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Study of morphological variation of *Nostoc punensis* and *Desmonostoc salinum* isolated from soils around lakes of Golestan province (N Iran) in different culture media

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Abstract

Studying the cyanobacteria strains grown on different culture media has led to controversies when they are identified by classic keys. Hence, the main target of this study is to identify cyanobacteria strains and compare their distribution in different soil, then identification the culture media with the highest stability and least effect on their variations. In this regard, soils samples of Ala gol, Aji gol, and Alma gol lakes belong to Golestan province (north of Iran), were collected and cyanobacteria taxa were isolated, and identified. In addition, an attempt was made to determine their incidence with some physicochemical characteristics of the soils. Morphological observation showed that five different strains were identified in Ajigol, four different strains in Alagol, and two different strains in Alma gol. Results of morphological identification showed that, *Nostoc punensis* and *Anabaena* sp. are common in Ala gol and Aji gol lakes, except Alma gol lake, where its EC was the highest and *Dulcicalothrix alborzica* and *Neowestiellopsis persica* were the only species found in the stands. Then two strains of *Nostoc punensis* and *Desmonostoc salinum* which had very variable morphology in culture media, were grown on Z8 and BG-11 culture media without nitrogen, and morphological variations of cells were measured from the inoculating time up to the end of the stationary phase of their growth. In addition, morphological variations showed that, vegetative cells and akinetes of both strains changed into spiral form and large and heterocytes also had a bony form on BG-11 medium but retained their natural form in Z8 medium. Accordingly, the Z8 medium is suggested to be used for its stability as preferred culture media to provide less morphological variations.

Keywords: Heterocystous cyanobacteria, morphological variations, morphometric analysis, Z8 medium

مطالعه تنوع ریخت‌شناختی *Nostoc punensis* و *Desmonostoc salinum* جدا شده از خاک‌های اطراف دریاچه‌های استان گلستان

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خلاصه

در مطالعه سویه‌های سیانوباکتریایی، تنوع ریخت‌شناختی سلول‌ها در هنگام رشد در محیط‌های کشت مختلف اغلب منجر به شکل‌گیری اختلاف نظرهای بسیاری در هنگام شناسایی آرایه‌ها با استفاده از کلیدهای شناسایی کلاسیک می‌شود. از این رو، هدف اصلی این مطالعه شناسایی سویه‌های سیانوباکتریایی و مقایسه توزیع آن‌ها و سپس تعیین بهترین محیط کشت با بیشترین ثبات و کمترین تأثیر بر تغییرات ریخت‌شناختی آن‌ها است. در این راستا، نمونه‌هایی از خاک اطراف دریاچه‌های آلاگل، آجی‌گل و آلماگل (استان گلستان) جمع‌آوری و آرایه‌های سیانوباکتریایی موجود در آن‌ها جداسازی و شناسایی شدند. به علاوه، ارتباط حضور سویه‌های مختلف سیانوباکتریایی با ویژگی‌های فیزیکوشیمیایی خاک نیز بررسی گردید. براساس نتایج به دست آمده، در مجموع، پنج سویه از آجی‌گل، چهار سویه از آلاگل و دو سویه از آلماگل گزارش شد. همچنین، نتایج بررسی‌ها نشان داد که دو سویه *Nostoc punensis* و *Anabaena* sp. در آلاگل و آجی‌گل به صورت مشترک و دو سویه *Dulcicalothrix alborzica* و *Neowestiellopsis persica* تنها در خاک منطقه آلماگل با بیشترین میزان EC حضور داشتند. سپس، دو سویه *Nostoc punensis* و *Desmonostoc salinum* که دارای بیشترین تغییرات ریخت‌شناسی در محیط کشت بودند، جهت ادامه مطالعات انتخاب شدند و این تغییرات در دو محیط کشت Z8 وBG-11 فاقد نیترات از زمان تلقیح تا پایان مرحله ایستایی رشد اندازه‌گیری گردید. نتایج حاصل از بررسی تغییرات ریخت‌شناختی نشان داد که سلول‌های رویشی و آکاینت هر دو سویه در محیط کشت BG-11 مارپیچ، بزرگ و هتروسیست‌ها استخوانی شکل شدند، اما شکل طبیعی خود را در محیط کشت Z8 حفظ کردند. بر این اساس، پیشنهاد می‌شود از محیط کشت Z8 به عنوان محیط کشت ترجیحی برای حفظ ثبات سلول‌ها و تغییرات ریخت‌شناختی کمتر استفاده شود.

واژه‌های کلیدی: آنالیز مورفومتریک، تنوع ریخت‌شناسی، سیانوباکتری‌های هتروسیست‌دار، محیط کشت Z8

Introduction

Soil algae including cyanobacteria have been employed widely in agronomical biotechnology. The great importance of heterocystous cyanobacteria is their role in the fundamental process of biological nitrogen fixation, which has great agronomical importance (Nowruzi *et al.* 2021).

Cyanobacteria are an important component of saline soils, including the surface crust that sometimes covers extensive areas in semiarid regions and mine spoil wastes (Whitton 2003). They are also abundant in many areas, which are wet or submerged for part of the year, especially rice-fields. Most cyanobacteria strains have sheaths or mucilage and these exopolysaccharides has important effects on the saline soil, mostly beneficial, such as improved saline soil structure, but sometimes adverse where a dense surface layer impedes drainage (Nowruzi *et al.* 2013). Cyanobacteria, are also added to the saline soils as bio-fertilizers (Rocha *et al.* 2020) that cause improving the soil condition by increasing its nitrogen, agglomeration of the particles, and retaining its water (Piotrowski *et al.* 2016, Singh *et al.* 2016, Abioye *et al.* 2021). Taxonomy of cyanobacteria has been a subject of contradictory among the scientists and it is necessary to consider the relevant problems more precisely. Part of the problem concerns with the growth of different isolates on various culture media on which their morphology changes, so that decision on characteristic traits of cyanobacteria may lead to a vague (Mareš *et al.* 2019). Therefore, it is necessary to study the morphological variation of the species on different culture media toward providing most proper keys as well as determining the most stable culture media for each isolate to meet less morphological variation. Totally, their morphological traits as gelatinous sheath, presence or absence of specialized cells like akinetes, heterocysts and the shape of apical cells of trichomes, and the size of vegetative cells are employed in taxonomic keys to identify them (Bongale 2002).

The growth and establishment of cyanobacteria in a given habitat are generally influenced by physical and chemical factors such as soil texture, humidity, pH, and amount of soil nutrients (Zhang *et al.* 2011). These factors can influence the distribution, frequency, and abundance of heterocystous cyanobacteria (Nisha *et al.* 2007). Despite the many studies, it is still difficult to comment on the importance of different environmental factors and any selective effect these may have on the occurrence or abundance of particular species (Bertos-Fortis *et al.* 2016).

Srivastava *et al.* (2008) found that, low salinity favoured the presence of heterocystous cyanobacteria, while very high salinity mainly supported the growth of non-heterocystous genera. High nitrogen content in the low salt soils is proposed to be a result of reduced ammonia volatilization compared to the high salt soils. Although many environmental factors could potentially determine the microbial community present in these multidimensional ecosystems, changes in the diversity of cyanobacteria in rice fields was correlated to salinity (Srivastava *et al.* 2008). However, studies on cyanobacteria of soils around lakes of Golestan province (north of Iran) in different culture media of Iran have been neglected (Etemadikhah *et al.* 2017). Despite the important role of cyanobacteria few investigations have been carried out on their taxonomy in soils around Ala gol, Aji gol, and Alma gol lakes of Golestan province. To approach the use of these microorganisms in agronomical biotechnology preliminary studies of their distribution and taxonomical status in the soils of the area are necessary (Nowruzi *et al.* 2012). In this study, the relationship between the presence of heterocystous cyanobacteria and the amounts of macro elements, EC, and pH of the soils around of three lakes of Ala gol, Aji gol, and Alma gol have been investigated. Moreover, morphological variation of two cyanobacteria strains were observed on two different culture media Z8 and BG-11. The preferred media of better growing and less variation of the strain is suggested.

Materials and Methods

- Collection, isolation, and purification

Since Sept. to Dec. 2020, three distinctly soils samples of Ala gol, Aji gol, and Alma gol lakes of Golestan province (34° 24' 32" N, 47° 00' 17" E) situated in north of Iran were collected. These areas are located at agro-ecological zones of the Golestan province (north of Iran). Since the presence of vegetation cover, affects the light intensity in agricultural zones, it is needed to explain the general condition of sampling sites. Sampling was carried out in autumn where the crops had been harvested and the fields had not been irrigated for two months and no fertilizer had been added to the Ala gol soils. Aji gol soils had not also received any fertilizers and some debris was left on the land following the harvest. However, a deep layer of humus had covered the Alma gol soils. In autumn 2020, soil samples were collected from the surface of the soils up to five cm deep by using sterilized spatula after removing debris from the surface (Liu *et al.* 2014). Three replicates of each site were collected and soil samples were put in plastic bags and transferred to the laboratory.

- Macro element soil analysis

Soils were analysed for organic carbon, total nitrogen, available phosphorous, potassium, EC and pH following standard methods (Bogunovic *et al.* 2017). Data analysis are shown as charts in figure 3.

- Culture collection, colony count and identification

Soils were sieved and 5 gr of each sample was added to petri dishes containing sterilized liquid nitrate free BG-11 medium with pH 7.4 from which nitrogen was excluded (Rippka *et al.* 1979). Petri dishes were kept in growth chamber at 28 °C and 50–55 μmol photons m^{-2} s^{-1}, light intensity (Nowruzi *et al.* 2013). After two weeks grown colonies of cyanobacteria were visible in petri dishes and the number of colonies in each petri dishes were counted (Table 1).

Grown colonies (Fig. 1 A) were re-cultured in solid nitrate free BG-11 medium for their purification. After some growth for a week or two they were sub-cultured on agar plates (Fig. 1 B) and if found unialgal after further growth, were transferred to solid agar culture tubes. A set of slant cultures were also prepared of each species, which were kept in refrigerator (Nowruzi *et al.* 2019). Species were identified according to specific keys (Komárek 2016 & 2018). The list of the species from each ecosystem was presented (Table 1).

- Study of morphological variation

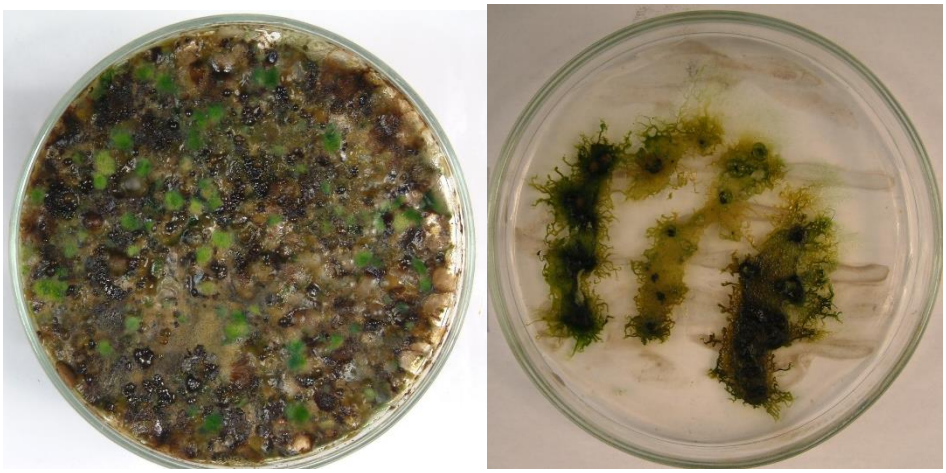
Two strains of *Nostoc punensis* (*N. punensis* Singh, sp. nov.) and *Desmonostoc salinum* (*D. salinum* de Alvarenga sp. nov.) had very variable morphology in culture media, that's why their morphological traits including their including their dimensions, presence of gelatinous sheath, the size of heterocysts, akinetes and the size of vegetative cells were measured on Z8 and nitrate free BG-11 medium. One gram of the colonies was transferred in to three conical flasks of 500 ml volume containing either modified. From day 1 to day 20 samples of each conical flask were studied under the microscope. Length and width of heterocysts, length and width of akinetes, length and width of vegetative cells of filaments in the samples were measured. The measurements were carried out in three replicates for each species grown in two culture media based on a factorial experiment (2 × 20 × 2), and a completely randomized design, where 2 shows the number of species, 20 represents days of experiment, and 2 shows the number of culture media, respectively. The results were analyzed by one-way ANOVA and compared by Duncan comparison test, and finally represented as dendrograms. The trend of increase or decrease of cell dimensions and frequencies of data were demonstrated using Excel software.

Results

- Colony count of cyanobacteria strains on soil fulfilled by liquid nitrate free BG-11 medium

Colony counts per 5 gr soils cultured by 20 ml liquid nitrate free BG-11 medium in Aji gol and Ala gol showed highest number (92 ± 3.71 and 72 ± 5.72) while Alma gol had the lowest colony number in liquid culture

media (54.8 ± 5.31). Results are Means ± SE. Morphological observation showed that, totally five different strains were identified in Aji gol, four different strains in Ala gol, and two different strains in Alma gol (Table 1).



A **B**

Fig. 1. Colonies of cyanobacteria grown on soil fulfilled by liquid nitrate free BG-11 medium (A) and on the solid nitrate free BG-11 medium (B).

Table 1. Representing indigenous heterocystous cyanobacteria strains identified in Aji gol, Ala gol, and Alma gol

Alma gol	Ala gol	Aji gol
Dulcicalothrix alborzica	*Nostoc punensis*	*Aliinostoc persicum*
Neowestiellopsis persica	*Anabaena* sp.	*Nostoc punensis*
-	*Desmonostoc salinum*	*Anabaena* sp.
-	*Fischerella* sp.	*Fischerella* sp.
-	-	*Desmonostoc salinum*

- Morphological characterization

A. *Dulcicalothrix alborzica*: The filaments appear curved. Trichomes with a firm sheath, not ending into hairs, sometimes opened at the apical end or in the middle (a). In some cases, hormogonia separate from the rest of the trichomes by a necridial cell which detaches to move smoothly and continuously along of the sheath. The cells show a marked tendency to aggregate into filaments (b).

B. *Aliinostoc persicum*: Heterocytes (a), vegetative cells (b), and akinetes cells (c).

C. *Neowestiellopsis persica*: Microscopical observation of the materials allowed the identification of long true branching filaments typical of the *Hapalosiphonaceae* family.

With increasing age, there are significant increases in the number of main and branching filaments terminating in an empty sheath (a), and intercalary heterocyst (b). Intercalary cylindrical and subspherical or even compressed (shorter than broad) heterocysts could be find more on branches, near the branch and main filamentous, D. *Desmonostoc salinum*: At the onset of incubation, variously-sized hormogonia tended to prevail (a). Degenerate vegetative cells, which eventually disintegrated or became detached from the filaments, resulting in filament fragmentation (b). Detached heterocysts are as single (c) or attached together as a group (d) were also observed. Akinetes was found in the middle of filaments in chain (e), E. *Anabaena* sp.: Heterocytes (a) in the filaments, F. *Fischerella* sp.: Heterocytes (a) and vegetative cells (b) in the filaments, and G. *Nostoc punensis*: Heterocytes (a), vegetative cells (b), and akinetes cells (c) (Fig. 2 A-G).

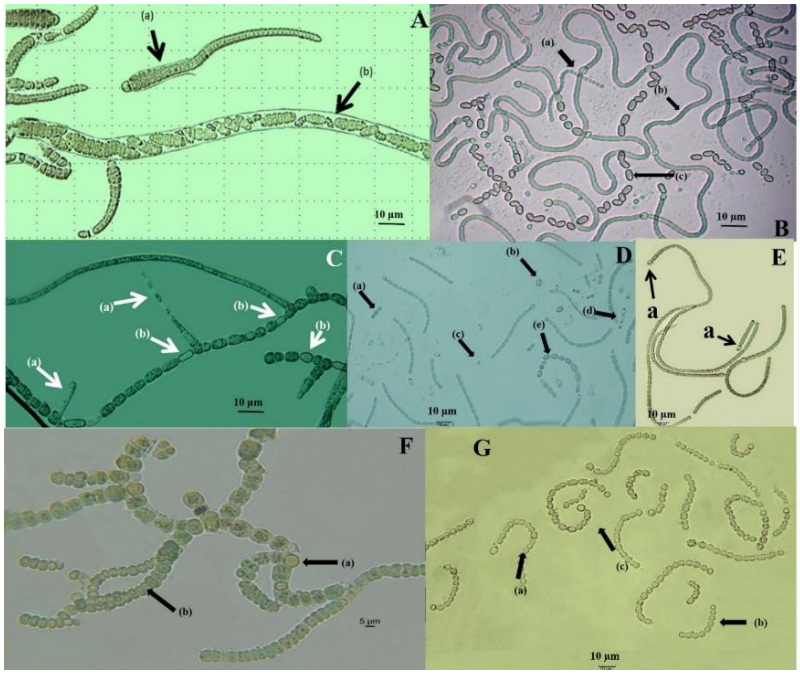


Fig. 2. Different light micrographs (A-G) of cyanobacteria strains. Details are indicated with lowercase letters according to the text: A. *Dulcicalothrix alborzica*, B. *Aliinostoc persicum*, C. *Neowestiellopsis persica*, D. *Desmonostoc salinum*, E. *Anabaena* sp., F. *Fischerella* sp., G. *Nostoc punensis.*

- Results of analysis of micro-elements

Analysis of N, P, K, EC, pH, and organic carbon of the soils showed that, the number of species and colonies had high positive correlation with the levels of available phosphorus, potassium, carbon, and nitrogen but were not highly correlated with EC and pH. *Nostoc punensis* and *D. salinum* had high frequency only in Alma gol with the highest level of EC and lowest rate of phosphorus, potassium, carbon and nitrogen. The Aji gol soils which had a higher level of phosphorus, potassium, carbon and nitrogen, than other sites but differed mainly in diversity of cyanobacteria (Fig. 3 A-E).

- Results of data analysis of morphological variation

Data analysis of morphological traits of *Nostoc punensis* and *Desmonostoc salinum* measured during 20 days showed significant difference between cell dimensions in different days. Results obtained from Duncan comparison test showed that, the fluctuations of heterocysts and akinetes diameters and length and diameter of vegetative cells of *N. punensis* were higher in modified nitrate free BG-11 medium than in Z8's while morphological variations of the length and diameter of Akinetes and the length of vegetative cells of *D. salinum* were higher in Z8 medium than in nitrate free BG-11 medium. In general, *N. punensis* showed more morphological changes in nitrate free BG-11 medium but *D. salinum* showed more changes in Z8 culture media (Table 2). The results of variations of the measured dimensions are represented as dendrograms of each species grown in two culture media, some of dendrograms were showed (Fig. 4 A-H).

The variations are grouped in separate clusters that indicate the presence of variation during the life cycle of the species. In general, with increasing culture days and decreasing nutrients in culture medium, cell size decreases significantly, however, morphological changes in cell dimensions in Z8 culture medium are much less noticeable. In addition, the results obtained from the frequency of cell dimensions showed that this rate is more frequent in the early growth period and before reaching the static phase than other days. Measurements of the dimensions of *N. punensis* and *D. salinum* showed decline in the size of all dimensions (Fig. 5 A-H). The results obtained from the frequency of the cellular dimensions are represented in figure 6.

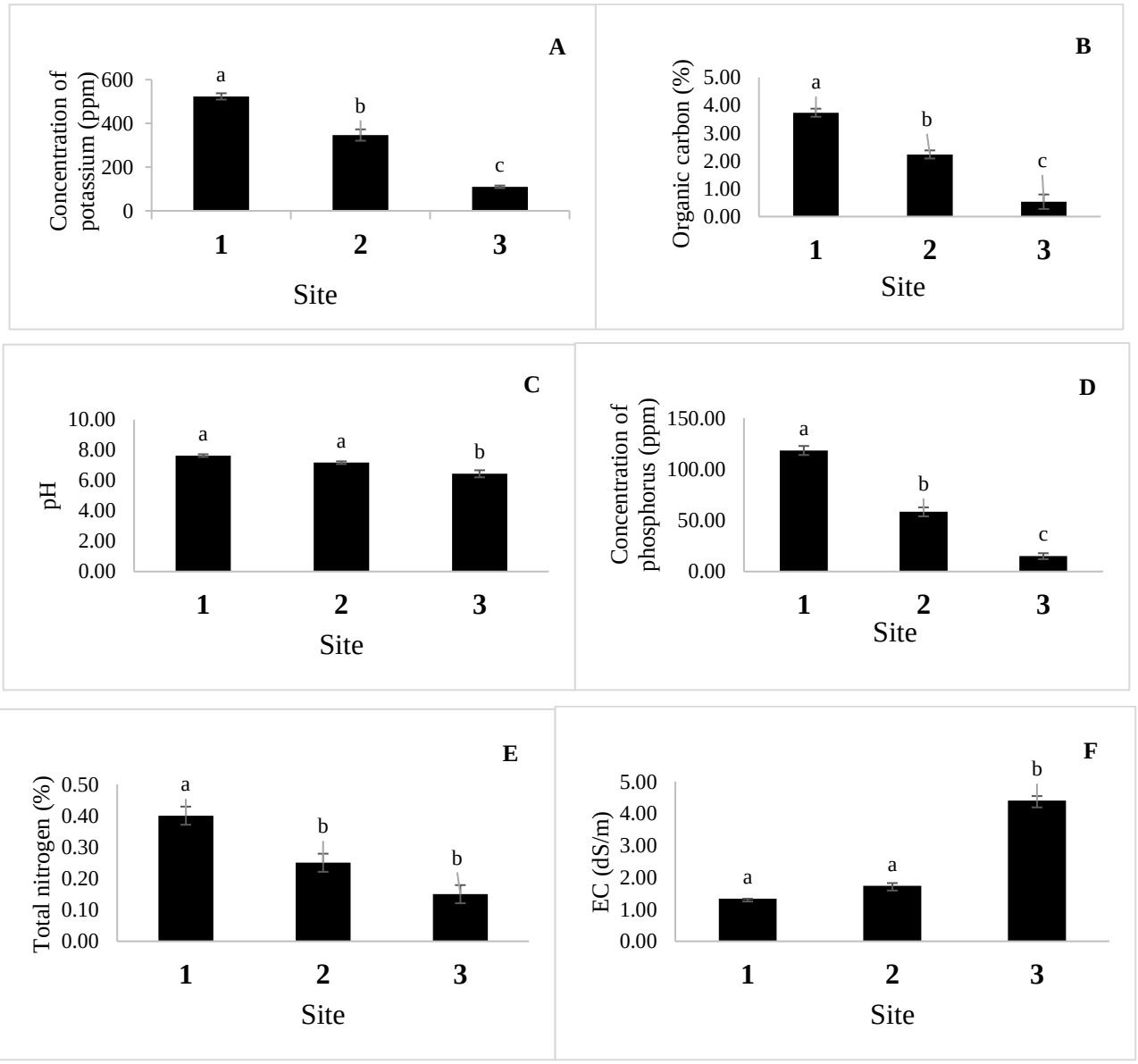


Fig. 3. Charts indicate the amount of micro-elements (A-F) in the soils of Aji gol (Site 1), Ala gol (Sites 2), and Alma gol (Sites 3): A. Potassium, B. Organic Carbon (%), C. pH, D. Concentration of phosphorus (ppm), E. Total nitrogen (%), F. EC (dS/m). Each column has an average of at least three repetitions. SE error lines are averages. Columns with similar letters are not significantly different from each other (P<0.05).

Table 2. The results of one-way ANOVA of parameters measured in *Nostoc punensis* and *Desmonostoc salinum* grown in modified Z8 and nitrate free BG-11 medium (p<0.05). H.D = heterocyst diameter, H.L = heterocyst length, A.L = Akainet length, A.D = Akainet diameter

Mean square							
***D. salinum* grown in modified nitrate free BG-11 medium**		***N. punensis* grown in modified nitrate free BG-11 medium**		***D. salinum* grown in modified Z8 culture medium**		***N. punensis* (grown in modified Z8 culture medium)**	
*4.491± 0.317	H.L	*5.032±1.346	H.L	*5.482±1.381	H.L	*7.418 ±1.413	H.L
*35.015±1.887	H.D	*6.301±0.516	H.D	*0.572±1.580	H.D	*0.337±1.167	H.D
*11.331±0.636	A.L	*16.098±1.984	A.L	*2.200±0.268	A.L	*2.137±0.351	A.L
*1.529	A.D	*2.140±0.247	A.D	*0.800±1.884	A.D	*1.952±0.517	A.D

* Significant difference

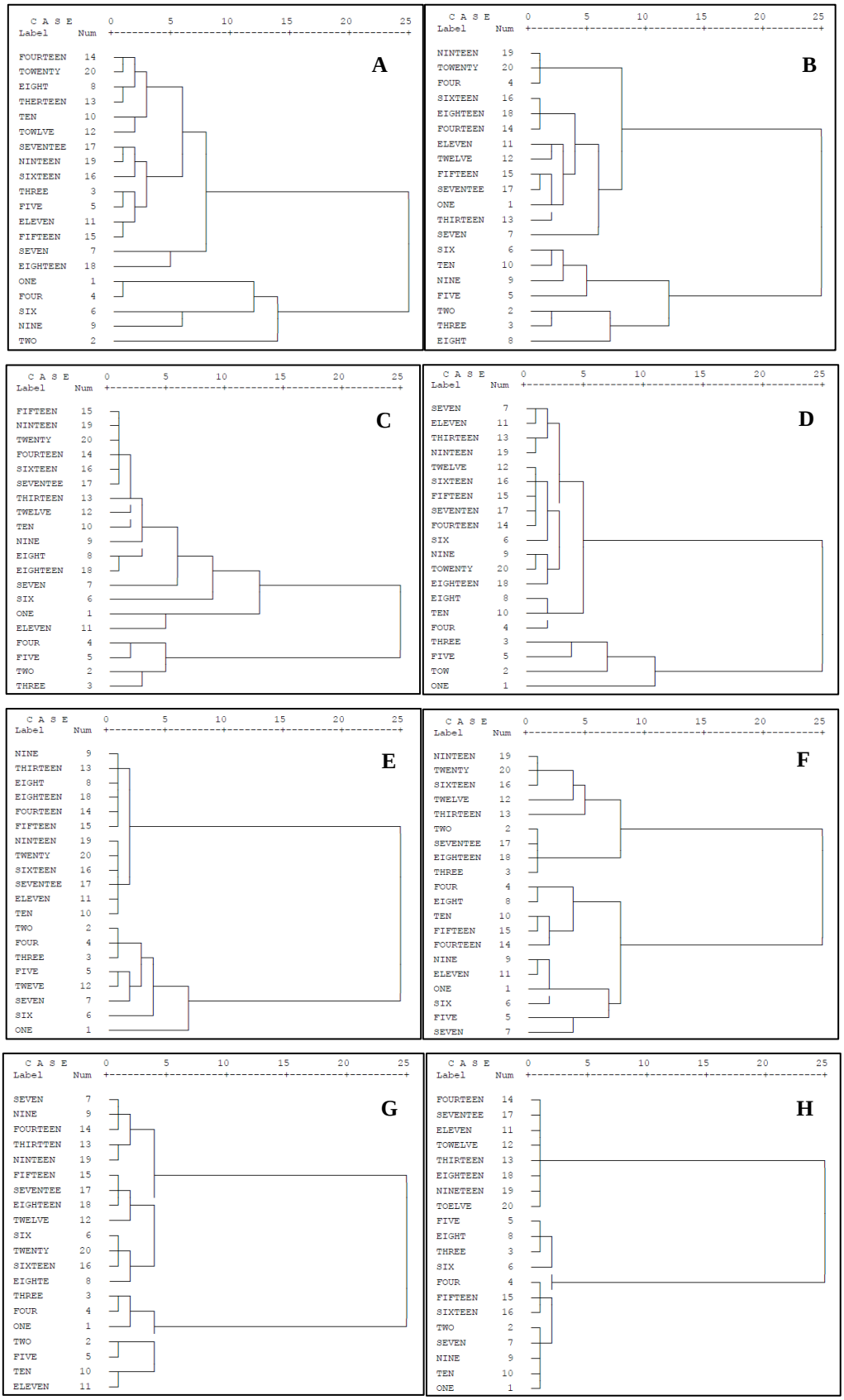


Fig. 4. Denderogram representing the relationship between different growing days (A-H) of cyanobacteria strains: A. Length of heterocysts of *Nostoc punensis* grown in nitrate free BG-11 medium, B. Width of vegetative cells of *N. punensis* grown in Z8 culture media, C. Length of akinetes of *Desmonostoc salinum* grown in Z8 culture media, D. Width of heterocysts of *N. punensis* grown in Z8 culture media, E. Width of akinetes of *D. salinum* grown in nitrate free BG-11 medium, F. Length of vegetative cells of *N. punensis* grown in nitrate free BG-11 medium, G. Length of heterocysts of *D. salinum* grown in nitrate free BG-11 medium, H. Width of akinetes cells of *N. punensis* grown in nitrate free BG-11 medium.

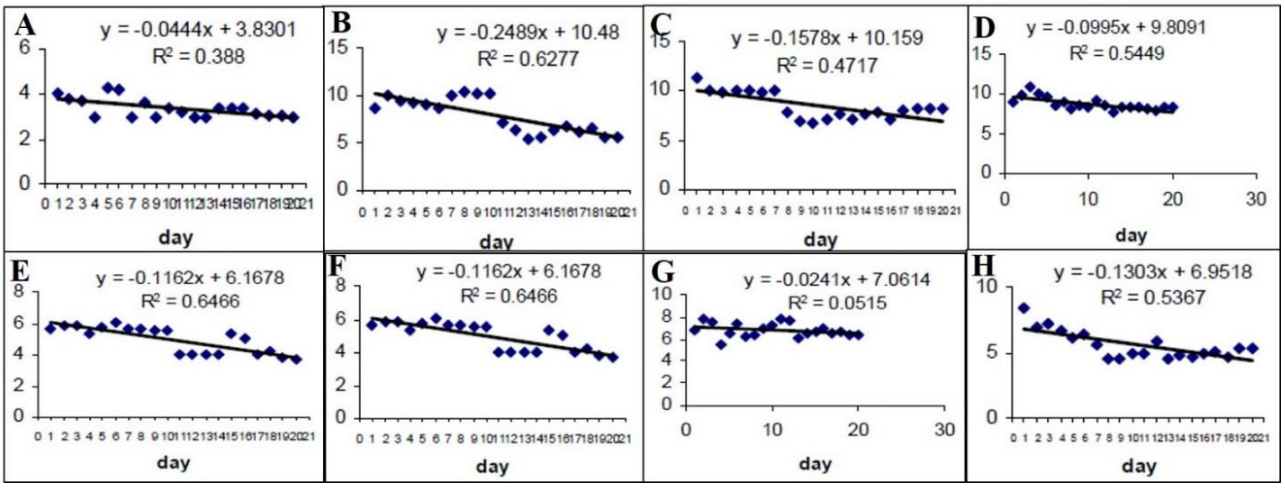


Fig. 5. Liner charts represents the decrease of cell's dimensions until the end of stationary phase (A-H): A. Length of heterocysts of *Nostoc punensis* grown in nitrate free BG-11 medium, B. Width of vegetative cells of *Nostoc punensis* grown in Z8 culture media, C. Length of akinetes of *Desmonostoc salinum* grown in Z8 culture media, D. Width of heterocysts of *Nostoc punensis* grown in Z8 culture media, E. Width of akinetes of *Desmonostoc salinum* grown in nitrate free BG-11 medium, F. Length of vegetative cells of *Nostoc punensis* grown in nitrate free BG-11 medium, G. Length of heterocysts of *Desmonostoc salinum* grown in nitrate free BG-11 medium, H. Width of akinetes cells of *Nostoc punensis* grown in nitrate free BG-11 medium.

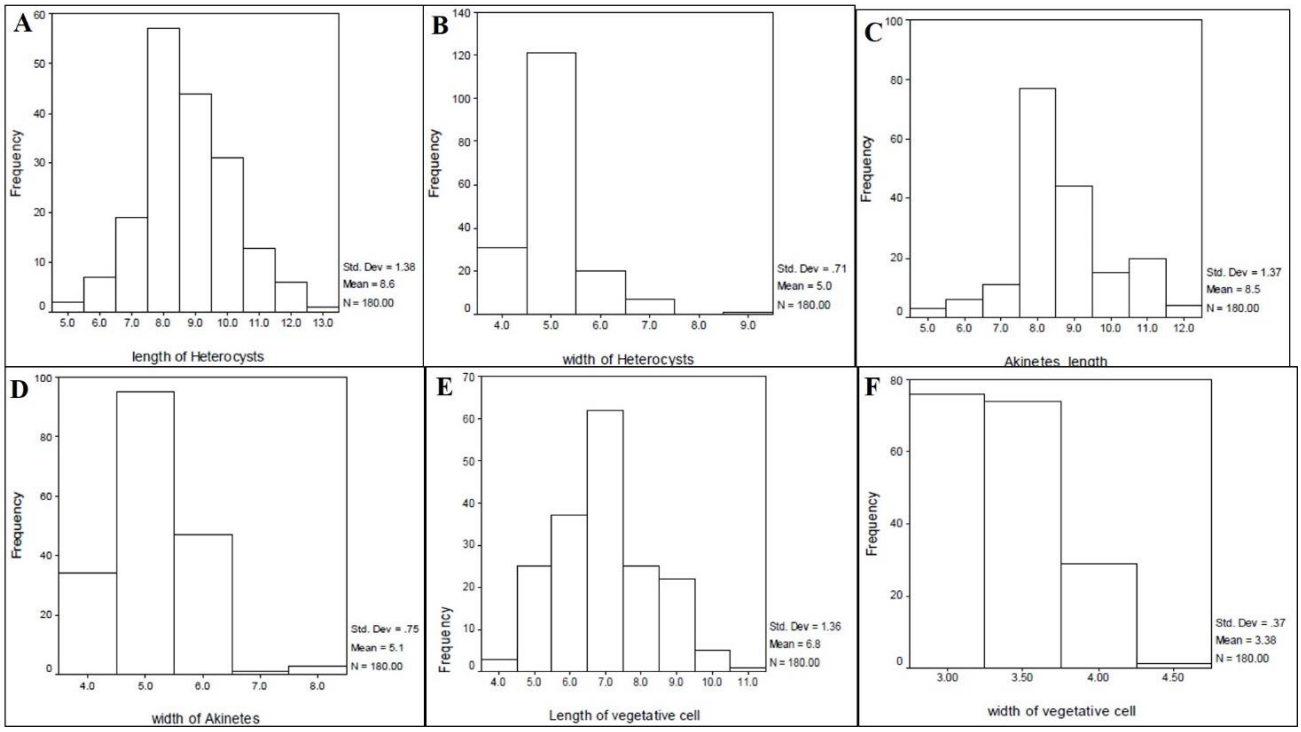


Fig. 6. Histograms represents the frequency of *Nostoc punensis* in Z8 culture media: A. Length of heterocysts, B. Width of heterocysts, C. Length of akinetes, D. Width of akinetes, E. Length of vegetative cells, F. Width of vegetative cell.

Discussion

Different cyanobacteria strains were recorded in all studied soils but the populations varied markedly due to the changes in soil properties while Aji gol lake had the highest number of heterocystous cyanobacteria strains. Many investigations have shown that, environmental conditions such as humidity, mineral, availability including N, P, K, and C amounts of the soil, pH etc. are limiting factors in cyanobacteria distribution (Singh *et al.* 2018). The comparison of the species between three soils of Ala gol, Aji gol and Alma gol lakes showed that, some of species are presented only in Aji gol. These species seems to have preferences for the Aji gol soils, which provides a favourable environment for the growth of cyanobacteria strains with respect of their requirement for high water and nutrient available (Mischke 2003). This may be accounted for the higher abundance of cyanobacteria strains in paddy fields than other cultivated soils. It is obvious that, interactions between various environmental conditions cause changes in the structure of cyanobacteria communities. For instance studies of algal flora of some Indian paddy fields have shown that, a kind of successional stages happens that affects the frequency of different species.

During the rainy season *Aphanothece*, *Microcoleus*, *Aulosira*, and some species of *Scytonema*, *Cylindrospermum* cover the fields surface, which are followed, by species of *Microcoleus* together with *Anabaena*, *Tolypothrix* and *Fischerella*, and after that, *Aulosira fertilissima* becomes the dominant species (Mehda *et al.* 2021). Although, many investigations have been carried out on cyanobacteria taxonomy and ecology, less attention has been paid to their distribution in soils around Ala gol, Aji gol, and Alma gol lakes. Totally, among the identified genera, *Nostocaceae* family had the highest frequency. Our autumnal results in all sites showed that, species of *Nostoc punensis* and *Anabaena* sp. have been reported to be ubiquitous in Ala gol and Aji gol except to Alma gol where its EC was significantly different and the amount of C, N, K, and P were lowest. This extreme condition may be the reason for the existence of *Dulcicalothrix alborzica* and *Neowestiellopsis persica.* It is suggested that, these strains are probably resistance to high EC. It has been indicated that, the abundance of cyanobacteria can affect the EC of the soils so that, their population increases the EC declines. For instance it has been reported that, cyanobacteria take-up sodium from the soil which is subsequently metabolized in the body cells and as the result sodium concentration decreases which might be one of the reasons for the reduction of EC by isolated cyanobacteria (Green *et al.* 2017). The lower diversity of species in Alma gol can also be addressed to the deficiency of phosphorus and carbon where other species of cyanobacteria are not able to cope with the deficiency.

There was not any correlation between number of species and number of colonies with the amount of to have either no effect or a depressive effect on algal growth (Garcia *et al.* 2015). The amount of N in paddy fields is usually correlated with the abundance of cyanobacteria strains. This can be due to the release of nitrogen from cyanobacteria during their autolysis. More than 50% of the fixed and released nitrogen is used by rice (Irisarri *et al.* 2001). In general, diversity of cyanobacteria strains in Alma gol was very low and branching species was found on this ecosystem. In addition, branched species had lower distribution in Ala gol and Aji gol than non-branched species. The scarcity of N_2, fixing cyanobacteria strains in Alma gol can be probably limited by low pH in comparison to other soils. Among the soil properties, pH is certainly the most important factor determining the algal flora composition. Study of pH in different soils showed that, Ala gol and Aji gol with pH between 7.63–7.17, have higher variety and frequency of heterocystous cyanobacteria comparing to Alma gol (pH = 6.43). Under alkaline conditions, cyanobacteria strains grows preferably in neutral environment with neutral pH (Waterbury 2006).

From field observation in this study it was inferred that, most cyanobacteria strains comparing other to preferred an alkaline or near neutral pH (7.83) but in the Alma gol, branching strains were capable of thriving

over a lower range 6.43. The most decisive factor next to pH was phosphorous content. Phosphorous plays a major role in the most cellular process particularly those involved in energy transfer and in nucleic acid synthesis. The role of phosphorous in algae metabolism, has also attractive the attention of ecologist since this element is frequency limited for algae growth in nature (Wu *et al.* 2012) also reported a positive relationship between available phosphorous and cyanobacteria population in some Bangladesh soils. The results obtained here are compatible of theses indications. However, it is important to evaluate the quantitative features of N fixing in different species concerning with the environmental conditions.

Morphological variations occurred in both species but of *Nostoc punensis* on nitrate free BG-11 medium was more than on Z8's, while variations of *Desmonostoc salinum* was observed more on Z8 than nitrate free BG-11 medium. These results can be explained on the basis of genetic differences between two species and suggests more studies to be carried out on this area. Identification of the genes responsible for permanent or fixed morphological characteristics is needed.

In addition, more study should be done with cultures generated from single cells because the genome of the cells in the strands used as inoculums is not clear to be unique. The variations showed another aspect where different clusters of dendrograms were formed at different intervals for different measured characters. This situation may be due to the reflection of both genetically differences of the cells and independence of the genes responsible for the traits. However, Z8 culture medium is richer than nitrate free BG-11 medium both in case of macro- and micro-nutrients, the condition that can influence cells growth and morphology. Investigations on the effect of environmental factors including the nutrients on cyanobacteria morphology in natural habitats and comparing such results with the results of growing them on artificial conditions, may lead to more satisfactory outcome for providing confidential basis to prepare new keys.

The results of statistical analysis also showed a decrease in the size of dimensions of the cells until the end of stationary phase. However, the decrease was more or less according to the different characters measured. It has been indicated that, during the stationary phase the number of the cells increases and deficiency of light, CO_2 and photosynthetic pigments happens. At this condition, the ability of the cells in assimilation of nitrogenous compounds declines and cyanophycin granule of the cells, which are cyanophycin and phycocyanin, are consumed by the cells and subsequently the cell size decreases.

It has been proved that, adding the nutrients into the medium affects both the size and the growth rate of the cells. Other factors such as light and temperature influence on these characters. Studies has showed the relation between temperature and the decrease of cell size in subspecies of *Anabaena* (Giordanino *et al*. 2011). These studies revealed that, at different temperature regimes the cells elongate and as temperature changes decrease of the width of the cells is more evident than their length. In other studies Carey *et al.* 2012 showed that, morphological variations of some species of cyanobacteria was influenced by light intensity and at low intensities the length of the cells increased but their width decreased (Carey *et al.* 2012). It is needed to investigate the dependence of cell size on environmental factors in natural conditions, where changes in altitudes and latitudes may influence morphological variations in cyanobacteria via different light intensities, which can lead to adaptations of these organisms to different habitats (Makhalanyane *et al.* 2015).

Changes of the forms of the heterocyst into bony and banana shapes in nitrate free BG-11 medium observed in this study are remarkable signs for choosing the convenient culture media with more stability. Hence, Z8 culture media is suggested as the superior medium. It is also suggested cyanobacteria to be grown and studied in both solid and liquid culture media toward writing taxonomic keys and to avoid morphological variations of the species as little as possible.

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