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CYANOBACTERIAL DIVERSITY AND PHYSICOCHEMICAL CHARACTERISTICS OF THERMAL SPRINGS IN THE KÜTAHYA PROVINCE OF TURKEY

SEVİLAY ÖZTÜRK*

Department of Biology, Faculty of Sciences and Letters, Manisa Celal Bayar University, Manisa, Turkey

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Abstract

Thermal springs are very difficult environments for organisms due to the high temperature, and physicochemical parameters. Cyanobacteria, which are photosynthetic prokaryotes, are best adapted to these environments. Kütahya is an important thermal area in Turkey. The aim of the study was to determine the cyanobacterial flora with a morphologic and ecologic approach in the 11 thermal. The physicochemical properties of the thermal springs in Kütahya province were measured. The thermal springs are alkaline (pH>6) with an average temperature of 52°C. As a result, 54 cyanobacteria taxa were identified. Oscillatoriales were the predominant order in terms of taxa diversity (24 taxa) and biomass size. Statistical analyses were conducted to reveal the physicochemical properties of the thermal springs and the distribution of cyanobacteria in detail. According to these analyses, the thermal springs were classified into two main groups with a Piper. As a result of the RDA analysis under CANOCO 5.0, the total variation was 55.45455, and the first two axes explained a total of 57.43% of the variance. There was a significant difference (P<0.001) in the comparison of the physicochemical parameters including pH, EC, TDS, and temperature values of the thermal springs in the Kruskal Wallis tests.

Introduction

Cyanobacteria are ecologically important because of their role in oxygen production and in assimilation of carbon and nitrogen. Although life is difficult in thermal springs, the cyanobacteria are the most adapted organisms for this environment. Cyanobacteria are the most commonly reported microbial groups constituting thermophilic mats and considered the major primary producers in these type of habitats (Castenholz, 1973). Due to their abilities, the determination of diversity of cyanobacteria in thermal springs is gaining importance. Studies of thermal springs allow us to know which cyanobacterial taxa can adapt to the thermal environment. The diversity of cyanobacteria in thermal springs depends on two basic factors: i) the temperature of the thermal spring, ii) the dissolved chemicals in the thermal spring.

There are more than 600 thermal springs in Turkey (Özsahin and Kaymaz, 2013). Despite this, studies of the biology of thermal springs are very limited (Adıgüzel *et al*., 2009; Yedier *et al*., 2016). Biodiversity studies of thermal algae in Turkey began in Pamukkale with collected algae (Regel and Skuja, 1937) and continued with Güner (1966, 1967, 1970); Aysel *et al*. (1992); Pentecost *et al*., (1997); Ünal (1996); Ulcay Öztürk *et al*., (2006; 2007); Yüksel *et al*., (2009); Demirel and Sukatar, (2011); Ulcay and Kurt, (2014a,b,c), Altunoz *et al.*, (2016); Öztürk Ulcay and Kurt (2017); Öztürk Ulcay *et al*., (2017); Kalkan *et al*., (2020) and Öztürk, (2020).

Corresponding author, E-mail: seviozturk@yahoo.com

Numerous cyanobacteria taxa have been reported in thermal springs throughout the world. Regarding their morphotypes characteristics, Sompong *et al*. (2005) identified 19 genera and 36 cyanobacteria taxa from nine thermal springs (30–80°C) in northern Thailand. Debnath *et al*. (2009) reported 18 taxa distributed in 12 genera at three geothermal springs in Bakreswar in India. In total, 43 taxa belonging to 20 genera of the planktonic cyanobacteria were identified at four hot springs in Iran by Heidari *et al*. (2013). Roy *et al*. (2015) identified 16 taxa spread over 14 genera in the Bakreswar geothermal springs in India. Based on morphology, the distribution of 31 cyanobacteria taxa (30–38.2°C) from Thermopylae thermal spring in Greece were identified by Kanellopoulos *et al*. (2016). Singh *et al*. (2018b) reported 22 taxa under 11 genera based on the morphology at nine thermal springs in the northwestern Himalayas.

The aim of the study was to determine the cyanobacterial flora of thermal springs in the Kütahya province, an important thermal area in Turkey including morphological and ecological aspects. In this context, the results obtained by morphological methods have been studied in an attempt to determine species diversity. Additionally, the thermal springs in Kütahya province measured to determine their physicochemical properties. The piper diagram provided ease in classification and comparison of the thermal springs in Kütahya province where the anion and cation of the water taken from 11 thermal springs was compared. In addition to all this, the relationship between the physicochemical parameters, the sampling sites, and the taxa were explored with the redundancy analysis (RDA) (CANOCO 5.0.). Kruskal-Wallis tests were applied under Statistical Package for the Social Sciences (SPSS) to reveal the statistical significance of differences or similarities of the physicochemical parameters and the taxa numbers of the sampling sites.

Materials and Methods

Sampling sites

Kütahya is situated on major fault lines in the western Anatolian region of Turkey. In this study, a large number of sampling sites with different physicochemical characteristics were selected from 11 thermal springs (Fig. 1). These sites were scattered over an area of approximately 2500 km^2 ranging from an altitude of 588 m to 1462 m, and most of them had thermal spring facilities like spas or thermal hotels.

Physicochemical characteristics of the thermal springs

Water samples were collected in sterile glass bottles from the sampling sites while collecting cyanobacteria samples. The temperature (T-°C), pH, conductivity (EC- mS/cm), and total dissolved solids (TDS-mg/l) were measured using a Hanna HI 9812-5 Portable pH/EC/TDS/Temperature Meter (Europa-Romania) in-situ. The water samples were labelled and transported to the laboratory for chemical analysis. Fluoride (F^-), chloride (Cl^-), bromine (Br^-), nitrite (NO_2^-), nitrate (NO_3^-), phosphate (PO_4^{-3}), sulphate (SO_4^{-2}) analyses were performed by DIONEX ICS-5000 Ion Chromatography/ppm. Other chemical analyses were performed by Perkin Elemer Optima 8000/mg/L for other chemical factors including calcium (Ca^{++}), ferrous (Fe^{2+}), potassium (K^+), magnesium (Mg^+), sodium (Na^+), silicon (Si), and manganese (Mn^+). Ammonium (NH_4^+) analyses were performed by Nesslerizasyon/ppm. All chemical analysis were performed at the Manisa Celal Bayar University-Applied Science Research Center (Manisa, Turkey).

A Piper diagram provides convenience in the classification and comparison of natural springs. The similarities and differences of these thermal springs were investigated with the Piper diagram, and the eleven springs were classified according to their chemical composition with the Piper diagram using GW Chart Software (USGS) and Microsoft Excel 2016 (Piper, 1944; Winston, 2020).

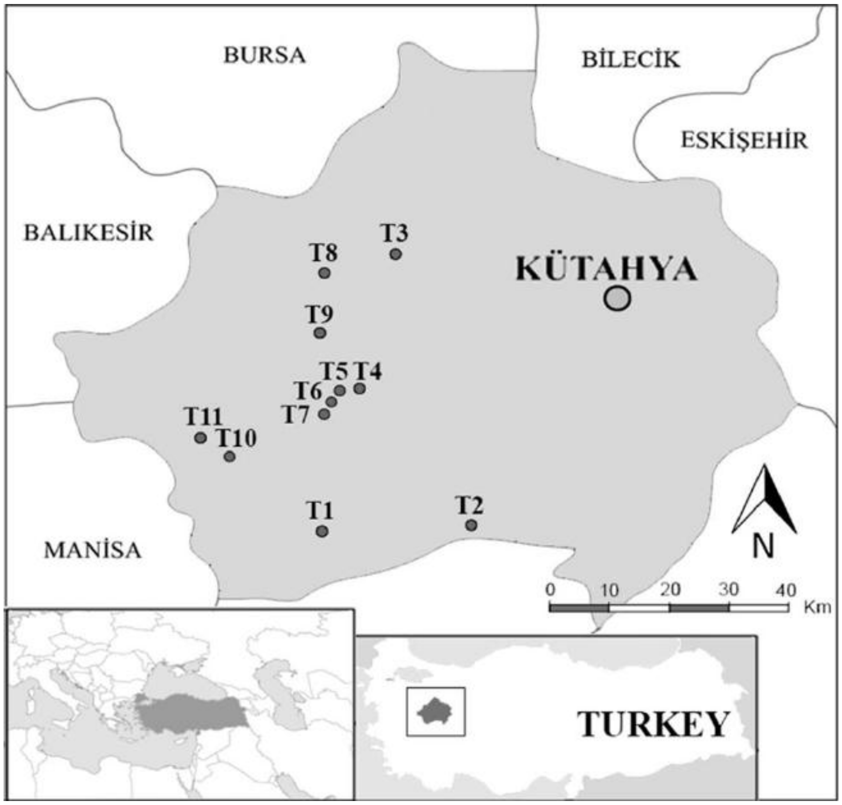

Fig. 1. Thermal Spring Sample Site Names and Locations in Kütahya Province. T1- *Gediz-Ilica* (38°56'22"N 29°15'31"E), T2- *Gediz-Murat Mountain* (38°57'19"N 29°37'14"E), T3- *Tavsanli-Göbel* (39°29'51"N 29°26'17"E), T4- Esire (39°12'08"N 29°16'53"E), T5- Sarpasan (39°12'10"N 29°16'40"E), T6- *Hisarcik-Hamam* (39°12'07"N 29°16'35"E), T7- *Hisarcik-Sefaköy* (39°10'33"N 29°15'37"E), T8- *Günlüce-Dereli* (39°27'46"N 29°15'55"E), T9- *Emet* (39°20'32"N 29°15'12"E), T10- *Simav-Eynal* (39°07'38"N 28°59'33"E), T11- *Naşa* (39°08'37"N 28°57'39"E).

Sampling and identification of cyanobacteria

Cyanobacteria samples were collected between February 2014 and January 2015. Collected samples were placed in 50 ml falcon tubes for morphological identification. All samples were labeled and transported to the laboratory. Collected samples were divided into two parts in the laboratory, one used in direct observations, and the other part fixed with 4% formalin solution to prevent degradation of the characteristics of the taxa.

Microscopic studies were conducted in the laboratory using an Olympus BX 50 (phase-contrast) microscope, and taxonomical characteristics were determined and photographed using the Sony DSC-TX7 camera for morphological identification. The identification of the taxa was made according to previous studies including Komárek and Anagnostidis (2000, 2005), John *et al.*, (2002) and Komárek, (2013). The nomenclature was checked on the AlgaeBase database (Guiry and Guiry, 2021).

Statistical analysis

The relationship between the physicochemical variables of the thermal springs and the distribution of cyanobacteria taxa was assessed by redundancy analysis (RDA) and detrended correspondence analysis (DCA). The analysis was carried out using CANOCO 5.0 software for Windows (Ter Braak and Smilauer, 2012). Initially, a DCA was performed to determine the gradient length and which model (linear or unimodal) the studied gradient is suitable for. According to the DCA results, it was seen that the available data was suitable for RDA analysis. To obtain gradients not associated with the coverable, a forward selection of the environmental variables was performed. Physicochemical properties were determined through a Monte Carlo test (499 permutations), taking into account all canonical axes.

Kruskal-Wallis tests were performed using SPSS 20.00 software to determine whether the differences (in terms of physicochemical parameters) between the thermal springs were statistically significant. Kruskal-Wallis is a non-parametric test and is used for multiple data comparisons. The Kruskal-Wallis test was used to determine the importance of pH, EC, TDS, and temperature values of the eleven thermal springs.

Results and Discussion

Physicochemical characteristics of the thermal springs

Some chemical parameters of the eleven thermal springs in Kütahya as well as the annual average pH, temperature, and EC and TDS measurements are shown in Table 1. The results indicate that the thermal springs were alkaline (pH>6), the average temperature was 52°C, and they were transparent. In addition, it was found that nutrient elements were high, and sulphate and ammonia were below the measurable values in the thermal springs. These physicochemical parameters explain the abundance of cyanobacteria diversity in the thermal springs.

In the Piper diagram, which is the most acceptable method in classifying and comparing natural springs and ground waters, anions and cations are shown in two separate triangles while all ions are shown from a quadrilateral, and this diagram makes classification and comparison of waters easier (Piper, 1944). Based on the Piper diagram, the thermal springs of the Kütahya province were classified into two main groups, one (T2, T4, T5, T6, T7, T9) Ca-Mg-SO_4, the other one (T1, T3, T8, T10, T11) Na-HCO_3-SO_4 (Fig. 2).

Cyanobacteria taxa

Collected samples were identified based on morphological characteristics. As a result, the 54 cyanobacteria taxa identified were distributed in five orders (Table 2). Among the identified taxa, Oscillatoriales were dominant with 25 taxa. In this study, *Pseudanabaena minima* had the highest diversity in the thermal springs of Kütahya. Among the sampling sites, Gediz Ilıca (T1) with 10 taxa and Naşa (T11) with one taxon represented the highest and the lowest species diversity, respectively. The most abundant genera was *Leptolyngbya,* which almost dominated in the nine thermal springs. The most common taxon was *Pseudanabaena minima,* which was identified from the three thermal springs.

Statistical analysis

The relationships between the physicochemical parameters, the sampling sites, and the taxa were explored with an RDA using CANOCO 5.0 software for Windows. Firstly, DCA was performed to find a suitable analysis and gradient lengths were assessed (Axis 1: 0.00; Axis 2: 0.00). Among the physicochemical parameters analyzed, five were included in the forward selection (temperature, pH, TDS, EC, and PO_4). In the RDA, the physicochemical parameters (T, pH, TDS, EC, and PO_4), the sampling sites, and the taxa were used as explanatory variables. The

Table 1. Physicochemical parameters of the thermal springs.

Parameters \ Sampling Sites	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11
pH	7.7	7.8	6.7	8.1	7.2	7.7	8	7	7.6	8.7	8.1
T (°C)	72	37	33	39	75	55	55	36	42	55	55
EC (mS/cm)	0	0	410	1000	1010	1060	1020	1460	630	0	1700
TDS (mg/l)	1530	1150	230	490	490	520	500	710	310	1340	860
F^- (ppm)	0.93	0.20	0.3	0.44	0.49	0.47	0.11	1.11	0.72	5.95	3.29
Cl^- (ppm)	95.13	3.12	7.14	9.72	10.10	9.36	11.73	36.13	14.62	63.27	49.85
NO_2^- (ppm)	*	*	*	*	*	*	*	*	*	*	0.03
Br^- (ppm)	0.28	0.05	0.04	0.07	0.07	0.07	0.07	0.17	0.07	0.20	0.17
NO_3^- (ppm)	*	1.13	*	0.69	0.75	2.09	0.27	*	0.55	*	0.45
PO_4^{-3} (ppm)	1684.71	2057.86	35.18	551.4	584.91	407.9	591.73	107.3	152.3	653.62	570.58
SO_4^{-2} (ppm)	1045.46	1340.86	13.82	467.65	410.52	395.54	388.87	69.45	133.83	587.78	436.13
$HCO_3^-+CO_3^-$	508.12	89.13	188.12	148.64	153.42	189.16	178.12	440.34	145.65	412.85	328.50
Ca^{++} mg/l	153.5	540.0	126.43	179.7	175.5	172.1	190.8	177.7	93.19	14.22	63.05
Fe^{2+} mg/l	0.77	0.177	0	0	0	0.069	0.049	0.014	0.034	0.283	0.102
K^+ mg/l	97.81	3.726	1.82	4.081	4.182	3.328	3.886	12.04	4.609	66.62	39.43
Mg^+ mg/l	60.22	79.09	22.33	45.86	44.06	39.3	43.89	40.38	29.47	0.383	8.818
Na^+ mg/l	674.48	6.209	11.02	12.85	12.63	10.19	12.27	155.8	13.01	520.88	379.1
Si^+ mg/l	41.28	17.49	22.16	24.75	24.01	21.35	23.56	15.96	16.49	143.4	87.32
Mn^+ mg/l	0.038	0.026	0	0	0	0	0.002	0.018	0.005	0.02	0.337
NH_4^+ mg/l	<1 ppm	<1 ppm	<1 ppm	<1 ppm	<1 ppm	<1 ppm	<1 ppm	<1 ppm	<1 ppm	<1 ppm	<1 ppm

* Value below the measuring limit

significance of their effect was supported by a Monte Carlo permutation test (499 permutations, *F*-ratio = 1.3, *P*-value = 0.026). As a result of the RDA analysis, the total variation was 55.45455, and the first two axes explained a total of 57.43% of the variance (Fig. 3).

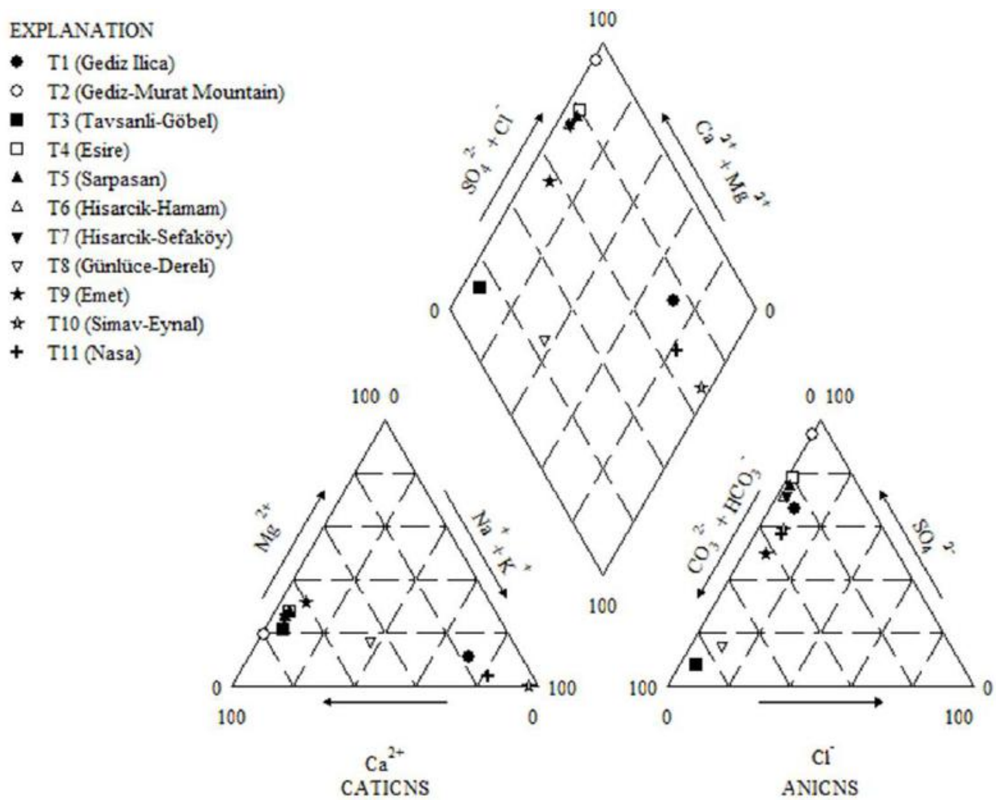

Fig. 2. Piper diagram showing the anions-cations and comparison of the thermal springs.

In the comparison of physicochemical parameters that cause species diversity and the taxa differences of the thermal springs, the question of whether the difference between them was significant with the Kruskal Wallis tests was examined. There was a significant difference (P<0.001) in the comparison of physicochemical parameters including pH, EC, TDS, and temperature values of the thermal springs in this study (Fig. 4). Kruskal Wallis tests showed variations in the physicochemical parameters of the thermal springs and in the cyanobacteria diversity (Fig. 5). The frequency of distribution of taxa according to pH, TDS, T, and EC values can be seen in Fig. 6 (P<0.001). Besides, the manganese (Mn) values of the sampling sites were compared with the Kruskal-Wallis test in SPSS (P<0.001) as a remarkable value (Fig. 7).

As a result, 54 cyanobacteria taxa were identified based on morphological characteristics. The physicochemical properties of the thermal springs were measured. In addition, statistical analyses were made to reveal in detail the physicochemical properties of the thermal springs and their comparisons with the cyanobacterial flora.

The piper diagram was provided for convenience in the classification and comparison of thermal springs in the Kütahya province; thus, the anions and cations of the water taken from the thermal springs (Fig. 2) were compared. According to the Piper diagram, sampling sites T1, T3, T8, T10, and T11 were classified as a Na-HCO_3-SO_4 type, and sampling sites T2, T4, T5, T6, T7, and T9 were classified as a Ca-Mg-SO_4 type. In addition, these thermal springs in Kütahya were classified with the Piper diagram by different researchers (Gemici *et al.*, 2004; Güneş, 2006; Bello *et al.*, 2014). In the literature, it is noteworthy that the Piper diagram has been used less frequently in the determination of cyanobacteria in thermal springs (Singh *et al.*, 2018b).

Table 2. Cyanobacteria taxa and sampling sites.

Taxa code	Cyanobacteria taxa	Thermal spring
	Chroococcales	
1	*Gloeocapsa* sp.	T6
2	*Gloeocapsopsis cyanea* (Krieger) Komárek & Anagnostidis	T3, T10
3	*Chroococcus membraninus* (Meneghini) Nägeli	T9
4	*Cyanosarcina thermalis* (Hindák) Kovácik	T6
	Synechococcales	
5	*Anathece clathrata* (West & G.S.West) Komárek, Kastovsky & Jezberová	T1
6	*Arthronema* sp.	T5
7	*Romeria chlorina* Böcher	T1
8	*Planktolyngbya contorta* (Lemmermann) Anagnostidis & Komárek	T1
9	*Leptolyngbya boryana* (Gomont) Anagnostidis & Komárek	T3
10	*L. tenerrima* (Hansgirg) Komárek	T8
11	*L. gelatinosa* (Woronichin) Anagnostidis & Komárek	T7,T10
12	*L. granulifera* (J.J.Copeland) Anagnostidis	T1
13	*L. thermarum* (Woronichin) Anagnostidis & Komárek	T10
14	*Leptolyngbya* sp. 1	T6
15	*Leptolyngbya* sp. 2	T5
16	*Leptolyngbya* sp. 3	T4
17	*Leptolyngbya* sp. 4	T4
18	*Pseudanabaena minima* (G.S.An) Anagnostidis	T1,T7,T10
19	*P. lonchoides* Anagnostidis	T8
20	*P. thermalis* Anagnostidis	T10
21	*P. limnetica* (Lemmermann) Komárek	T10
22	*Pseudanabaena* sp.	T5
23	*Limnothrix mirabilis* (Böcher) Anagnostidis	T1
24	*Trichocoleus sociatus* (West & G.S.West) Anagnostidis	T2
	Spirulinales	
25	*Spirulina subsalsa* Oerstedt ex Gomont	T1
26	*S. subtilissima* Kützing ex Gomont	T9
27	*S. labyrinthiformis* Gomont	T5
	Oscillatoriales	
28	*Geitlerinema* nematodes (Skuja) Anagnostidis	T1
29	*Anagnostidinema amphibium* (C.Agardh ex Gomont) Strunecký, Bohunická, J.R.Johansen & J.Komárek	T5
30	*Planktothrix clathrata* (Skuja) Anagnostidis & Komárek	T8

Table 2 Contd.

Taxa code	Cyanobacteria taxa	Thermal spring
31	*Microcoleus autumnalis* (Gomont) Strunecky, Komárek & J.R.Johansen	T3
32	*M. lacustris* Farlow ex Gomont	T8
33	*M. paludosus* Gomont	T2
34	*Kamptonema jasorvense* (Vouk) Strunecký, Komárek & J.Smarda	T1
35	*K. okenii* (C.Agardh ex Gomont) Strunecký, Komárek & J.Smarda	T9,T10
36	*K. cortianum* (Meneghini ex Gomont) Strunecký, Komárek & J.Smarda	T9,T10
37	*Phormidium incrustatum* Gomont ex Gomont	T8
38	*P. terebriforme* (C.Agardh ex Gomont) Anagnostidis & Komárek	T5
39	*P. thermobium* Anagnostidis	T7,T8
40	*P. chalybeum* (Mertens ex Gomont) Anagnostidis & Komárek	T9
41	*Phormidium* sp.	T4
42	*Oscillatoria subcapitata* Ponomarev ex Elenkin	T8
43	*O. proboscidea* Gomont	T1,T5
44	*O. princeps* Vaucher ex Gomont	T10,T11
45	*O. subbrevis* Schmidle	T5
46	*O. curviceps* C.Agardh ex Gomont	T2
47	*O. sancta* Kützing ex Gomont	T8
48	*Lyngbya martensiana* Meneghini ex Gomont	T3
49	*L. thermalis* Kützing ex Gomont	T2
50	*Limnoraphis hieronymusii* (Lemmermann) J.Komárek, E.Zapomelová, J.Smarda, J.Kopecký, E.Rejmánková, J.Woodhouse, B.A.Neilan & J.Komárková	T4
51	*Blennothrix* sp.	T4
	Nostocales	
52	*Nostoc* sp.	T7
53	*Calothrix* sp.	T3
54	*Hapalosiphon* sp.	T10

Although the T1 and T2 springs appear to be close to each other, the T2 source is located at a much higher altitude than the others. When the physicochemical parameters of the T1 and T2 are examined (Table 1), it is seen that the pH and Mg values are similar. However, the other parameters, particularly the temperature, are quite different. Based on their own studies, Singh *et al*. (2018b) stated that the close proximity of the hot springs does not mean that they may have similar physical and chemical characteristics. However, the physicochemical parameters of the T4, T5, T6, and T7 springs are quite similar, so they are seen to be in the same class in the Piper diagram and close to each other in the study area (Fig. 2).

It has been reported that there are different species compositions in different thermal springs depending on the substratum and the physicochemical parameters of springs (Ward and Castenholz, 2000; Papke *et al.*, 2003). In the literature, there are many studies of the cyanobacterial flora in thermal springs (Sompong *et al*., 2005; Debnath *et al.*, 2009; Heidari *et al.*, 2013; Roy *et al*., 2015; Kanellopoulos *et al*., 2016; Singh *et al*., 2018a, Singh *et al*., 2018b). When compared with the literature, it may be seen that more cyanobacteria taxa were determined in this study. The main reason for this may be that there are high numbers of thermal springs and sampling sites in this study. Another possible reason may be that the thermal springs in the sampling area have different physicochemical properties.

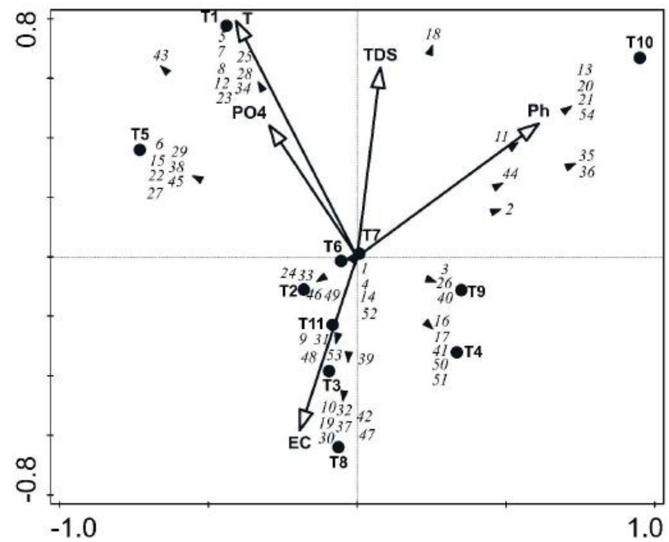

Fig. 3. RDA Diagram showing the relationship between the cyanobacteria taxa (with full triangle), the thermal springs (with full circle), and the physicochemical variables of thermal water (with arrow) [the cyanobacteria taxa code and the thermal springs where they were sampled are given in Fig. 1 and Table 2] T: temperature, EC: conductivity, TDS: total dissolved solid, and PO_4^{-3}:phosphate.

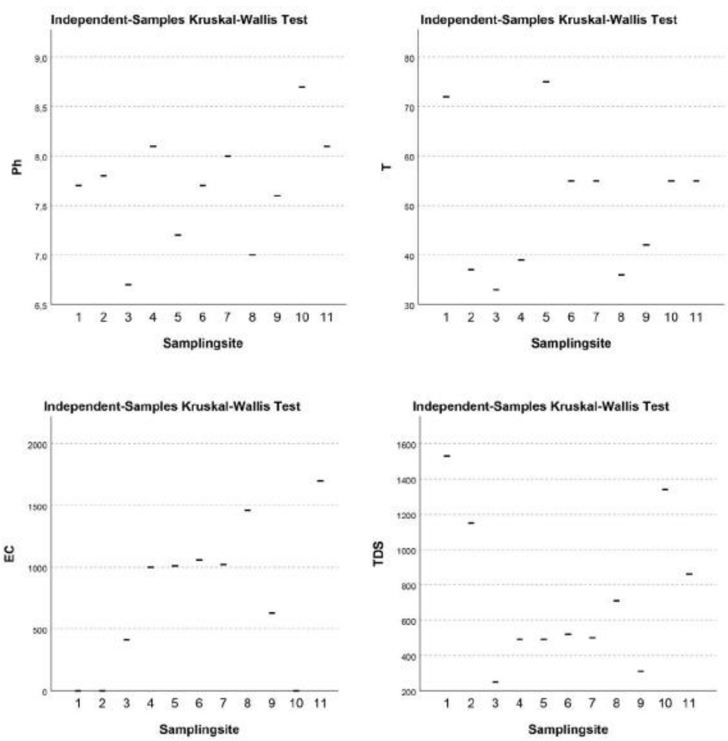

Fig. 4. Comparison of the pH, TDS, T, and EC values of the sampling sites with the Kruskal-Wallis test in SPSS (P<0.001).

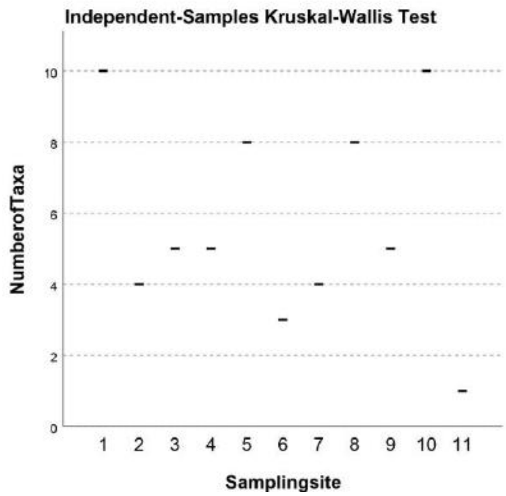

Fig. 5. Comparison of the number of taxa and the sampling sites with the Kruskal-Wallis test in SPSS.

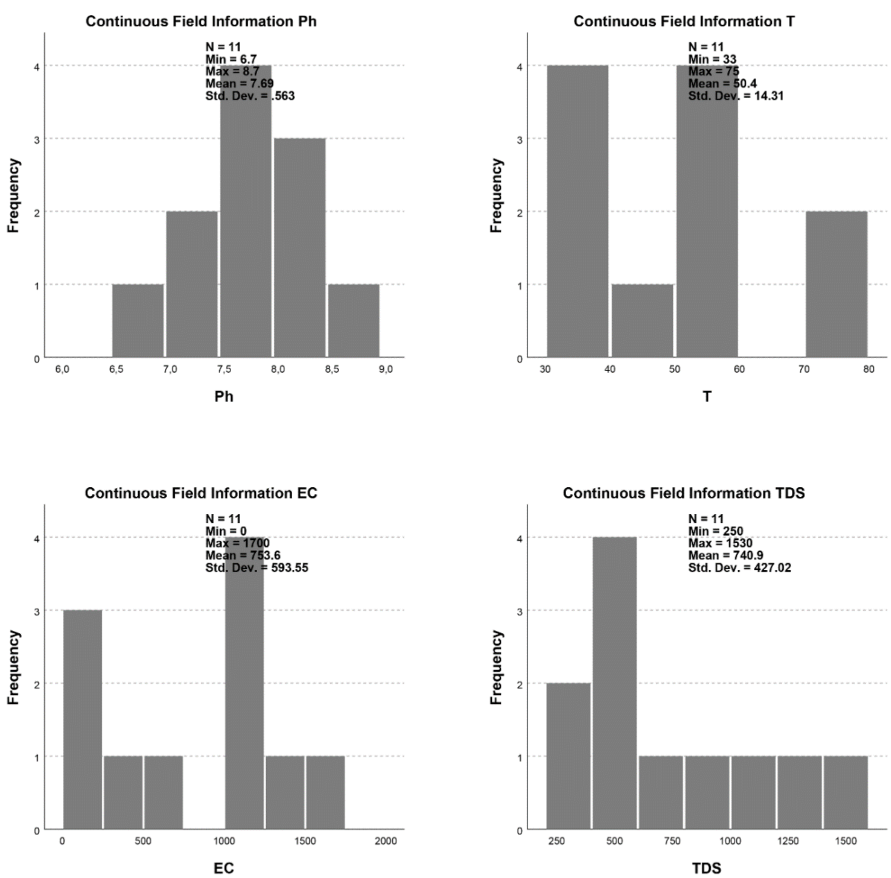

Fig. 6. Frequency of pH, TDS, T, and EC between the taxa (P<0.001).

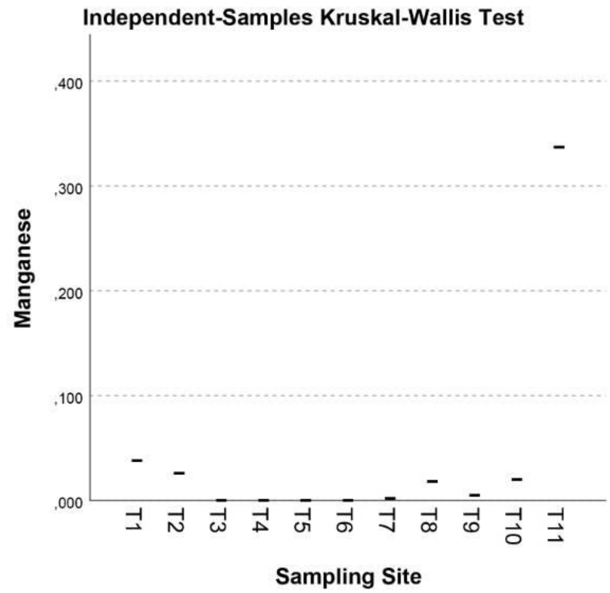

Fig. 7. Comparison of the manganese (Mn) values of the sampling sites with Kruskal-Wallis test in SPSS ($P<0.001$).

Oscillatoriales were a predominant order with taxa diversity (24 taxa) in this study. Similarly, a major component of the thermal spring's cyanobacterial flora worldwide belongs to order Oscillatoriales (Pentecost *et al*., 1997; Sompong *et al*., 2005; Mcgregor and Rasmussen, 2008; Ionescu *et al*., 2010; Arman *et al*., 2014). Nevertheless, *Leptolyngbya* (order Synechococcales) were determined frequently in the thermal springs of the Kütahya province. Also, this taxon is one of the most frequently reported taxa observed in thermal springs (Ulcay Öztürk *et al*., 2006; Mcgregor and Rasmussen, 2008). Commonly identified *Pseudanabaena* and *Spirulina* taxa in this study were also reported in other thermal springs (Heidari *et al*., 2013; Arman *et al*., 2014; Roy *et al*., 2015). According to the RDA analysis, the affinity of *Spirulina subsalsa* and *Spirulina subtilissima* with temperature was completely different in this study. While *S. subsalsa* was related to temperature, *S. subtilissima* was not. Krienitz *et al*. (2003) reported that *S. subsalsa* and *S. subtilissima* are closely related and have a wide ecological distribution. In addition, both taxa occur in thermal springs and in mesophilic brackish and marine habitats (Geitler, 1932).

According to the results of the RDA analysis, the presence of cyanobacteria taxa in thermal springs was related to the physicochemical parameters (Fig. 3). The RDA analysis showed that the most highly determining factor affecting the distribution of the taxa is the temperature (T) in this study (Fig. 3). Similarly, Roy *et al*. (2015) reported that temperature has been one of the most important factors as far as the distribution and diversity of cyanobacteria are concerned in geothermal springs. Also, there are a lot of studies concerning this subject in the literature (Sompong *et al*., 2005; Debnath *et al*., 2009; Singh *et al*., 2018b).

Pseudanabaena thermalis was collected at 50°C and below from sampling site T10. Similarly, this taxon was collected by McGregor and Rasmussen (2008) at 48.6°C from Innot Hot Springs in Australia. *Planktolyngbya contorta* was clearly associated with temperature and PO_4 values in this study. Similarly, *P. contorta* was sampled in the thermal springs in Himachal Pradesh, India by Singh *et al*. (2018a).

Lyngbya thermalis was sampled in the form of dark green and thin mats at 36°C (close to spring mouth) and at 29°C (where the water was discharged) from the sampling site T2. Similarly, Lukavsky *et al*. (2011) reported that a deep blue-green growth washed directly with water of 43°C was colonized with *L. thermalis;* in addition, *L. thermalis* also dominated near the outlets of hot water of 22°C. Arman *et al*. (2014) sampled *L. thermalis* at two different thermal springs from a temperatures range of 37–42°C. Castenholz (1969; 1973) stated that changes in species composition with concomitant changes of temperature occurred along the gradient from the mouth of the thermal springs.

Based on the RDA analysis, *Gloeocapsa* sp., *Cyanosarcina thermalis*, *Leptolyngbya* sp. 1, and *Nostoc* sp. had an affinity with low pH, T, TDS, EC, and PO_4 in this study (sampling sites T6 and T7). Despite the results of the RDA analysis, *C. thermalis* was sampled at 42–38°C from the T6 in this study. Actually, *C. thermalis* is known as a common taxon at thermal springs (Rueda and Monroy, 2009; Komárek and Anagnostidis, 2000; Arman *et al*., 2014; Šaraba and Krunić, 2017).

It has been reported that *Spirulina labyrinthiformis* has a high tolerance for sulfides (Pentecost and Coletta, 2007; Ward *et al.*, 2012). *S. labyrinthiformis* was not collected in sampling sites with higher sulfate values, but it was sampled from sampling site T5 with 410.52 ppm SO_4^{-2} in this study (Tables 1 and 2). Similarly, Pentecost and Coletta (2007) reported that the dominance of this taxon might be related to its tolerance of dissolved sulfide although *Spirulina* is scarce in sampling sites with the highest sulfide. In some hot springs in Yellowstone Park (52°C or below), a sulfide-utilizing *S. labyrinthiformis* morphotype predominates near the sulfide-rich source (Ward *et al*., 2012).

Temperature, in combination with the availability of combined nitrogen, phosphorus and other nutrients, and/or a concentration of free sulfide also determines the cyanobacteria composition (Ward and Castenholz, 2000; Singh *et al*., 2018b). Sulfide rich thermal springs usually contain sulfide tolerant and sulfide utilizing *Oscillatoria* (Castenholz and Utkilen, 1984; Ward and Castenholz, 2000; Singh *et al*., 2018b). *Oscillatoria princeps* were sampled from sampling sites T10 and T11 with sulfate values of 587.78 and 436.13 ppm SO_4^{-2}. In the literature, *O. princeps* has similarly been sampled from high sulphate values in thermal springs (Heidari *et al*., 2013; Arman *et al*., 2014). However, this taxon has been recorded in thermal springs with relatively low sulphate values (Debnath *et al*., 2009; Roy *et al*., 2015). In addition, *O. princeps* was the only taxon detected in sampling site T1, and it had formed large mats. Also, this taxon was sampled from T10 (Table 2). According to the literature, *O. princeps* is perhaps cosmopolite (not marine) (Komárek and Anagnostidis, 2005). When compared to the physicochemical parameters of the sampling sites, the high manganese (Mn) value of T11 drew attention. A comparison of the Mn values of the sampling sites with the Kruskal-Wallis test showed significant differences ($P<0.001$) (Fig. 7). The reason it was the only taxon in T11 may be that *O. princeps* can tolerate a high manganese value. Mn is an essential micronutrient that may become toxic if present at a high concentration (Moura *et al*. 2019).

Ward and Castenholz (2000) and Sompong *et al*. (2005) reported that pH is as important as temperature for cyanobacteria in thermal springs. In the RDA analysis, a negative correlation of *Pseudanabaena limnetica* with EC and a positive correlation with pH was determined in this study. In contrast, Altunöz *et al*. (2016) stated that *P. limnetica* has the highest affinity with EC and a negative correlation with other environmental variables including pH. Cyanobacteria can be considered alkaline since they grow optimally between pH 7.5 and above (Brock, 1973). *Gloeocapsopsis cyanea* was sampled from the two sampling sites with the lowest and the highest pH values in this study (T3, pH 6.7; T10, pH 8.7). In conclusion, it can be concluded that the ecological valence of the taxon for the pH demand is wide. In the literature, this taxon was reported from different environments (Lamprinou *et al*., 2012; Arman *et al*., 2014; Ozturk Ulcay *et al*., 2017; Davydov, 2018). Also, Grimmett and Lebkuecher (2017) reported that *G. cyanea* was among the taxa determined as potential indicators of nutrient-rich areas based on their own data.

Phormidium incrustatum is known as the common taxa of the limestone/travertine/calcareous substrata (Pentecost, 2005; Couradeau *et al*., 2013; Kanellopoulos *et al*., 2016). *P. incrustatum* was sampled from T8, one of the stations with high carbonate and bicarbonate values in this study. Kanellopoulos *et al*. (2016) noted that trichomes of *P. incrustatum* are surrounded by a firm sheath of extracellular polymeric substances (EPS), constituting the locus of intensive calcification.

Because many taxa cannot tolerate high temperatures, thermal springs are extreme habitats for living organisms (Ozturk Ulcay and Kurt, 2017). Thermal springs are very difficult environments for organisms because of high temperatures and physicochemical parameters. Yet, thermal springs create special living environments. The group that has best adapted to these environments is cyanobacteria, which are photosynthetic prokaryotes. In this case, it is important to determine the diversity of cyanobacteria. However, in the literature, no sufficient study of the biodiversity of thermal springs in Turkey was found.

The Kütahya province is very rich in thermal springs, and the primary objective of this study was to determine the cyanobacterial diversity in these springs. In this study, numerous different statistical analyses were performed to reveal the physicochemical properties of the thermal springs and compare them with the cyanobacterial flora. Comparison between the physicochemical parameters and the cyanobacteria taxa was made with RDA analysis, the Piper diagram, and Kruskal Wallis tests. Since physicochemical parameters are important in understanding the ecology of aquatic habitats, many parameters were measured in this study. It is important to perform these statistical analyses to understand the ecology of the thermal springs and the cyanobacteria that prefer these environments.

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