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DISTRIBUTION OF CYANOBACTERIA IN TWO SIRCH HOT SPRINGS WITH REGARDS TO THE PHYSICOCHEMICAL TRAITS OF WATER

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Abstract. Cyanobacteria live in diverse ecosystems including hot springs. In the present study, cyanobacterial flora of two hot springs located in Kerman, Iran was investigated for the first time. After sample collection, species were identified based on their morphological traits. To understand the fundamental patterns of species distribution; Physico–chemical environmental factors and species frequency were measured using standard methods and thereafter Canonical Correspondence Analysis (CCA) of species frequency and environmental factors was carried out by Canoco program. Twenty cyanobacteria were identified. Results showed an ordered distribution of the species along the environmental factors. Results also showed the presence of mesothermophilous and facultative thermophile cyanobacteria in types of unicellular and filamentous in the two studied springs. Results also suggest that some species are adapted and resistant forms to sulfide. Diazotrophic strains were found to align in lowest compound nitrogen level, where others were removed from competition.

Keyword: Cyanobacterial flora, *Aphanocapsa*, Kerman, Sirch hot spring.

Introduction

Cyanobacteria are widespread organisms found from Polar Regions to hot water springs and can photosynthesis and live in harsh environments [BRIAN and WITTHON, 2002]. It has been indicated that today's hot springs environment are similar to the conditions of Precambrian era, time of emergence of cyanobacteria.

Conditions such as high temperature, low free oxygen levels and high concentrations of sulfur [CASTENHOLZ, 1969].

Long evolutionary history of cyanobacteria caused their high tolerance to harsh environmental conditions [MILLER and CASTENHOLZ, 2000].

The hot springs also have been identified as a great source of heat resistant enzymes such as DNA polymerases, proteases and amylases [VIEILLE and ZEIKUS, 2001].

The discovery of new and endemic species could be a step to investigate on such issues.

Cyanobacteria of world hot springs have been studied by many researchers [CASTENHOLZ, 1969; SOMPONG *et al.*, 2008; WARD *et al.*, 1998]. There are many thermal springs in Iran and few investigations have been carried out on their cyanobacterial flora [GHADI et al., 2012, HEIDARI et al., 2014].

Only in Kerman Province, there are 11 known thermal springs with volcanic and fault origins.

The aim of this study was to investigate the cyanobacterial flora of Sirch hot springs through collecting and their identification on axenic cultures and to consider the relationship between species distribution and physical and chemical environmental factors by CCA.

Material and methods

Sirch hot springs are located in 50 Km of southeast of Kerman city center, Iran with coordinates of 30° 09´ 44.2˝ N and 57° 35' 46.5˝ E and the second placed in 30° 09 ' 43.3˝ N and 57° 35 ' 53.4˝ E (Figure 1).

They are formed due to tectonic activities caused the appearance of hot water flow through Gowk (Golbaf) fault as springs [HASHEMI and NEGARESTANI, 2010].

These springs have got therapeutic benefits and are a source of tourist attractions. Phytobenthos and periphyton cyanobacterial samples were collected from bottom and walls of each spring in a 15 x 15 cm quadrate following Debnath and Mandal [DEBNATH and MANDAL, 2009].

Four sampling sites of each spring were selected and three replicates were collected from each site.

The samples were kept in containers separately and transferred to Lab. Samples of collected cyanobacteria were grown on BG11 culture medium according to Padmasree [PADMASREE, 2011].

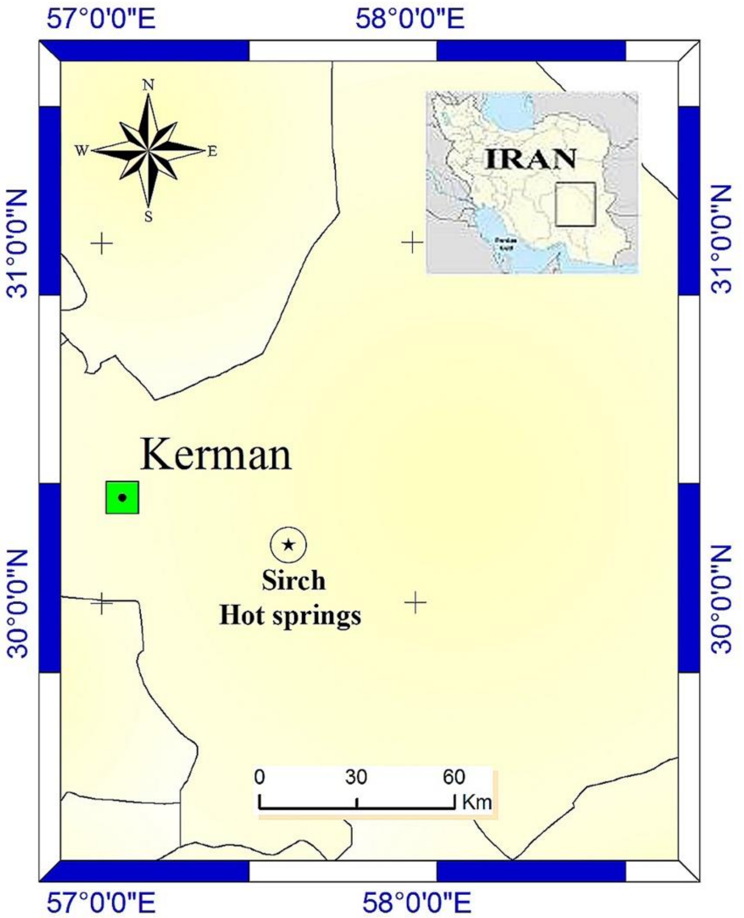

Figure 1. Map showing the location of two studied hot springs in Sirch region Kerman, Iran.

Semi–permanent slides of each species were prepared and kept in the biology department of Shahid Bahonar University of Kerman, Iran.

Species were identified by international identification keys of other authors [PRESCOTT, 1962, DESIKACHARY, 1959, JOHN *et al.*, 2002] by Olympus light microscope BH–2 and Olympus Vanox–T AH–2 fluorescent microscope (Olympus, Tokyo, Japan) based on the morphological characters of colonies, vegetative cells, heterocysts and akinetes.

To calculate the frequencies of species in every site, each sample was mixed thoroughly and 6 replicates of 10 µL of each were poured in hemocytometer (improved neubauer).

According to standard protocol cyanobacteria were counted under light microscope [LOBBAN *et al.*, 1988].

Physico–chemical traits of hot springs' water were measured at the sampling site where necessary.

Temperature was measured by a digital thermometer; pH, EC, TDS and salinity of water by pen type portable sets of Az Inc. instruments 8686 pH pen, 8361 Cond. & TDS pen and 8371 Salinity pen respectively.

The content of sulfate (SO_4^{2-}), Sulfide (S^{2-}, HS^-, H_2S) and nitrate (NO_3^-) were also measured using standard methods of turbidimetric, iodometric and UV spectrophotometry respectively [CELESCERI *et al.*, 2005].

Prior the measuring of SO_4^{2-} amounts the aqueous samples were diluted 5 times to be adjusted to the standard method. Dissolved oxygen was measured by Winkler method [PRADYOT, 2010, RODINO, *et al.*, 2014, BUTNARIU, *et al.*, 2015].

The free Chloride (Cl^-) content was measured by Karizab Inc. kit of 4444 model and total hardness also measured by Karizab Inc. kit of 4210 model based on calcium carbonate.

Three replicates of each parameter were measured and the mean of resulted data were recorded in Table 2.

Some of each these factors have linear or exponential relation (Data have not given).

To investigate the fundamental patterns of the correlation of the environmental controls with species distribution, Canonical Correspondence Analysis (CCA) which is a novel method in ordination among statistical methods was carried out to analysis sets of multivariate data by using Canoco version 4.5 (Biometris–Plant Research Internatitonal, Wageningen, Netherlands) [LEPS and SMILAUER, 2003].

Results and discussion

Twenty cyanobacteria species of 11 genera were found (Table 1). The species *Aphanocapsa rivularis*, *A. delicatissima* introduced as novel species for Iran flora Salajegheh Ansary and collab. [SALAJEGHEH ANSARY *et al.* 2014].

Identified species of sirch hot spring belong to 5 families of Chroococcaceae, Nostocaceae, Scytonemataceae, Oscillatoriaceae and Synechococcaceae.

According to the results of species counting (Table 1) in each sampling site of two hot springs one or two cyanobacteria were dominant.

Among these species *Oscillatoria* with 6 species is dominant genera and after that *Microcoleus*, *Aphanocapsa*, *Chroococcus*, each with 2 and *Aphanothece, Gloeocapsa*, *Merismopedia*, *Nodularia*, *Scytonema*, *Spirulina, Lyngbya* and *Stigonema* with 1 species had the highest numbers.

Table 1.

Representing frequencies of the species in 8 stations.

Species / *Stations*	1	2	3	4	5	6	7	8
Aphanothece microscopica Nägeli					++	+		
Aphanocapsa delicatissima West & G.S.West	++	++				+	++	
Aphanocapsa rivularis (Carmichael) Rabenhorst	++						+	
Chroococcus dispersus (Keissler) Lemmermann		+	++		+	+		
Chroococcus turgidus (Kützing) Nägeli		++			++	+		
Gloeocapsa punctate Nägeli						+		
Lyngbya aestuarii Liebman ex Gomont	++				+		++	
Merismopedia elegans A. Braun ex Kützing		+	++++		+	+		
Microcoleus acutissimus Gardner		++						
Microcoleus paludosus Gomont						++		
Nodularia spumigena Mertens ex Bornet & Flahault					++			
Oscillatoria curviceps C. Agardh	+	+			++	+++	++++	
Oscillatoria formosa Bory de Saint–Vincent ex Gomont					+			
Oscillatoria limnetica Lemmermann					++++			
Oscillatoria princeps Vaucher	++++	+++	++	+++	+	++	++	++++
Oscillatoria prolifica Gomont		+++			+		++	++
Oscillatorianigra Vaucher		++	++	+++	+		+	
Scytonema sp.	++				+	++		
Spirulina major Kützing		++						
Stigonema sp.					++			

+, ++, +++, ++++ are signs of relative frequency of species, 1–5%, 5–25%, 25–50% and 50–90% respectively.

Results of measured physico–chemical environmental factors are presented in table 2. The ordination plot (Figure 2) was obtained from Canoco program. According to eigenvalues, the first axis (by 56 % variations) and the second axis (42.8 % variations) are the most significant among four axes. The first axis is significantly (p=0.01) correlated with temperature (temp., r=0.4365), pH (pH, r=0.12), dissolved oxygen (D.O., r=0.94), chloride (Cl^-, r=0.0997), Sulfate (SO_4^{2-}, r=0.466).

The second axis is also significantly (p=0.04) correlated with EC (EC, r=0.986), TDS (TDS=0.3278), salinity (salinity, r=0.2799), hardness (hardness, r=0.2799) and nitrate (NO_3^-, r=0.2799).

The results of CCA showed that cyanobacteria species are well distributed along both axes indicating different physico–chemical demands of different species.

Whereas parameters of NO_3^-, EC, TDS, salinity, hardness and S^{2-} are negatively correlated with this group of cyanobacteria, distribution of species *O. prolifica*, *Gloeocapsa punctate, Aphanocapsa*, *Microcoleus acutissimus* are favoured by higher values these parameters of hot spring water.

Table 2.

The Results of physico–chemical Parameters measured in two hot springs. Each data represents the mean of 3 replicates

Factors	1st spring sampling sites				2nd spring sampling sites			
	1	2	3	4	5	6	7	8
Temp (°C)	42.0	41.4	41.3	28.7	44.9	42.1	40.7	41.6
pH	6.47	6.54	6.57	7.54	6.25	6.40	6.36	6.30
EC (µS)	1618.33	1620.33	1618.67	1503.67	1620.00	1617.67	1636.00	1630.33
TDS (ppm)	809.67	811.00	814.00	753.00	813.00	818.33	816.00	806.33
Salinity (ppt)	0.78	0.78	0.79	0.72	0.77	0.79	0.78	0.74
Hardness (mg/L)	13.88	13.88	14.00	12.82	13.71	14.00	13.82	13.23
D.O. (ppm)	16.6	41.0	32.0	36.6	29.6	30.6	34.6	30.6
Cl^- (mg Cl_2/L)	0.03	0.03	0.00	0.07	0.00	0.00	0.00	0.00
S^{2-} (mg/L)	5.20	2.47	3.47	1.13	5.67	1.33	2.53	2.47
SO_4^{2-} (mg/L)	21797.92	17905.76	18602.44	20032.98	20599.62	19447.76	20506.72	19131.93
NO_3^- (mg/L)	0.08	0.06	0.18	0.39	0.02	0.29	0.75	0.55

Species *Lyngbya* and *Aphanocapsa* compared to other species show more positive relation to high sulfide levels.

Occurrences of species *A. microscopica*, *O. formosa*, *Chroococcus* and *Spirulina major* suggests that they are compatible with the average concentrations of sulfate and nitrate in springs' water.

Species of cyanobacteria in Sirch hot springs form compact mats and placed in the category of mesothermophilous because they live in temperature below 45°C.

In stations 2, 3, 4, 5 and 7 cyanobacterial species *Oscillatoria prolifica*, *Merismopedia elegans*, *O. princeps* and *O. nigra*, *O. limnetica*, *O. curviceps* were dominant respectively (Table 1).

Some authors [SOMPONG *et al.*, 2005, DEBNATH *et al.*, 2009] have reported other species as dominant in this temperature range.

The species *O. princeps* is known as a facultative thermophile cyanobacterium which found in both freshwater resources and the hot springs [DEBNATH et al., 2009].

In 44–45°C *Oscillatoria limnetica* was dominant but it was the only species which present in all stations (from 28–45°C) (Table 1).

It is proved that this species can use sulfide as an electron donor source in photosynthesis [SALAJEGHEH ANSARY *et al.*, 2014].

Physico–chemical factors like temperature, sulfide and nitrate are well justify the distribution of species among sampling stations.

Generally, the most significant factor of hot spring species distribution is temperature [WARD *et al.*, 2012]. This result is consistent other investigations [DEBNATH *et al.*, 2009; SKIRNISDOTTIR *et al.*, 2000].

Studies showed that amounts of sulfide between 0.6–1.2 mM heavily prevent photosynthesis of non–adapted cyanobacteria species [CASTENHOLZ, 1977].

Sulfide level measured at stations of Sirch hot springs ranged between 38–150 µM indicating possible relative strength and adaptation of these cyanobacteria to sulfide ions.

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However some species of cyanobacteria may be resistant to sulfide toxicity, but do not use it in photosynthesis [CASTENHOLZ and UTKILEN, 1984].

In stations where amounts of combined nitrogen are maximum, most members of Oscillatoriaceae were found.

In contrast, nitrogen–fixing species of *Nodularia spumigena* and *Scytonema sp*. were found only at stations which measured concentrations of nitrate is at lowest level.

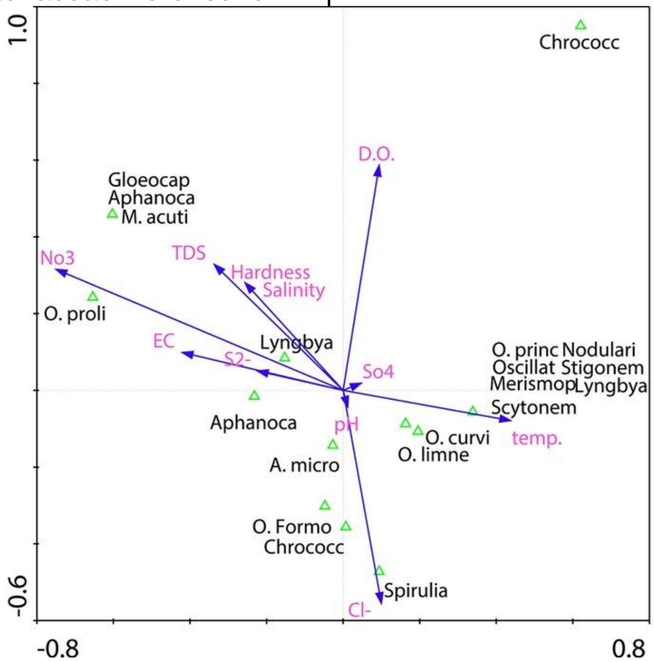

Figure 2. Diagram representing the CCA biplot of frequency of each species and environmental factors

CCA plot graph also shows that the nitrogen–fixing species are negatively correlated with nitrate ion and are located (Figure 2). These findings are consistent with [DEBNATH *et al.*, 2009; MONGRA, 2012].

Although it is suggested that nitrogen–fixing species make another species of nutritional help and support they are removed from the contest by non–nitrogen–fixing cyanobacteria in places where there are sufficient levels of nitrogen [WARD and CASTENHOLZ, 2002].

Conclusions

In the present study, also CCA data of physico–chemical factors and species frequency show that temperature (r=4.365) is one of the most effective factors in the distribution of cyanobacteria in the two hot springs.

Another important factor that cyanobacteria involved with in hot springs is high levels of sulfur compounds.

It has been indicated for a long time that most of cyanobacteria are very sensitive to sulfide toxicity and exposing to low concentrations of sulfide, lead to complete and irreversible cessation of light CO_2 fixation (24).

Levels of measured nitrate in water samples were variable.

CCA plot graph also shows that the nitrogen–fixing species are negatively correlated with nitrate ion and are located in the opposite direction of NO_3^- vector, indicating that the ability of these species to colonize where the nitrate level is low.

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