Chapter-5

Discussion
Cyanobacteria colonize successfully almost any illuminated environment on Earth, many of which are considered to be hostile for life (Stal, 2007). Typical examples of extreme environments include those that are exceptionally dry, hot, cold, salty, acid or alkaline. Cyanobacteria play a prominent role in many of these extreme environments (Stal, 2007). A solution to the question as to what really represents extreme may be approached not from the environmental conditions per se, but rather from the biodiversity that is present in the environment. Thus, species richness and species abundance may be the critical parameters to decide the extremeness of environment. One would expect low species richness and low structural complexity of the truly “extreme” environments (Seckbach et al., 2007). Biologically, the organisms that inhabit extreme environments are important not only because of the unique species they represent, but also because of their unusual physiological and biochemical properties (Seckbach, 2007). Extremophilic cyanobacteria are potential candidates for many biotechnological applications (Thajuddin and Subramanian, 2005). Hot water springs and cold environments are two contrast extreme habitats where cyanobacteria grow predominantly. Since cyanobacterial diversity of hot water springs and cold desert area of North-Western Himalayas is unexplored and extremophilic cyanobacteria from these areas may be a good source of biotechnologically important extremozymes/proteins and other biomolecules, The present study was undertaken so that naturally occurring cyanobacterial species from these habitats are identified using polyphasic approach and to maintain them in pure culture so that these are turn out to be source material for biotechnological applications in future.

5.1 Identification of cyanobacteria

Initially, Cyanophytes (cyanobacteria), prokaryotic phototrophic organisms, were traditionally studied together with eukaryotic algae as blue-green algae, since their functional position in nature constituted as important primary producers in almost all biotopes of the biosphere (Anagnostidis and Komárek, 1985). The cell structure of cyanophytes reveals both bacterial and plant characters. Bacterial features include absence of morphologically limited nucleus and plastids and the structure of cell wall, and plant features are presence of chlorophyll a, the structure of thylakoids, O₂ evolving photosynthesis and function as primary producers in nature (Anagnostidis and Komárek, 1985). Although prokaryotic in nature, botanists still consider that
taxonomic treatment of cyanobacteria along-with bacteria is not fully justified (Anagnostidis and Komárek, 1985; Komárek and Anagnostidis, 2005).

Identification of cyanobacterial species by microscopy without culturing or by culturing them is practical and widely used. Their structural simplicity provides the taxonomist very useful, although very few, characters those are determinable by microscopy. Although cyanobacteria are comparatively rich in features when compared to other prokaryotes, many morphological features are highly variable, and often dependent on environmental factors or culture conditions (Pearson and Kingsbury, 1966). Simple cyanobacteria such as *Synechococcus* and *Cyanothece* are especially difficult to identify and classify (Castenholz, 1992; Komárek *et al.*, 2004). Komárek and Anagnostidis (1989) estimated that a large number of the cyanobacterial strains in culture collections have been misidentified.

Classification of cyanobacteria is still being resolved by easily applicable morphological and ecological features. New scientific period yields new methods mainly in molecular biology. New data from ultrastructure studies, ecological analyses and particularly from molecular biology have considerably changed the cyanobacterial taxonomy in last few years. Modern combined approach allows greater recognition and more exact definition of the width of cyanobacterial diversity. Unfortunately, in spite of big amount of new molecular data, there is a lot of misinformation and errors. The data are cumulated in on-line databases (NCBI, The European ribosomal RNA database) not providing an easy survey. Misidentifications are common and data about taxonomy and ecology are missing in these database. Even known mistakes are not corrected. In most cases, new information is added beside the wrong one making the whole situation more complicated. Despite this fact, on-line databases offer a huge supply of under used or unused information (but a critical view is needed for using these data).

Confusion in cyanobacterial nomenclature is being caused by an effort to place nomenclature of Cyanobacteria under the rules of International Code of Nomenclature of Bacteria (Stanier *et al.*, 1978). Initially, only botanical code was valid, bacteriological code was validated later and used simultaneously. Taxa validly described under the bacteriological code are summarized in Bergey’s manual of Systematic Bacteriology (Castenholz, 2001b), where 63 form-genera are listed. This means that most of the Cyanobacteria still do not have valid bacteriological name. Up to date, total number of validly described cyanobacterial genera under botanical code
is 265 (Komárek and Hauer, 2013). This number is only a small part of worldwide diversity, especially as many of the very diverse habitats remain unexplored (Palinska and Surosz, 2014). The GenBank database has, complete and partial 16S rRNA gene sequence of only 135 genera to date (July, 2014), from axenic, non-axenic unicellular cyanobacterial cultures, as well as from natural populations.

Molecular data seem to be very important for taxonomy and further phylogenetic investigations, revealing necessity of separation of polyphyletic taxa into a number of narrower monophyletic genera or cryptogenera (Giovannoni et al., 1988). Acquirement of 16S rDNA cyanobacterial sequences is limited by the difficulty of cyanobacteria to grow in culture or absence of their natural occurrence in monotypic colonies, which cause problem for cyanobacterial DNA isolation. These conditions are usually hard to achieve and therefore 16S rRNA gene of few cyanobacteria are sequenced so far. The molecular studies pertaining to uncultured cyanobacterial diversity in various habitats are many, but often it is not clear what is meant under the taxonomic designations assigned as ‘uncultured cyanobacterial clone’ to the studied organisms (Frias-Lopez et al., 2003; Becker et al., 2004; Komárek and Anagnostidis, 2005; Zwart et al., 2005; Taton et al., 2006b; Willame et al., 2006). In most studies only molecular diversity is being discussed, with no reference to morphology (Geiss et al., 2004; Kim et al., 2004; Fourcans et al., 2004; Nagy et al., 2005). In addition, there are bulk of cyanobacterial sequences available in NCBI database with no morphological identity (Wilmotte and Herdman, 2001) or/and of many misidentified cyanobacterial strains under culture collections (Komárek et al., 1994). However, the need for molecular tools to study the genotypic relationships of cyanobacteria, to reconstruct their evolution, and to improve their taxonomy has been recognized (Wilmotte, 1994, Komárek and Anagnostidis, 2005). Despite the undisputed importance of molecular data, morphology continues to play an important role in cyanobacterial characterization (Komárek, 2010, Kauff and Budel, 2011). The combination of both molecular and morphological approaches for modern cyanobacterial taxonomy is therefore essential (Komárek, 2006, 2010a).

During the present study, cyanobacterial diversity of cold desert area (Lahaul-Spiti) and hot water springs of North-Western Himalayas has been studied identifying cyanobacterial species following “polyphasic” approach. Total 625 cyanobacterial isolates from 150 samples were isolated from hot water springs and 460 isolates were obtained from 220 samples collected from cold desert area. Each isolate was observed
under light microscope and identified on the basis of morphological taxonomic features (Table 8 and 9). All the isolates from hot water springs were represented by 22 species of 11 genera while isolates from cold desert area were represented by 38 species of 16 genera. Cyanobacterial diversity of hot water springs and cold desert area was represented by 60 species of 21 genera, 5 genera were present exclusively in hot water springs and 10 genera were present exclusively in cold desert area while 6 genera were common in both hot water spring and cold desert area (Table 10). These genera were belonging to 12 families of 4 orders of class Cyanophyaceae. Genotypic diversity of cyanobacterial isolates of both habitats was investigated using amplified 16S ribosomal DNA restriction analysis (ARDRA), identification of most of cyanobacterial species was confirmed based on their morphological features combined with 16S rRNA gene sequences. These species were further characterized by rbcL gene and cpcBA-IGS region sequence analysis. In the present study, the botanical code of nomenclature has been followed, and therefore, the most appropriate and traditional name with the suffix-phyceae (Cyanophyceae) has been used to represent this group. Cyanobacterial diversity of these regions was analyzed by employing diversity indices. Physico-chemical characteristics of sampling sites were determined and correlated with occurrence of cyanobacteria in these sites so as to understand the relationship between occurrence of cyanobacteria and physico-chemical variables.

5.2 Cyanobacterial diversity
5.2.1 In hot water springs
Thermal environments are created by: solar heating, combustion processes, radioactive decay and geothermal activity, which results in the formation of hot springs and fumaroles that may provide suitable environment for growth of thermophilic organisms (Castenholz, 1969). Hot springs have been documented at temperatures from 45 ºC to boiling point of water. Thermophilic cyanobacteria exist in the range 45-74 ºC. Some other bacteria and archaea can survive up to boiling water temperatures of hot water springs (Castenholz, 1973).

Hot water issuing from the earth’s surface has been a subject of special interest to human being since the dawn of humankind. Ancient civilizations revered thermal springs because these were believed to have supernatural and healing powers (LaMoreaux and Tanner, 2001). Archaeological evidences also show that thermal springs were used as bathing facilities in the ancient city of Mohenjo-Daro, India
before 2000 BC, the royal palaces of Knossos in Crete, Greece built between 1700 BC and 1400 BC, and the Egyptian royal city of Tall al ‘Amarinah that was built about 1350 BC. During the Western Zhou Dynasty, Emperor Xuanzong built a palace at the Huaqing Hot Spring near the city of Xian in China. This is still functioning as a major tourist resort. Asian countries such as Japan have widespread hot spring resorts cater this to Japanese passion for bathing in hot springs (Ohno et al., 2003). The eastern and central provinces of Saudi Arabia are also developing hot spring resorts for recreational purposes (Arif, 1997). Hot spring bathing is also extremely popular throughout the Philippines, Taiwan, and other Asian regions. Although issues on health risks associated with bathing in natural hot spring have been raised, no reports of toxic thermophilic cyanobacteria that may pose harmful effects to humans have been recorded (Arif, 1997). However, ecological studies on hot springs in Yellowstone National Park show that facultative gram-negative bacilli Legionella species, a known causative agent of Legionnaires’ disease, inhabit hot spring waters with temperature ranging from 25-60 °C (Sheehan et al., 2005). Furthermore, few species of free-living vahlkampfiid amoebae, that cause diseases such as amoebic meningoencephalitis and which can also be a potential host of Legionella, have also been isolated from Nymph Creek hot spring in Yellowstone National Park (Sheehan et al., 2003a). The thermotolerant Naegleria fowleri which causes meningoencephalitis, has been recovered from hot springs with temperature and pH ranges of 30-41°C and 2.2-8.3, respectively in Grand Teton National Park (Sheehan et al., 2003b).

The hot water springs of North-Western Himalayas belong to Northwest Himalayan Geothermal sub-province of Himalayan Geothermal Province which is one of the seven major Geothermal Provinces of India (Cinti et al., 2008). The water of these springs has meteoric origin and is of peripheral nature, it is hot due to shallow circulation, due to a gravity-controlled descent cold water towards greater depth through faults and fractures of rocks and a heating linked to an anomalous geothermal gradient causing the rapid movement upwards of deep fluids towards the surface (Cinti et al., 2008). Most of hot water springs studied presently are famous for their recreational value and for tourism. The hot water springs are usually mineralized to greater or lesser extent depending on the characteristics of the geological formations associated with the circulating water (Todd, 1980).
During the present study, maximum water temperature (60-90 °C) was observed at Manikaran whereas minimum (40-45 °C) was at Janki Chatti and Gauri Kund hot water springs. Cyanobacteria grow abundantly in these hot water springs in the form of microbial mats. In the present study, total 625 cyanobacterial isolates were obtained from samples collected from the selected hot water springs, which comprised 105 unicellular (16.8%), 388 non-heterocystous filamentous (62.08%) and 132 heterocystous filamentous branched (21.12%) forms (Table 8 and Fig. 33). Although the temperature of these hot water springs ranged from 45 °C to 90 °C, temperature of water where cyanobacterial mats were present was below 75 °C. Samples collected from water above 75 °C did not contain cyanobacteria. Previous reports have suggested that highly developed cyanobacterial mats are common at temperature below 74°C and neutral/alkaline pH (Sompong et al., 2008; Debnath et al., 2009). Results revealed that cyanobacteria of hot water springs were represented by 22 species belonging to 11 genera of 8 families and 3 Orders of class Cyanophyceae (Table 23). Debnath et al. (2009) reported 18 species of 12 cyanobacterial genera from Bakreswar hot water spring, West Bengal, India. Sompong et al. (2005) recorded 19 genera and 36 species from nine thermal springs (30–80 °C) in northern Thailand. Similarly, Hindak (2001) described 19 cyanobacteria taxa from hot springs on the shore of lake Bogoria, Kenya.

The restriction fragment length polymorphisms (RFLPs) of particular PCR products can provide signature profiles specific to the genus, species, or even the strain (Lyra et al., 1997). Genetic characterization of cyanobacterial isolates from hot water springs has been undertaken using RFLPs of the 16S rRNA gene (16S-ARDRA). The dendrogram generated from the banding pattern of 16S rRNA gene PCR-RFLP of 220 isolates from hot water springs revealed that all the isolates belonged to 22 ARDRA groups (Table 11, Figs. 7-9). Forty cyanobacterial isolates, at least one representative from each ARDRA group (highlighted, Table 11), were randomly selected as the representatives of their respective ARDRA group for 16S rRNA gene sequencing. A BLASTn search of generated partial 16S rRNA gene sequences of most of selected representatives showed >97% similarity, except representatives isolates of 3 ARDRA group (II, VI and XXI, marked with asterisk), with their nearest relatives taxa in the NCBI GenBank database. Representative isolates of ARDRA group XXI showed 95% similarity with Leptolyngbya sp. BN44, while on the basis of morphological characters these isolates were placed near type
species *L. thermalis*. Representative isolates of ARDRA groups, II and VI showed 93% similarity with *Phormidium* sp. KS and *P. tergestinum* CCALA 155, respectively while on the basis of their morphological characters these were placed near type species *P. thermobium* and *P. tergestinum*, respectively (Table 13). Such isolates where 16S rRNA gene sequence showed less than 97% similarity with 16S rRNA gene sequences available in database indicated that these may be new candidate taxa for delimitation of new species/genus after further characterization.

There was a good correlation between phenotypic identification and 16S rRNA gene sequence based identity of cyanobacterial isolates from hot water springs at genus level, while at species level, identity of only 6 ARDRA group (highlighted in Table 13) representatives was confirmed by 16S rRNA gene sequence based identification (Table 13). Morphology based species level identification of other ARDRA groups (III, IV, V, XI, XII, XIII, XIV, XVI, XVII, XVIII, XIX, XX and XXI) representatives did not match with identification based on 16S rRNA gene sequence analysis (marked with double asterisk, Table 13).

Characteristics of cyanobacteria such as morphological features, development (type of reproduction and division pattern), physiology (chromatic adaptations, salinity tolerance, vitamin requirements) show variation with changing environmental and culture conditions (Rippka *et al*., 1979; Dor and Hornhoff, 1985; Castenholz and Waterbury, 1989; Palinska *et al*., 1996; Otsuka *et al*., 2000; Lyra *et al*., 2005; Rajaniemi *et al*., 2005). In addition, cyanobacteria show the phenomenon of “cryptic species,” i.e. organisms seem to belong to a particular species from a morphological point of view but are genetically distinct (Ward *et al*., 1998; Casamatta *et al*., 2005; Taton *et al*., 2006a; Marquardt and Palinska, 2007).

The constrains in between 16S rRNA gene sequence based identity and morphology based identity in the present study were possibly due to the unavailability of gene sequences of strains with identity at species level in GenBank database. On searching GenBank database, whether the 16S rRNA gene sequences of these species, which have been identified at species level on morphological features, is available in the database or not, it was observed that 16S rRNA gene sequence of these cyanobacterial species identified at species level are not present in database. Thus the 16S rRNA gene sequences of these 13 cyanobacterial species from hot water springs with proper morphology based species identity were submitted to GenBank database for the first time during present study. All cyanobacterial species of hot water springs
were assigned taxonomic identity on the basis of morphology and 16S rRNA gene sequence analysis and are being maintained in pure cultures under the code PUPCCC in our culture collection (Table 14).

The 16S rRNA genes are the most widely used markers for the identification of bacteria and cyanobacteria due to their conserved nature and universal presence. Several researchers have exploited the conserved regions of the 16S rRNA gene for phylogenetic analysis of cyanobacteria (Nübel et al., 1997; Crosbie et al., 2003; Salomon et al., 2003; Premanandh et al., 2006). However, the application of 16S rRNA gene to identify organism at the species level and below has been contested (Fox et al., 1992). As a result, researchers have targeted other variable regions such as the rbcL gene (Morden and Golden, 1991; Gugger et al., 2002; Tomitani, 2006; Moro et al., 2010; Singh et al., 2014) and the intergenic spacer region (IGS) of the phycocyanin (PC) locus (Neilan et al., 1995; Scheldeman et al., 1999; Iteman et al., 2000; Ballot et al., 2004; Kim et al., 2006; Premanandh et al., 2006; Six et al., 2007).

Two other Molecular markers i.e rbcL gene and cpcB-cpcA intergenic spacer (PC-IGS) were used to further characterize cyanobacterial species from hot water springs. Plastid-encoded rbcL gene is a very important functional gene present in cyanobacteria. Apart from being a single copy gene, approximately of 1,430 base pairs in length and it is also known to have a fairly conservative rate of evolution (Singh et al., 2014). The form I enzyme, found predominantly in plants, eukaryotic algae, and cyanobacteria, contains large (rbcL) and small (rbcS) subunits that have been shown to assemble into a complex hexadecameric structure, (L2)4(S4)2 (Tomitani, 2006; Singh et al., 2014). During the present study, a BLASTx search of partial rbcL gene sequence for nearest relatives in the NCBI GenBank database revealed that 7 cyanobacterial species (highlighted in Table 17) from hot water springs sustained same identity as inferred from 16S rRNA gene sequence analysis, while from rest 15 species, 7 at species level (marked with #, Table 17) and 8 at genera level (marked with asterisk, Table 17), showed >96% similarity with the amino acid sequence of ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit of varied taxa (Table 17). On searching GenBank database, whether the rbcL gene sequences of these species, which have been identified at species level on the basis of morphological features are available or not, it was observed that rbcL gene sequence of these cyanobacterial species identified at species level are not present in database. So the differences in the rbcL gene sequence based identity and identity
based on the 16S rRNA gene sequences and morphology features was due to the fact that sequences of the \textit{rbcL} gene of these species are not available in GenBank database. The \textit{rbcL} gene sequence of these species was obtained for the first time in the present study and submitted to GenBank (Table 21). Reports on characterization and phylogenetic assessment of cyanobacteria based on \textit{rbcL} gene are very few and do not encompass many of the cyanobacterial genera (Morden and Golden, 1991; Gugger \textit{et al}., 2002; Tomitani \textit{et al}., 2006, Moro \textit{et al}., 2010 Sciuto \textit{et al}., 2011; Singh \textit{et al}., 2014).

The \textit{cpcBA} locus has also been used for the study of cyanobacterial diversity and phylogeny (Neilan \textit{et al}., 1995; Bolch \textit{et al}., 1996; Robertson \textit{et al}., 2001; Kim \textit{et al}., 2006; Ivanikova \textit{et al}., 2007; Six \textit{et al}., 2007; Haverkamp \textit{et al}., 2008). The partial sequence of approximately 700 bp of \textit{cpcB-IGS-cpcA} and flanking coding regions of phycocyanin locus were obtained for 10 cyanobacterial species (Table 19). The \textit{cpcB-IGS-cpcA} region of cyanobacterial isolates belonging to genera \textit{Geitlerinina}, \textit{Leptolyngbya} and \textit{Pseudanabaena} could not be amplified employing all primer sets reported in literatures even after all the modification in PCR conditions suggested by various workers (Neilan \textit{et al}., 1995; Bolch \textit{et al}., 1996; Robertson \textit{et al}., 2001; Kim \textit{et al}., 2006; Ivanikova \textit{et al}., 2007; Six \textit{et al}., 2007; Haverkamp \textit{et al}., 2008). A BLASTn search of PC-IGS sequence revealed that PC-IGS region sequence of these 10 cyanobacterial species showed 84-100% similarity with the PC-IGS region sequences of diverse taxa in GenBank database (Table 19). The identity of only 3 species (\textit{Gl. gelatinosa}. PUPCCC 009.2, \textit{Micro. chthonoplastes} PUPCCC 120.4 and \textit{S. elongatus} PUPCCC 010.5) as revealed by PC-IGS sequences analysis (highlighted, Table 19), matched exactly with identity based on 16S rRNA gene sequence and \textit{rbcL} gene sequences. However, the identity of other 7 species, 2 at species level (marked with #, Table 19) and 5 at genera level (marked with asterisk, Table 19), did not match with identity based on either 16S rRNA or \textit{rbcL} gene. The \textit{cpcB-IGS-cpcA} region sequence of these species was obtained for first time in the present study and these sequences have been submitted in GenBank database (Table 21).

The morphological features of Oscillatorialean genera, of both cultured isolates as well as natural populations, have been shown to be highly variable and dependant on growth and cultivation conditions (Hindak, 1985; Jeeji Bai, 1985; Komárek and Lund, 1990). A major component of the hot water springs cyanobacterial flora worldwide belongs to the Oscillatoriales (Dadheech \textit{et al}., 2013).
This is also true for the present study. Unfortunately, this order is widely considered amongst the taxonomically most problematic, and within the order, *Leptolyngbya* is recognized as a poorly defined genus (Dadheech *et al.*, 2013). Historically, the members of Oscillatoriales including the genera *Oscillatoria, Lyngbya, Phormidium, Schizothrix* and *Plectonema* have been taxonomically characterized by sheath characteristics and the presence of false branching (Gomont, 1892; Geitler, 1932). Some species of these genera with simple morphology and fine trichomes (up to 3 mm wide) were considered problematic taxonomically by Rippka *et al.* (1979) and transferred them into the LPP-group B (*Lyngbya, Phormidium, Plectonema*) despite the fact that species of these genera displayed a high level of variability in important taxonomic features (Anagnostidis and Komárek, 1985, 1988). Anagnostidis and Komárek (1988) subsequently proposed the new genus *Leptolyngbya* with 75 new species based on features including presence of fine sheath, cell shape, trichome immobility, thylakoids arrangement, cell wall constrictions, and trichome fragmentation. Many of these diagnostic features were later shown to be environmentally variable, thus not phylogenetically informative (Albertano and Kovacik, 1994). Further work has demonstrated the stability of some of these features (e.g. the arrangement of thylakoids) for a limited number of species (Komárek and Caslavksa, 1991; Casamatta *et al.*, 2005), however, morphological (Albertano and Kovacik, 1994) and phylogenetic data (Payne, 2001) suggested that the genus *Leptolyngbya* is not a monophyletic taxon. Our results have again demonstrated that the molecular and morphological features used to define species of genera belonging to family Pseudanabenaceae require reconsideration.

In the present study, of the total cyanobacterial species diversity in the hot water springs, 78.88% were represented by non-heterocystous cyanoprokaryotes, whereas heterocystous cyanoprokaryotes comprised only 21.12%. Non-heterocystous taxa belonged to Chroococcales and Oscillatoriales while Stigonematalean taxa represented heterocystous group. In terms of number of species and abundance, Oscillatoriales was the most dominant order as it contributed 16 species (72.74%) followed by order Stigonematales and Chroococcales, being represented by 3 species each [13.63+13.63% (27.26%)] (Fig. 34). Genus *Leptolyngbya* (order- Oscillatoriales) was dominant at species level with 9 species (40.90%) followed by *Phormidium* with 3 species (13.63%) (Fig. 35). Studies from other continents on similar habitats have revealed dominance of oscillatorialean members in microbial mats of hot water
springs, particularly when the pH is >6.0 and the temperature is below 72 °C (Castenholz, 1976; Ward et al., 1998; Sompong et al., 2005; Roeselers et al., 2007).

The samples collected from the Tattapani showed most diverse species assemblages, followed by those of Kheer Ganga, Surya Kund, Vashisht, Manikaran, Kasol, Gauri Kund, Janki Chatti and Kalath hot water springs (Table 25). Tattapani, Kheer Ganga, Surya Kund and Vashisht hot springs, having water temperature in the range of 50-80 °C, had higher cyanobacterial diversity. Temperature is one of the important factor for cyanobacterial diversity in microbial mats (Kullberg, 1968; Brock, 1978; Castenholz, 1978; Miller and Castenholz, 2000; Sompong et al., 2005). Kalath hot spring, which has the lowest temperature (45-50 °C) among all the hot water springs also had less cyanobacterial diversity as compared to the other springs with high temperature. This is due to the fact that this spring has been converted into a permanent and cemented bathing spot with regular cleaning. This results in damage to the natural biodiversity of the spring with the result less cyanobacterial species diversity was observed in this spring. **Leptolyngbya** sp. PUPCCC 112.22 is the most abundant species with 9.92% relative abundance followed by **M. laminosus** PUPCCC 515.6, **F. thermalis** PUPCCC 510.2 and **S. elongatus** PUPCCC 010.5 with relative abundance of 9.44%, 7.36% and 6.88%, respectively (Table 25). The distribution and density of other taxa varied significantly from site to site, may be due to their different biological demands. The unicellular forms like **Synechococcus** grow abundantly in cyanobacterial mats at the thermal gradient from 50 °C to 75 °C. The cyanobacterial mats occurring at 40-50 °C are generally dominated by morphologically defined filamentous cyanobacteria like **Phormidium**, **Oscillatoria**, **Pseudanabaena**, **Calothrix** and **Fischerella** (Ward and Castenholz, 2000; Sompong et al., 2005; Debnath et al., 2009).

Distribution of thermophilic cyanobacteria in hot springs around the world is uneven (Castenholz, 1996). Most cyanobacterial species have distinct distribution pattern among different hot springs, depending on their ability to tolerate high temperatures and physico-chemical conditions. Species of the genus **Synechococcus** have been reported to ubiquitously inhabit hot springs of North America, Asia, Africa and possibly Europe, but are absent from hot springs in Iceland, Alaska and the Azores (Castenholz, 1996). However, further studies have shown that the morphology of **Synechococcus** in New Zealand was different from that in European hot springs (Castenholz, 1969). Moreover, the upper temperature limit for growth of
Synechococcus spp. shifted from 72 °C in North American hot springs to 63 °C in Japanese, New Zealand, Italian and African hot springs. Geographic isolation has also been suggested as one of the most important factors in determining cyanobacterial diversity in hot springs worldwide (Papke et al., 2003).

Diversity indices are the mathematical representation of diversity in a community. Diversity indices provide important information about the rarity and commonness of the particular type in a community (Prasanna and Nayak, 2007). Diversity indices take into account both species richness and the relative abundance of each species to quantify how well species are represented within a community. The Shannon-Wiener and Simpson’s diversity indices are the two most widely used diversity indices for examining overall community characteristics. The Shannon-Wiener index (H) takes both species richness and the relative abundance of each of these species in a community into account to determine the uncertainty that an individual picked at random will be of a given species. Biologically realistic H values range from 0 (only one species present with no uncertainty as to what species each individual will be) to about 4.5 (high uncertainty as species are relatively evenly distributed). Simpson’s diversity index takes into account the number of species present, as well as the abundance of each species (Prasanna and Nayak, 2007; Nongbri and Syiem, 2012). During the present study, the calculated diversity indices revealed that cyanobacterial assemblage was more diverse at Tattapani hot water springs and less diverse at Kalath hot water springs and order Oscillatoriales were more abundant in the selected hot water springs. Debnath et al. (2009) also reported that the members of order Oscillatoriales were dominant in Bakreswer hot water spring, West Bengal, India. Temperature between 40 °C-60 °C supports a range of different cyanobacterial mats comprising filamentous and unicellular taxa and the occurrence of these is determined by temperature plus combined nitrogen and free sulphide levels (Hongmei et al., 2005). Spring water having low concentration of nitrogen supports mats of diazotrophic cyanobacteria, usually Fischerella or Calothrix and/or Pleurocapsa at lower temperatures, whereas those rich in nitrogen support Synechococcus and Phormidium/Oscillatoria mats (Ward and Castenholz 2000). Mats in sulphide rich waters comprise Oscillatoria species that are sulphide tolerant/utilizing (Ward and Castenholz 2000). The diversity indices of the selected hot water springs revealed that the cyanobacterial diversity of these springs was influenced by the physico-chemical characteristics of water.
5.2.2 In cold desert area

The vast regions of the earth remain at temperatures near or below freezing. Extreme cold is a defining feature of High Arctic, Antarctic and high alpine zones, which are separated by large distances and climatic barriers. The biodiversity of these cryoenvironments is mostly microbial, and the existence of a perennially cold terrestrial biosphere has implications for microbial speciation, dispersal, biogeography and gene exchange at a planetary scale (Jungblut et al., 2010). Cyanobacteria are common throughout the terrestrial North and South Polar Regions, where they form benthic mats and films at the bottom of lakes, ponds and streams (Zakhia et al., 2007). These communities often dominate total ecosystem biomass and productivity, and must contend with persistent low temperature, repeated freeze–thaw cycles and highly variable light, nutrient and osmotic regimes (Vincent, 2000). In general, high latitude and high altitude cyanobacteria tend to be cold-tolerant (psychrotrophs), with suboptimal growth under low temperatures, rather than psychrophiles that grow optimally at low temperature (Tang and Vincent, 1999). Earlier work on psychrophilic cyanobacteria, employing both morphological and molecular methods, has been mostly carried out in the Antarctic and Arctic regions, where cosmopolitan and endemic taxa have been reported (Komárek, 1999; Taton et al., 2003, 2006a, b; Jungblut et al., 2005; Comte et al., 2007).

High-elevation ecosystems, above the highest reaches of vegetation, are among the most extreme terrestrial environments on Earth (Swan, 1992; Costello et al., 2009; Schmidt et al., 2011). In fact, the high Himalayas have been called as ‘third pole’ because of the extreme physical environment coupled with the logistical difficulties involved in exploring these regions (Dyhrenfurth, 1955). The highest soil ecosystems on Earth are characterized by low oxygen pressure, low levels of available water, high levels of radiation and extreme temperature cycling across the freezing point (Swan, 1992; Schmidt et al., 2009, 2011). During the present study, cyanobacterial diversity of soil/flowing water, glaciers and high altitude lakes of cold desert area (Lahaul-Spiti) of North-Western Himalayas has been studied for first the time. Climatic conditions of this area are typical of dry temperate and alpine zones type. Altitudinal range in this region is between 2,400 m-6,400 m, rainfall is scanty, and the area remains covered with snow for more than 6 months in a year. Temperature during winter falls to nearly -30 °C and even the temperature of summer
nights is sub zero. Except for periods of rain or snowfall, the air is dry and strong winds blow most of the year. In the present study, total 220 samples were collected from three different habitats (150 samples from soil/mats in flowing water, 16 samples from Glaciers and 54 samples from four Lakes) of Cold desert area. After enrichment in culture medium, total 460 cyanobacterial isolates (355 isolates from Soil and Mats in flowing water, 15 isolates from Glaciers and 90 isolates from four high altitude Lakes) were obtained which comprised 57 unicellular (12.40%), 121 heterocystous filamentous (26.30%) and 282 non-heterocystous filamentous (61.30%) forms (Table 29).

Cyanobacterial diversity of soil/mats in flowing water of this area was represented by 37 species of 15 genera belonging to 7 families of 3 orders class Cyanophaceae (Table 30). In the High Arctic (Svalvard, 78°N), 18 different cyanobacterial species belonging to seven genera including *Leptolyngbya* and *Phormidium* were enumerated from barren soils, (Kastovska et al., 2005). Fifteen cyanobacterial isolates represented by 7 species of three non heterocystous filamentous genera were obtained from 16 samples collected from two glaciers of this area, (Table 35). The above species were also reported from samples collected from the soil/mats in flowing water of Lahaul-Spiti area of North-Western Himalayas during the present study. Cyanobacterial diversity of the lakes of this area was represented by 20 species belonging to 11 genera of 5 families and 3 Orders of the class Cyanophyaceae (Table 30). All the species reported from the lakes of this area were also common in soil/mats flowing in water and glaciers of this area, but *Gloeocapsopsis pleurocapsoides* PUPCCC 008.2 which was reported only from the Chandra Tal, high altitude lake of this area (Table 30). Morpho-species belonging to Oscillatoriales were the most abundant taxa at all the sites, followed by those of Chroococcales and Nostocales. Thus the microbial mat communities were made up of morpho-species within orders Oscillatoriales, Nostocales and Chroococcales, and were similar to member of Antarctic microbial mats (Howard-Williams et al., 1989; Taton et al., 2003, 2006a, b; Jungblut et al., 2005). Filamentous, mucilage-producing Oscillatoriales are responsible for much of the cyanobacterial biomass of these polar mat consortia. They have been shown to tolerate a wide range of conditions and to maintain slow net growth despite the frigid ambient temperatures (Tang et al., 1997). Schmidt et al. (2011) reported that there are remarkable similarities between microbial life of arid soils of Antarctica and the high Himalayas. In particular,
morpho-species belonging to *Leptolyngbya*, *Pseudanabaena*, *Phormidium*, *Oscillatoria* and *Nostoc* are characteristic of polar mats and form their overall structure (Vincent, 2000). The morphological diversity of Chroococcales from Lahaul-Spiti area was similar to freshwater ponds in the Larsemann and Vestfold Hills region; Antarctica. However cyanobacterial communities at McMurdo Ice Shelf and in the McMurdo Dry Valleys, lack any Chroococcalean morphotypes (Taton *et al*., 2003; Jungblut *et al*., 2005). Similar to this observations, species belonging Chroococcales was not reported from samples collected from glaciers of Lahaul-Spiti area of North-Western Himalayas.

Molecular characterization of cyanobacterial isolates from cold desert area was done using RFLPs of the 16S rRNA gene (16S-ARDRA). Restriction analysis of 16S rRNA gene of 226 cyanobacterial isolates from cold desert area revealed 38 ARDRA groups (Table 12, Figs. 12-15). The 16S rRNA gene PCR-RFLP results of isolates from cold desert area were consistent with the species identity based on their morphological features. A comparison of 16S rRNA gene sequences of these ARDRA group representative through BLASTn with 16S rRNA gene sequences present in GenBank database revealed that most of representative showed >97% similarity, while representative 6 ARDRA groups (marked with asterisk) showed <97% similarity with diverse taxa (Table 15). The identity of representatives of 14 ARDRA groups (highlighted, Table 15) was same as inferred from their morphological features. For other 18 ARDRA groups there was good correlation between 16S rRNA gene sequence based identity at genus level with identity based on morphological features, but at species level identity of these species did not match with morphology based identity at species level (Table 15). The mismatch between 16S rRNA gene sequence based identity of representatives of these 18 ARDRA groups and morphology based identity was mainly due to the unavailability of the GenBank database entries of these taxa with species names. Therefore, during the present study, the 16S rRNA gene sequences of these taxa with proper morphology based species identity were submitted to GenBank database for the first time (Table 22). The 16S rRNA gene sequence of representative isolates of six ARDRA groups (II, III, XV, XXVIII, XXXIV and XXXV) showed <97% similarity with *Planktothrix* sp. PCC 9214 (95%), *Plank. mougeotii* HAB626 (90%), *L. boryana* UTEX 'B 488' (96%), *Nostoc* sp. CENA88 (96%), *Nostoc* sp. 'Mollenhauer 1:1-067' (96%), *Leptolyngbya* sp. BN44 (96%), while on the basis of their morphological features
these placed near type species *Plank. agardhii*, *Plank. prolifica*, *L. boryana*, *N. paludosum*, *N. piscinale* and *L. tenuis*, respectively (Table 15). Such isolates where 16S rRNA gene sequences showed low similarity (<97%) with 16S rRNA gene sequences available in database indicated that these are new candidate taxa for delimitation of new species/genus after further characterization. All cyanobacterial species from cold desert area were assigned taxonomic status on the basis of morphology and 16S rRNA gene sequence analysis, and are being maintained as pure cultures under the code PUPCCC in our culture collection (Table 16).

The 16S rRNA gene sequence analysis is the most widely applied strategy for assessing cyanobacterial diversity in nature (Palinska and Surosz, 2014). For phylogenetic studies, sequence data from the 16S rRNA gene sequences are commonly used due to their efficacy in indistinguishing higher-level taxonomic groups, as well as traditional species (Nübel et al., 1997; Crosbie et al., 2003; Salomon et al., 2003). However, it is argued that there are some pitfalls in the use of 16S rRNA gene sequences for studying microbial biodiversity (Fox et al., 1992; Premanandh et al., 2006). The application of 16S rRNA to identify an organism at the species level and below has been contested (Fox et al., 1992). Moreover, the conserved nature of 16S rRNA and the lower evolutionary rate in variation compared to the protein-encoding genes makes it less useful for phylogenetic studies of closely related organisms. Additionally, the 16S rRNA sequences, in spite of having hyper variable and extremely informative regions for close relationship, is often not divergent enough to give good separation in close relation, e.g. species of the same genus (Normand et al., 1996).

Two other molecular markers, *rbcL* gene and *cpcB-cpcA* intergenic spacer (*PC-IGS*), were used to further characterize these cyanobacterial species. A BLASTx search of partial *rbcL* gene sequences of 38 cyanobacterial species from cold desert area for nearest relatives in the NCBI GenBank database revealed that sequences of these cyanobacterial species showed 94-100% similarity with the amino acid sequence of Rubisco large subunit of their nearest relatives in GenBank database (Table 18). The *rbcL* gene sequence analysis further revealed that 6 cyanobacterial species (highlighted, Table 18) sustained same identity as inferred from 16S rRNA gene sequence analysis and morphological features, while other 32 species, 24 at species level (marked with #, Table 18) and 8 at genera level (marked with asterisk, Table 18) showed similarity with the amino acid sequence of Rubisco large subunit of
different but closely related taxa. On searching GenBank database, to see whether the rbcL gene sequences of these species are available or not, which have been identified at species level on the basis of morphological features, it was observed that rbcL gene sequences of these cyanobacterial species, identified at species level, are not present in database. Thus, the differences in the rbcL gene sequence based identity and identity based on the 16S rRNA gene sequence and morphology was due to the fact that rbcL gene sequences of cyanobacterial species identified at species level had not been submitted yet by any worker. Thus rbcL gene sequence of these species was obtained for the first time in the present study and submitted to GenBank (Table22). Reports on the phylogenetic assessment of cyanobacteria based on the rbcL gene are very few and do not encompass many of the cyanobacterial genera (Morden and Golden, 1991; Gugger et al., 2002; Tomitani et al., 2006, Moro et al., 2010 Sciuto et al., 2011; Singh et al., 2014).

Genetic diversity of cyanobacteria has been further characterized by determining the DNA polymorphism within the phycocyanin (PC) locus (Neillan et al., 1995). The distribution of PC in cyanobacteria makes the study of PC gene sequence heterogeneity ideal for the classification of cyanobacteria. The PC operon contains genes coding for 2 bilin subunits and 3 linker polypeptides (Whitton, 1992). The intergenic spacer (IGS) between the 2 bilin subunit genes, designated (cpcB) and (cpcA) of the PC operon is considered as a potentially highly variable region of DNA sequence useful for the identification of cyanobacteria to the strain level (Ishida et al., 1997; Nübel et al., 1997; Tillett et al., 2001). The cpcBA locus has been widely used for the study of cyanobacterial diversity and phylogeny (Ivanikova et al., 2007; Six et al., 2007; Haverkamp et al., 2008). With the help of this approach diversity among Anabaena, Aphanizomenon, Cylindrospermopsis, Microcystis, Nostoc, Nodularia and Oscillatoria has been assessed (Neillan et al., 2002). The cpcB-IGS-cpcA and flanking coding region of phycocyanin locus of 24 cyanobacterial species was amplified during the present study and partial sequence of approximately 700 bp were obtained. BLASTn result of obtained sequences revealed that PC-IGS region sequence of cyanobacterial species from cold desert area showed similarity in the range of 84-100% with PC-IGS region sequence of diverse taxa in NCBI GenBank database (Table 20). Only 6 species (P. autumnale PUPCCC 118.4, Syn. pevalekii PUPCCC 062.1, Nod. sphaerocarpa PUPCCC 420.1, Nod. spumigena PUPCCC 420.5, Tolypothrix sp. PUPCCC 415.2 and A. variabilis PUPCCC 410.2) exhibited same
identity as inferred from 16S rRNA gene sequence analysis (highlighted, Table 20), while other 18 species, 16 at species level (marked with #, Table 20) and 2 at genera level (marked with asterisk, Table 20) did not match with identity based on either 16S rRNA or rbcL gene. The cpcB-IGS-cpcA region sequence of these species was obtained for first time in the present study and these sequences have been submitted in GenBank database (Table 22). The cpcB-IGS-cpcA region of cyanobacterial isolates belonging to genera Geitlerinema, Leptolyngbya and Pseudanabaena could not be amplified employing all primers sets reported in literatures, even after all the modification in PCR conditions suggested by various workers (Neilan et al., 1995, 2002; Bolch et al., 1996; Robertson et al., 2001; Kim et al., 2006; Ivanikova et al., 2007; Six et al., 2007; Haverkamp et al., 2008).

During the present study, although cyanobacterial species reported from soil/mats in flowing water, glaciers and high altitudes were common, but their distribution as well as abundance varied with sampling site. In soil/mats in flowing water of cold desert area non-heterocystous filamentous forms were dominant (69.02%) followed by heterocystous filamentous forms (30.98%). Non-heterocystous taxa belonged to Oscillatoriales and Chroococcales while Nostocalean taxa represented heterocystous group. Cyanobacterial species belonging to three orders Chroococcales, Oscillatoriales and Nostocales have been frequently reported from Antarctic soils (Cavacini, 2001; Wood et al., 2008). In terms of number of species and abundance, Oscillatoriales was the most dominant order as it contributed 22 species (59.45%) followed by order Nostocales (32.43%) and Chroococcales (8.1%), being represented by 12 and 3 species, respectively (Fig. 37). Humidity has been postulated as the main factor limiting the development of these edaphic cyanobacteria, but the chemical composition of soil may also play an important role, controlling the rate of supply of limiting elements and of toxic elements (Wood et al., 2008). The occurrence of cyanobacterial taxa seems to be related to the extent and duration of liquid water conditions in the cold climate conditions (Vincent, 1988). In mineral soils periodically flushed with water, Nostocales tend to dominate, while in moist to wet soils Oscillatoriales are the most frequent. Chroococcales are associated with other taxa in moist but unflushed sites (Quesada and Vincent, 2012). Proximity to the inoculum source is another factor affecting the development of edaphic communities (Wood et al., 2008). Genus Leptolyngbya (order- Oscillatoriales) was dominant at species level with 10 species (26.31%) followed by Nostoc with 8 species (21.05%) (Fig. 38). L.
benthonica PUPCCC 112.5 was most abundant species with 6.19% relative abundance followed by Syn. pevalekii PUPCCC 062.1 (5.63%), Micro. acremanii PUPCCC 120.2 (5.07%), as these species were isolated from the maximum number of samples and have maximum distribution (Table 32). Floristically, in most of the investigated soils, Leptolyngbya and Phormidium belonging to Oscillatoriales are the most common genera (Cavacini, 2001; Mataloni et al., 2000). This distribution is related to the desiccation tolerance of each taxon, which is related to amount of EPS produced and its characteristics (Wynn-Williams, 2000). Yergeau et al. (2007) demonstrated in an Antarctic latitudinal gradient, extending from the sub-antarctic Falkland Islands (51°S) to the Ellsworth Mountain Range (79°S), that both diversity and species richness of microbial communities decreased as a function of increasing latitude. However, the proportion of cyanobacterial operational taxonomic units was higher at higher latitudes (Yergeau et al., 2007). The distribution and density of other taxa varied significantly from site to site. Some of the species were found to be site specific e.g. L. antarctica PUPCCC 112.2 was recorded only from Koksar, whereas Leptolyngbya sp. PUPCCC 112.9 only from Keylong and Kunzam, L. cebennensis PUPCCC 112.4 only from Gramphoo and Koksar, Leptolyngbya sp. PUPCCC 112.7 from Gramphoo and Losar, P. autumnale PUPCCC 118.4 from Gramphoo and Kaza, Nod. epilithica PUPCCC 111.2 from Trilokinath and Keylong, and Nostoc sp. PUPCCC 405.8 from Kaza and Trilokinath. Gondla and Trilokinath exhibited almost the same pattern of taxa formation having 16, and 17 taxa, respectively (Table 32).

Cyanobacteria make most of the biovolume of phototrophs in Himalayan soils, and their prevalence is regulated by a combination of factors, particularly high soil pH, undeveloped and unstable soil substrate, and high UV radiation (Řeháková et al., 2011). Numerous studies on cyanobacteria in freshwater and soil indicate that their diversity and abundance are greatest at higher pH values, though the reasons for their success under these conditions are still unclear (Whitton and Pott, 2000). Other factor seems to be CO$_2$-concentrating mechanisms; since cyanobacteria, unlike microalgae, are able to effectively use HCO$_3^-$ as a source of carbon dioxide (Gordiano et al., 2005). Another factor influencing the cyanobacterial species composition is high UV radiation in the Himalayas. Many cyanobacteria tolerate high levels of UV radiation and produce a wide range of UV protectants (scytonemin, carotenoids, or mycosporine-like amino acids) (Seckbach et al., 2007). The ability of cyanobacteria to grow on an unstable substrate of successionaly young or undeveloped soils has
been reported from de-glaciated soils and sub-glacial sediments at Svalbard, from soils without vegetation in Antarctic, and from arid and semi-arid regions of the western USA (Flechtner et al., 1998; Kaštovská et al., 2005, 2007; Nemergut et al., 2007). Nostocales are usually thought to be able to colonize young undeveloped soils because of their ability to fix nitrogen, which may be the limiting nutrient in this type of soil (Whitton and Pott, 2000; Řeháková et al., 2011). Oscillatoriales prevailed in alpine meadows (which had relatively high organic matter and fine soil texture), while Nostocales dominated in the sub-nival zone and scree. Eukaryotic microalgae together with cyanobacteria of the order Chroococcales were mostly present in the sub-nival zone in Dry Mountains of Ladakh, NW Himalaya (Řeháková et al., 2011).

During the present study, 15 cyanobacterial isolates were obtained from 16 samples collected from fore-fields of two glaciers of Lahaul-Spiti. These were represented by 7 species of three non heterocystous filamentous genera (L. antarctica PUPCCC 112.2, L. foveolarum PUPCCC 112.8, L. frigida PUPCCC 112.1, L. lurida PUPCCC 112.6, Leptolyngbya sp. PUPCCC 112.11, P. autumnale PUPCCC 118.4 and Pseudanabaena sp. PUPCCC 106.7) (Table 35). Cyanobacteria make up a significant proportion of the microbial communities in barren arctic environments and on arctic glacier surfaces (Kastovska et al., 2005; Stibal et al., 2006; Řeháková et al., 2011). All cyanobacterial species reported from the two glaciers during the present study were related to cyanobacterial species reported from river, lakes or rocks in the Arctic, Antarctic and Himalayas (Taton et al., 2003; Strunecky et al., 2010; Wong et al., 2010; Jungblut et al., 2010; Khan et al., 2011; Schmidt et al., 2011; Singh et al., 2014). The presence of these uncultured psychrophilic and/or psychrotolerant cyanobacteria in the extreme environments has been identified only by culture-independent approaches such as 16S rRNA gene clone library screening or automated ribosomal ITS analysis fingerprinting (Wood et al., 2008; Xiang et al., 2009; Jungblut et al., 2010; Schmidt et al., 2011; Zeglin et al., 2011; Kleinteich et al., 2012). But during the present study, all these species have been successfully cultured. Previous studies on other glaciers, cold dry valleys in the Himalayas and lithic environments in the Antarctic reported that the psychrophilic cyanobacteria belong to the orders Oscillatoriales, Chroococcales and Nostocales (Wood et al., 2008; Xiang et al., 2009; Segawa and Takeuchi, 2010; Khan et al., 2011; Schmidt et al., 2011; Řeháková et al., 2011). This is also confined during the present study.
High altitude lakes characterized by low temperature, generally low buffering capacity and low level of nutrients act as reference systems for global climate change (Psenner, 2002; Catalan et al., 2006). High altitude lakes have received little attention so far in terms of their limnology, biodiversity, conservation and water management, but they are becoming increasingly important due to the possible consequences of the global climate change (Bhat et al., 2011). Information on biological diversity of any aquatic ecosystem may provide a more sensitive time-integrated assessment of a water body than physical or chemical variables. Of the total cyanobacterial diversity in four high altitude lakes of this area, 80% diversity was represented by non-heterocystous cyanoprokaryotes (Chroococcales and Oscillatoriales), whereas 20% diversity comprised heterocystous cyanoprokaryotes (Nostocales). Members of Oscillatoriales were dominant, followed by members of Nostocales. It is reported that Oscillatoriales and Nostocales are commonly present in polar benthic habitats (Vincent and James, 1996; Broady and Weinstein, 1998; Singh and Elster, 2007). During the present study it was observed that taxa belonging to Oscillatoriales were the most abundant in all the four lakes, followed by member of Nostocales and Chroococcales. Genus *Leptolyngbya* with 6 species was dominant at species level (30%) followed by *Nostoc* with 3 species (15%), and *Phormidium* and *Planktothrix* with 2 species each (10%) (Fig. 41). In particular, morpho-species of *Leptolyngbya*, *Pseudanabaena*, *Phormidium*, Oscillatoria and Nostoc are characteristic of polar mats (Vincent, 2000). The diversity of Chroococcales observed during the present study was similar to freshwater ponds in the Larsemann and Vestfold Hills region, Antarctica (Wharton et al., 1983; Taton et al., 2003; Jungblut et al., 2005).

Diversity indices provide important information about the rarity and commonness of the particular biotype in a community. Sissu Lake, which is comparatively rich in nutrients with average temperature higher than other three lakes, exhibited high values of Shannon’s index, Simpson’s index and species richness index. The calculated ecological indices of these lakes revealed that the cyanobacterial diversity of these lakes was influenced by the physico-chemical characteristics of water. In shallow, clear water polar and high altitude lakes, benthic phototrophs are major phototrophs (Ellis-Evans, 1996; Tang et al., 1997). In cold water ecosystems, mat-forming cyanobacteria of orders Chroococcales, Oscillatoriales and Nostocales are the conspicuous members (Howard-Williams et al., 1989; Taton et al., 2003, 2006a, b; Jungblut et al., 2005).
5.3 Phylogenetic analysis

The 16S rRNA sequence data have increasingly been used in cyanobacterial systematics and are essential for determining its phylogeny (Casamatta et al., 2005; Komárek, 2010; Loza et al., 2013). A cut-off point of 97.5% and 95% 16S rRNA gene sequence similarity have been suggested for prokaryotic species and genus definition, respectively (Stackebrandt and Goebel, 1994; Tindall et al., 2010). Subsequent researches have led to the threshold for defining a species being raised to a range above 98.7%–99.0% (Stackebrandt and Ebers, 2006). Molecular phylogenetic analyses have shown that cyanobacteria form a monophyletic cluster among eubacteria (Woese, 1987; Garrity and Holt, 2001). The cyanobacterial cluster also contains the plastids of eukaryotes (Giovannoni et al., 1988; Wilmotte and Golubic, 1991; Turner, 1997). Based on the phylogenetic analysis of the 16S rRNA gene sequences, it has been concluded that the diversification of cyanobacteria occurred within a short time (Giovannoni et al. 1988; Wilmotte and Herdman, 2001). Another important evolutionary event is the recognition of polyphyletic nature of the Prochlorophyta (Urbach et al., 1992) and its clustering with cyanobacteria (Wilmotte, 1994; Palenik and Swift, 1996). This suggests that the Prochlorophyta and cyanobacteria shared a common ancestor and the recognition of Prochlorophyta as a separate group (Palenik and Swift, 1996). Phylogenetic analysis of Cyanobacteria based on 16S rDNA indicated that Chroococcales (Section I) and Oscillatoriales (Section III) are polyphyletic (Nelissen et al., 1996; Ishida et al., 1997; Honda et al., 1999; Turner et al., 1999; Garcia-Pichel et al., 2001). In 2001, the monophyly of Pleurocapsales (Section II) was denied (Ishida et al., 2001). This is not consistent with their traditional classification. Heterocysts forming Nostocales (Section IV) and Stigonematales (Section V) were found to be monophyletic (Giovannoni et al., 1988; Wilmotte, 1994; Nelissen et al., 1996; Turner 1997; Turner et al., 1999; Wilmotte and Herdman 2001; Lyra et al., 2001; Gugger and Hoffmann 2004). Nowadays, skepticism to resolve phylogenetic branching of big clusters by sequencing of 16S rDNA gene has appeared (Casamatta et al., 2005). A number of workers have put forward proposals for the recognition of phylogenetic lineages or clusters that are not consistent with the classification under ICBN or with classification of Ripkka et al. (1979) or the Bergey’s Manual. Wilmotte and Herdman (2001) recognized 14 phylogenetic clusters among cyanobacteria, mainly based on 16S rRNA gene
sequencing. This formed a part of Bergey’s Manual. Other workers recognized seven (Honda et al., 1999), ten (Turner et al., 1999) and five (Seo and Yokota, 2003; Tomitani et al., 2006) phylogenetic clusters among cyanobacteria. Casamatta et al. (2005) suggested that it is too early to make conclusions about how many cyanobacterial lineages are there. Schirrmeister et al. (2011a) constructed a phylogenetic tree based on 16S rRNA gene sequences of 1254 taxa and suggested that cyanobacteria may share a common ancestor, which was unicellular. Phylogenetic tree further indicated that Gloeobacter violaceus, which has light harvesting system at the outer membrane of the cell rather than internal thylakoids, is the nearest living organism to that ancestor.

5.3.1 Phylogeny of cyanobacteria from hot water springs

Phylogeny of uncultured cyanobacteria from hot water springs around the world have been intensively studied using molecular methods such as DGGE and cloning (Papke et al., 2003; Lau et al., 2005; Ward et al., 2006; McGregor and Rasmussen, 2008; Sompong et al., 2008; Oren et al., 2009; Ionescu et al., 2010; Dadheech et. al, 2013). During the present study, cyanobacterial diversity of the hot springs of the North-Western Himalayas was studied using culture based approach. The phylogenetic relationship among cyanobacterial species isolated from the hot water springs of North-Western Himalayas was analyzed on the basis of 16S rRNA gene, rbcL gene and cpcB-IGS-cpcA phycocyanin locus sequences. The 16S rRNA gene sequences of all cyanobacterial species from hot water springs were blasted against the sequences in NCBI GenBank database, and those sequences found to share a high level of similarity with generated sequences were used to resolve alignment ambiguities and to establish relationships for the sequences obtained in this study. During BLASTn search, the sequences of uncultured organisms were not taken into account.

The phylogenetic tree, generated for sequences obtained during present study and the related sequences from the NCBI database is shown in Fig. 16. The 16S rRNA sequence of Gloeobacter violaceus was used as the out-group to root the tree. The phylogenetic tree revealed 8 distinct clusters (I-VIII), which were defined as monophyletic groups with bootstrap values equal or higher than 70%. In general, the phylogenetic distribution is congruent with the classification results based on the morphology. Moreover, a species or genus attributed to the isolates in the morphological analysis was in agreement with the species/genus of the reference
strains they clustered with. Several authors have hypothesized that geographical isolation of hot springs leads to occurrence of indigenous thermophilic cyanobacteria (Castenholz, 1996; Papke et al., 2003; Miller et al., 2007; Finsinger et al., 2008; Dadheech et. al., 2013). Phylogenetic analysis of cyanobacteria from hot water springs during the present study revealed that most of cyanobacterial communities of North-Western Himalayan hot springs were phylogenetically distinct from those of the thermal/hot springs of other continents. This is most pronounced in Synechococcus sp. where C1, C9 and A/B lineages have been described (Papke et al., 2003). The C9 lineage has been recorded in most sampling sites, with the exception of cyanobacteria from northern Italy, while the A/B lineage have been recorded in North America (Papke et al., 2003; Ionescu et al., 2010; Dadheech et. al., 2013). Phylogenetic relationship among strains of Synechococcus obtained through 16S rRNA gene sequence was congruent with relationship on the basis of rbcL gene and cpcB-IGS-cpcA phycocyanin locus sequences (Fig. 24 and 29). It is noteworthy that S. elongatus PUPCCC 010.5 isolated from NW Himalayan hot water springs did not cluster with any of the other lineages described earlier.

In the present study, the cluster I of phylogenetic tree belonged to genus Phormidium and comprised three cyanobacterial species identified as P. ambiguuum PUPCCC 118.1, Phormidium sp. PUPCCC 118.2 and Phormidium sp. PUPCCC 118.3. In sub-cluster (Ia) species identified as P. ambiguuum PUPCCC 118.1 grouped with their globally distributed mesophilic counterpart such as P. ambiguuum IAM M-71, Oscillatoria sp. MMG-2, P. irriguum CCALA 759 and Phormidium, sp. DVL1003c (Ishida et al., 1997; Sciuto et al., 2012; Shishido et al., 2013). While in sub-cluster (Ib) Phormidium sp. PUPCCC 118.2 and Phormidium sp. PUPCCC 118.3, which showed <97% similarity with the closest relatives in the NCBI database, were grouped together, separately from their nearest relative strains Phormidium sp. KS, P. tergestinum CCALA 155 and P. autumnale UTEX 1580. Phylogenetic relationship among these two strains of Phormidium obtained through 16S rRNA gene sequence was congruent with relationship obtained on the basis of rbcL gene and cpcB-IGS-cpcA phycocyanin locus sequences (Fig. 24 cluster I; Fig. 29 cluster III). So these species represent the candidate taxa to define new species of genus Phormidium after further characterization. In cluster II one species identified as Micro. chthonoplastes PUPCCC 120.4 showed close relationship with mesophilic strain Microcoleus sp. PCC8701 (Fig. 16). Our observations therefore indicate that there may be
considerable degree of endemism/speciation of most of the cyanobacterial species inhabiting the hot springs of North-Western Himalayas.

On the other hand, some of the cyanobacterial species from hot water springs showed phylogenetic relationship with thermophilic species found in other biogeographical regions. This indicates the presence of cosmopolitan species in the hot water springs of North-Western Himalayas. There are a few examples of thermophilic cyanobacteria that have global distribution patterns one of the best known and most thoroughly characterized genus is *M. laminosus* Cohn. This ‘weedy’ species has been identified in almost all hot spring sites below an upper temperature limit of about 58 °C (Castenholz, 1996) and appears to have superior dispersive ability conferred by the production of akinetes which tolerate drying and freezing (McGregor and Rasmussen, 2007). In the present study, in cluster III *G. gelatinosa* PUPCCC 009.2 clustered with thermophilic counterpart *Gloeocapsa* sp. PCC 7428. *Gloe. thermalis* PUPCCC 008.4 grouped with *Gloeocapsopsis* sp. LEGE 06123 and *Gloeocapsopsis* sp. AAB1 (Ramos et al., 2010). In cluster IV taxa belonging to the heterocystous filamentous branched Stigonematalian genera were grouped with their globally distributed counterparts. In Cluster IV species identified as *Chlorogloeopsis fritschii* PUPCCC 505.4 grouped with reference strains *Chl. fritschii* PCC 6718 and *Chl. fritschii* PCC 6918, while in Cluster V species identified as *M. laminosus* PUPCCC 515.6 grouped with reference strains *M. laminosus* CCAP 1447/3 and *M. laminosus*. *F. thermalis* PUPCCC 510.2 grouped with reference strains *F. major* NIES-592, *Fischerella* sp. MV9, *Fischerella* sp. RV14 and *Fischerella* sp. MV11 (Fig. 17). *Fischerella*-like cyanobacteria are a frequent and major constituent of natural populations at thermal sites (Jing et al., 2005; Miller et al., 2006). In cluster VI species belonging to two genera *Leptolyngbya* (nine isolates) and *Geitlerinema* (one isolate) grouped together with species of thermophilic cyanobacteria from hot water springs worldwide and showed close phylogenetic relationship among themselves. Hot water springs of North-Western Himalayas are dominated by *Leptolyngbya* as evidenced by microscopic studies and molecular characterization (Fig. 35), although this taxon is currently described as polyphyletic and there is need of revision (Albertano and Kovacik, 1994; Nelissen et al., 1994; Turner, 1997; Ishida et al., 2001; Casamatta et al., 2005; Komárek and Anagnostidis, 2005; Taton et al., 2006; Johansen et al., 2008; Bohunicka et al., 2011; Dadheech et. al., 2013). In cluster VII *Pseud. limnetica* PUPCCC 106.2 and *Pseud. thermalis* PUPCCC 106.4
were grouped with thermophilic cyanobacterial species *Pseudanabaena* sp. 0411 and *Leptolyngbya* sp. 0412 isolated from Hot Spring of the Baikal Rift, Russia (Sorokovikova *et al.*, 2008). Our phylogenetic analysis based on 16S rRNA gene sequence suggests that the cyanobacterial species of these hot water springs of North-Western Himalayas, belonging to order Stigonematales are monophyletic, whereas species belonging to order Chroococcales and Oscillatoriales are polyphyletic, as suggested by other workers (Giovannoni *et al.*, 1988; Wilutzky and Herdman, 2001; Gugger and Hoffmann 2004). The results of phylogenetic analyses based on *rbcL* gene (Fig. 24) and *cpcB*-IGS-*cpcA* phycocyanin locus sequences (Fig. 29) were congruent with the phylogenetic analysis based on 16S rRNA gene sequence.

Endemic species can evolve when the rate of evolution is faster than the rate of dispersal, and in contrast, cosmopolitan species evolve when the rate of evolution is slower than the dispersal rate (Padisak, 2009). Many explanations have been put forward to explain the selection of ecological species during the course of evolution. The nature of the substrate, climatic and geographical conditions influence microbial community structure (Ragon *et al.*, 2012; Dadheech *et al.*, 2013). The theory of Baas-Becking, later clarified by Wit and Bouvier (2006), stated that ‘everything is everywhere but nature selects’ emphasizing the global distribution of phylotypes (Dadheech *et al.*, 2013). Another theory based on concepts of genetic speciation suggested that strong chemical/physical boundaries prevent gene outflow and result in genetic lineages which diverge from their relatives (Wright, 1931, 1943). Ionescu *et al.* (2010) argued that the non-uniform biogeographical patterns in a complex cyanobacterial community, in which species similar to other thermal environments in the world and endemic species occurring in a single sampling site of a hot spring in Jordan (the Zerka Ma’in) can only be explained by a combination of the two, principally contradicting theories mentioned above. Variation in the rates of dispersal as compared to the rates of evolution depends on the geographical isolation of a given area and the dynamics of the local cyanobacterial communities (Ionescu *et al.*, 2010; Dadheech *et al.*, 2013).

On the basis of phylogeographic analysis of cyanobacteria from hot water springs of North-Western Himalayas, it is concluded that these hot water springs are another example of a habitat harbouring endemic and cosmopolitan cyanobacterial species alike. Three cyanobacterial species which showed less than 97% 16S rRNA gene sequence similarity with their closest relatives in GenBank are candidates as new
taxa from the area. Cyanobacterial species *P. ambiguum* PUPCCC 118.1, *Micro. chthonoplastes* PUPCCC 120.4 and *S. elongatus* PUPCCC 010.5 which showed similarity with the strains which have been earlier reported habitats from other than thermal habitats, have been reported first time from the hot water springs of North-Western Himalayas in this study. Other species like *Chloro. fritschii* PUPCCC 505.4, *F. thermalis* PUPCCC 510.2, *M. laminosus* PUPCCC 515.6, *G. gelatinosa* PUPCCC 009.2, *Gloeo. thermalis* PUPCCC 008.4, *Pseud. limnetica* PUPCCC 106.2, *Pseud. thermalis* PUPCCC 106.4, *G. sulphureum* PUPCCC 110.2, *L. carnea* PUPCCC 112.15, *L. copelandii* PUPCCC 112.16, *L. thermarum* PUPCCC 112.23, *L. orientalis* PUPCCC 112.18, *L. laminosa* PUPCCC 112.17, *L. gelatinosa* PUPCCC 112.19, *L. thermobia* PUPCCC 112.20 and *L. ramosa* PUPCCC 112.21 represent the cosmopolitan thermophilic cyanobacterial species reported from thermal water from all over the world. Thermophilic cyanobacterial species like *Gloeo. thermalis* PUPCCC 008.4, *Pseud. limnetica* PUPCCC 106.2, *Pseud. thermalis* PUPCCC 106.4, *G. sulphureum* PUPCCC 110.2, *L. carnea* PUPCCC 112.15, *L. copelandii* PUPCCC 112.16, *L. thermarum* PUPCCC 112.23, *L. orientalis* PUPCCC 112.18, *L. laminosa* PUPCCC 112.17, *L. gelatinosa* PUPCCC 112.19, *L. thermobia* PUPCCC 112.20 and *L. ramosa* PUPCCC 112.21 have been reported for the first time from Indian hot water springs.

### 5.3.2 Phylogeny of cyanobacteria from cold desert area

The phylogenetic relationship among cyanobacterial species isolated from the cold desert area of North-Western Himalayas was analyzed on the basis of 16S rRNA gene, *rbcL* gene and *cpcB*-IGS-cpcA phycocyanin locus sequences. The 16S rRNA gene sequences based phylogenetic tree revealed eighteen consistent clusters (I-XVIII), which were defined as monophyletic groups with bootstrap values equal or higher than 70% (Fig. 19-22). In addition, the phylogenetic distribution is congruent with the classification based on the morphological features. In the present study, species identified as *Nostoc commune* PUPCCC 405.1 and *Nostoc sp.* PUPCCC 405.6 were grouped closely in cluster I, with less than 97% sequence similarity to the nearest relatives in the database, and these two taxa were separated phenotypically by the morphology of their vegetative cells and akinetes. Therefore, their similarity at genetic level does not recapitulates the morphological differences found between them and their subsequent classification as separate species. Similarly, *N. muscorum*
PUPCCC 405.4 and A. variabilis PUPCCC 410.2, were well separated phenotypically on the basis of morphology of their vegetative cells, heterocysts and akinetes, but these were clustered together in cluster (V) with Nostoc sp. PCC 7120. The alignment of Nostoc sp. PCC 7120 with the GenBank strain Anabaena sp. CCAP/4A in the tree generated during present study once again pointed towards the genetic relatedness of the important strain PCC 7120 with the genus Anabaena. Phylogenetic relationship among strains PCC 7120 and Anabaenal/Nostoc obtained through 16S rRNA gene sequence was congruent with relationship on the basis of rbcL gene and cpcB-IGS-cpcA phycocyanin locus sequences (Fig. 25 cluster I; Fig. 30 cluster I). Thus, phylogenetic analysis during the present study once again re-ignite the continuous debate over the placement of strain PCC 7120 in Nostoc or Anabaena. This observation is consistent with earlier studies on 16S rRNA gene sequences analysis of various Nostoc strains, which also showed great genetic heterogeneity, suggesting that the Nostoc cluster comprises more than one genus (Hrouzek et al., 2003; Rajaniemi et al., 2005, Komárek, 2010; Mateo et al., 2011; Loza et al., 2013).

In the present study, cluster I (Fig. 20) comprised species of Nostoc, which grouped with species reported from geographically distinct regions and with species having symbiotic association with fungus Peltigera (O’Brien et al., 2005). Many symbiotic Nostoc isolates are referred to as N. punctiforme (Mollenhauer et al. 1996, Rasmussen and Svenning 2001), and it is open to debate how similar are all the strains whom this name is given, as other species can produce stages during their life cycle resembling N. punctiforme when grown on artificial medium (Rasmussen and Svenning 2001, Whitton, 2011). Further characterization of these species is required to confirm the actual species identity. Two species of genus Nodularia were isolated from cold desert area and were identified as Nod. sphaerocarpa PUPCCC 420.1 and Nod. spumigena PUPCCC 420.5 during the present study. These grouped together in cluster II with Nodularia sp. PCC 73104/1, Nod. sphaerocarpa BECID36, Nodularia sp. PCC7304/1, Nod. sphaerocarpa BECID36, Nod. sphaerocarpa HKVV and Nod. sphaerocarpa UTEX B 2092 reported as an isolate of brackish water (Lehtimaki et al., 2000), and Nod. spumigena PCC 73104 which has been reported to show symbiotic association with fungus Peltigera (Lyra et al., 2005; O’Brien et al., 2005).

In cluster III Tolypothrix sp. PUPCCC 415.2 grouped with Tolypothrix sp. PCC 7504 isolated from Mexico (Dominguez-Escobar et al., 2011). In cluster IV Nostoc sp. PUPCCC 405.3 showed close relationship with Nostoc sp. LCRSM-10 reported from
Indian paddy fields, while *N. linckia* PUPCCC 405.2, whose 16S rRNA gene sequence showed <97% similarity with Brazilian isolate *Nostoc* sp. CENA 88, formed an independent branch (Fig. 20).

The species *Plank. clathrata* PUPCCC 108.8 showed close relationship with two strains *Planktothrix* sp. CYN61 and *Planktothrix* sp. VUW25, isolated from Waitaki River, New Zealand (Wood *et al.*, 2010). Two species identified as *Planktothrix* sp. PUPCCC 108.5 and *Planktothrix* sp. PUPCCC 108.6, whose 16S rRNA gene sequence showed <97% similarity with sequences in database formed an independent branch. The species which were showing less than 97% similarity, on the basis of 16S rRNA gene sequences, are candidate taxa to propose as new species after further characterization. In cluster VIII *Gloeo. pleurocapsoides* PUPCCC 008.2 clustered with *Gloeocapsopsis* sp. AAB1, an extremely desiccation-tolerant cyanobacterium isolated from the Atacama Desert (Azua-Bustos *et al.*, 2014). Two species of *Phormidium*, *P. autumnale* PUPCCC 118.4 and *P. chalybeum* PUPCCC 118.8 were grouped together in cluster IX and showed close relationship with fresh water isolates *P. autumnale* SAG35.90, and *Phormidium* sp. DVL1003c, respectively (Siegesmund *et al.*, 2008). Two species of genus *Microcoleus*, identified as *Micro. acremanii* PUPCCC 120.2 and *Micro. vaginatus* PUPCCC 120.5 were grouped together with taxa reported from near-polar habitats (Casamatta *et al.*, 2005). *Micro. vaginatus* has been reported to occur in Arctic, Alpine and Antarctic soils (Broady, 1996; Cowan and Tow, 2004). In cluster X *Chro. cubana* PUPCCC 005.5 grouped with *Chro. cubana* SAG 39.79, *Chro. thermalis* PCC7203 and *Chroococcidiopsis* sp. SAG2025. One species identified as *G. acutissimum* PUPCCC 110.4 grouped with fresh water planktonic cyanobacterial strains *G. carotinosum, Geitlerinema* sp. Sai004 and *G. splendidum* (Willame *et al.*, 2006). In cluster (XII) *Syn. pevalekii* PUPCCC 062.1 clustered with *Synechocystis* sp. PCC 6805, *Synechocystis* sp. PCC 6803, *Synechocystis* sp. PCC 6714, *Synechocystis* sp. MMG-8 and *Synechocystis* sp. LMECYA 68 (Fig. 21, Cluster XII). The most of the references strains from GenBank database, showing close relationship with the cyanobacteria isolated during present study, were unpublished and database entries are without any detail on isolation source/habitats.

In the present study, genus *Leptolyngbya* is represented by ten species, which grouped into three major clusters (XIII, XIV and XVII) in the phylogenetic tree constructed on the basis of partial 16S rRNA gene sequences (Fig. 21), while in the
phylogenetic tree generated on the basis of rbcL gene sequences these ten species were grouped in two major clusters (IV and V) (Fig. 25). Our results are in agreement with the earlier reports which currently describe Leptolyngbya as polyphyletic and suggest that there is need for its revision (Albertano and Kovacik, 1994; Nelissen et al., 1994; Turner, 1997; Ishida et al., 2001; Casamatta et al., 2005; Komárek and Anagnostidis, 2005; Taton et al., 2006b; Johansen et al., 2008; Bohunicka et al., 2011; Dadheech et al., 2013). In cluster XIII of phylogenetic tree constructed on the basis of 16S rRNA gene sequences during the present study (Fig. 21), species identified as L. foveolarum PUPCCC 112.8 showed close relationship with L. foveolarum and L. boryana UTEX B 488, and species identified as Leptolyngbya sp. PUPCCC 112.9, which showed <96% similarity with L. boryana UTEX B 488 and formed an independent branch. Phylogenetic trees of earlier studies also had cluster corresponding to the L. boryana sequences but these clusters also included other taxonomic identities, such as L. foveolarum, L. tenerrima, and L. angustata, which have been considered as synonyms of L. boryana (Cuzman et al., 2010). The cluster XIV of phylogenetic tree (Fig. 22), included five species representing genus Leptolyngbya and showed close relationship with species earlier reported to be distributed worldwide in cold environments including polar region and high altitudinal lakes (Taton et al., 2006; Furtado et al., 2009; Cuzman et al., 2010). In cluster XVII four species of Leptolyngbya and one species of Nodosilinea were grouped with their globally distributed closest reference strains. Species identified as L. subtruncata PUPCCC 112.10 showed relationship with species reported from Portuguese temperate estuaries Leptolyngbya sp. LEGE 07296 (Lopes et al., 2012). Nodosilinea epilithica PUPCCC 111.2 was closely related to Nodosilinea epilithica, which was previously reported from Italy, as green biofilm on the wall of a house (Casamatta et al., 2010). Species L. cebennensis PUPCCC 112.4 showed relationship with Leptolyngbya sp. 0BB19S12 and Leptolyngbya sp. MX1, and species identified as L. lurida PUPCCC 112.6 and Leptolyngbya sp. PUPCCC 112.7 showed close relationship with Siberian permafrost soil isolates Leptolyngbya sp. 690.AC125 and Phormidium sp. 195-A12 (Vishnivetskaya, 2009). High similarity of 16S rRNA gene sequence in both Leptolyngbya and Nodosilinea (Fig. 22, Cluster VII), and close phylogenetic relationship of Nodosilinea with member of Nostocales in the present study (Fig. 26, cluster III), suggest the need of analyzing multiple gene sequences to understand the phylogenetic relationships within these genera. This study indicated
that while the 16S rRNA gene sequence is very useful for phylogenetic analysis and recognition of monophyletic clusters of species, it was not useful enough at the species level within the genus *Leptolyngbya*. This observation is in agreement with other studies in which species were not resolved well within clades representing species/genera of family Pseudanabena (Johansen *et al*., 2008, 2011; Perkerson *et al*., 2011).

The cluster XV represented by genus *Cyanobium*, included only one species identified as *C. parvum* PUPCCC 007.1 which grouped with *Cyanobium* sp. UAM406, *C. gracile* PCC6307 and *Synechococcus* sp. SM0708 (Fig. 22). Our results are in agreement with earlier reports that also reported similar grouping (Loza *et al*., 2013). Rippka and Cohen-Bazire (1983) proposed a new genus designated as *Cyanobium* on the ground that some strains assigned to *Synechococcus* were genetically distinct from members of this genus and were sensitive to various cyanophages. *Cyanobium* was validated as a genus by Komárek *et al*. (1999), on the basis that phenotypic diacritical characters were found to coincide well with the molecular markers. However, the relationship between this genus and similar unicellular cyanobacteria (*Synechococcus, Prochlorococcus, Anathece*) needs to be investigated further using molecular approaches (Komárek, 2011). In cluster XVI of phylogenetic tree species *Limn. redekei* PUPCCC 116.2 grouped with *Limn. planktonica, Limn. redekei, Limnothrix* sp. CENA545 and *Limnothrix* sp. NQAJF306. The cluster XVIII represented genus *Pseudanabaena*, in which species identified as *Pseudanabaena* sp. PUPCCC 106.7 grouped with fresh water *Pseudanabaena* sp. ABRG5-3, *Pseudanabaena* sp. ACCS013 and *Pseudanabaena* sp. Sai011 (Nishizawa *et al*., 2010) (Fig. 22).

The results of phylogenetic analyses based on *rbcL* gene (Fig. 25) and *cpcB*-IGS-*cpcA* phycocyanin locus sequences (Fig. 30) were congruent with the phylogenetic analysis based 16S rRNA gene sequence. As expected, the heterocystous cyanobacteria formed a coherent genetic cluster, whereas the unicellular and filamentous non-heterocystous genera were intermixed. These results are consistent with other studies in which phylogenetic analyses provided evidence of the monophyly of heterocystous cyanobacteria (Lyra *et al*., 2001, Hoffmann *et al*., 2005, Berrendero *et al*., 2011). Conversely, unicellular and simple filamentous forms (with no further cell differentiation, e.g., heterocysts, akinetes) are heterogeneous and belong to several distinct evolutionary lineages (Turner *et al*., 1999, Hoffmann *et al*.,

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The question of endemism and distribution of cyanobacterial taxa is still a topic of much debate. Castenholz (1992) commented that slow rates of speciation in cyanobacteria together with their large dispersal abilities, suggests that endemism is likely to be rare amongst polar cyanobacteria. Phylogenetic analysis of cyanobacterial communities of cold desert area (Lahaul-Spiti) of North-Western Himalayas during present study revealed that cyanobacteria of this area resembled with globally distributed psychrotrophic (cold-tolerant) cyanobacteria. Cold, barren ecosystems, such as the sub-nival zone, are ideal for testing biogeographic hypotheses concerning the global distribution and biogeography of microbes because they are essentially isolated islands of the cryosphere surrounded by vast expanses of warmer ecosystems (Martiny et al., 2006; Green et al., 2008; Schmidt et al., 2011). This is especially true of the Himalayas, where the highest mountains on Earth arose out of a sea of tropical and subtropical biomes to the south (Schmidt et al., 2011). Schmidt et al. (2011) reported that there are remarkable similarities between microbial life of arid soils of Antarctica and the high Himalayas. In accordance with this, results of the present study revealed that cyanobacterial communities of cold desert area showed resemblance to those described for Antarctic microbial mats, in particular in the McMurdo Ice Shelf, McMurdo Dry Valleys and Larsemann and Vestfold Hills (Taton et al., 2003, 2006a; Jungblut et al., 2005; Singh and Elster, 2007). Further, future comparative studies, based on molecular characterization of cultured as well as uncultured cyanobacterial diversity of this area, with other cold environment are required so as to understand phylogeography of psychrotrophic cyanobacteria of North-Western Himalayas.

From the above observation it is concluded that although most of the cyanobacterial species characterized during the present study showed resemblance with the species reported from the cold environments from all over the world, but few species were reported for the first time from the high mountain cold environment. The cyanobacterial species belonging to Nostocales like Anabaena variabilis PUPCCC 410.2, Tolypothrix sp. PUPCCC 415.2, Nostoc sp. PUPCCC 405.8, Nostoc sp. PUPCCC 405.6, Nostoc sp. PUPCCC 405.3, Nostoc muscorum PUPCCC 405.4, Nostoc sp. PUPCCC 405.2, Nostoc sp. PUPCCC 405.9, Nostoc commune PUPCCC 405.1, Nostoc sp. PUPCCC 405.7 represent a group which is generally reported from fresh water and agriculture soil of India, but have been reported for the first time from
the study area. Six cyanobacterial species from the study area showed less than 97% similarity of 16S rRNA gene sequence with the closest relatives in the GenBank and thus represent the candidate taxa as new species from the study area. Cyanobacterial species *C. parvum* PUPCCC 007.1, *Gloeocapsa pleurocapsoides* PUPCCC 008.2, *Chroococclopsis cubana* PUPCCC 005.5, *L. antarctica* PUPCCC 112.2, *L. benthonica* PUPCCC 112.5, *L. cebennensis* PUPCCC 112.4, *L. frigida* PUPCCC 112.1, *L. subtruncata* PUPCCC 112.10, *L. subtilis* PUPCCC 112.11, *Nodothrix epilithica* PUPCCC 111.2, *P. chalybeum* PUPCCC 118.8 and *Planktothrix clathrata* PUPCCC 108.8 have been reported first time from study area as well as from India.

### 5.4 Physico-chemical characteristics

#### 5.4.1 Hot water springs

Water of hot water springs has a diverse chemical composition; pH in particular differs among hot springs. The most common hot springs are those with alkaline pH, which are associated with volcanic or tectonic activity; near-neutral pH springs are less common and acid pH springs, such that of the Yellowstone caldera, are associated with active volcanism or shallow magma pools (Castenholz, 1996). The solute concentration of hot springs also varies greatly. The majority of thermal springs, in general, have constant chemical composition at the point of emergence. However, natural disturbances and seasonal changes can probably have a short term effect on hot spring water characteristics (Castenholz, 1969; 1973).

The water temperature of the selected hot springs during the present study was in the range of 40 °C to 90 °C. The highest water temperature (90 °C) was noted at Manikaran while lowest (40 °C) at Gauri Kund and Janki Chatti hot water springs. Manikaran hot water spring is a sprout from the joints of Rampur quartzite nearby the Parvati river bed and chemical analysis of water revealed that it is Na\(^+\)+K\(^+\)(Ca\(^{2+}\))-Cl\(_2\) type. The higher discharge temperature of the Manikaran hot water spring, compared to Kalath and other hot water springs, probably reflects higher depth of penetration of cold meteoric water along the faults system in a crust affected by an anomalous geothermal gradient (Cinti *et al.*, 2009). The seasonal variations in the temperature of source water of each hot water spring was less than 5 °C, suggesting that the thermal gradient downstream of each hot water spring was a stable and persistent physical feature of the environment. All the hot water springs have neutral to alkaline pH (7.0-8.0). Highest pH range (7.6-8.0) was noted at Kheer Ganga whereas minimum (6.8-7.2) at Kalath (Table 40). The difference between the pH value of the selected springs
is small (less than ±1), indicating that these springs are neither corrosive nor depositional in nature.

The variability in temperature and pH of springs results in significant differences in quantities of hydro-chemical parameters (Hsu et al., 2006). For example, concentration of bicarbonate ions is regulated by pH of water and these ions predominantly are present within the pH range of 4 to 10 (Chen and Sung, 2009). Moreover, high temperature of spring water reduces concentration of bicarbonate ions due to low CO₂ dissolution (Alfaro and Wallace, 1994; Afşin et al., 2006). High temperature of spring water also tends to increase dissolution quantities of certain ions, such as K⁺, Na⁺ and Cl⁻ (Zhu and Yu, 1995; Yee et al., 2003).

The conductivity of all hot water springs was in the range of 145 µs cm⁻¹ to 460.2 µs cm⁻¹ with lowest conductivity (145 ±10.5 µs cm⁻¹) at Gauri Kund hot water spring and highest (460.2 ±14.25 µs cm⁻¹) at Tattapani hot water spring. The conductivity of water depends on temperature and the dissolved ions or total component concentration in the water (Raksaskulwong, 2000). It appears that concentration of magnesium, sodium and potassium ions contributed much to the conductivity of hot springs. Maximum alkalinity (580.0 ±18.24 mg L⁻¹) was measured at Tattapani, whereas minimum (324 ±8.25 mg L⁻¹) at Kheer Ganga. The alkalinity of water body is influenced by surface water and geological dissolved substances such as nutrients and inorganic carbon forms (Hudak and Sanmanee, 2003).

Based on composition of their major ions, springs can be classified into different water types (Minissale et al., 1997; Mariner et al., 2003; Afşin et al., 2006). A piper diagram is useful in illustrating the chemical characteristics of hot water springs and in identifying the degree of correspondence between the source areas of the springs. The Piper diagrams are widely used in geo-hydrology for the interpretation of the genesis of the chemical character of groundwater in an aquifer. A piper diagrams is a tri-linear diagram in which the concentrations of the major ions are plotted as percentages, with each point representing a chemical analysis. The two base triangles reflect the sample anion and cation concentrations, respectively. Based on piper diagram, it is concluded that water of Kasol and Gauri Kund was Ca²⁺+Mg²⁺-Cl₂ type, Surya Kund and Kheer Ganga was Na⁺(Ca²⁺)+K⁺(Mg²⁺)-Cl₂ type, Tattapani, Kalath and Janki Chatti was Na⁺+K⁺-Cl₂ type, Manikaran was Na⁺+K⁺(Ca²⁺)-Cl₂ type and Vashisht was Mg²⁺+CO₃²⁻+HCO₃ type (Fig. 42). A dendrogram generated from the co-relation matrix of physico-chemical characteristics based on UPGMA
clustering algorithms revealed same type of grouping of selected hot water springs (Fig. 45). Cluster analysis was successfully used, for instance, to classify lake samples into geochemical facies (Jaquet et al., 1975). Alther (1979), Williams (1982), and Farnham et al. (2000) also applied cluster analysis to classify water-chemistry data.

The chemical composition of Manikaran hot water springs differentiates it from other hot water springs, while at Vashisht, the chemical composition is the distinguishing feature. Manikaran, Kheer Ganga and Kasol hot water springs are sprout from Manikaran geothermal field in Parvati Valley but Manikaran hot water spring differs from Kasol which is only 5 Km apart from it. Although Surya Kund (Uttarakhand) and Kheer Ganga (Himachal Pradesh) are more than 100 Km (aerial distance) apart but these are almost identical in the chemical characteristics of their water. From the above, it is evident that proximity of springs to each other does not mean that they may have identical physical and chemical characteristics. HCO$_3^-$, Cl$^-$, SO$_4^{2-}$, Na$^+$, K$^+$, Ca$^{2+}$, and Mg$^{2+}$ are the major ions in spring water which originate as a result of dissolution and mineralization of rocks (Davisson et al., 1994; Minissale et al., 1997; Afşin et al., 2006; Stambuk-Gilijanovic, 2008). Thus, geological conditions determine water quality of springs and the hydrochemical composition of spring water may indicate its geological origin (González-Partida et al., 2005; Sanada et al., 2006; Tarits et al., 2006).

Principal Component Analysis (PCA) is a factor analysis that can be performed on any kind of scientific data to establish a pattern of variations among variables or reduce large data sets into factors for easy handling and interpretation. The total number of factors generated from a typical analysis indicates the total number of possible sources of variation in the data. Factors are ranked in order of merit. The first factor or component has the highest eigen-vector sum and represents the most important source of variation in the data. The last factor is the least important process contributing to the chemical variation. PCA can be applied to chemical data to extract the principal factors corresponding to different sources of variation in the data. PCA has been widely used in biological, physical, social and hydrologic sciences (Yu and Zou, 1993).

The correlation matrix obtained from the PCA of the 14 physico-chemical variables of the Hot water springs studied presently revealed that only few variables exhibited significant correlation (Table 41). The output from the PCA is presented in ordination diagram or two dimensional scatter plot. Typically, two dimensional
graphs of the first two factors are plotted because the first two factors accounted for most of the variance in the data set. The third factor, as well as other factors, are assessed, but rarely provide any new groupings of the spring waters. The eigenvalue of the ordination axis in PCA is used to show much variance and is accounted for by the axis (Shaw, 2003). The graph plotted was the scatter graph of axis 1 against axis 2, the greatest percentage of the overall variance was held by these axes. The eigenvalue for axis 1 was 5.382 and for axis 2 was 3.145. The cumulative percentage for both axes was 56.846, which indicated that two axes captured about 57% of total variance in the data set. The majority of the information content of the data could be described by using only the first axis (Table 42).

The PCA ordination diagram showed that factor F1 which contributed to 35.87% of the total variance of the PCA analysis was determined in positive scale of F1 by TP, Na\(^+\), K\(^+\), EC, TH, TA and TDS variables and on negative scale by NO\(_3^-\). The factor F2 that contributes 20.97% of total variance, is determined in the positive scale by Temp, pH, NH\(_4^+\), Mg\(^{2+}\), Cl\(^-\), SO\(_4^{2-}\) and Ca\(^{2+}\). (Fig. 43).

The PCA correlation biplot of selected hot water springs and their physico-chemical variables showed that these were grouped into two major groups. The first group included three hot water springs: Tattapani, Kalath and Janki Chatti on positive scale of factor F1 showing correlation with TP, Na\(^+\), K\(^+\), EC, TH, TA and TDS, while Vashisht hot water spring on negative scale of factor F1 showing correlation with NO\(_3^-\). The second group included four hot water springs: Surya Kund, Gauri Kund, Kheer Ganga and Kasol on the positive scale and Manikaran on negative scale of Factor F2, showing correlation with Temp, pH, Cl\(^-\), NH\(_4^+\), Mg\(^{2+}\), Ca\(^{2+}\) and SO\(_4^{2-}\) (Fig. 44).

5.4.2 High altitude lakes

The water temperature and ambient air temperature of the sampling sites were close to one another’s, mainly due to the standing nature of the water in the lakes. The water temperature in streams generally shows variation with respect to air temperature because of the running nature of water (Hynes, 1979; Williamson et al., 2008). Further, the temperature of water was dependent upon the altitude of the lake with the lowest temperature (6 ± 2°C) being that of Suraj Tal, located at a height of 4883 m above sea level, and the highest temperature (18 ± 2°C) being that of Sissu Lake, situated at 3010 m above sea level. The pH of all the lakes was alkaline, in the range
8.5 ±0.2 to 9.1 ±0.1 (Table 43). Since all the lakes have clear water without any macrophytic growth, it seems that geological features of the catchment and basin areas, and the evaporation pattern of water from the lake surface, rather than photosynthesis, are responsible for alkaline pH of water. Usually pH is on the alkaline side when the CO$_3^-$ system prevails in water bodies and total alkalinity is higher than the HCO$_3^-$ system (Toma, 2011). The high pH of studied lakes is also not ascribable to the release of carbohydrates from sediments and their conversion to bicarbonates by carbonic acid formed by decomposition of organic matter as these lakes do not have high amount of organic matter (Hannon et al., 1979). The alkaline pH of the lakes in the present study is also supported by high alkalinity (208–480 mg L$^{-1}$). Zutshi et al. (1980) reported decrease in water conductivity with the increase in altitude, which holds true for the present study also. Conductivity and alkalinity of water bodies are strongly influenced by the nature of incoming water and its periodicity (Kiplagat et al., 1999). The source of water in high altitude Himalayan lakes is mostly snowmelt, usually low in ionic content, and scantly rains. The cation progression in the lakes was Ca$^{++}$>Mg$^{++}$> Na$^+$>K$^+$. Zutshi et al. (1980) have reported that calcium is generally the dominant cation in Himalayan lakes of Kashmir region because of the predominance of lime-rich rocks in the catchment areas. Ammonium-nitrogen, nitrate-nitrogen and total phosphate are good indicators of anthropogenic pressures (Zutshi et al., 1984). Sissu Lake is comparatively rich in these nutrients compared to other lakes because of human activity. Vass (1980) and Zutshi et al. (1980) have reported that the distribution of nitrate, ammonium and phosphate are governed by physical factors such as input source to the lake (catchment characteristics), changes in mixing depth during stratification and complete mixing during isothermal conditions. The nitrogenous compounds in the water bodies are derived to an appreciable degree from the atmosphere, whereas ammonium is the chief product of decomposition of plant and animal proteins (Sharma, 2000). As the decomposition of organic matter in these lakes, except Sissu Lake, is negligible, catchment characteristics (geological features), atmosphere and closed nature of the lakes seem to be responsible for observed level of nitrogen, ammonium and phosphate.

The physico-chemical parameters of selected lakes were used to perform Principal Component Analysis (PCA) and cluster analysis to find out correlation or relationship between lakes. The correlation matrix obtained from the PCA of 14
physico-chemical parameters of lakes revealed that majority of the parameters exhibited significant correlation (Table 44). The cumulative percentage for both the axes, axis 1 and axis 2, was 91.554%, which indicated that two axes capture about 92% of total variance in the data set. (Table 45). The PCA ordination diagram showed that factor F1 that contributed 78.62% of the total variance of the PCA analysis was determined in positive part of diagram by majority of variables. The factor F2 that contributed 12.94% of total variance, was determined in the positive part of diagram by pH and Ca$^{2+}$ (Fig. 46). The proximity of majority of variables, witnessed a good correlation between these variables. This fact was confirmed by correlation matrix.

The PCA correlation biplot of lakes and their physico-chemical variables showed that three collection sites (SS1, SS2 and SS3) of Sissu Lake are grouped on the positive scale of factor F1, showing positive correlation with majority of physico-chemical variables, whereas collection sites of Chandra Tal on the negative scale of factor F1, showing negative correlation with these variables. Suraj Tal on positive scale of factor F2 showing correlation with pH and Ca$^{2+}$, while Deepak Tal on negative scale of factor F2 showing negative correlation with these variables (Fig. 47). On the basis of nutrient content, Sissu Lake is classified as mesotrophic while other three are ultra-oligotrophic.

5.5 Relationship between physico-chemical parameters and cyanobacterial diversity

5.5.1 Hot water springs

Environmental factors such as temperature, water chemistry and some biological factors such as grazing may affect the distribution of photosynthetic organisms in geothermal springs (Castenholz and Utkilen, 1984; Ward et al., 1989; Dillon and Castenholz, 2003). Cyanobacteria are not usually observed in hot springs with pH below 4.0, although Synechococcus spp. has been observed in Yellowstone National Park hot springs with pH as low as 5.2 (Ward and Castenholz, 2000). Temperature, coupled with nitrogen availability and sulphide concentration in hot spring water, also determines the composition of cyanobacterial mats. In springs with temperature lower than 60 °C nitrogen fixing Calothrix occurs (Castenholz, 1969; Ward et al., 1989). Sulphide is toxic to primary metabolism, thus springs with high level of free sulphide may be dominated by sulphide-tolerant species such as Oscillatoria limnetica and Cyanidium caldarium which have the ability to use sulphide as photosynthetic electron donor (Castenholz, 1969; Ward et al., 1989). Some of the well-studied
cyanobacterial mats are from Hunter’s Hot Spring, Oregon and Octopus Spring, Yellowstone National Park. Cyanobacterial mats from Hunter’s spring are composed of green top layer of *Synechococcus lividus* at 54-55 °C. A dark red-brown layer of *Oscillatoria* cf. *terebriformis* was also observed. Octopus Spring cyanobacterial mat on the other hand is made up of *Phormidium* and *Synechococcus* at ~57 °C (Ward and Castenholz, 2000).

During the present study, CCA was employed to study the relationship between the cyanobacterial species composition and environmental variables. The arrows in CCA ordination diagram represent the direction of maximum change in the value of environmental variables. The angle between two arrows (axes) indicates the correlation between the length of the arrow which is equal to the maximum value of the variables. In the ordination diagram, the species which are located at greater distance from the centre indicate relevant significance which may be specific to sampling sites than other species which are closer to centre. Cyanobacterial species from hot water springs studied presently are well separated along both the axes indicating different physico-chemical demands for different species (Fig. 49). The CCA data were differentially distributed, which allowed separation of the cyanobacterial species into two groups based on water quality parameters of the hot water springs. Group 1 included two globally distributed heterocystous filamentous branched themophilic species of Stigonematalean genera, *F. thermalis* PUPCCC 510.2, *M. laminosus* PUPCCC 515.6, unicellular species *S. elongatus* PUPCCC 010.5 and *Gl. gelatinosa* PUPCCC 009.2 as well as non-heterocystous filamentous species *G. sulphureum* PUPCCC 110.2, *Leptolyngbya* sp. PUPCCC 112.22, *L. thermarum* PUPCCC 112.23, *L. orientalis* PUPCCC 112.18, *L. ramosa* PUPCCC 112.21, *L. thermobia* PUPCCC 112.20, *Phormidium* sp. PUPCCC 118.2 and *Micro. chthonoplastes* PUPCCC 120.4, placed on right side of the graph, with high temperature, electrical conductivity, high content of total nitrogen, sodium, sulphate, and low pH and total phosphate. In contrast Group 2 included *L. copelandii* PUPCCC 112.16, *L. laminosa* PUPCCC 112.17, *L. carnea* PUPCCC 112.15, *L. gelatinosa* PUPCCC 112.19, *Chloro. fritschii* PUPCCC 505.4, *Phormidium* sp. PUPCCC 118.3, *P. ambiguam* PUPCCC 118.1, *Gloeo. thermalis* PUPCCC 008.4, *Pseud. limnetica* PUPCCC 106.2 and *Pseud. thermalis* PUPCCC 106.4 plotted on left side of graph with high pH, total phosphate, low temperature and other nutrient level. The distribution of the cyanobacterial species was determined by environmental
parameters of hot water springs indicating that environmental conditions play important role in their distribution. *Synechococcus, Fischerella* and *Mastigocladus* are one of the best documented genera of cyanobacteria from thermal springs and are able to grow at 55 °C globally (Ward *et al.*, 1998; Miller and Castenholz, 2000, Ramsing *et al.*, 2000; Papke *et al.*, 2003; Sompong *et al.*, 2005, 2008). Earlier studies on the cyanobacterial diversity of hot water springs have revealed that besides temperature other factors like pH, nitrogen and sulphide also effect species distribution of hot water springs (Ward and Castenholz, 2000; Sompong *et al.*, 2005; Debnath *et al.*, 2009).

### 5.5.2 High altitude lakes

CCA is useful in qualitative analysis of interactions between ecological factors and planktonic communities (Barinova and Tavassi, 2009; Liu *et al.*, 2011; Tian *et al.*, 2012; Loza *et al.*, 2013). It was observed during the present study that cyanobacterial species are well separated along both axes of CCA biplot, indicating different physico-chemical demands of different species (Fig. 50). The CCA data were differentially distributed, which allowed separation of the cyanobacterial species into three groups on the basis of water quality parameters at the sampling sites. Group 1 included the heterocystous species *Nostoc* sp. PUPCCC 405.2, *Nostoc* sp. PUPCCC 405.6 and *Nod. sphaerocarpa* PUPCCC 420.1 as well as non-heterocystous species *G. acutissimum* PUPCCC 110.4, *Limn. redekei* PUPCCC 116.2, *Planktothrix* sp. PUPCCC 108.6 and *Plank. clathrata* PUPCCC 108.8, placed on the right side of the graph, with high temperature, high nutrient levels and low pH. In contrast, Group 2 included *Gloeo. pleurocapsoides* PUPCCC 008.2, *L. antarctica* PUPCCC 112.2, *L. frigida* PUPCCC 112.1, *Pseudanabaena* sp. PUPCCC 106.7 and *Nostoc* sp. PUPCCC 405.8 plotted on the left side of the graph with high pH, low temperature and low nutrient levels. Group 3 comprised *C. parvum* PUPCCC 007.1, *Syn. pevakekii* PUPCCC 062.1, *L. benthonica* PUPCCC 112.5, *L. foveolarum* PUPCCC 112.8, *L. lurida* PUPCCC 112.6, *Leptolyngbya* sp. PUPCCC 112.7, *P. autumnale* PUPCCC 118.4 and *P. chalybeum* PUPCCC 118.8, and are located in the middle of the graph. Distribution of species of this group could not be associated with a specific environmental condition or sampling site. Conductivity and lake trophy have been identified as the main factors regulating species composition of benthic communities (Vincent and James, 1996; Roberts *et al.*, 2001; Sabbe *et al.*, 2004), but in the present
study pH, temperature and nutrient level of lakes seem to be major factors which influence distribution of cyanobacterial species.

**Salient features of present study**

- Non-heterocystous filamentous forms dominate in both hot water springs and cold desert area of North-Western Himalayas.
- Oscillatoriales is the dominant order in both habitats in terms of number of species and abundance.
- Cyanobacterial diversity of hot water springs and cold desert area was represented by 60 species of 21 genera, 5 genera were present exclusively in hot water springs and 10 genera were present exclusively in cold desert area while 6 genera were common in both hot water spring and cold desert area.
- The heterocystous cyanobacterial forms were represented by the Stigonematales in hot water springs while members of Nostocales are present in cold desert area.
- Genus *Leptolyngbya* is dominant at species level, in hot water springs (9 species) as well as cold desert area (11 species).
- Three cyanobacterial species from hot water springs and 6 cyanobacterial species from cold desert area are candidate taxa as new species.
- The following gene sequences have been submitted for the first time to Gen Bank database:

<table>
<thead>
<tr>
<th>Gene</th>
<th>Number of species</th>
<th>Habitat</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S rRNA</td>
<td>15</td>
<td>Hot water springs</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>Cold desert area</td>
</tr>
<tr>
<td><em>rbcL</em></td>
<td>15</td>
<td>Hot water springs</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>Cold desert area</td>
</tr>
<tr>
<td><em>cpcBA</em>-IGS</td>
<td>7</td>
<td>Hot water springs</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>Cold desert area</td>
</tr>
</tbody>
</table>

- The hot water springs of North-Western Himalayas are moderately alkaline with varied nutrient content. On the basis of distribution of cyanobacterial species in relation to water chemistry of hot water springs, it is observed that Temperature, pH, conductivity, and content of nitrogen, sulphur and phosphate in water are the major factors affecting distribution of cyanobacteria in these hot water springs.
Three lakes of cold desert are ultra-oligotrophic while Sissu Lake is mesotrophic in nature. Occurrence of cyanobacteria in these lakes is influenced by Temperature, pH and nutrient level of the lake.

The present study has contributed to the knowledge on distribution, diversity, characterization and germplasm of cyanobacteria from hot water springs and cold desert area of North-Western Himalayas. The important leads generated for future studies include:

- Characterization by additional molecular markers, biochemically and electron microscopically the interesting taxa for defining new species/genera;
- Whole genome sequencing of thermophilic and psychrophilic cyanobacteria to understand their functional adaptability to extreme environments; and
- Exploration of germplasm collection for biotechnological applications and value added products.