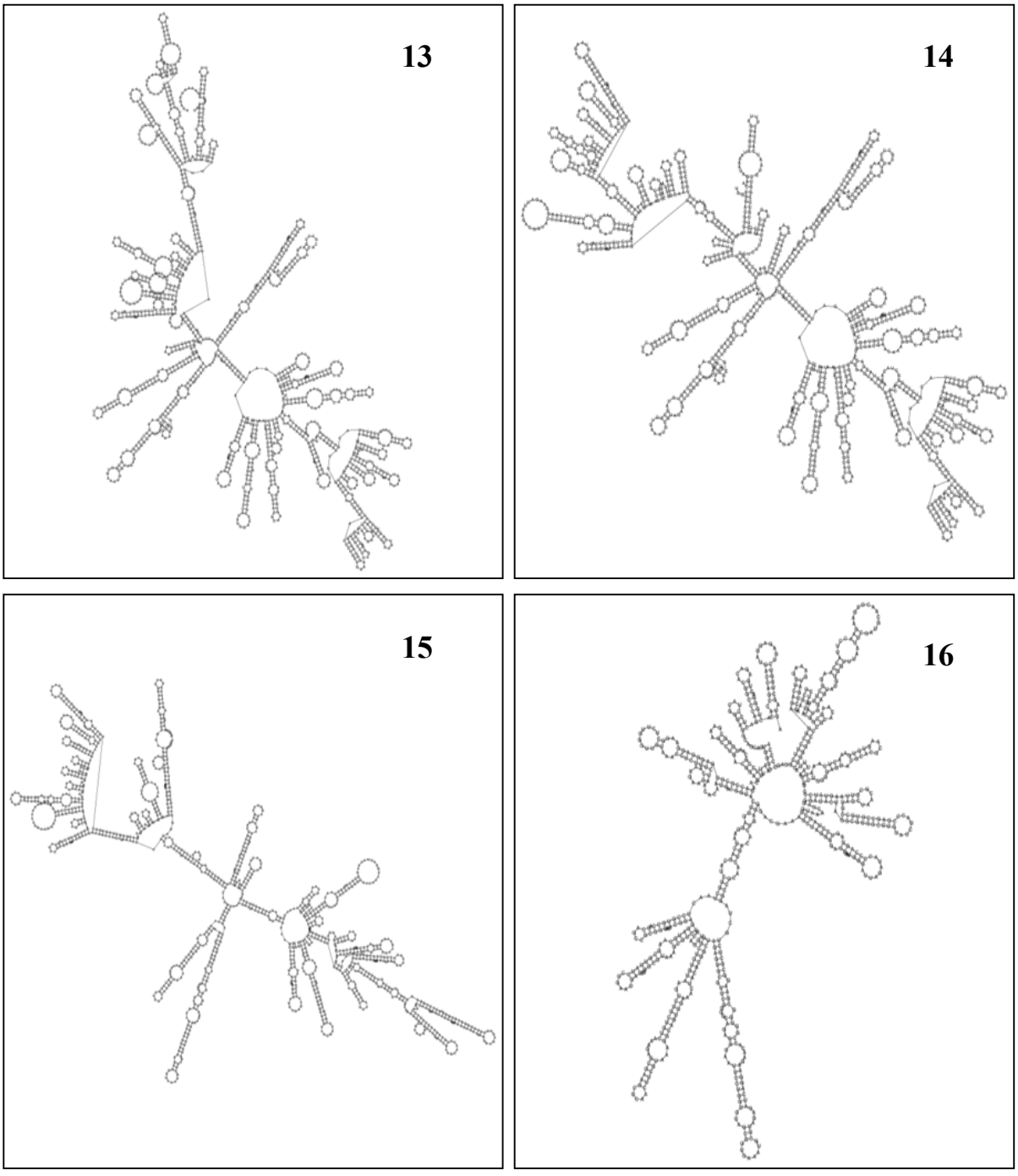

Figure 50. Secondary structures of 16S rRNA gene sequences isolated from north hills regions of Chhattisgarh. (13) *Calothrix* sp. 13ss, (14) *Calothrix* sp. 14ss, (15) *Anabaena* sp. 15ss, (16) *Nostoc* sp. 16ss

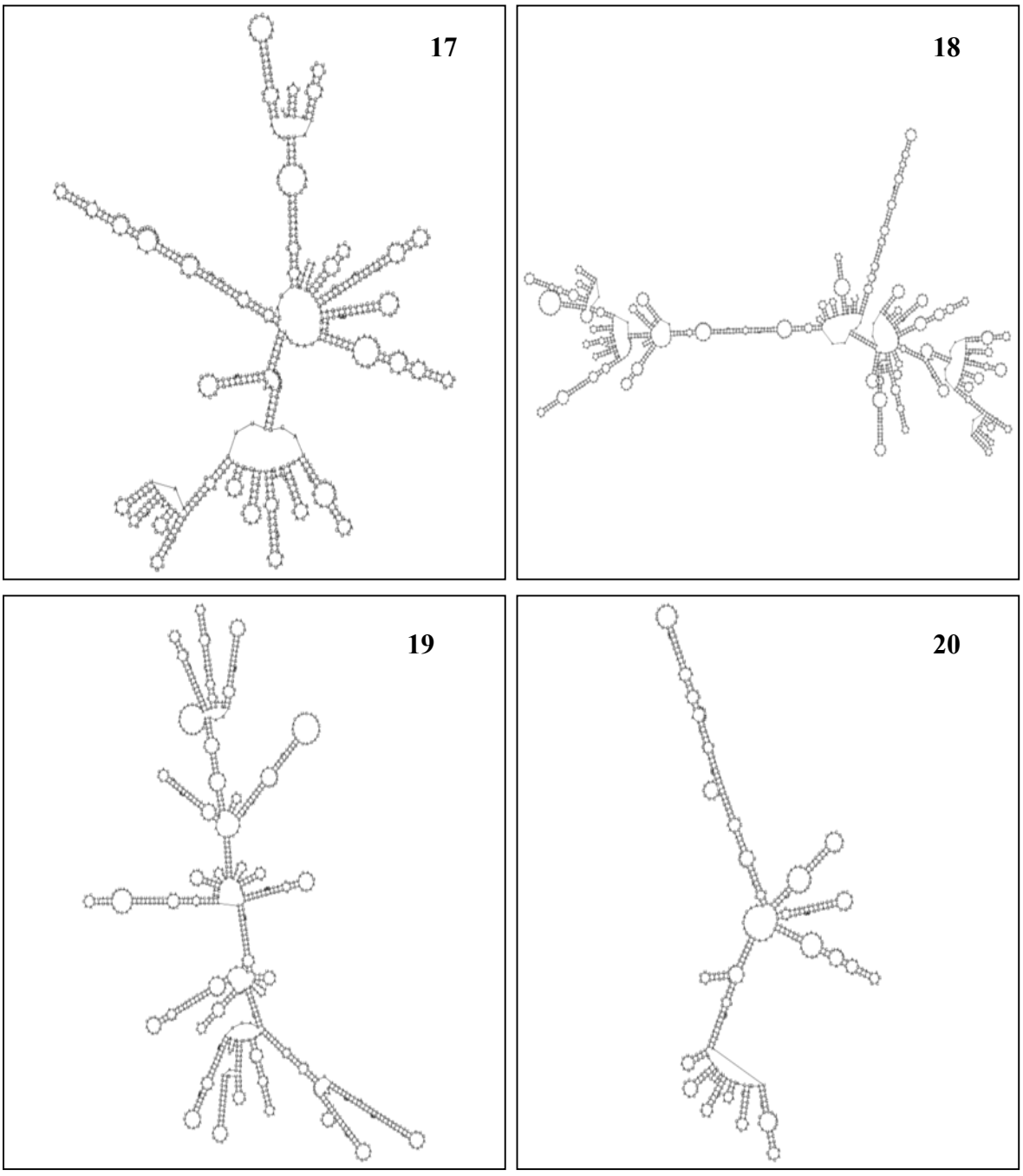

Figure 51. Secondary structure of 16S rRNA gene sequences isolated from north hills regions of Chhattisgarh. (17*) Nostoc* sp. 17ss, (18) *Scytonema* sp.18ss, (19) *Anabaena* sp. 19ss (20) *Anabaena* sp. 20ss

CHAPTER-5

Discussion

5.1 PHYSICOCHEMICAL CHARACTERIZATION OF SOIL SAMPLE COLLECTED FROM DIFFERENT SITES OF CHHATTISGARH

Natural ecosystem including soil is complex and dynamic environment consists of a variety of physical, biological and chemical factors that affects the abundance and diversity of microorganisms (Garbeva *et al.,* 2004., Bonilla *et al.,* 2005; Meliani *et al.,* 2012). Physical and chemical parameters are directly related to the survival and growth of the microorganisms, animals or plants in a wide range of conditions. Moreover, microorganisms have played an important role in transformation the soil nutrients into an available form for the plants and animals and also paid much attention in the maintenance of soil health which decide the health and survival of the microorganisms, animals and plants. Cyanobacteria, gram negative prokaryotes comprise a diverse group and commonly found in paddy fields. They play a major role in sustaining the fertility of an ecosystem and also regulating the soil biological activities (Prasanna and Nayak, 2007).

It is well known that the physicochemical parameters such as temperature, salinity, pH, dissolved oxygen content help to determine the biological activities in any ecosystem. Therefore, in the present study, analysis of the different physico-chemical properties of the soil sample were done and results showed different combinations of all physico-chemical parameters or in other words they were varied from each and every area therefore it may be possible reasons for the maximum abundance of cyanobacterial diversity in plain regions as well as North hill regions.

Among soil properties, pH is a very important factor for the growth of cyanobacterial diversity, which has generally been reported to prefer neutral to slightly alkaline pH for optimum growth (Cho *et al.,* 2016). Acidic soils are therefore one of the stressed environments for cyanobacteria and they are normally absent at pH

values below 4 or 5 **(**Singh, 1961). In the present study, the pH was ranged from 9.30 (Turkadih paddy field) to 6.83 (Navagarh and Dhamtari). Biogeographically, the observed range of pH value provides favorable environmental condition for specific cyanobacterial community (Giraldez-ruiz *et al.,* 1997; Maier *et al.,* 2009).

Similarly, temperature was also observed in the plain and North Hills regions. Temperature of the different soils ranged from 26°C to 37°C in plain regions where as in the field of North Hills, the temperature was observed which ranged between 28°C to 31°C. In present investigation, *Nostoc commune* grew up to maximum temperature.

Soil salinity refers to the salts content in the soil and salts adversely affect the plant and microbial growth. An excess of salt concentration determines the sensitivities and survival of microorganisms in terms of metabolic activities imposed by the need for stress tolerance mechanisms (Mackey *et al.,*1984; Yan *et al.,* 2015; Pade and Hagemann, 2015). The maximum salinity was observed in the paddy field of Turkadih (142.83 ± 2.0 g L^{-1}) whereas the lowest was observed in Somri (31.27 ± 1.50 g L^{-1}). The cyanobacteria grew at high salt concentrations is surprising. This is in accordance with the results previously reported for *Nostoc* and *Anabaena* isolated from saline soil and may play a role in maintaining fertility and productivity of saline soil **(**Zhang and Fang, 2008). In spite of these our results agreed with low salinity which favored the presence of heterocytous cyanobacteria, while very high salinity mainly supported the growth of non-heterocytous genera (Srivastava *et al*., 2009).

Dissolved Oxygen is the amount of gaseous oxygen (O_2) dissolved in the water. Dissolved oxygen has a link with temperature and affects levels of dissolved oxygen (Ugwu *et al.,* 2006; Singh *et al.,* 2014).

In the present study the DO range varied from 132.0-21.2 ppm in plain as well as north hill regions of Chhattisgarh.

Electrical Conductivity is a useful tool to evaluate the purity of water (Acharya *et al.,* 2008). Water becomes a conductor of electrical current when

substances are dissolved in it and the conductivity is proportional to the amount of dissolved substances. In the present study, the electrical conductivity ranged of the soils was varied between 33.92 ± 0.93 µ Seimen cm^{-1} to 140.33 ± 2.5 µ Seimen cm^{-1}. The source of EC is due to poor irrigation management, minerals from rain water runoff, or other human activities (Singh *et al.,* 2013; Khalil *et al*., 2015; Kekane *et al.,*2015).

The physico-chemical changes in the environment may affect particular species and induce the growth and abundance of other species which ultimately leads to the succession of several species in a course of time. The maximum number of Nostocales members such as *Anabaena, Nostoc and Scytonema* prevalent in almost all the selected sites of Chhattisgarh, India which proved the strong ability of these genera to adapt readily to the changed surroundings. Overall, a single physicochemical parameter does not affect the growth of cyanobacterial community isolated from the selected sites. But the specific combinations of tested physicochemical parameters in both regions (Plains as well as North hills of Chhattisgarh) suggested different combinations of cyanobacterial community which represented cyanobacterial diversity.

5.2 MORPHOLOGICAL CHARACTERIZATION OF CYANOBACTERIA

Forty cyanobacterial strains were characterized on their shape and size of the vegetative cell, shape and size of heterocyte and heterocyte frequency, akinites and their position of the filament. They show the remarkable variation on the morphology and might be considered for identification and characterization of cyanobacterial community (Rippka *et al*., 2001; Chonudomkul *et al.,* 2004; Palinska *et al.,*2011; Singh *et al*., 2014; Basavaraja & Naik, 2018). Previous reports related to the different cyanobacterial genus based on the morphological characters help to ecological categorization (Palinska *et al.,* 2006; Nayak *et al.,* 2007).

In the present investigation, most of the cyanobacterial species formed straight filament and shape of the vegetative and heterocyte cells were ranged from cells show

the barrel oval, ellipsoidal, spherical and cylindrical and size of the vegetative cell reported range between 7.5-3.5 µm in all tested cyanobacterial strains which is in accordance with the earlier reports (Rajaniemi *et al*., 2005; Willame *et al*., 2006; Mishra *et al.,* 2013; Singh *et al.,* 2014).

It is well known that the cyanobacteria possesses the special type of compartment know as heterocyte which is responsible for the atmospheric nitrogen fixation. Our observation proved that the shape, size and frequency of the heterocytes varied from species to species whereas the maximum heterocyte was found in *Anabaena sphaerica* 1e isolated from the plain region of Chhattisgarh. The regulation of heterocyte differentiation depends on several factors like nitrogen source deprivation, light quality, cell physiology and also on the intercellular communication (Golden and Yoon, 1998; Adams 2001., Haselkorn, 2007). Cyanobacteria are almost found at all possible habitats therefore they possess the tremendous capacity for survival strategies (Witton and Potts 1997). Heterocyte generally are formed under the nitrogen starvation. Some reports were available related to the heterocyte differentiation in the cyanobacteria (Golden *et al.,* 1985; Singh and Montgomery, 2011; Kumar *et al.,* 2010).

Akinetes are thick walled spore and non-motile cell, differentiated from the vegetative cells and for surviving in unfavorable conditions (Adam and Duggan, 1999). The position of akinete along the trichome varied among cyanobacterial species where in some cases heterocytes were reported to influence their location (Wolk 1966). Whereas the characteristic feature of akinetes share the other prokaryotic organisms and indicator of mesoproterozoic biosphere evolution (Errington 2003; Srivastava 2005).

In the present study, different types of akinetes were observed in the order Nostocales members including genus *Anabaena* and it was rarely found in *Nostoc* sp.

Similarly in our study akinetes shape (sphere to barrel sometimes oblate or spheroid) showed the variation among all the cyanobacterial strains in plain as well as North Hills regions. The resting spores was found in *Anabaena doliolum* 1a, *Anabaena sphaerica* 1e, *Anabaena* sp. 2a and *Nostoc* sp. 4 whereas rest of the sixteen cyanobacterial species did not show. Only two cyanobacterial species of the north hill regions showed the resting spore *viz. Anabaena* sp. 7ss and *Anabaena* sp. 12ss. The akinete shape was varied among species to species and their distribution and position within a filament was also used as a taxonomic feature (Mishra *et al.,* 2013).

Earlier, a few reports are available related to akinetes which are developed immediately adjacent to heterocyte in *Anabaena cylindrica* but the same are developed several cells away from the heterocyte in *Anabaena circinalis* and in some other planktonic species (Fay *et al.*, 1984; Li *et al.,* 1997). In most cases, akinetes develop into strings, showing a gradient of decreasing maturity. Adams and Duggan, (1999) explained the akinete placement in relation to heterocyte by the need to accumulate large amounts of cyanophycin. Akinetes undergo various metabolic and morphological changes during their development and maturation. Our study also proved that the differentiation of akinetes among the experimental cyanobacteria might be due to environmental condition, lack of nutrient and limitation of light (Li *et al*., 2001; Meeks *et al.,* 2002; Tompson *et al.,* 2009; Selvi *et al*., 2012).

Earlier reports supported our findings that all the morphological characters including heterocyte frequency varied from species to species and helped to identify and characterize the cyanobacterial strains (Gorcia–pichel, 2001; Mishra *et al*., 2012; Mishra *et al*., 2013). But sometimes same species has different morphology (Mur *et al*., 1999; Gercia–pichel *et al.,* 2001). A few reports are available which deal systematically with the effect of environmental factors on cell differentiation and morphology (Singh and Montgomery, 2011).

According to our findings, the morphological characters of cyanobacteria have more plasticity, so, more unstable features have been observed in the selected cyanobacterial strains. The relationship between morphology and genetic characters

of heterocytous cyanobacteria exhibit that shape, size and position of the vegetative cells and heterocyte within trichome and germination of akinetes are determined features which justify the validity of several taxonomic criteria and help to novel approach to develop criterion based on transcriptional data for the plasticity and it was the first hypothesis which provide evidence for its occurrence during the complex evolution of colony morphology in the cyanobacteria (Srivastava, 2005; Koch *et al*., 2015).

The cluster analysis helps to decipher the proper relationship among the tested cyanobacterial strains. The cluster analysis among all the cyanobacteria isolated from plain as well as north hill regions was carried out on the basis of shape and size of the vegetative cells, shape and size of heterocyte, heterocyte frequency, presence and absence of akinetes.

The constructed dendrogram showed *Nostoc* sp. 9f, *Nostoc* sp. 9i, *Nostoc* sp. 12, and *Scytonema* sp. 12c in to cluster I and it was also proved by the similarities in shape and size of the vegetative cells (Figure. 2).Based on the closeness of vegetative cell (shape and size), heterocyte (shape and size) and also the intercalary position of heterocyte, *Nostoc* sp. 1d, *Nostoc* sp. 9ma1 and *Nostoc* sp. 9ma2 were grouped in to sub cluster IIA2. *Anabaena sphaerica* 1e, *Nostoc* sp. 4, and *Anabaena* sp. 2a were placed in same cluster IIB1 by sharing the same attributes or similar pattern of phenotypic characters.

Similarly, cluster IIB2 comprised of *Tolypothrix* sp. 9k, *Desmonostoc muscorum* 9a, *Anabaena* sp. 1n, *Scytonema* sp. 1f, *Nostoc* sp. 1h, *Nostoc* sp. 1c, *Nostoc* sp. 9j and *Anabaena* sp. 2b and suggested the existence of close relationship between false branching cyanobacterial to filamentous cyanobacteria.

On the other hand, cluster analysis of North hills cyanobacteria also followed the similar pattern as observed among the plain regions (Figure.3). Cluster analysis revealed that *Calothrix* sp.13ss and *Anabaena* sp. 20ss showed the close relationship

might be due to the phenotypic characters *i.e.* vegetative cell and heterocyte. *Anabaena* sp. 7ss showed the separate cluster in II A1.

Similarly the cluster IIA2 showed the intermixed relationship among the cyanobacterial strains *Anabaena* sp. 12ss, *Anabaena* sp. 19ss, *Scytonema* sp. 18ss, *Scytonema* sp. 1ss, *Anabaena* sp. 14ss, *Nostoc* sp. 17ss *and Calothrix* sp. 3ss. Whereas *Tolypothrix* sp. 8ss, *Scytonema* sp. 6ss, *Anabaena* sp. 2ss, *Anabaena* sp. 16ss, *Anabaena* sp. 15ss and *Anabaena* sp. 9ss were grouped into cluster IIA 2a.

Apart from these, cluster IIB was clearly a distinct and separate group which was formed due to the unicellularity pattern of *Aphanothece* sp. 4ss, *Aphanothece* sp. 5ss and *Aphanothece* sp. 10ss.

5.4 PHYSIOLOGICAL CHARACTERIZATION

Cyanobacteria one of the richest repertoire of physiological, biochemical and molecular activities, provide a basis for ecological processes such as biogeochemical cycles and food chains, as well as maintaining vital relationships among the other microorganisms (Panizzon *et al*., 2015). Furthermore, the physiological heterogeneity play an important role in microbial as well as cyanobacterial community and generally appear that the microbial populations contain substantial cell-to-cell differences in physiological parameters such as growth rate, resistance to stress and other physiological activities and introduce to the inter-relatedness of microbial activity including biochemistry and genetic behaviors in context of function of cells (Lidstrom and Konopka, 2010).

Physiological attributes were also studied among the tested cyanobacterial strains including growth pattern (in terms of OD), chlorophyll a content, carotenoid, phycobilin protein, protein content and carbohydrate content. The growth pattern of cyanobacteria is usually much lower than that of many algal species (Hoogenhout and Amesz, 1965; Reynolds, 1984). Each cyanobacterial strains showed at least similar growth pattern in terms of direct absorbance at 663 nm vs. incubation time. The observed differences in growth behavior suggested that the physiological diversity

may be the result of the inoculums added *i.e.* age, temperature and also the nutrient composition as reported earlier (Fiore *et al*., 2005). One thing was also clear that the specific growth pattern from the physiological observations was the result of the distribution and diversity or ecology, occurrence of cyanobacteria in a specific environment *i.e.* Plain as well as North hills of Chhattisgarh.

Cyanobacteria are ecologically important photosynthetic organisms which serve as a popular model organism for photosynthetic molecular and ecological studies whereas the monitoring of photosynthetic analysis can provide non invasive measures of physiological behaviour in a wide range of cyanobacteria (Campbell *et al.,* 1998). Cellular photosynthetic pigments concentration is one of the central physiological parameter which is routinely followed in many research areas ranging from stress physiology to biotechnology.

In present study Chlorophyll a content was varied among different cyanobacterial strains isolated from Plain and North Hills regions of Chhattisgarh. Earlier findings suggested that carotenoid concentration was also related to the other cellular stress and combined pigments assessment provide useful insight into cellular physiological status.

Phycobiliproteins (PBPs) were varied among tested cyanobacterial strains according to the requirement because they are large water soluble supramolecular protein aggregates involved in light harvesting among cyanobacteria and contain 40–60 % of the total soluble protein (Bogorad, 1975; Kumar *et al*., 2014). These can be divided broadly into three classes based on their spectral properties *i.e*. Phycoerythrin (565 nm), phycocyanin (620 nm) and allophycocyanin (650 nm). Phycocyanin is widely found in cyanobacteria (Huang *et al.,* 2007; Yoshikawa and Belay, 2008; Thangam *et al.,* 2013).

The basic nutrients are required for the growth and maintenance of metabolic functions by any microorganisms. Cyanobacteria can derive energy from

carbohydrates and metabolize simple sugars such as cyanophycin starch, a special type of food storage materials (Sandle, 2015; Gunina & Kuzyakov, 2015).

Furthermore, the observed variation in cellular constituents such as Chlorophyll a, carotenoid content, phycobilin protein content, protein content and carbohydrate content might be due to the differences in the nutrient availability, light, temperature, pH and culture condition.(Schwarz and Forchhammer, 2005; Kenesi *et al*., 2009; Ashokkumar and Anand, 2010; de Figueiredo *et al*., 2011; Mishra *et al.,* 2013). Cyanobacteria composed of multiple differentiated cells which possess different functions and behavior due to the certain environmental condition *i.e.* starvation of nitrogen source. Cyanobacteria, one of the excellent organisms known for nitrogen fixing ability, possess multiple specialized cell *i.e.* heterocyte (Adams, 2000; Kumar *et al*., 2009). Cyanobacteria have developed strategies to protect the nitrogenase from oxygen inhibition. On the basis of phenotypic nature of cyanobacteria, they reported the ability of nitrogen fixation in filamentous heterocytous, non heterocytous and unicellular. In heterocytous cyanobacteria vegetative cells are morphologically differentiated into the special compartment knows as heterocyte (Rippka and Waterbury, 1977; Berman Franke *et al.,* 2003). Heterocyte and vegetative cells are mutually interdependent. Heterocytes lack component of oxygen evolving pigment system II and have a unique physiology. (Thiel *et al*., 1995; Golden and Yon 2000). Cyanobacterial heterocyte possess highly conserved enzyme complex known as Dinitrogenase enzyme which is activated under anaerobic condition and convert molecule nitrogen into ammonia (Rippka and Stanier 1977; Bohme, 1998) whereas the fixed nitrogen exported the into neighbor cells to heterocyte. There are many cyanobacterial genera including nonheterocytous and unicellular cyanobacterial strains *e.g. Oscillatoria, Trichodesmium* and *Gleothece*, *Aphanothece* posses nitrogen fixation (Leon *et al.,* 1986; Haung and Chow, 1988).

In the present investigation, there was a great variation in nitrogenase activity of the cyanobacterial species isolated from hills as well as plain regions of Chhattisgarh.

In our results, an interesting fact were observed that the low amount of nitrogenase activity was found in unicellular cyanobacteria *Aphanothece* sp. 4ss *Aphanothece* sp. 5ss and *Aphanothece* sp. 10ss. It might be due to the maximum exposure of oxygen dependent nitrogen fixation ability (Singh, 1973; Leon *et al*., 1986; Reddy *et al*., 1993).

Glutamine synthetase plays a central role in nitrogen metabolism of prokaryotes. In cyanobacteria, ammonium assimilation takes place mainly by sequential action of GS-GOGAT pathway (Meeks *et al.,* 1978; Melida *et al.*, 1991). In recent time, the regulation of GS synthesis has been well characterized in different cyanobacterial strains *i.e. Anabaena* species strain PCC 7120 and *Synechocystis* strain PCC 6803 (Media *et al.,* 1991; Meeks and Elhai, 2002). Earlier reports suggested that the highest GS activity was reported in heterocytes in comparison to vegetative cells and in the presence of abundant carbon source, nitrogen deficiency results high level of GS activity (Merrick and Edwards, 1995; Garcia-Dominguez *et al*., 1999; Galmozzi *et al.,* 2007). The GS-GOGAT pathway represents the connecting step between C and N metabolism and is tightly regulated in many organisms including cyanobacteria. In cyanobacteria, GS is modulated at transcriptional and post-transcriptional level, depending on carbon and nitrogen supply (Marida *et al*., 1991; Muro-Pastor and Florencio 2003; Muro-Pastor *et al*., 2005).

Over all, comprehensive study of enzymatic activity in *Anabaena* species showed the highest GS activity (Renström-Kellner *et al*., 1990; Merida *et al*., 1991; Reyes and Florencio, 1995; Nayak *et al*., 2007; Mishra *et al.*, 2013). Results also suggested that this enzyme is the check point for assimilation of ammonium (Sanz *et al*., 1995). In filamentous cyanobacteria, early studies demonstrated that GS from *Anabaena* sp. strain PCC 7120 is controlled neither by adenylylation nor by feedback inhibition by glutamine. However, levels of glutamine synthetase are lower in ammonium grown cells than in cells grown using nitrate or dinitrogen as the nitrogen source (Orr and Haselkorn, 1984).

The reduction of nitrate to nitrite by the enzyme nitrate reductase is the first step in the mechanism of nitrogen metabolism and it is considered as rate limiting step in the nitrate assimilation process (Berges and Harrison, 1995; Lopes *et al*., 2002). Localization of NR activity in the vegetative cells and not in the heterocyte has been confirmed by several workers (Ramaswamy *et al*., 1996; Kumar *et al*., 1985; Guerrero and Lara, 1987). Initially, in the present experiment, NR activity was determined on the 8^{th} day of incubation in the nitrate (5mM) grown cultures.

The varied activity of Nitrate reductase among different cyanobacterial species may be fulfill the complete N-demand in their cells for survival under specific conditions. Result also supported the earlier findings where NR has been found to be a substrate inducible enzyme and NO^{3-} played a positive role in regulating NR activity of filamentous cyanobacteria (Herrero *et al*., 1981; Martín-Nieto *et al*., 1989; Solomonson and Barber, 1990; Flores and Herrero, 1994; Sanz *et al*., 1995; Thaivanich and Incharoensakdi, 2007). This required demand for nitrate depends on the growth phase of the strains at which the activities are determined (Sanz *et al*., 1995; Lopes *et al*., 2002; Singh *et al*., 2008).

Overall our results revealed that the enzymatic activities such as Nitrogenase, NR activities and GS activities were strain specific attributes and were dependent on the phase, conditions and culture media used for the cyanobacterial cells. Indeed, in batch culture, the nitrogen concentration decreases at dissimilar rates as a function of its initial concentration and cell density in the medium and for this reason diverse result might have been obtained.

5.4 BIOCHEMICAL CHARACTERIZATION OF CYANOBACTERIA BASED ON FATTY ACID ANALYSIS

The analysis of the fatty acid profile and differences in their composition suggested that these differences may be responsible for phenotypic differences and also help in systematic of microorganisms (Gokart *et., al* 1991). There are many diverse approaches to cyanobacterial chemotaxonomy. Among these, fatty acid

profiling is one of the more popular method in chemotaxonomy (Tornabene, 1985; Romano *et al.* 2000; Shukla *et al.,* 2012). Assessment of the taxonomic position of heterocytous cyanobacteria on the basis of fatty acid composition and also comparison of the morphological attributes of cyanobacteria validated the application of fatty acid profile as a important marker for diversity assessment and suggested that particular groups of organisms may be characterized by particular fatty acid that can be used as a biological marker (Rezonka *et al.,* 2003; Shukla *et al.,* 2012). In recent time, so many workers studies on diversity of major and minor fatty acids profile in the bacterial and cyanobacterial species and suggested that it may be useful for the taxonomic relationships among these species (Li *et al*., 2001; Liu *et al.,* 2003; Tamina *et al*., 2007). Cellular fatty acid composition permitted to assign the cyanobacteria subsection II order Pleurocapsales (Sallal *et al.,* 1990; Caudales *et al.,* 2000).

Most of the cyanobacterial strains showed a good correlation between morphological properties and fatty acid composition whereas the dendogram based on fatty acid composition also proved the results (Li and Watanebe, 2004). Chemotaxonomic studies based on fatty acid composition also used as an alternative taxonomic approach to assure resolution of the taxonomy of cyanobacterial species with an environmental concern.

Our present studies based on Fatty acid methyl esters (FAME) provide the expected conformation that each strain has a great diversity and the varied length of carbon chains (C11:C26) in comparison to other cyanobacterial species (Table 5). Our results also proved the strain specificity based on FAME profiling either isolated from Plain regions or from North Hills regions.

Our data based on the fatty acid composition was agreed with the previous reports though there were differences which may be attributed to the provided culture conditions (Cohen and Vonshak, 1991; Cohen *et al*., 1995; Walsh *et al*., 1997; Tamina *et al*., 2007; Galhano *et al*., 2011; Shukla *et al*., 2012).

Few fatty acids were found as species representative of some cyanobacteria isolated from plain regions among all the twenty heterocytous cyanobacterial strains which is in accordance with the earlier reports (Walsh *et al*., 1997; Řezenka *et al*., 2003; Li and Watanabe, 2004; Tamina *et al*., 2007). Signature fatty acids were also found in many cyanobacteria such as polyunsaturated fatty acids (16:2, 18:2 and 18:3) which have also been detected among our tested cyanobacterial species isolated from plain regions (Jungblut *et al*., 2009). On the basis of present investigation, among the tested cyanobacterial strain Tetradecanoic acid (14:0), Hexacosanoic acid (26:0), 11,14-Eicosatetraenoic acid (20:5), 11,14-Eicosadienoic acid (20:2), 5,8,11,14,17-Eicosapentaenoic acid (20:5), 8,11-Octadecadienoic acid (18:2) and 13-Tetradecynoic acid (13:1) can be used as a chemotaxonomic marker in order to differentiate the strains at genus or species level rather than major fatty acids which occurred almost in every species with slight variation on the total percentage of fatty acids.

In case of *Scytonema,* both the *Scytonema* sp. showed different fatty acids. In *Scytonema sp.*1f, Pentadecanoic acid, 13-Docsenoic acid, Cyclopropentoic acid, and Undecanoic acid were present whereas in the *Scytonema* sp.12c, Tetracosanoic acid, 11-Octadecanoic acid and Undecanoic acid have been reported. The presence of Gama linolenic acid in *Desmonostoc muscorum 9a* was found to be in agreement with the findings of Gugger *et al*. (2002a).

Polyunsaturated fatty acids are important source of lipids of cyanobacteria (Řezenka *et al*., 2003; Řezenka and Dembitsky, 2006; Tamina *et al*., 2007). Unsaturated and polyunsaturated fatty acids play an important role in maintaining the fluidity and proper functioning of cell and organelle membrane and helps in acclimation to the environmental stresses (Chapman, 1975; Chi *et al*., 2008). The other major function of PUFA is to protect the photosynthetic machinery from photo inhibition under stress condition (Gombos *et al*., 1997). Most of the Polyunsaturated fatty acids *i.e.* linoleic acid, α-linolenic acid (ALA) and γ- linolenic acid (GLA) are important in pharmaceutical industry and γ-linolenic acid is known for promising therapeutic agent for numerous health disorders and serve as an important compound.

It is necessary for reducing inflammation and in treatment of heart disease, Parkinson disease, multiple sclerosis, plasma cholesterol levels, dermatitis, diabetes, and pre-menstrual syndrome (Biagi *et al*., 1991; Wainwright *et al*., 1996; Ghazala *et al*., 2005; Tran *et al*., 2009).

Our results based on the fatty acid composition and ratio of MUFA, SAFA and PUFA were supported by the earlier studies (Caudalance and wells, 1992; Cohen *et al*., 1995; Vargan *et al.,* 1998; Li and Watanebe, 2001; Liu *et al.,* 2005; Sharthchndra and Rajashkar, 2011).

The percentage of Saturated fatty acid (SAFA) was also calculated and maximum was found in *Nostoc* sp. 1c followed by *Tolypothrix* sp. 9k. Similarly, maximum percentage of Monounsaturated fatty acid (MUFA) was reported in *Anabaena doliolum* 1a followed by *Anabaena* sp. 1n. Minimum was found in *Nostoc* commune 9n. Maximum and equal percentage of Polyunsaturated fatty acid (PUFA) was found in *Anabaena* sp. 2b and *Nostoc* sp. 9ma2 whereas minimum was reported in *Nostoc* sp. 4. The results were supported by the high PUFA content of *Anabaena* and *Nostoc* species (Candale *et al.,*1995; Tamina *et al.,* 2007; Li and Watanebe, 2004). The ratio of MUFA+PUFA/ total fatty acid were calculated and found that all the cyanobacterial strains showed the significant changes of fatty acids. It was found that the ratio was ranged between 0.45 to 0.72%. Similarly, the ratio of MUFA/PUFA was ranged between 0.32 to 6.6% and the ratio of SAFA/MUFA ranged between 0.53 to 3.46%.

Our data based on the fatty acid composition and fatty acid ratio proved a strain dependent features and PUFA indicated a valuable biochemical signature of the species. The variation of fatty acids among the tested strains might be due to the differences in demand of lipid content for the formation of membrane for maintaining the membrane fluidity (Wada *et al.,* 1993). This analysis also revealed that the variation may be due to the changes of metabolic and biochemical processes which help in the adaptation with the environment changes and also maintain the membranes fluidity (Los and Murata, 2004; Yang *et al*., 2011; Maulucci *et al*., 2016). The

comparative study of these cyanobacterial strains based on FAME analysis also suggested that it may be considered as the species specific chemotaxonomic marker whether isolated from plain regions or from North Hill regions.

Cluster analysis using Euclidean distance based on FAME profile suggested that *Nostoc* sp. 4 formed a clearly separate cluster which might be due to the minimum percent of Saturated fatty acid as compared to another cyanobacterial species. *Nostoc* sp. 4 of cluster I was distinctly related to the other cyanobacterial species of cluster II.

Finally, it was concluded that all the cyanobacterial species intermixed in the generated dendrogram based on the FAME profiling and other comprehensive parameters and cluster analysis indicated that the fatty acid profiling of cyanobacterial species might be used in the assessment of cyanobacterial diversity at the species level.

Conclusively, it can be suggested that the fatty acid composition and percentage or amount of each fatty acid detected in cyanobacterial species might reflect the evolutionary changes and cluster analysis also indicated that fatty acid profile facilitated the distribution among the different species to species taken for the taxonomical characterization.

The fatty acids of cyanobacteria are either saturated or unsaturated. They can also tolerate environmental stresses such as heat, cold, desiccation, salinity etc. (Sinha, *et. al*., 1996; Agarwal *et al*., 2002; Mahajan, 2005; Pushparaj *et al.,* 2008; Dadheech, 2010; Horváth *et al.,* 2012).

Whole cell protein pattern analysis has been established as an excellent marker for grouping large number of closely related strains of bacteria and cyanobacteria which yielded information only at or below species level (Sánchez *et al*., 2003; Nayak *et al*., 2007; Ezhilarasi and Anand, 2010). The high protein content of various cyanobacterial species is one of the key reason to consider them as an unconventional source of protein (Benson and Hanna, 1983; Lanoot *et al*., 2002; Zahran *et al*., 2003;

Koort, 2006; Kim *et al*., 2010; Singh *et al*., 2010). Several observations were also made in the recent past based on the application of the protein (whole cell protein) as biochemical marker for assessing inter and intra-species diversity of the genera *Anabaena* (Lyra *et al*., 1997; Nayak *et al*., 2007; Ezhilarasi and Anand, 2010). SDS-PAGE is a useful technique for analyzing those protein which are used as taxonomical tool and provide offers the advantages of being fairly fast and easy and of having a good level of taxonomic resolution at species or subspecies level when performed under highly standardized conditions (Devriese *et al*., 1995; Sánchez *et al*., 2003). On the contrary, the use of protein as a reliable marker has been controversial because they represent the expressed part of the genome, which in turn is influenced by the growth and the environmental factors (Sood *et al*., 2007).

In present study, SDS-PAGE was also considered as a taxonomic tool to classify and identify the proteins among isolated species of cyanobacteria. Different cyanobacteria showed different banding pattern in protein profiling and showed variation in the protein bands of high molecular weight while considerable similarity was found among the bands of lower molecular weight. Some unique protein bands were also observed in certain cyanobacterial species. (Figure. 36 and Figure. 37)

A dendogram constructed on the basis of variable protein profile by using Jaccard's similarity coefficient 0.11-1.00, showed clearly separate major cluster I and II (Figure. 38). The cluster I comprise only single species of *Scytonema* sp.1f found in separate and distinct cluster due to the unique protein bands. The grouping of false branching *Scytonema* sp. 12c and *Tolypothrix* 9k with *Anabaena* sp. 1h due to the similar protein band observed in protein profiling.

Similarly, protein profiling was also done among the twenty cyanobacterial species isolated from North Hill regions of Chhattisgarh. The cluster I comprise single species *Nostoc* sp. 16ss and cluster II grouped rest of the cyanobacterial species isolated from north hill of Chhattisgarh. The cluster I comprise only single species *Anabaena sp. 16ss*. The separate cluster was due to the presence of some unique protein bands. The grouping of the false branching *i.e. Scytonema* sp. 18ss and

Scytonema sp. 6ss with the other member of order Nostocales *Anabaena* sp.7ss, *Anabaena sp*. 12ss, *Anabaena* sp. 1ss and *Anabaena* sp. 14ss is due to the similar protein band observed in protein profiling. Apart from these the grouping of *Calothrix* sp. 3ss and *Calothrix* sp.13ss with the member of Nostocales *Anabaena* sp. 2ss, *Anabaena* sp. 20ss and *Nostoc* sp. 11ss, *Nostoc* sp. 17ss was also observed.

Our results also confirmed the heterogeneity present in the genera *Anabaena* and *Nostoc* and the grouping of *Anabaena* species with *Nostoc* sp. might be due to the presence of both common and unique bands generating in the protein profiling. These observed unique bands may be responsible for the interspecies and intergenic diversity of the cyanobacteria. Dissimilarity of protein bands in protein profiling might be due to the differences in the level of expressions. Further, to confirm the position of *Scytonema* sp.1f, *Anabaena* sp. 1h, *Scytonema* sp. 18ss, *Scytonema* sp. 6ss, *Calothrix* sp. 3ss and *Calothrix* sp.13ss some more markers *viz*. chemical and genetically should be taken and assessed.

Cyanobacteria are prokaryotic photoautotroph's of great evolutionary importance with exceptional physiological and biochemical plasticity. The identification of these organisms is traditionally based on morphological features which are useful for broad classification up to phenotypic level but are also open to doubt due to their phenotypic changes under different environmental conditions. However there is a distinct need for diverse data for identification of species level of cyanobacteria and the present study directly help to evaluating the cyanobacterial diversity based on polyphasic approach.

5.7 *Molecular characterization based on 16S rRNA*

Bacterial 16S ribosomal RNA (rRNA) genes contain nine "Hyper variable regions" (V1–V9) that demonstrate considerable sequence diversity among different bacteria. Species-specific sequences within a given hyper variable region constitute useful targets for investigations. In recent time, to proper systematic studies based on the different molecular markers *viz*. 16S rRNA and *nif* gene along with other

molecular markers reflect the systematic position of cyanobacteria and help to arrive at the correct classification (Rajaniemi *et al*., 2005; Mishra *et al*., 2013; Komarek and Johansen, 2014, Singh *et al*., 2017)

In the present investigation the aim was to check the molecular diversity using the heterocytous cyanobacteria belonging to Nostocales members isolated from plains region of Chhattisgarh. For the proper comparative analysis of genetic relatedness we also used a few other genera more distantly related to cyanobacterial strains available from NCBI database (Figure 40).

In the molecular assessment based on 16S rRNA gene, the phylogenetic tree showed that the genus *Anabaena* is scattered in pattern. On the basis of the cluster formation in the Phylogenetic tree, *Scytonema* sp. 1f and *Scytonema singhii* might be termed as cospecific because both shared 94% sequence similarity and a high bootstrap support (94%, 100% respectively). Results suggested that these cyanobacterial species are phylogenetically linked and are of similar genetic makeup but appeared differently due to varied physiological conditions (Castenholz and Norris, 2005).

Cluster II of Clade 1 & 2 includes *Anabaena doliolum1a, Anabaena sphaerica* 1e, *Nostoc* sp. 1d and *Nostoc* sp. 4 but the bootstrap support for this cluster formation is found to be high, which represented the heterogeneity present within the genus. Previously, it was also reported that the work showed the heterogeneity between genus *Anabaena and Nostoc* was also reported earlier (Rajaniemi *et al.,* 2005; Svenning *et al.,* 2005; Halinen *et al*., 2008, Shukla *et al*., 2013). The grouping pattern also confirmed that the distinction between the two genera was not reflective of their evolutionary relationships. Although the observed sequence similarities between the two strains on the basis of 16S rRNA gene sequence were low *i.e.* only 70% thus supporting the low similarity percent observed on the basis of the dendrogram obtained using protein profiling patterns. This further confirmed that these were two different genera and the cluster.

Earlier reports had that confirmed that the most of the Nostocales members showed genetic heterogeneity viz. *Anabaena*, *Aphanizomenon*, *Nostoc*, *Trichormus* and thus suggested the taxonomic revision of these genera (Iteman *et al*., 2002; Hrouzek *et al*., 2005; Rajaniemi *et al*., 2005; Svenning *et al*., 2005; Willame *et al*., 2006; Thomazeau *et al*., 2010; Singh *et al*., 2016)

Scytonema sp. 12c a false branching genera of the order Nostocales and family Scytonemataceae had a sequence similarity of 91% with the sequence of other species of the genera *Scytonema* present in NCBI. On the basis of low sequence similarity it might be concluded that the genera *Scytonema* is heterogeneous. Clustering of *Scytonema* sp. 12c with *Nostoc* sp. 9i along with the different sub cluster which showed the intermixed genetic behavior of these cyanobacterial strains. The reason for this differentiation can be attributed to the difference in the culture conditions or due to the site of isolation for the particular strain. (Komarek *et al.,* 2013)

An important finding was the placement of *Desmonostoc muscorum* 9a with the *Anabaena variabilis* with highest bootstrap value. In cluster V, the clustering of *Desmonostoc muscorum* supported the separation from the genus *Nostoc* thus suggesting the existence of a unique phylogenetic coherent clade. It supported the single monophyletic cluster concept related to *Nostoc* (Hrouzek *et al*., 2005; Rehakova *et al*., 2008; Mateo *et al*., 2011). High sequence similarity (99.9%) was observed between the strains *Anabaena sp.* 2b and *Anabaena cylindrica* from the NCBI database which was also well supported by high bootstrap value (100) (Figure 40). On the other hand, a low bootstrap support and also low sequence similarity has been observed between *Anabaena sp.*1n and the database strain of *Anabaena oscillarioides*. The reason might be due to the differences in the extent of adaptation towards different environmental and culture conditions and appearance different in their physiological behavior (Gugger *et al*., 2002, Kust *et al*., 2015). Further, our results also confirm the polyphyletic origin of the all the tested cyanobacterial species belonging the order Nostocales based on the clustering patterns *i.e.* clusters I II III and IV

Similar investigations of twenty cyanobacteria isolated from North Hills regions showed five clusters strongly supported by bootstrap value in all the constructed phylogenetic tree *viz.* NJ, ML, MP. (Figure 41) It also confirmed the monophyletic origin of Clusters I whereas cluster II, III and IV proved and confirmed the polyphyletic origin of the order Nostocales.

Cluster I was highly supported and it comprised the three different unicellular cyanobacterial species belonging to the order Chroococcales *i.e. Aphanothece* sp. 4ss, *Aphanothece* sp. 5ss and *Aphanothece* sp. 10ss. The present study confirmed the monophyletic origin and they shared the sequences similarity together (93%, 92%). The grouping of these results conclave with the earlier findings (Giovannoni *et al*., 1988; Hoffmann and Castenholz, 2001; Fewer *et al.,* 2002; Litvaitis 2002).

Similar observations were also found in cluster II, *Scytonema* genus having the false branching belonging to the family Scytonematacae. All the tested *Scytonema* sp. *i.e*. *Scytonema* sp. 6ss, *Scytonema* sp. 18ss followed by *Scytonema* sp. 1ss and *Scytonema* sp. 8ss showed the highest sequences similarity between the earlier reports of new strains of *Scytonem*a sp. from Chhattisgarh *viz. Scytonema singhii* and *Scytonema bilaspurensis.* The strains *Scytonema* sp. which had three representatives in the present study was found to be diverging even within itself and separate to other isolates viz., *Scytonema* sp. 1ss, *Scytonema* sp. 18ss *and Scytonema* sp. 6ss. This divergence of three *Scytonema* strains was found to be incoherent with the other studies and it also indicated towards the possible effect of environmental conditions on the genetic diversity of heterocytous cyanobacteria (Komarek *et al*., 2013; Hentschke and Komarek, 2013; Singh *et al*., 2016; Singh *et al*., 2017; Minj *et al.,* 2017).

However, in cluster III the strain *Nostoc* sp. 16ss showed affinities towards relatives *Anabaena* and *Nostoc* thus making the clustering tendency of this genus showed the highest sequences similarities and they comprise in same cluster with high boot strap value in all the constructed Phylogenetic tree (Svenning *et al.,* 2005; Nayak *et al.* 2007; Kust *et al.* 2015; Singh *et al*., 2016).

The Phylogenetic clustering of *Nostoc* sp. 16ss cluster III showed that the Nostoc species exhibited the maximum genetic heterogeneity and the *Anabaena* strains showed conflicting affiliations in both intra-generic and inter-generic perspectives.

On the other hand in the Cluster IV, presences of *Calothrix* sp.3ss, *Calothrix* sp. 13ss and *Calothrix* sp.14ss showed the high sequences similarities and high bootstrap value between their relatives obtained from NCBI database.

Nostoc sp. 9ss is a heterocytous filamentous genera belonging to order Nostocales & showed a high sequence similarity to the sequence of other species of *Aliinostoc morphoplasticum* submitted to the NCBI database and it also formed a separate cluster which was phylogenetically dissimilar from other Nostocales and supported the distinction between the genera *Nostoc* and *Anabaena*, as shown previously in studies of 16S rRNA gene sequences (Wilmotte & Herdman, 2001). Our results also, support the earlier reports and the taxonomic status of *Nostoc* as has been widely discussed earlier (Rajaniemi *et al*., 2005, Singh *et al.,* 2016; Minj *et al*., 2017). Overall conclude that our result also confirm the monophyletic origin of the order Nostocales and the member of family rivulariaceae *viz. Calothrix* sp. 14ss, *Calothrix* sp. 13ss and *Calothrix* sp. 3ss based on the clustering pattern *i.e*. clusters I III and IV whereas false branching cyanobacteria *i.e. Scytonema* sp. 8ss*, Scytonema* sp. 1ss*, Scytonema* sp. 18ss and *Scytonema* sp. 6ss of the family Scytonematacae were found to be polyphyletic origin (cluster II).

These secondary structures were used to assess the phylogenetic relationship among the members of intrageneric cyanobacterial strains. The secondary structure of 16S rRNA proved that there were clear differences even in the different species of the same genus. The structural differences among the secondary structure of 16S rRNA in terms of length of stem, no. of loop, presence and absence of unilateral loop and size of loop (large and small), suggested that there was large variation in loop structure and length of sequence in the stem which also clearly observed among all the forty cyanobacterial strains (Figure. 42-51).

Some of the cyanobacterial strains of plain region *i.e. Anabaena* sp.2b and *Anabaena* sp. 1n showed only slight changes in the secondary structures and rest of the cyanobacterial strains showed a high degree of variation in secondary structure.

Furthermore, based on 16S rRNA secondary structure of twenty different cyanobacterial strains isolated from north hill regions of Chhattisgarh, it was found that all the constructed secondary structures were dissimilar in terms of length of the stem, no. of loops, size of loop large and small etc. The comparison between the intrageneric level showed the slightly variation in their secondary structure. The members of Chroococcales *i.e. Aphanothece* sp. 4ss, *Aphanothece* sp.5ss and *Aphanothece* sp.6ss showed maximum similar in their secondary structure. Similar observation was also found in *Scytonema* sp.1ss, *Scytonema* sp. 6ss and *Scytonema* sp. 18ss. Only short length of stem showed the variation among the secondary structure of 16S rRNA of the above three species of *Scytonema*. Finally, it was concluded that the secondary structures clearly differentiate all the cyanobacterial strains.

Over all on the basis of the sequence similarity of the 16S rRNA gene sequences studies not only, it is not sufficient for the taxonomic validation of the cyanobacterial species belonging to closely related genera and thus use of more than one molecular criteria is used for proper systematic of cyanobacterial taxonomy (Mishra *et al*., 2013; Singh *et al*., 2017).

Molecular markers are strong tools for research on cyanobacterial diversity, identification, and evolution. However, the small subunit ribosomal RNA (16S rRNA) gene, which is the most popular molecular marker in reconstructing Phylogenetic relations among cyanobacteria, is not sufficient for studies at the sub generic level because it is highly conserved among closely related species and strains.

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