American-Eurasian J. Agric. & Environ. Sci., 18 (5): 258-268 2018
ISSN 1818-6769

DOI: 10.5829/idosi.aejaes.2018.258.268

# Anthropogenic and Climatic Influence on Cyanobacteria Biodiversity in Sundry Thermal Springs, Southwestern Region, Saudia Arabia

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**Abstract:** Thermal springs are valuable public resources with unique thermophiles used in a broad range of biotechnological applications. Thermal springs in the southwest region of Saudi Arabia were investigated to identify thermophilic cyanobacteria, analyze their thermal habitat. Ten springs are located in widely detached areas (350 $Km^2$), Gizan region. A total of 39 cyanobacteria species recorded. *Oscillatoria* genus are essential mate-forming cyanobacteria, Oscillatoriales, Nostocales and Stigonematales are principal components of the community due to their excellent adaptation. *Chroococcus* and *Synechococcus* represent a highly pervasive genera. Twenty four parameters were monitored to understand factors governing diversity. Multivariate analyses were provide a picture of these complex habitats. Ecological factors, such as human remodeling, brutal floods, transforming a natural wall of springs to artificially cemented stones, altered species biodiversity. Stable wall surface was permits enough stability for cyanobacteria to colonize with significantly high biological diversity. Substantial recovery and establishment of cyanobacteria community observed in two months, are indicative that cyanobacteria are powerful thermophiles quickly. Fully protected spring enhanced cyanobacteria to reach steady state. These data are crucial to conserving and manage thermal habitats in Saudi Arabia that might apply to other thermal ecosystems in a different part of the world.

**Key words:** Hot Springs · Thermophiles · Blue Green Algae · Anthropogenic Impact · Flood · Gizan · Saudi Arabia

## INTRODUCTION

In Saudi Arabia ten thermal spring recorded in Saudi Arabia, six of these observed in Gizan area [1], Ain Khulab, Ain Khulab Quwa, Ain Mijara Quwa, Ain ad Damad, Ain al Wagrah and Ain al Wagrah Dam. Only, Wagrah Dam, Ain al Wagrah and Ain Khulab are known for public and their sites prepared by the government for recreational activities. Hot springs in Gizan are of volcanic origin [2], covered with cyanobacteria mats most of the year [1]. To date, only 16 species of cyanobacteria were recorded in Gizan high temperature (65 °C - 80 °C) hot springs, while only ten species recorded in low-temperature springs (48 °C - 52 °C) that are mat-forming [1]. *Microcoleus vaginatus* and *Synechococcus lividus* are abundant species in hot spring of Saudi Arabia. However, *Chroococcus minutus*, *Fischerella thermalis*, *Oscillatoria limosa*, *Phormidium tenue*, *Plectonema boryanum*, *P. mucicola* and *Schizothrix calcicola* are species restricted to the high temperature [1]. These taxa are common in thermal habitats worldwide [3-6]. *Synechococcus* is native thermophiles that are well-adapted to an extreme environment [7, 8]. Cyanobacteria of the order Oscillatoriales are well known to inhibit high temperature (up to 66 °C) and high level of sulfur [5, 9-12]. Indeed, hot spring growth conditions are selective for particular members of thermophilic photosynthetic prokaryotes. Nostocales and Stigonematales represent the primary producer of organic nitrogen from molecular nitrogen in hot spring in Saudi Arabia and another part of the world [6, 13]. However, to date no clear literature record of planktonic form cyanobacteria in Saudi thermal

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springs. Generally in thermal springs, extremophiles are exposed to extreme variations in pH, temperatures, light intensities, salt concentrations and sometimes high level of heavy metals [3-6]. Therefore, these microorganisms are tuned to these extreme microhabitats within thermal spring ecosystem [7, 8]. Non-photosynthetic thermophiles species of bacteria were isolated from Al-Khobah and Al-Aridah hot springs in Saudi Arabia [14, 15]. Interestingly, intensive study for ten geothermal springs in Al Lith and Gizan led to the isolation of fifteen isolates of thermo-aerobic bacteria [15]. Indeed, thermophiles are attractive bio-factories for biotechnological and industrials uses. This study aims to identify cyanobacteria and analyze their ecological conditions to deploy them in varies biotechnological purposes.

## MATERIALS AND METHODS

**Study Sites:** The study area is present in the southwestern region of the Saudia Arabia between latitude 17.0354- 19.038944 and longitude 41.0679194-43.219215 (Fig. 1). This area includes Ahad Al-Tharban, Al Haridhah and Gizan. Tharban springs (Harrah) was not described before that is a new record and described here for the first time. It is 445 km from Jeddah (Fig. 1 & 2 The-A&B). In Haridhah governorate, there is a small thermal spring's named Labibah (Fig 2 Lab-A&B) that is 685 km south of Jeddah and novel record in this study. Interestingly, the Labibah spring was remodeled by Al Haridhah governorate between winter and spring of 2015. Gizan area is the southwest corner of Saudia Arabia that characterized by seven thermal springs located in three main location, wadi Gizan, Wadi Dammed and Fayfa Mountain. In Wadi Gizan, Aridah springs are the oldest famous recreational springs located in AL Aridah governorate and characterized by five sources (Fig. 2 Ar1 A&B to 5A&B). Remodeling and regulatory development in this site are quickly evident. In Al Qowah governorate, there are two thermal springs previously known as Ain Khulab that named Kobah (Fig 2 Kob-A&B) and Ain Khulab Qowah named here Qowah (Fig. 2 Qow-A&B). Qowah hot spring is 8.3 km east of Khobah (publically known as Al Ain Al Harrah). Finally, in Al Dayer governorate (Fayfa Mountain), Bin Malik thermal spring exists (Fig. 2 Bin-A&B).


Fig. 1: The map shows thermal springs locations in the southwestern region of Saudi Arabia, insert in the top right corner shows the study area position relative to the Red Sea coast, Saudi Arabia.


Fig. 2: Images of thermal springs investigated in this study. Each thermal spring is named with short names listed in Table 1. Images provide ecological characteristics of thermal spring such as topography, drainage net and cyanobacteria mats.

**Physicochemical Parameters:** On-site measurement of physical parameters carried out by EXO2 Multi-Parameter Water Quality Sonde (Yellow Spring, OH, USA). Sonde equipped with ROX dissolved oxygen, pH, conductivity/temperature, chlorophyll/BGA and turbidity probes. Each water quality datum is associated with a date, time and GPS coordinates (NAD83). Indeed, temperature, pH, Salinity, conductivity, turbidity, chlorophyll and cyanobacteria concentration were measured directly in the running thermal spring water to ensure accuracy and reliable measurements.

**Macronutrient and Micronutrients Analyses:** Chemical species of relevance to cyanobacteria analyzed to correlate water chemistry with species diversity. Three replicates collected from different site surrounding each thermal spring, subsurface water samples were taken at all locations in pyrogenic free one-liter bottles [17]. Macronutrient and micronutrients analyzed with YSI 9500 Photometer and its kits (Yellow Spring, OH, USA) according to the manufacturer protocols.

**Cyanobacteria Productivity and Species Abundance:** Regular seasonal collections were carried out to the studied sites from winter 2015 to autumn 2016. For qualitative studies of phytoplankton species, collected samples was fixed and concentrated using Lugol's solution [17]. Qualitative analysis was carried out using the preserved as well as fresh specimens. These were examined microscopically for the identification of the present genera and species.

**Identification of Cyanobacteria Taxa:** Species identification, as well as the standing crop carried out using a phase contrast microscope (Carl Zeiss, Jena). The characteristics of the species (length, width, thickness and diameter) measured using an ocular and slide micrometer (Craticules, LTD, Tonbridge, Kent). Receipt materials deposited in Microbial Biotechnology Laboratory (MBL), Biology Department, Faculty of Science & Arts and Jeddah University, Saudi Arabia. Photographs will take using an inverted microscope (OLYMPUS series 1 x 70 equipped with the SC35 camera (type 12) and a color video monitor Panasonic, Tc-1470Y. The primary references used for identification of phytoplankton species are [18-27].

**Statistical Analyses:** Community Analysis Package Version 5 used for Principle component analysis and cluster analysis [28].

## RESULTS

Thermal springs are valuable resources that support novel thermophiles. In the southwest region of Saudi Arabia, a highly diverse group of thermal springs are located in widely detached in areas of approximately 350 $Km^2$ (Fig. 1). High-resolution images identify these thermal springs general characteristics (Fig 2) and document their ecological conditions. Geographical information, short names and physical characteristics listed in Table 1. Chemical analyses of thermal water for each spring provided in Table 2A-C. Interesting ecological events that dramatically impacted these thermal springs such as human remodeling (Labibah and Aridah) to severe floods (in Khobah and Qowah) were observed during the study period and discussed.

**Physicochemical Characteristics of Gizan Thermal Springs:** In Tharban, high visitor's pressure is a key factor that may affect its physicochemical characteristics. Macronutrients are at a low level; however, micronutrients were similar to most of the Gizan springs (Tab. 2). Micronutrients reach 10-times their concentration in the BG11 medium. Labibah springs macronutrients are below the level in BG11 medium, but micronutrients show very high level compared to the artificial growth medium (BG11) of cyanobacteria. Magnesium is 7-times compared to BG11. Copper, zinc and molybdenum vary from 4 times to 13 times of their level in BG11. Bromine, nickel and aluminum are mid-average of the other hot springs. Labibah shows a dramatic decrease in turbidity and BGA-PE after remodeling. Where remolding provides more stable substrate and in turn cyanobacteria mats become firmly attached to the cement surface that minimized turbidity and BGA. The temperature of Aridah springs varies between 45ºC and 57ºC (Table 1). Specific conductivity of all Aridah springs are similar that imply a

Table 1: Thermal springs full names, short names, geographical locations, elevations and physical factors (Temp: Temperature, SPCond: Specific conductivity, TDS: Total dissolved salts, ODO: Dissolved oxygen, BGA: Blue green algae, Baro: Pressure). Bolded and underlined values are seasonal variations recorded in four seasons.

| Thermal spring | SN | Location lat/long | Elev. m | Temp°C | SPCond µS/cm | TDS mg/L | pH | ODO mg/L | BGA µg/L | Baro mmHg |
|---|---|---|---|---|---|---|---|---|---|---|
| Tharban | The | 19.038944 | 220 | 47.30 | 4, 924.27 | 2, 586.50 | 8.46 | 10.14 | 0.10 | 553.43 |
| | | 41.679194 | | 4.46 | 1, 053.52 | 47.30 | 0.75 | 17.62 | 0.39 | 369.21 |
| Labibah | Lab | 18.001492 | 123 | 41.26 | 7, 328.61 | 4, 020.23 | 7.38 | 2.05 | 11.29 | 744.77 |
| | | 42.056008 | | 0.60 | 976.49 | 133.80 | 0.30 | 0.97 | 20.34 | 2.86 |
| Aridah 1 | Ar1 | 17.035116 | 172 | 56.73 | 4, 345.85 | 2, 824.71 | 7.28 | 6.89 | 0.60 | 741.27 |
| | | 42.989716 | | 1.10 | 88.35 | 57.35 | 0.23 | 1.70 | 0.28 | 3.53 |
| Aridah 2 | Ar2 | 17.035400 | 172 | 54.39 | 4, 369.63 | 2, 840.23 | 7.61 | 32.14 | 2.30 | 741.24 |
| | | 42.989532 | | 1.29 | 86.83 | 56.45 | 0.39 | 12.99 | 2.13 | 3.67 |
| Aridah 3 | Ar3 | 17.035467 | 172 | 46.33 | 4, 353.88 | 2, 829.92 | 7.84 | 30.29 | 0.85 | 741.28 |
| | | 42.989384 | | 2.77 | 101.27 | 65.66 | 0.39 | 13.90 | 0.74 | 3.57 |
| Aridah 4 | Ar4 | 17.035900 | 172 | 46.94 | 4, 342.43 | 2, 822.63 | 7.81 | 30.45 | 1.30 | 741.29 |
| | | 42.989319 | | 2.14 | 91.39 | 59.49 | 0.56 | 21.05 | 1.67 | 3.57 |
| Aridah 5 | Ar5 | 17.036066 | 172 | 50.47 | 4, 022.05 | 2, 614.15 | 7.43 | 28.81 | 0.45 | 741.32 |
| | | 42.989201 | | 2.62 | 551.15 | 358.28 | 0.28 | 25.75 | 0.29 | 3.36 |
| Khobah | Kob | 16.764383 | 163 | 61.20 | 4, 350.07 | 2, 095.75 | 7.78 | 38.95 | 1.41 | 743.80 |
| | | 43.129601 | | 7.62 | 1, 409.23 | 513.68 | 0.72 | 50.35 | 1.02 | 5.08 |
| Qowah | Qow | 16.796267 | 245 | 59.35 | 3, 833.66 | 1, 673.16 | 7.66 | 2.38 | 3.04 | 736.32 |
| | | 43.200481 | | 9.48 | 957.14 | 129.45 | 0.34 | 1.57 | 3.84 | 4.62 |
| Binmalik | Bin | 17.269768 | 657 | 45.02 | 2, 631.32 | 1, 237.33 | 7.35 | 3.00 | 0.54 | 705.3 |
| | | 43.219215 | | 0.38 | 17.47 | 1.89 | 0.21 | 1.77 | 0.13 | 3.47 |

Table 2A: Macronutrients concentration in thermal springs. Ca: Calcium, K: potassium, $PO_4$: Phosphate, $NO_3$: Nitrate, $SO_4$: Sulfate, Mg: Magnesium. Standard deviation values are added as plus and minus next to the average values of three sampled analyzed for each spring.

| Springs | Ca ug/L | K mg/L | $PO_4$ ug/L | $NO_3$ ug/L | $SO_4$ mg/L | Mg mg/L |
|---|---|---|---|---|---|---|
| Tharban | 707±188.1 | 5±0.2 | 240±84.9 | 1±0.1 | 365±21.2 | 0.0±0.0 |
| Labibah | 451±4.2 | 8±0.1 | 325±63.6 | 1±0.0 | 365±21.2 | 62±3.1 |
| Khobah | 389±1.4 | 7±0.1 | 465±21.2 | 1±0.2 | 375±21.2 | 0.0±0.0 |
| Qowah | 583±42.0 | 12±3.8 | 275±07.1 | 1±0.1 | 375±7.1 | 0.0±0.0 |
| bin Malik | 215±103.2 | 7±0.6 | 230±70.7 | 4±0.2 | 249±9.9 | 13±2.3 |
| Aridah 1 | 263±4.2 | 12±0.5 | 440±14.1 | 1±0.0 | 367±32.5 | 0.0±0.0 |
| Aridah 2 | 275±7.1 | 11±0.1 | 305±49.5 | 1±0.2 | 335±49.5 | 0.0±0.0 |
| Aridah 3 | 312±62.2 | 11±1.4 | 450±42.4 | 3±0.4 | 344±50.9 | 0.0±0.0 |
| Aridah 4 | 250±2.8 | 10±0.1 | 325±07.1 | 2±0.5 | 365±35.4 | 0.0±0.0 |
| Aridah 5 | 310±60.2 | 12±1.4 | 350±45.4 | 2.5±0.4 | 348±50.9 | 0.0±0.0 |

Table 2B: Micronutrients concentration in thermal springs. Mn: Manganese, Cu: Cupper, Zn: Zinc, Mo: Molybdenum, Ni: Nickel. Standard deviation values are added as plus and minus next to the average values of three sampled analyzed for each spring

| Springs | Mn ug/L | Cu ug/L | Zn ug/L | Mo ug/L | Ni ug/L |
|---|---|---|---|---|---|
| Tharban | 23±1.2 | 220±13.4 | 506±58.6 | 460±112.7 | 116±28.9 |
| Labibah | 45±7.0 | 150±9.2 | 643±100.7 | 683±116.5 | 150±50.0 |
| Khobah | 35±4.2 | 160±9.8 | 746±32.1 | 733±117.9 | 123±25.2 |
| Qowah | 42±1.2 | 40±2.4 | 343±20.8 | 2443±105.9 | 133±57.7 |
| bin Malik | 37±6.1 | 10±0.6 | 520±98.5 | 1836±295.7 | 283±76.4 |
| Aridah 1 | 0.0±0.0 | 80±4.9 | 323±25.2 | 373±198.9 | 316±104.1 |
| Aridah 2 | 0.0±0.0 | 80±4.9 | 373±126.6 | 820±100.7 | 300±100.0 |
| Aridah 3 | 0.0±0.0 | 60±3.7 | 446±45.1 | 786±140.1 | 346±50.3 |
| Aridah 4 | 0.0±0.0 | 100±6.1 | 580±43.6 | 526±92.5 | 350±150.0 |
| Aridah 5 | 0.0±0.0 | 70±3.7 | 456±45.1 | 755±140.1 | 355±50.3 |

Table 2B: Micronutrients concentration in thermal springs. $NO_2$: Nitrite, Si: Silicon, $SO_3$: sulfite, Br: Barium, Al: Aluminum. Standard deviation values are added as plus and minus next to the average values of three sampled analyzed for each spring.

| Springs | $NO_2$ ug/L | Si mg/L | $SO_3$ mg/L | Br ug/L | Al ug/L |
|---|---|---|---|---|---|
| Tharban | 5±1.4 | 52±5.1 | 10±2.8 | 520±113.1 | 85±21.2 |
| Labibah | 3±0.1 | 61±1.2 | 7±1.4 | 640±0.0 | 50±14.1 |
| Khobah | 37±3.5 | 67±4.0 | 7±1.4 | 575±35.4 | 115±7.1 |
| Qowah | 8±0.0 | 118±9.5 | 6±2.8 | 445±148.5 | 77±4.2 |
| bin Malik | 2±0.0 | 80±2.0 | 7±1.4 | 485±205.1 | 100±28.3 |
| Aridah 1 | 5±2.1 | 103±8.7 | 9±1.4 | 415±21.2 | 86±19.8 |
| Aridah 2 | 1±0.0 | 92±4.7 | 11±1.4 | 305±91.9 | 60±0.0 |
| Aridah 3 | 3±2.1 | 100±4.9 | 7±1.4 | 415±7.1 | 80±0.0 |
| Aridah 4 | 6±0.7 | 97±5.8 | 5±1.4 | 455±35.4 | 50±14.1 |
| Aridah 5 | 26±2.1 | 88±4.9 | 8±1.4 | 425±7.1 | 75±0.0 |

shared source of thermal water. Low level of the chemical species is the general trend of the five Aridah springs. Calcium, zinc, molybdenum and copper in Aridah springs are among the lowest level compared to all analyzed hot springs in Gizan. The Wadi Dammed springs show the highest seasonal fluctuation in temperature that is of course due to flood activity (Tab. 1). The pH increases from 7.15 to 9.06 during the spring season. Cyanobacteria has washed away during winter but bloomed again in the spring season. In Khobah (Table 2A-C), phosphate has the highest level observed during this work that is 6.64 % of the BG11 medium. Zinc is 15-times its concentration in the BG11 medium. Flood is frequent in Qowah because it located in the bed of Wadi Dammed that causes continuous erosion to the cyanobacteria mats in Qowah. Potassium in Qowah is 71% that present in BG11. Bin Malik thermal spring is characterized by moderate temperature, low salinity and slightly alkaline water (Table 1). It is water flow rate is enough to support large recreational site that separated from the spring. Zinc and molybdenum in this spring are as high as 10-times the level in the BG11 that may exert physiological pressure on cyanobacteria (Tab. 2B&C).

Table 3: Species list recorded in thermal springs, short names are listed in Table 1. Plus sign (+) is indicative of species recorded at least once during study period, species absence is indicated by minus sign (-). Species Frequency (Freq.) was calculated. Total number of species recorded in each springs are listed at the end for each thermal spring

| | | Gizan thermal springs | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Species name | Freq. | Qow | Ar 1 | Ar 4 | Ar 2 | Kob | Ar 3 | Lab | Thr | Ar 5 | Bin |
| *1.* *Lynqbya aerugino-coerulea* (Kutz.) Gomont | 10 | + | + | + | + | + | + | + | + | + | + |
| *2.* *Lyngbya nigra* (Gomont) Anagnostidis & Komárek | 10 | + | + | + | + | + | + | + | + | + | + |
| *3.* *Oscillatoria brevis* Gomont | 10 | + | + | + | + | + | + | + | + | + | + |
| *4.* *Oscillatoria limosa* (Roth) C. Agardh | 10 | + | + | + | + | + | + | + | + | + | + |
| *5.* *Oscillatoria proboscidea* Gomont | 10 | + | + | + | + | + | + | + | + | + | + |
| *6.* *Oscillatoria willei* Gardn. | 10 | + | + | + | + | + | + | + | + | + | + |
| *7.* *Phormidium cebennese* Gomont S | 10 | + | + | + | + | + | + | + | + | + | + |
| *8.* *Chroococcus ansuyensis* Vasishta | 9 | + | + | + | + | + | + | + | + | + | - |
| *9.* *Chroococcus minor* (Kützing) Nägeli | 9 | + | + | + | + | + | + | + | + | + | - |
| *10.* *Chroococcus yellowstonensis* Copeland | 9 | + | + | + | + | + | + | + | + | + | - |
| *11.* *Gloeocapsa gelatinosa* Kutzing | 9 | + | + | + | + | + | + | + | + | + | - |
| *12.* *Microcoleus vaginatus* Gomont ex Gomont | 9 | + | + | + | + | + | + | + | + | - | + |
| *13.* *Oscillatoria cortiana* Gomont | 9 | + | + | + | + | + | + | + | + | + | - |
| *14.* *Phormidium tenue* Gomont S | 9 | + | + | + | + | + | + | + | + | + | - |
| *15.* *Phormidium terebriformis* (C.Agardh ex Gomont) | 9 | + | + | + | + | + | + | + | + | + | - |
| *16.* *Synechococcus bigranulatus* Skuja | 9 | + | + | + | + | + | + | + | + | + | - |
| *17.* *Aphanocapsa grevillei* (Berkeley) Rabenhorst | 8 | + | + | + | + | + | + | - | - | + | + |
| *18.* *Lyngbya diqueti* (Kutz.) Gomont | 8 | + | + | + | + | + | - | + | + | - | + |
| *19.* *Oscillatoria princeps* Vaucher and Gomont | 8 | + | + | + | + | + | + | + | + | - | - |
| *20.* *Synchococcus elongatus* (Nägeli) | 8 | + | + | + | - | + | + | + | + | + | - |
| *21.* *Anabaena variabilis* Komárek & Anagnostidis | 7 | + | + | + | + | - | + | + | - | + | - |
| *22.* *Calothrix thermalis* Hasngirg ex Bornet & Flahault | 7 | + | + | + | + | - | + | - | + | - | + |
| *23.* *Cyanothece* sp. C. Agardh | 7 | - | + | - | + | + | - | + | + | + | + |
| *24.* *Mastigocladus laminosus* Cohn ex Kirchner | 7 | + | + | - | + | + | + | + | - | - | + |
| *25.* *Merismopedia tenuissima* Lemmermann | 7 | + | + | + | + | + | + | + | - | - | - |
| *26.* *Microcystis protea* J.J.Copeland | 7 | + | + | + | - | + | + | + | + | - | - |
| *27.* *Nostoc* sp VAUCHER | 7 | + | + | + | + | + | + | - | - | + | - |
| *28.* *Oscillatoria filiformis* J.Copeland | 7 | + | + | + | + | + | + | - | + | - | - |
| *29.* *Schizothrix calcicola* Gomont | 7 | - | + | + | - | + | + | - | + | + | + |
| *30.* *Spirulina subsalsa* Oersted ex Gomont | 7 | + | - | + | + | + | - | + | - | + | + |
| *31.* *Thermosynechococcus elongatus* Robert E. Bruccoleri | 7 | + | - | + | + | + | + | + | - | + | - |
| *32.* *Rivularia* sp. C. Agardh | 6 | - | + | + | - | + | + | - | + | - | + |
| *33.* *Scytonema leptobasis* Ghose | 6 | - | + | + | + | - | - | + | - | + | + |
| *34.* *Plectonema notatum* Anagnostidis & Komárek | 5 | - | + | + | + | - | - | - | + | - | + |
| *35.* *Anacystsis* sp C. Agardh | 1 | + | - | - | - | - | - | - | - | - | - |
| *36.* *Cyanothece aeroginosus* (Nägeli) Komárek | 1 | + | - | - | - | - | - | - | - | - | - |
| *37.* *Oscillatoria amphibia* C.Agardh ex Gomont | 1 | + | - | - | - | - | - | - | - | - | - |
| *38.* *Oscillatoria rubescens* De Candolle ex Gomont | 1 | + | - | - | - | - | - | - | - | - | - |
| *39.* *Oscillatoria rosea* Batters | 1 | + | - | - | - | - | - | - | - | - | - |
| Total species present | | 34 | 32 | 32 | 30 | 30 | 29 | 27 | 26 | 24 | 18 |

**Species Diversity and Frequency:** A total of 39 cyanobacteria species recorded in Gizan thermal springs. The majority of these species are the inhabitant of Qowah spring (Tab. 3). Five species are observed only in Qowah (*Anacystsis* sp., *Cyanothece aeroginosus*, *Oscillatoria amphibian*, *Oscillatoria rubescens*, *Oscillatoria rosea*). There are no recreational activities found in this spring due to the absence of a natural and man-made water pool. In Labibah, only two species recorded before remodeling, however, after remodeling 18 species colonized the cemented surface. Cyanobacteria well represented in Aridah springs with 32 species (Table 3), where, Chroococcales are dominant and Oscillatoriales are co-dominant species. Remodeling activity is active in Aridah and high demand of tourists characterizes the site. In Khobah, the flood was active in winter 2015 that dramatically destroyed the cyanobacteria mats. However, after the flood a vigorous recovery and substantial coverage of cyanobacteria mats observed (Fig. 2 kob-A&B) that is identical scenario occurred in Qowah

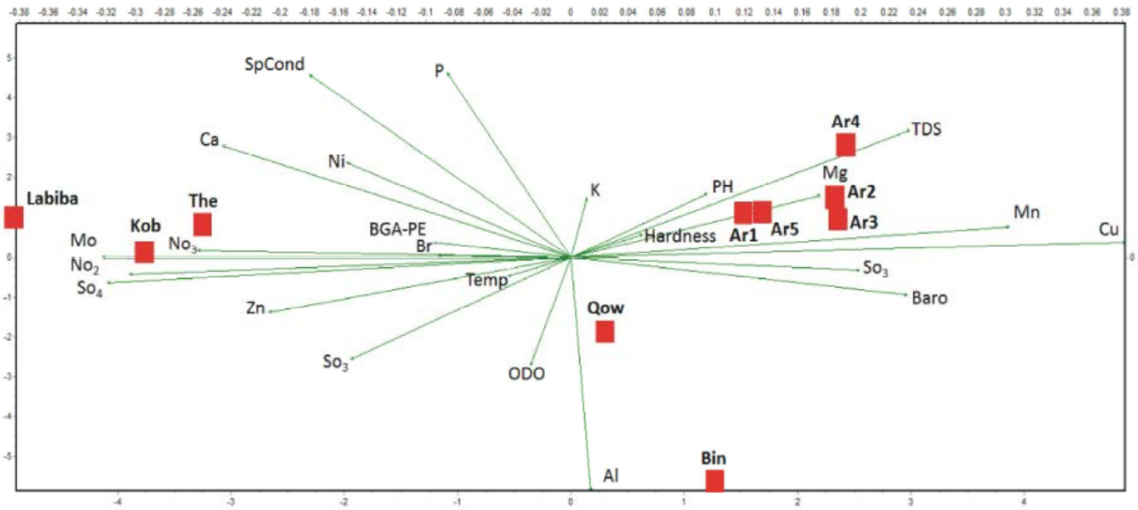


Fig. 3: Ordination plot of physicochemical parameters are represented by arrows, length and direction of these arrows are the factor of their ecological strength and closely related factors are oriented in the same directions. Gizan thermal springs are marked with red squares named according to their short names in Table (1), their locations are indicative of their ecological relation relative to the physicochemical factors. Physical factors abbreviations are as follow: Temp: Temperature, SPCond: Specific Conductivity, TDS: Total dissolved salt, ODO: Dissolved oxygen, BGA: Blue-green algae, Baro: Pressure. Standard chemical species (Ca: Calcium, K: potassium, PO4: Phosphate, NO3: Nitrate, SO4: Sulfate, Mg: Magnesium, Mn: Manganese, Cu: Cupper, Zn: Zinc, Mo: Molybdenum, Ni: Nickel, NO2: Nitrite, Si: Silicon, SO3: Sulfite, Br: Barium, Al: Aluminum).

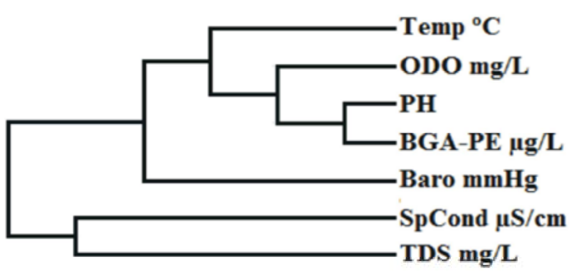


Fig. 4: Cluster analysis of physical factors analyzed in Hot Springs.

(Fig. 2 Qow-A&B), species appear healthy under the microscope that is indicative of the favorable renewed environment. In contrast, the lowest species diversity recorded in the fully protected (fenced) Bin Malik thermal spring (Table 3), only 18 species recorded that is less than half of the recorded species. Chroococcales were absent and Oscillatoriales observed in low density. Indeed, flooding enhances species diversity nonetheless the protected thermal habitat did not.

**Multivariate Analysis of Gizan Thermal Springs:** Ordination of the physicochemical factors: 24 ecological factors that extracted to understand the factors governing species diversity in Gizan thermal springs. Indeed multivariate analyses were applied as powerful statistical tools that provide a simplified picture of the compound data. Ordination plot of physicochemical parameters and thermal springs shown (Fig. 3), ecologically related factors oriented in the same directions. Arrows length is indicative of their contribution power to species biodiversity. Thermal springs are grouped (Red squares) in an unusual order. Aridah springs clustered grouped. While, the ordination of Khobah, Tharban and Labibah indicate that these springs are ecologically related. However, Qowah and Bin-Malik are separated from human impacts springs. Indeed, PCA ordered thermal springs based on the anthropogenic impact.

**Cluster Analyses of Physicochemical Analyses:** To understand the ecological correlation between physicochemical parameters, cluster analysis extracted the ecological relatedness between them (Fig. 4) that is informative and ecologically relevant. Temperature, dissolved oxygen and pH clustered with cyanobacteria. However, specific conductivity and total dissolved salt are separated into a single group.

**Pca Ordination of Thermal Springs:** PCA analyses of Gizan thermal springs based on the chemical analysis (Fig. 5A) and species composition (Fig. 5C) are relevant

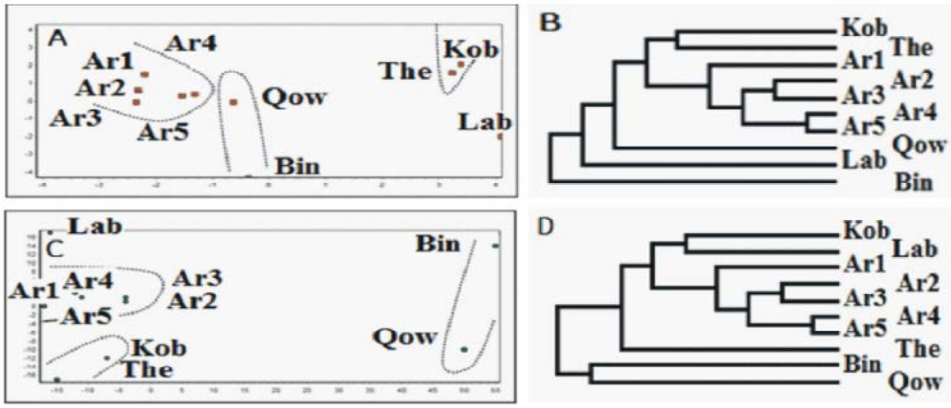


Fig. 5: PCA ordination and cluster analyses of the Gizan springs based on chemical analyses (A and B respectively), based on species composition (C and D). Aridah springs (Ar1-Ar5), Qow: Qowah spring; Bin: Bin Malik spring; Kob: Khobah spring, The: Tharban; Lab: Labibah spring. Dotted lines show thermal springs groups in PCA ordinations.

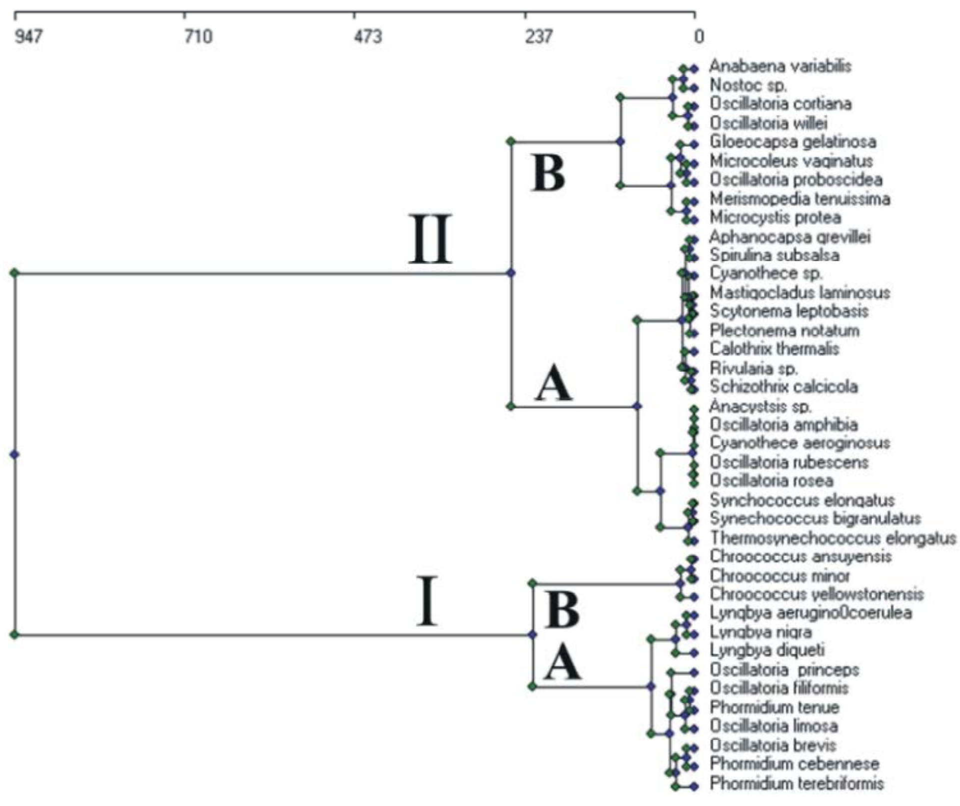


Fig. 6: Cluster analysis of species recorded in Gizan thermal springs based on their complete linkage

with field observation. Aridah springs are unique that always grouped together. Khobah and Tharban are highly similar but chemically distinct from Aridah springs. Labibah has positioned far from all springs. Qowah and Bin are different from other springs. However, they still separate from each other; Qowah has the highest species diversity and Bin-Malik has the lowest species diversity. Interestingly, PCA ordination based on species diversity reordered thermal springs. Thermal springs that are heavily impacted by human positioned in the left side (Fig. 5B) versus low or no anthropogenic affected springs on the opposite side. Indeed, human impact is the primary factors controlling species biodiversity in thermal springs of the southwest region, Saudi Arabia.

**Cluster Analysis of Gizan Thermal Springs:** Cluster analyses based on chemical compositions provides additional information for thermal springs (Fig. 5B) that is comparable with studies based on physicochemical factors and species compositions (Fig 5D). These analyses resulted in similar output; Aridah springs gathered one cluster in both analyses that may be due to similar ecological conditions in Aridah site. Khobah is chemically close to Labibah. However, it clustered with Tharban when physical and species compositions are involved. The most obvious factors are the tourist pressure in both springs. Again, Qowah and Bin-Malik stayed away from human-disturbed thermal springs. Therefore, these two springs ecologically unique regardless of the variation in species compositions.

**Species Cluster Analysis:** Cluster analysis of the species compositions based on the average linkage of species frequency was achieved by Twinspan Community Analysis Package (Fig. 6). In this dendrogram, species were clustered into two main groups I & II, split into two sub-clusters. Cluster IA represent members of Oscillatoriales order, within this sub-cluster, *Lyngbya* species formed a separate cluster. Chroococcales represented by three species in Gizan springs grouped in one pure sub-cluster (IB). Indeed, the order Chroococcales are associated with mat-forming highly adapted filamentous cyanobacteria species of the genus Oscillatoriales in the cluster (I). Interestingly, ten species of Oscillatoria genus are the major component of cyanobacteria mats in Gizan thermal springs. These species systematically scattered throughout the clusters. A highly diverse group observed in IIA; however, it still has slightly homogenous sub-clusters. Indeed, species distribution in this dendrogram reflects a commensalism relationships based on an optimized genetic system that generates cooperative association between cyanobacteria species.

## DISCUSSION

High biodiversity of cyanobacteria in the southwestern thermal springs is due to moderate water temperature and alkaline pH. These growth conditions are common in tropical thermal springs [5, 6, 13] as well as springs in temperate region [7]. Species of the order Oscillatoriales are known to dominate in moderate temperature Springs (40 - 66 °C) that are probably the key factor controlling mat-forming thermophile cyanobacteria [5]. *Synechococcus* is widely distributed in hot springs worldwide including Gizan, Saudi Arabia [3-6]. This genus is naturally optimized to extreme temperatures and different chemistry [7, 8] that are common in thermal springs [3-6]. Indeed, thermal habitats elect specialized microorganisms to dominate particular microhabitat variations within thermal spring ecosystem. Interestingly, all species of cyanobacteria recorded in this study are common in Al-Lith springs (Unpublished data).

The success of *Oscillatoria* genus in Gizan springs is due to its adaptability to colonize anaerobic, sulfide-containing habitats [9] and ferment glycogen formed from the daytime photosynthetic activity [12] that provides it with growth advantage to dominate. Oscillatoriales members in sulfur-rich environments are well documented [9,10]. *Oscillatoria*. *Phormidium* and *Lyngbya* seem to adapt well in Gizan springs that are filaments mat-forming cyanobacteria. Therefore, Oscillatoriales, Nostocales and Stigonematales are the major components of the mat-forming cyanobacteria due to their high adaptability to thermal habitat [6,13]. Further, filamentous life form developed unique thick exopolysaccharide sheath that serves as the backbone of the microbial mat in general [11].

*Chroococcus* and *Synechococcus* represent a highly pervasive genus in thermal habitat. The unicellular planktonic form was not recorded before in thermal springs of Saudi Arabia. However, these two genera are associated with members of filamentous mat-forming orders (Oscillatoriales, Nostocales and Stigonematales). Therefore, they live in mat-forming microhabitat and rarely present as a free floating in the thermal water. Therefore, filamentous cyanobacteria are crucial in the thermophilic ecosystem to protect unicellular species [3-6]. *Synechococcus* is well-known to adapt to extreme temperatures [7] and is optimized to live in chemically diverse hot springs [8].

Human transformation of Thermal springs: it was an opportunity to observe the ecological impact of the remolding in Labibah and Aridah springs and its effects on species biodiversity. Before remodeling, cyanobacteria present as a fragile mat on the soil surface of Labibah. However, after remodeling cyanobacteria mat becomes attached to the cemented rock wall that provides a stable surface. Only two species recorded before remolding, however, a total of 18 species colonized and form intricate mat after remodeling. Indeed, remodeling is a key factor that causes a dramatic increase in species composition because stable cemented-wall seems to be essential for mat-development that allows enough time for the filamentous species to colonize and build up healthy habitat for diverse species.

Flooding was quite frequent due to torrential rains on the mountain that forms massive water canals. Because Khobah and Qowah located wadi bed that frequently flooded. During this work, the flood was robust enough to remove any cyanobacteria mats colonized in the drainage net of these two springs. Although, these two spring were equally flooded but ecologically distinct due to high anthropogenic impact in Khobah while Qowah naturally protected. Indeed, Qowah is a virgin that supports the highest species diversity of cyanobacteria in all thermal springs investigated so far. Although Bin Malik artificially protected, it supports the lowest species diversity in Gizan. Indeed, natural protection is advantageous over the artificial protection because artificial protection stabilizes thermal community for an extended period that causes it to reach maturation and steady state that elects limited biodiversity. However, ecological disturbance due to flood prevented community maturation and increased habitat diversity that promotes high biological diversity. This observation reflects the strength of cyanobacteria to recolonize and invade such habitat in a short period. *Microcoleus vaginatus* is a well-known species that dominate desert soil crust and thermal springs that may be indicative of the role of desert crust in seeding hot springs after the flood.

The power of multivariate analyses was employed to generate a mathematical picture that provides ecologically relevant explanations. Gizan thermal springs were classified based on the degree of human interference. Tourists heavily visit Tharban, Khobah and Aridah. However, Qowah is naturally secured from anthropogenic interventions followed by the artificially-protected Bin-Malik spring. Indeed, the human effect is the key factor controlling cyanobacteria species diversity in southwest thermal springs, KSA. However, temperature, pH and all other factors are provided fine-tuning to these thermal ecosystems. Indeed, ordination analyses were compatible with cluster analyses that added additional evidence that human impact is the controlling factor of cyanobacteria biodiversity as long as the temperature is moderate and pH is neutral or slightly alkaline.

This work help extracts unique ecological data for thermal springs in Saudi Arabia that might generate rational decisions for the sustainable development of these valuable resources. Indeed, natural climatic conditions are compelling that promote biodiversity in thermal ecosystems. Qowah thermal spring is an ideal resource that requires rational management protection program to preserves virginity and biodiversity in parallel. Indeed, thermal ecosystems are valuable resources for thermophiles that are ruling a broad range of biotechnological applications.

## ACKNOWLEDGMENT

This project was funded by the National Plan for Science, Technology and Innovation (MAARIFAH)- King Abdulaziz City for Science and Technology - the Kingdom of Saudi Arabia- award number (AT- 34 - 169). The authors also, acknowledge with thanks, Science and Technology Unit, King Abdulaziz University and Faculty of Sciences, Khulais branch, Jeddah University for technical support.

## REFERENCES


1. Mohamed, Z.A., 2008. Toxic cyanobacteria and cyanotoxins in public hot springs in Saudi Arabia. Toxicon, 51: 17-27.
2. Lashin, A., M. Pipan, N. Al Arifi, A. Al Bassam, A. Mocnik and A. Forte, 2015. Geophysical exploration of the western Saudi Arabia geothermal province: First results from the Al-Lith area. Proceeding World Geothermal Congress. Melbourne, Australia: 19-25.
3. Arif, A., 1997. Composition of blue-green algal mats and water chemistry of the Bani Malik hot spring (Gizan Province), Saudi Arabia, Kuwait J. Sci. Eng., 24: 109-122.
4. Papke, R.T., N.B. Ramsing, M. Bateson and D.M. Ward, 2003. Geographical isolation in hot spring cyanobacteria, Environ. Microbiol., 5: 650-659.
5. Pentecost, A., 2003. Cyanobacteria associated with hot spring travertines, Can. J. Earth Sci., 40: 1447-1457.
6. Sompong, U., P.R. Hawkins, C. Besley and Y. Peerapornpisal, 2005. The distribution of cyanobacteria across physical and chemical gradients in hot springs in northern Thailand, FEMS Microbiol. Ecol., 52: 365-376.
7. Miller, S.R. and R.W. Castenholz, 2000. Evolution of thermotolerance in hot spring cyanobacteria of the genus synechococcus, Appl. Environ. Microbiol., 66: 4222-4229.
8. Ferris, M.J, A.L. Ruff-Roberts, E.D. Kopczynski, M.M. Bateson and D.M. Ward, 1996. Enrichment culture and microscopy conceal diverse thermophilic Synechococcus populations in a single hot spring microbial mat habitat, Appl. Environ. Microbiol., 62: 1045-1050.

9. Chorus, I. and J. Bartram, 1999. Toxic Cyanobacteria in Water:A guide to their public health consequences,monitoring and management. First published E & FN Spon, an imprint of Routledge, 400pp 242-246.
10. Elshahed, M.S., J.M. Senko, F.Z. Najar, S.M. Kenton, B.A. Roe, T.A. Dewers, J.R. Spear and L.R. Krumholz, 2003. Bacterial diversity and sulfur cycling in a mesophilic sulfide-rich spring, Appl. Environ. Microbiol., 69: 5609-5621.
11. Van Gemerden, H., 1993. Microbial mats: a joint venture, Mar. Geol., 113: 3-25.
12. Teske, A. and D.A. Stahl, 2002. Microbial mats and biofilms: evolution, structure and function of fixed microbial communities. In: J.T. Stanley and A.L. Reysenbach, Editors, Biodiversity of Microbial Life, Wiley-Liss Press, NewYork, NY, pp: 22-45.
13. Roeselers, G., T.C. Norris, R.W. Castenholz, S. Rysgaard, R.N. Glud, M. Kühl and G. Muyzer, 2006. Diversity of phototrophic bacteria in microbial mats from Arctic hot springs (Greenland), Environ. Microbiol., 9: 26-38.
14. Khlil, A., 2011. Isolation and characterization of three thermophilic bacterial strains ( lipase, cellulose and amylase producers ) from hot springs in Saudi Arabia. Afr. J. Biotechnol., 10(44): 8834-8839.
15. Mohammad, A.K., A.S. Ehab, M.S. Maher and H.B. Ali, 2012. Thermo-aerobic bacteria from geothermal springs in Saudi Arabia. African Journal of Biotechnology, 11: 4053-4062.
16. WHO, 1998. Guidelines for Drinking-Water Quality - Second Edition - Volume 2- Health Criteria and Other Supporting Information- Addendum, pp: 200.
17. APHA, 1995. Standard Methods for the Examination of Water and Wastewater (19th ed), American Public Health Association, Washington, pp: 150.
18. Anagnostidis, K. and J. Komarek, 1988. Modern approach to the classification system of cyanophytes. 3. Oscillatoriales, Algol. Stud., 58: 291-302.
19. Anagnostidis, K. and J. Komárek, 1990. Modern approach to the classification system of the cyanophytes 5: stigonematales, Algol. Stud., 59: 1-73.
20. Bourrely, P., 1968. Les algues d'eau douce II les algues Jaunes et bruns N. Boubee and Cie, Paris, 438 pp.
21. Desikachary, T.V., 1959. Cyanophyta. (ed.) Indian council Of Agriculture Research New Delhi, 686 pp.
22. Komárek, J., 1998. Cyanoprokaryota Teil 1 / Part 1: Chroococcales, Süßwasserflora von Mitteleuropa, Band 19/1.
23. Prescott, A.G.W., 1978. How to know the fresh water algae (Third eddition), pp: 293.
24. Komárek, J. and A. Anagnostidis, 2007. Cyanoprokaryota Part 2: Oscillatoriales. Süßwasserflora von Mitteleuropa, Band 19/2.
25. Komárek, J., B. Büdel, G. Gärtner, L. Krienitz and M. Schagerl, 2013. Süßwasserflora von Mitteleuropa, Bd. 19/3: Cyanoprokaryota: 3. Teil / 3rd part: Heterocytous genera.
26. Van Vuuren, S.J., J. Taylor, C. van Ginkel and A. Gerber, 2006. Easy identification of the most common freshwater algae. A guide for the identification of microscopic algae in South African freshwaters. North-West University (Potchefstroom Campus), pp: 2520.212.
27. Nienaber, M.A. and M. Steinitz-Kannan, 2018. A Guide to Cyanobacteria: Identification and Impact Kindle Edition. Kentachy, pp: 186.
28. Henderson, P.A. and R.M.H. Seaby, 2014. Community Analysis Package Version 5, Pisces Conservation Ltd, Lymington, UK