Diversity of Thermophilic Cyanobacteria in Maragheh Mineral Springs and Variable Environmental Factors

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Abstract

Cyanobacteria or blue-green algae are photosynthetic microorganisms, found in several habitats. Hot springs are considered as extreme habitats with "sub-cosmopolitan" geographical distribution, the biodiversity of which is unique due to their special conditions. Over the last century, these natural inhabitants have attracted the attention of biologists and tourists. In the present study, cyanobacteria were collected and identified from five hot and mineral springs located in East Azarbaijan province, Maragheh city during 2019. In this regard, the spring water was analyzed physic-chemically, the effect of some environmental parameters on algal communities was assessed, and the samples were fixed in 4% formaldehyde solution. Based on the results, 30 species were detected, which the highest and least diversity of cyanobacteria were respectively observed in Isty Bulakh (15 species) and Ghare Palchigh spring. In addition, *Phormidium* sp. possessed the maximum diversity among all of the identified taxa. Finally, environmental factors such as total dissolved solids (TDS) and turbidity were determined among the factors affecting biodiversity.

Keywords: Cyanobacterial diversity, Hot spring, Maragheh, *Phormidium* sp., Physico-chemical parameters

Introduction

Cyanobacteria are a group of ancient photosynthetic organisms with 2.8 billion years age (Dadheech et al., 2013). Due to the high compatibility of the microorganisms, they are found in extreme habitats such as saline waters and hot springs (Mishra et al., 2018). In general, they are considered as free-living microorganisms, which occur in symbiotic association with some eukaryotic plants, algae, fungi, gymnosperms, pteridophytes, angiosperms, and bryophytes, as well as the lower group of animals like ascidians (Anand et al., 2019). Additionally, the microorganisms can play an important role in atmospheric nitrogen fixation (Jeevanantham et al., 2019). The use of cyanobacteria, as an environmental indicator, is suggested during the recent decade (Barinova et al., 2017). In general, cyanobacteria are studied in aquatic ecosystems for various purposes

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(Dubey, 2019) such as floristic, ecological, physiological, or applied phycological ones. Thermo-mineral springs are a special group of aquatic ecosystems with particular characteristics. The aquatic ecosystems are highlighted as interesting habitats for evaluating algal microflora including the cyanobacteria, which are often plentiful and can live in different ecological niches (Singh et al., 2018). The issue makes them an appropriate candidate for different uses such as industrial processing, bottled water, power production, and health and well-being sector (Lai et al., 2019). Therefore, academic communities highlighted the examination of the natural resources significantly over the past decades (Durowoju et al., 2018).

Cyanobacteria have been detected in the thermal-mineral springs of several countries such as China (Tang et al., 2018), Sri Lanka (Medhavi et al., 2018), India (Singh et al., 2018), Russia (Gorlenko et al., 2019), Ireland (Shiels et al., 2019), Saudi Arabia (Yasir et al., 2019), Kenya (Ngetha et al., 2019), Brazil (Ramos et al., 2019), Indonesia (Prihantini et al., 2018), Turkey (Yilmaz-Sariozlu and Yilmaz-Cankilic, 2018), Australia (McGregor and Sendall, 2017), Pakistan (Amin et al., 2017), Japan (Martinez et al., 2019), Africa (Maree et al., 2018), and Iran (Heidari et al., 2013, 2018). In Iran, 149 springs exist in Sarab and Bostanabad, and 141 ones are found in Tabriz and Azarshahr area. Maragheh, as one of the most important tourist areas, is located in the northwest of Iran, one of the natural attractions of which includes the presence of several hot springs. So far, limited studies have been published regarding algal flora in East Azarbaijan. Thus, the present study aimed to assess cyanobacterial diversity in some of the springs in the area.

Material and methods

Study area and sampling sites

Samples were randomly taken from five thermal springs with different geographical situations located in Maragheh city, East Azerbaijan (Table 1, Fig. 1) during April-October 2019.

Further, benthic taxa were collected by scraping the algae from the bottom of the springs and the surfaces of marginal rocks, muddy sediments, aquatic plants, and macrophytes. All samples were fixed in 4% formalin, labeled, and transferred to laboratory in cool containers. In order to assess some of the physical and chemical properties of each sitre, 1.5 L of water without formalin was taken by using plastic bottles. Furthermore, the temperature was measured by using a mercury thermometer, and pH was determined using a portable pH meter WTW LF 320 EC meter, a Testo 320 pH meter, and a multi-parameter analyzer. The electrical conductivity, turbidity, alkalinity, TDS (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Cl^{-}, SO_4^{2-}, S^{2-}, NH_4^{+}, NO_3^{-}, NO_2^{-}) of water were determined by Arian Fan Azma Institute (WWW.AFA-Co.ir). Table 3 summarizes laboratory measurement methods.

Identification of cyanobacterial taxa

The semipermanent slides of colonies were prepared and morphometric study was performed by light microscopy (Olympus, Model BH-2) based on Desikachary (1959), Prescott (1970), Komárek and Anagnostidis (1986, 2005) and Komárek (2014). The most variable morphological characteristics were color and shape of colonies; color, shape and size of thallus, vegetative cells, apical cell shape,and presence or absence of mucilage sheath.

Results

Species diversity in Maragheh springs

In the present study, 30 taxa of cyanobacteria were detected and recorded from Maragheh springs, which the highest and least diversity was respectively determined in site two (Isty Bulakh) and five (Ghare Palchigh) with 15 and 5 species (Table 2, Fig. 2). In Oscillatoriales, *Phormidium* sp. with fila-

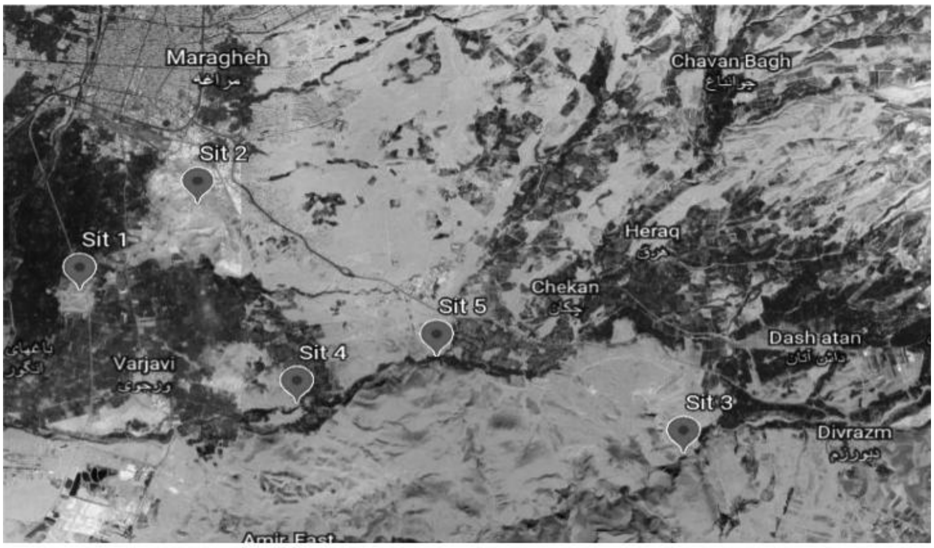

Fig. 1. Location of the springs examined in Maragheh. Sit 1 (Sari Sou); Sit 2 (Isty Bulakh); Sit 3 (Goshayesh); Sit 4 (Shour Sou); Sit 5 (Ghare Palchigh)

Table1. Geographical details of the sampling locations

Site No.	Spring name	Location	Height (m)	Temperature (°C)
1	Sari Sou	37° 20′ 57.3″ N 46° 13′ 38.3″ E	1390	45
2	Isty Bulakh	37° 20′ 14.8″ N 46° 17′ 38.4″ E	1493	31
3	Ghoshayesh	37° 23′ 12.7″ N 46° 14′ 41.1″ E	1579	25
4	Shour Sou	37° 19′ 46.1″ N 46° 16′ 5.6″ E	1495	19
5	Ghareh Palchigh	37° 21′ 52.4″ N 46° 14′ 58.0″ E	1432	28

Table 2. List of cyanobacterial species recorded from five hot springs in Maragheh

Taxon	Sit 1	Sit 2	Sit 3	Sit 4	Sit 5
Spirulina subsalsa Oersted ex Gomont	●			●	
Spirulina major Kützing ex Gomont	●				
Spirulina rosea Crouan			●		
Chroococcus turgidus (Kützing) Nägeli		●	●		
Chroococcus minutus (Kützing) Nägeli		●	●		
Chroococcus thermalis (Meneghini) Nägeli			●	●	
Chroococcus lithophilus Ercegovic		●	●	●	
Phormidum schroeteri (Hansgirg ex Hansgirg) Anagnostidis	●	●			
Phormidum lividum Nägeli in Kützing ex Gomont	●	●			
Phormidum cortianum (Meneghini ex Gomont) ex Komárek		●		●	
Phormidum toficola Gomont	●				
Phormidum lucidum Kützing ex Gomont	●	●			●
Phormidum articulatum (Gardner) Anagnostidis and Komárek		●			
Merismopedia sp.		●			
Merismopedia elegance A. Braun	●	●			
Merismopedia glauca (Ehrenb.) Nägeli		●		●	
Pseudanabena oblonga Kullberg	●			●	
Pseudanabaena limnetica (Lemmermann) Komárek		●			
Pseudanabaena firigida (Fritsch) Anagnostidis	●			●	●
Pseudanabaena minima (G.S.An) Anagnostidis					●
Geitlernema numidicum (Gomont) Anagnostidis			●		
Nodularia sp.			●	●	
Nostoc sp.			●	●	
Cyanabacterium minervae (Copeland) Komárek					●
Planktothrix isothrix (Skuja) Komárek et Komárková			●	●	
Planktolyngbya limentica (Lemmermann) Komárková - Leganerova & Cronberg		●		●	
Osillatoria subcapitata Ponomarev ex Elenkin		●			
Osillatoria prolifica (Gerv.) Gomont				●	●
Nostoc microscopicum Carmichael	●		●		
Gleocapsa punctate Nägeli		●			

Sit 1 (Sari Sou), Sit 2 (Isty Bulakh), Sit 3 (Goshayesh), Sit 4 (Shour Sou), Sit 5 (Ghare Palchigh)

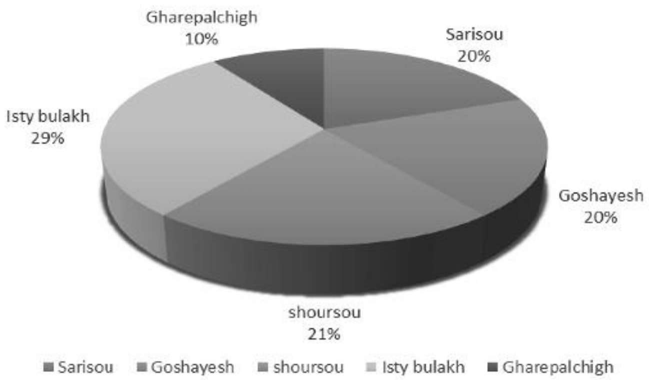

Fig. 2. Frequency percentage of cyanobacterial species in the studied mineral springs

mentous structure (six species) represented the maximum species diversity among the identified taxa.

Physicochemical parameters of water

Table 3 presents the results related to the physicochemical analysis of five springs in East Azerbaijan province (Maragheh city). The water physical parameters in aquatic ecosystems affect the life cycle and diversity of cyanobacteria. The factors provide accurate data for predicting the behavior of algae in aquatic ecosystems such as springs. Based on the results of the present study, water temperature varied between 19-45 ˚C so that the highest and least temperature was respectively observed in site one (Sari Sou) and four (Shour Sou). In addition, the maximum electrical conductivity, turbidity, pH, TDS, and salinity were obtained in Ghare Palchigh spring. Further, sulfate content was maximized and minimized in Ghare Palchigh and Shour Sou springs, respectively (Table 3).

The results indicated that a decrease in total dissolved solid (TDS) resulted in increasing the total hardness (TH), electrical conductivity (EC), and turbidity diversity of cyanobacteria. The results indicated that a decrease in total dissolved solid (TDS), the total hardness (TH), electrical conductivity (EC), and turbidity resulted in increasing diversity of cyanobacteria. Furthermore, the highest species diversity was determined in Isty Bulakh (TDS: 1380 $mg.L^{-1}$, TH: 721 $mg.L^{-1}$, Ca^{+2}: 437 $mg.L^{-1}$, Mg^{+2}: 284 $mg.^{L-1}$, Na^{+}: 79.9 $mg.L^{-1}$, K^{+}: 7.20 $mg.l^{-1}$, Cl^{-}: 65.3 $mg.L^{-1}$) and the lowest was recorded in Ghare Palchigh (TDS: 4310 $mg.L^{-1}$, TH: 1524 $mg.L^{-1}$, Ca^{2+}: 1011 $mg.L^{-1}$, Mg^{2+}: 513 $mg.L^{-1}$, Na^{+}: 690 $mg.L^{-1}$, K^{+}: 78 $mg.L^{-1}$, Cl^{-}: 891.8 $mg.L^{-1}$).

Discussion

The cyanobacterial diversity has been studied in several aquatic ecosystems of Iran, but there are limited reports on the diversity of these microorganisms in thermo-mineral springs of our country. Based on the results, the maximum diversity of taxa was obtained in Isty Bulakh at 31 ˚C. The thermophilic al-

Table 3. Physicochemical parameters in the studied springs

Parameters	Analytical Method	Scale	studied Sites				
			Site 1	Site 2	Site 3	Site 4	Site 5
Temperature	Laboratory and field	°C	45	31	25	19	28
EC	Platinum electrode	$\mu S.cm^{-1}$	4990	1825	3690	3260	6010
Turbidity	Nephelometric	NTU	51.5	0.50	3.37	3.23	187
pH	Electrometric	------	6.88	6.87	6.58	6.50	6.93
TDS	Electrical conductivity	$mg\ L^{-1}$	3680	1380	2815	2505	4310
TH	EDTA titrimetric	$mg\ L^{-1}$	1400	721	1570	1443	1524
Salt	Electrical conductivity	%	0.101	0.020	0.030	0.022	0.174
Ca^{2+}	EDTA titrimetric	$mg\ L^{-1}$	732	437	1057	960	1011
Mg^{2+}	EDTA titrimetric	$mg\ L^{-1}$	868	284	513	483	513
Na^{+}	Flame emission spectrometric	$mg\ L^{-1}$	401	79.9	117	85.9	690
K^{+}	Flame emission spectrometric	$mg\ L^{-1}$	57.30	7.20	16.25	10.20	78.00
OH^{-}	Titrimetric	$mg\ L^{-1}$	1765	730	1519	1463	1673
SO_4^{2-}	Turbidimetric	$mg\ L^{-1}$	208	93	186	70	380
Cl^{-}	Argentometric	$mg\ L^{-1}$	356.6	65.3	95.4	52.3	591.8
S^{2-}	Methylene blue	$mg\ L^{-1}$	3.28	-----	----	----	----
HCO_3^{-}	Titrimetric	$mg\ L^{-1}$	1765	730	1519	1463	1673

Sit 1. Sari Sou; Sit 2. Isty Bulakh; Sit 3. Goshayesh; Sit 4. Shour Sou; Sit 5. Ghare Palchigh; EC (Electrical Conductivity), TDS (Total Dissolved Solid), TH (Total Hardness).

gae can be divided into hypothermophilous (15-26 ˚C), mesothermophilous (26-45 ˚C), euthermophilous (45-65 ˚C), and hyperthermophilous (65˚C and more) communities. Additionally, the cyanobacterial taxa distributed in Sari Sou, Isty Bulakh, and Ghare Palchigh were mesothermophilous community, while those in Goshayesh and Shour Sou included hypothermophilous one. Temperature is an environmental factor which plays an important role in development of algal communities. Due to the impact of temperature on enzymatic reactions of living organisms, this environmental factor affects many critical biological mechanisms such as photosynthesis, respiration and cell division. In addition, temperature can also affect species diversity of algae in aquatic ecosystems (Aghashariatmadary et al. 2017).

Further, 30 cyanobacterial taxa were detected, and the results revealed that identified taxa belong to the several orders: Oscillatoriales (36.6%), Chroococcales (30%), Synechococcales (13.3%), Spirulinales (10%) and Nostocales (10%), respectively. Regarding Oscillatoriales, six species of *Phormidium* formed 20% of total taxa, which is the maximum diversity among the identified species. Accordingly, *Oscillatoria, Phormidium,* and *Lyngbya* genera seemingly adapted to the habitat and are the most dominant species of Maragheh springs. These cyanobacteria are filamentous and successful in mat-forming. In addition, species of the order Oscillatoriales appear in springs with the temperature ranging 25-45 ˚C (Pentecost, 2003, 2014) and their exopolysaccharide sheath is probably considered as the backbone of developed microbial mat (Abdelwahab and Amin, 2017).

The electrical conductivity of water indicates the amount of solutes in the water, which is a function of temperature and ions in the water. Among the studied stations, the lowest electrical conductivity is related to the station of Isty Bulakh with 1825 (µS cm^{-1}) and the highest electrical conductivity is related to Ghare Palchigh station with 6010 (µS cm^{-1}). Based on the results obtained in this study, there is an inverse relationship between EC and species diversity. This negative correlation between variability and EC was reported in some previous studies (Sheath and Cole,1992).

Moreover, salinity reduces productivity (Srivastava et al., 2005), as well as the phylogenetic diversity of some cyanophytes (Wang et al., 2011). According to the water analysis report, highest water hardness was 180 mg/L and classified as hard water (Li et al., 2017).

According to World Health Organization (WHO) standard, the highest desirable and permissible levels of water calcium content are 75 and 200 $mg.L^{-1}$, respectively (Cotruvo and Bartram, 2009). In the present study, the calcium content ranged 437-1057 $mg.L^{-1}$ in Maragheh springs, which is higher than the level of WHO standard (200 $mg.L^{-1}$) (Daghara et al., 2019).

Sulfate is relatively non-toxic, the range of which in the drinking water is up to 200 $mg.L^{-1}$ so that the level more than 500 $mg.L^{-1}$ produces a bitter taste (Gaglioti et al., 2019). Regarding the present study, sulfate level was determined 70-380 $mg.L^{-1}$ in Maragheh springs, while that of Ghare Palchigh and Sari Sou was obtained 380 and 208 $mg.L^{-1}$, respectively. Thus, the water of Ghare Palchigh and Sari Sou is not considered as suitable for drinking because of having sulfate content above the WHO standard level.

Based on the guideline for the chloride level allowed in water, the chloride range is set between 0 to 142 $mg.L^{-1}$, which is appropriate for irrigation, while the level above 355 $mg.L^{-1}$ is inappropriate for irrigation. Finally, the chloride content of the springs under study was measured 52.3-591.8 $mg.L^{-1}$. Consequently, the springs of Ghare Palchigh and Sari Sou are non-suitable for irrigation (Shafikhani et al., 2019).

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