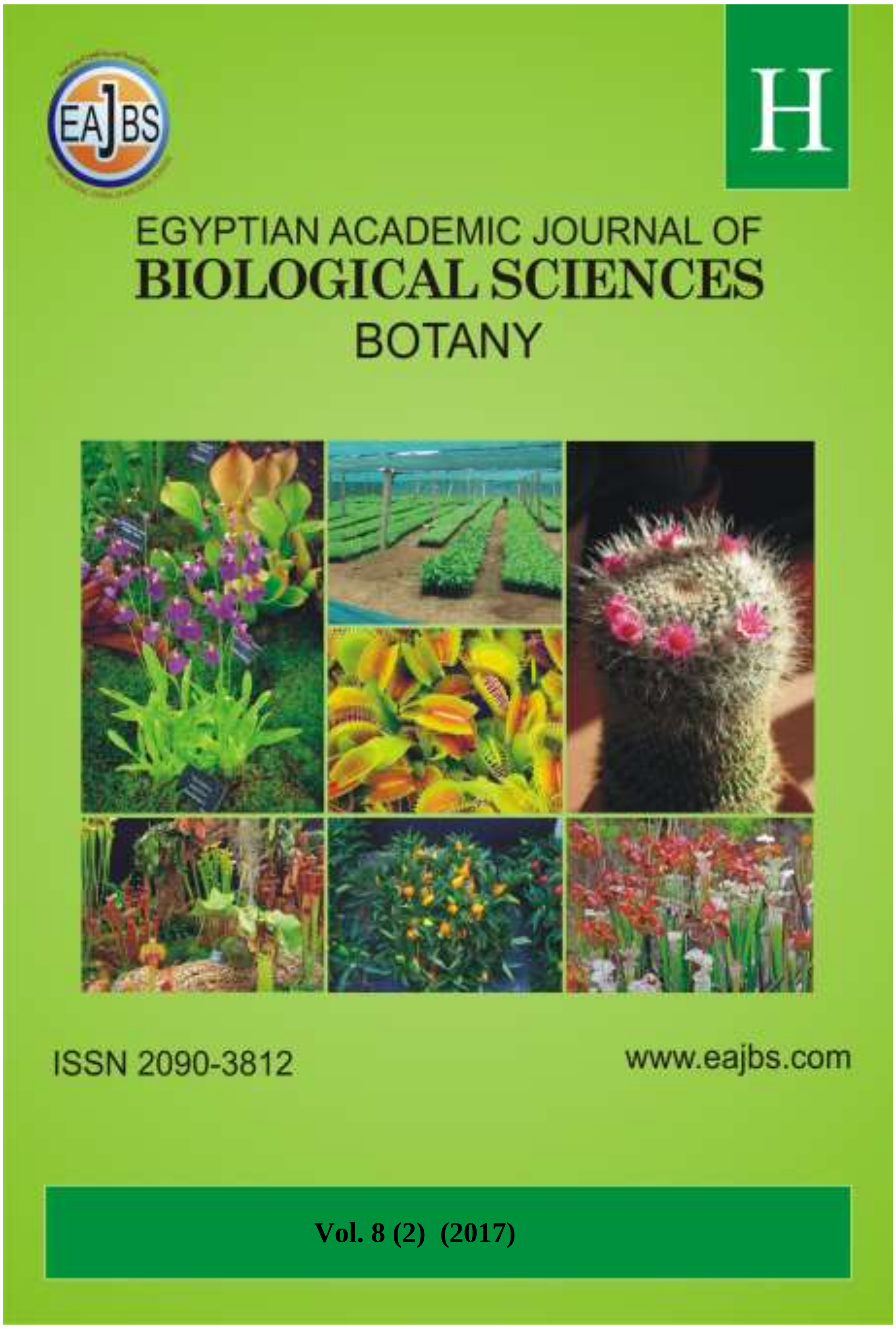

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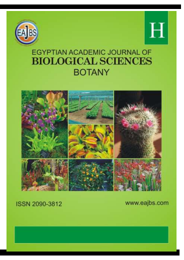
www.eajbs.eg.net

Citation :*Egypt. Acad. J. Biolog. Sci.* (H. Botany) *Vol.8(2)pp59-74(2017)*

Egypt. Acad. J. Biolog. Sci.,8(2):59–74 (2017)

Egyptian Academic Journal of Biological Sciences
H. Botany
ISSN 2090-3812
www.eajbs.eg.net



Diatoms Diversity of Thermal Springs in the Southwest Region, Saudi Arabia

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ARTICLE INFO
Article History
Received: 25/10/2017
Accepted: 21/11/2017

Keywords:
Bacillariophyta- extreme habitat- physicochemical factors- Gizan - Saudi Arabia.

ABSTRACT

Hot springs have received attention in thermal systems around the world. Particularly, as they relate to Diatoms. Diatoms biodiversity of thermal springs in the southwest region, Saudi Arabia was studied. Seasonal collections were carried out in Gizan thermal springs in six localities from winter 2015 to autumn 2016. A total of 23 species have been identified, recorded based on their frequency. Fifteen species were considered the most dominant species. The majority of it is the inhabitant of Qowah spring, Diatoms present in high abundance in 80% of thermal spring in Gizan region. This may indicate that diatoms have an essential biological role in the development and stability of the thermal spring ecosystem. Diatoms are the most frequent species present in Saudi Arabia thermal springs based on this study. Four species *Cocconeis pinnata*, *Cocconeis scutellum*, *Hantzschia amphioxys* , and *Melosira varians* were only recorded in Qowah and are the only rare diatoms species. In this study, rare species with respect to all sites were abundant in Qowah. Therefore, Qowah is a reservoir of those rare species. Accordingly, Qowah protection will help protect these rare species. Indeed, it is recommended to generate regulations to protect biodiversity in Qowah thermal spring

INTRODUCTION

Hot springs are one of the first sites of the discovery of the third domain of life, the Archaea (Barns et al, 1994, 1996). Extreme environments such as hot springs have become more interesting for those who are seeking to discover life on places other than earth within our own solar system, and beyond. Thus, hot springs may represent analog habitats that life forms can withstand; what we humans perceive to be “extreme,” with different chemical compositions (Seckbach and Kociolek, 2011).

Prokaryotes in these extreme environments, particularly Cyanobacteria are well documented, but considerably low record is accounted for eukaryotes. However, the thermal resistance of algal species occurs in water bodies with temperatures from 30 up to84°C. Diatoms are a group of eukaryotes that occupy a wide array of ecological niches, their species are known to occur in, on and around the ice, as well as extremely acidic and hot waters (Nikulina and Kociolek, 2011). Thermophilic algae species belongs to Chlorophyta, Bacillariophyta, Euglenophyta and Dinophyta. Research on diatoms has revealed that *Rhopalodia*, *Denticula*, *Mastogloia*, *Navicula*, *Nitzschia*, *Pinnularia*, *Caloneis* and *Achnanthes* are some of the genera found in thermal habitats (Stockner, 1967,1968; Jonker, 2013). Species diversity is influenced by the mineral composition and water temperature of the thermal spring (Sompong et al. 2005; Cantonati and Lange-Bertalot, 2010).

In Saudi Arabia ten thermal spring recorded, six of these observed in Gizan area (Mohamed, 2008), 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. Generally, in thermal springs, extremophiles are exposed to extreme variations in pH, temperatures, light intensities, salt concentrations, and sometimes high level of heavy metals (Arif, 1997; Papke et al. 2003; Pentecost, 2003; Sompong et al. 2005). Therefore, these microorganisms are tuned to these extreme microhabitats within thermal spring ecosystem (Miller and Castenholz, 2000; Ferris et al. 1996).

Stress factors such as pH, temperatures, light, salt concentrations, dissolved ions, and sometimes heavy metals are common in thermal springs (Arif, 1997; Papke et al. 2003; Pentecost, 2003; Sompong et al. 2005). Therefore, specialized organisms inhabit thermal springs that are adapted to this specific microhabitat (Miller and Castenholz, 2000; Ferris et al. 1996). This study aims to identify diatoms and analyze their ecological conditions chemical and biological components of the hot springs to understand the factors controlling growth and distribution of Diatoms that documents the general characteristics of thermal springs ecosystems.

MATERIALS AND METHODS

Study Sites:

This work is intended to investigate thermal springs in Saudi Arabia to search for ecological research. Sampling, identification of Bacillariophyta were done during 2015. To 2016. The study area is present in the southwestern region of Saudi 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 (Figs. 1 & 2 The-A&B). In Haridhah governorate, there is a small thermal spring's named Labibah (Figs. 2 Lab-A&B) that is 685 km south of Jeddah and novel record in this study. Interestingly, Labibah spring was remodeled by Al Haridhah governorate between winter and spring of 2015. Gizan area is the southwest corner of Saudi Arabia that characterized by seven thermal springs located in three main locations, 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 (Figs. 2 Ar1 A&B to 5A&B). Remodeling and regulatory development in this site are evident. In Al Qowah governorate, there are two thermal springs previously known as Ain Khulab that named Kobah (Figs. 2 Kob-A&B) and Ain Khulab Qowah named here Qowah (Figs. 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 (Figs. 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 eukaryotic species.

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, and chlorophyll concentration were measured directly in the running thermal spring water to ensure accuracy and reliable measurements.

Chemical species of relevance to Bacillariophyta 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 (W.H.O., 1998) Macronutrient and micronutrients analyzed with YSI 9500 Photometer and its kits (Yellow Spring, OH, USA) according to the manufacturer protocols. All chemical analysis of the macronutrient and micronutrients are analyzed by YSI kits used with YSI 9500 Photometer (Yellow Spring, OH, USA) according to the manufacturer protocols.

Bacillariophyta Productivity and Species Abundance:

Regular seasonal collections were carried out to the studied sites from winter 2015 to autumn 2016. For qualitative studies of Diatoms species, samples were fixed and concentrated using Lugol's solution (Utermohl, 1958). Concerning diatoms, it was necessary to clear it using conc. H_2SO_4. 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 and recorded based on their frequency.

Identification of Bacillariophyta Taxa:

Species identification, as well as the standing crop carried out using a phase contrast microscope (Carlzeiss, Jena). Photographs will take using an inverted microscope (OLYMPUS series 1 x 70 equipped by SC35 camera (type 12) and a color video monitor Panasonic, Tc-1470Y. The main references used for the identification of phytoplankton species were Hendey (1964); Bourrely (1970); Weber (1971). Vinyard (1975); Prescott’s (1978); Sykes (1981); McLaughlin (2012)

Statistical Analyses*:*

Community Analysis Package Version 5 used for Principle component analysis and cluster analysis (Henderson and Seaby, 2014).

RESULTS

Thermal springs are valuable resources that support diverse 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:

Temperature and pH value of Tharban is higher than other springs. Macronutrients are at a low level; however, micronutrients were similar to most of the Gizan springs (Table 1). In Labibah Hot Springs No significant changes in temperature during the sampled seasons (Table 1). Ions concentration are high, pH changed from slightly acidic during winter to slightly alkaline during the spring season. In the spring season, four water bodies recorded nearby Labibah. There was no rain or flood. It was surprising to see high variations of temperature, conductivity, pH and salinity. Only, magnesium shows an abnormally high level compared to all hot springs Bromine, nickel and aluminum (Tab.2 A-C) are comparable to the medium average of the hot springs tested so far.

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,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	Baro mmHg
Tharban	The	19.038944	220	47.30	4,924.27	2,586.50	8.46	10.14	553.43
		41.679194		**4.46**	**1,053.52**	**47.30**	**0.75**	**17.62**	**369.21**
Labibah	Lab	18.001492	123	41.26	7,328.61	4,020.23	7.38	2.05	744.77
		42.056008		**0.60**	**976.49**	**133.80**	**0.30**	**0.97**	**2.86**
Aridah 1	Ar1	17.035116	172	56.73	4,345.85	2,824.71	7.28	6.89	741.27
		42.989716		**1.10**	**88.35**	**57.35**	**0.23**	**1.70**	**3.53**
Aridah 2	Ar2	17.035400	172	54.39	4,369.63	2,840.23	7.61	32.14	741.24
		42.989532		**1.29**	**86.83**	**56.45**	**0.39**	**12.99**	**3.67**
Aridah 3	Ar3	17.035467	172	46.33	4,353.88	2,829.92	7.84	30.29	741.28
		42.989384		**2.77**	**101.27**	**65.66**	**0.39**	**13.90**	**3.57**
Aridah 4	Ar4	17.035900	172	46.94	4,342.43	2,822.63	7.81	30.45	741.29
		42.989319		**2.14**	**91.39**	**59.49**	**0.56**	**21.05**	**3.57**
Aridah 5	Ar5	17.036066	172	50.47	4,022.05	2,614.15	7.43	28.81	741.32
		42.989201		**2.62**	**551.15**	**358.28**	**0.28**	**25.75**	**3.36**
Khobah	Kob	16.764383	163	61.20	4,350.07	2,095.75	7.78	38.95	743.80
		43.129601		**7.62**	**1,409.23**	**513.68**	**0.72**	**50.35**	**5.08**
Qowah	Qow	16.796267	245	59.35	3,833.66	1,673.16	7.66	2.38	736.32
		43.200481		**9.48**	**957.14**	**129.45**	**0.34**	**1.57**	**4.62**
Binmalik	Bin	17.269768	657	45.02	2,631.32	1,237.33	7.35	3.00	705.3
		43.219215		**0.38**	**17.47**	**1.89**	**0.21**	**1.77**	**3.47**

The average temperature of Aridah springs is between 45ºC and 57ºC (Tab. 1). Specific Conductivity of springs in Aridah is similar to each other. In fact, it was observed that in Aridah, water flow in old heavily used spring start to decrease at the beginning until it completely stopped and new spring start to form in a close by the site. pH values are slightly alkaline during the winter season. However, in spring season most pH values increased to more alkaline values especially in Aridah 2 and 4. Low level of the chemical species is the general trend of the Aridah springs. Potassium and sulfur are within the average high of all springs (Tables 2 A-C). Nickel values in Aridah springs within the average middle compared to the other springs (Tables 2 A-C)

In Khobah (Tables 2A-C), phosphate and Zinc have the highest level observed during this work. Due to the presence of Khobah springs in wadi Dammed, long canal ended by large and deep Lake. Therefore, gradient measurement of physical parameters of Al Khobah were recorded to collect prices measurements and understand the nature of micro-environmental variations in this site. In the spring season, due to the absence of cool flood water, thermal spring water was able to keep its temperature above 43ºC that is close to or higher than that of many springs. The level of dissolved ions stayed high during the discharge time in the canal with slight change in the Lake. The original value of the pH from the spring source is slightly alkaline. pH did not increase during the winter season from the source until it reaches the reservoir (Lake). However, pH starts to increase from 7.15 to 9.06 during the spring season.

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 2C. 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

Flood is frequent in Qowah because it located in the bed of Wadi Dammed. Qowah is one of the high temperature thermal spring like Khobah. In Qowah spring shown in Potassium and silica Sulfate concentration values are the highest values recorded in all hot springs (Tables 2A-C). Molybdenum and Nickel concentration is the second highest level in it with respect to all hot springs is among the highest level similar shows the lowest level in Qowah (Tables 2A-C). Bin Malik thermal spring is characterized by moderate temperature, low salinity and slightly alkaline water Pressure measurements in this spring show low values. The flow rate of water in this spring is among the highest spring. (Tab. 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 high (Table 2B).

Chemical compositions of hot water from Bin Malik hot spring is shown in Tab. 2A-C. Chemical species are generally in the average range compared with other analyzed springs. Only, magnesium shows the second highest level among all springs. However, copper is the lowest concentration among all hot springs analyzed. Zinc and molybdenum are higher In addition to high level of aluminum and nickel.

Species Diversity and Frequency:

A total of 23 species of Bacillariophyta have been identified in Gizan thermal springs. The majority of these species are the inhabitant of Qowah spring (Table 3). Some of the most abundant species of Diatoms are *Achnanthes exigua* Grunow, *Synedra ulna* (Nitzsch) Ehrenberg, *Achnanthes grimmei* Krasske, *Caloneis bacillum* Grun, *Diploneis interrupta* (Kutz.) Cleve, *Epithemia zebria* (Ehr.) Kutz., *Gomphonema angustatum* (Kutz.) Rabenh., *Navicula cincta* (Ehr.) Kutz. *((*Table 4).

Table 3: Diatom species recorded in thermal springs of the southwest region of Saudia Arabia, thermal springs short names are according to table (1)

Species name	Freq.	Qow	Bin	Lab	Thr	Ar 1-4	Kob	Ar 5
1. *Achnanthes exigua* Grunow	20	+	+	+	+	+	+	+
2. *Achnanthes grimmei* Krasske	20	+	+	+	+	+	+	+
3. *Achnanthes pinnata* Hust.	20	+	+	+	+	+	+	+
4. *Caloneis bacillum* Grun.	20	+	+	+	+	+	+	+
5. *Diploneis interrupta* (Kutz.) Cleve	20	+	+	+	+	+	+	+
6. *Diploneis smithii* (Brébisson) Cleve	20	+	+	+	+	+	+	+
7. *Epithemia zebria* (Ehr.) Kutz.	20	+	+	+	+	+	+	+
8. *Gomphonema angustatum* (Kutz.) Rabenh.	20	+	+	+	+	+	+	+
9. *Navicula cincta* (Ehr.) Kutz.	20	+	+	+	+	+	+	+
10. *Navicula radiosa* Kutz.	20	+	+	+	+	+	+	+
11. *Nitzschia frustulum* Kutz	20	+	+	+	+	+	+	+
12. *Nitzschia obtusa* W. Sm.	20	+	+	+	+	+	+	+
13. *Pinnularia castor* Hohn et Hellerm	20	+	+	+	+	+	+	+
14. *Surirella ovalis* (Brébisson)	20	+	+	+	+	+	+	+
15. *Synedra ulna* (Nitzsch) Ehrenberg	20	+	+	+	+	+	+	+
16. *Achnanthes lanceolata* (Brébisson) Grun.	19	+	+	+	+	+	+	-
17. *Achnanthes minutissima* Kutz.	19	+	+	+	+	+	+	-
18. Cyclotella striata (*Kutzing*) *Grunow*	18	+	+	+	+	+	+	-
19. *Nitzschia thermalis* Kutz.	18	+	+	+	+	+	+	-
20. *Cocconeis pinnata* W.Gregory ex Greville	1	+	-	-	-	-	-	
21. *Cocconeis scutellum* Ehrenberg	1	+	-	-	-	-	-	-
22. *Hantzschia* amphioxys (Ehrenberg) Grunow	1	+	-	-	-	-	-	-
23. *Melosira varians* C.Agardh	1	+	-	-	-	-	-	-
Total number of species		23	19	19	19	19	19	15

In Harrah Tharaban a limited number of Bacillariophyta (Table 3) were recorded. Only one species *Navicula cincta* (Ehr.) Kutz. are considered as the most abundant species of diatoms while only *Navicula radiosa* Kutz. are the most dominant species were recorded in In Harrah Tharaban springs. Also, Bacillariophyta are present in low diversity compared to other spring in Labiba. However, seasonal changes in Bacillariophyta diversity is almost absent only *Achnanthes minutissima* Kutz. are the most abundant species was recorded in Labibah spring(Table 3).

Bacillariophyta was present with 19 species In Aridah spring (Tab. 3). These species are mostly concentrated in Spring 3 and 4, they show more abundance during the spring season. The most dominant species were recorded are *Caloneis bacillum* Grun, *Navicula cincta* (Ehr.) Kutz. *Navicula radiosa* Kutz.

While In Khobah spring Bacillariophyta as usual present in high diversity and frequency during the spring season (Table 3). The most abundant species of Bacillariophyta were *Caloneis bacillum* Grun., *Diploeis smithii* (Brébisson) Cleve, *Epithemia zebria* (Her.) Kutz., *Navicula cincta* (Her.) Kutz., *Navicula radiosa* Kutz., and *Surirella ovalis* (Brébisson) . However, diatoms are dominant by 19 species in Bin Malik spring (Table 3). All diatoms species are present in abundant quantities.

Table 4. Species recorded in this study are grouped based on their presence and absence

	Rare sp. (0-3%)	Frequent sp. (4-50%)	Common sp. (51-85%)	Dominant sp. (86-95%)
1		*Cocconeis pinnata* W.Gregory ex Greville	*Achnanthes lanceolata* (Brébisson) Grun.	*Achnanthes exigua* Grunow
2		*Cocconeis scutellum* Ehrenberg	*Achnanthes minutissima* Kutz.	*Achnanthes grimmei* Krasske
3		*Hantzschia* amphioxys (Ehrenberg) Grunow	*Cyclotella striata* (Kutzing) *Grunow*	*Achnanthes pinnata* Hust.
4		*Melosira varians* C.Agardh	*Nitzschia thermalis* Kutz.	*Caloneis bacillum* Grun.
5				*Diploneis interrupta* (Kutz.) Cleve
6				*Diploneis smithii* (Brébisson) Cleve
7				*Epithemia zebria* (Ehr.) Kutz.
8				*Gomphonema angustatum* (Kutz.) Rabenh.
9				*Navicula cincta* (Ehr.) Kutz.
10				*Navicula radiosa* Kutz.
11				*Nitzschia frustulum* Kutz
12				*Nitzschia obtusa* W. Sm.
13				*Pinnularia castor* Hohn et Hellerm
14				*Surirella ovalis* (Brébisson)

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.

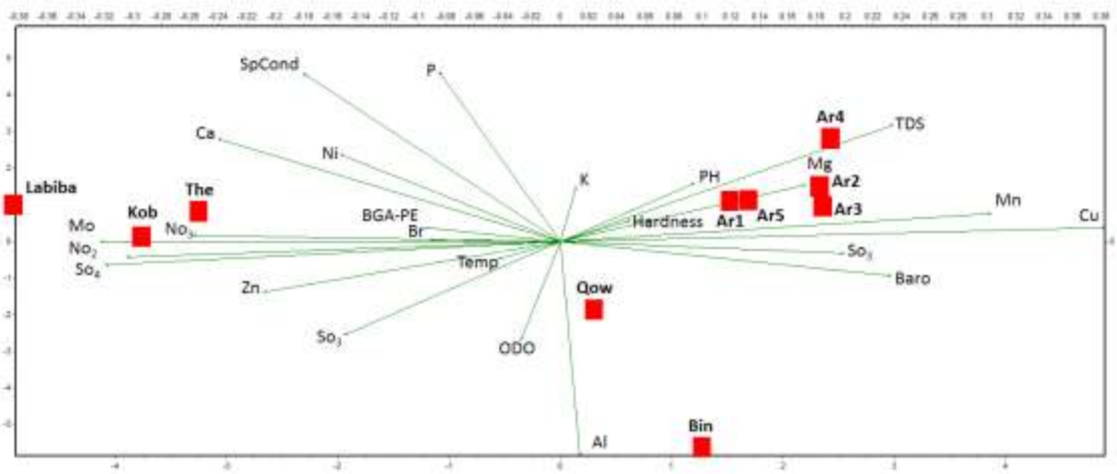

Fig. 3. Ordination plot of physicochemical parameters are represented by arrows, length, and direction of these arrows are the factor of their ecological strength and closely related factors are oriented in the same directions. Gizan thermal springs are marked with red squares named according to their 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, Baro: Pressure. Standard chemical species (Ca: Calcium, K: potassium, PO_4: Phosphate, NO_3: Nitrate, SO_4: Sulfate, Mg: Magnesium, Mn: Manganese, Cu: Cupper, Zn: Zinc, Mo: Molybdenum, Ni: Nickel, NO_2: Nitrite, Si: Silicon, SO_3: Sulfite, Br: Barium, Al: Aluminum).

Cluster Analyses of Physicochemical Parameters:

PCA ordination of thermal springs: PCA analyses of Gizan thermal springs based on the chemical analysis (Fig. 4A) and species composition (Fig. 4C) are relevant 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. 4B) 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. 4B) that is comparable with studies based on physicochemical factors and species compositions (Fig 4D). 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.

Cluster Analysis of Bacillariophyta Taxa.

Cluster analysis based on Diatoms presence and absence in thermal spring (Fig. 5) investigated that four new rarely present species named, *Cocconeis pinnata* W.Gregory ex Greville; *Cocconeis scutellum* Ehrenberg; *Hantzschia* amphioxys

(Ehrenberg) Grunow and *Melosira varians* C.Agardh were recorded for the first time only in Qowah thermal springs in comparison to all other studied hot springs. These species are not recorded before. All other Diatoms species are clustered according to their frequency percentage (Fig. 5)

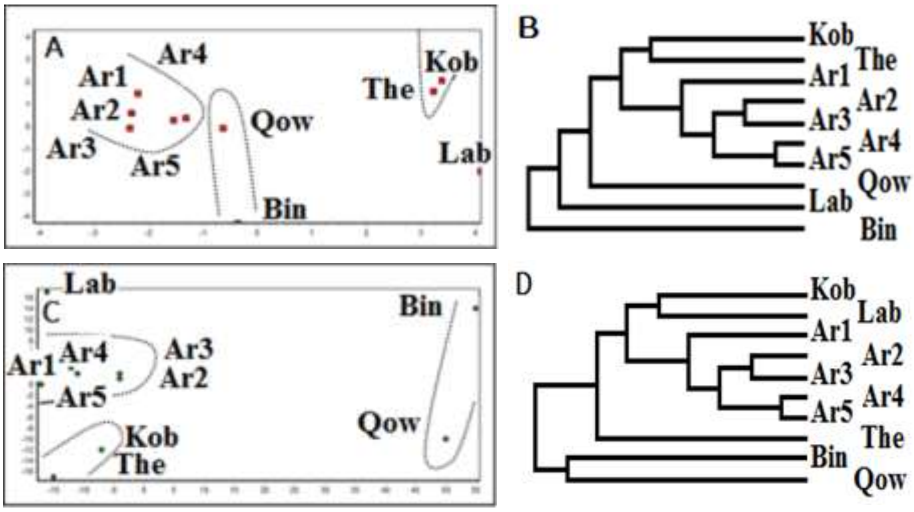

Fig. 4. PCA ordination and cluster analyses of the Gizan springs based on chemical analyses (A and B respectively), based on species composition (C and D). Aridah springs (Ar1-Ar5), Qow: Qowah spring; Bin: Bin Malik spring; Kob: Khobah spring, The: Tharban; Lab: Labibah spring. Dotted lines show thermal springs groups in PCA ordinations.

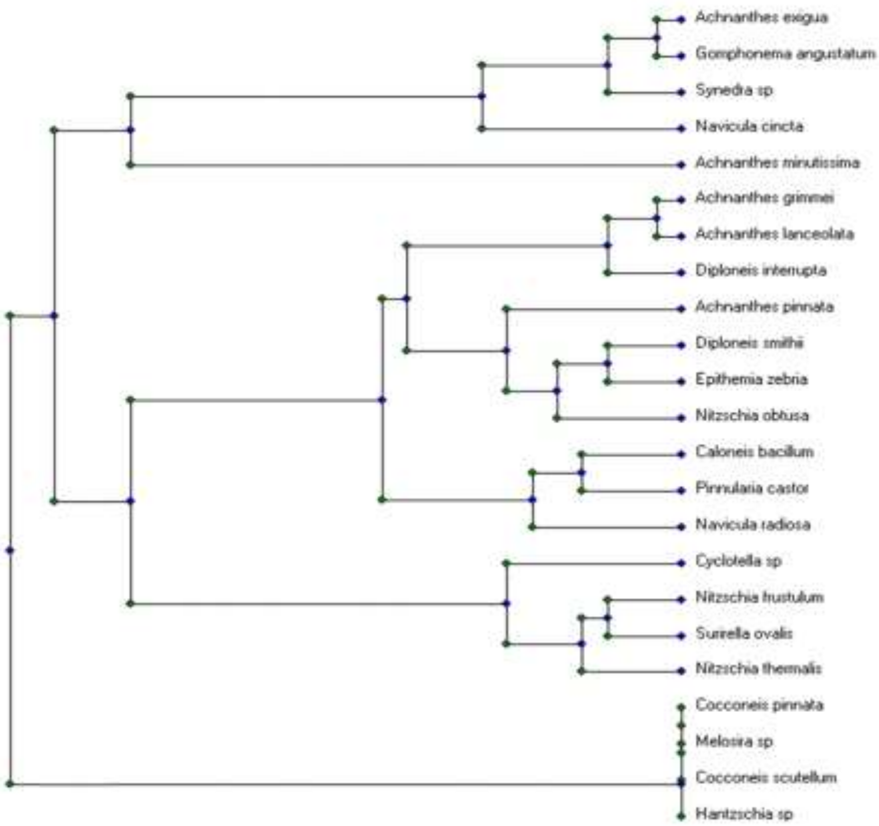

Fig. 5. Cluster analysis of Bacillariophyta taxa. It is based on their presence and absence in thermal spring investigated.

DISCUSSION

Algae are common in various habitats, at different geographic latitudes, and on all continents. They found in waters habitats with different degrees of temperatures, organic matter, hydrogen ions, and at various salinities. Research in recent years has shown that certain life forms can thrive under extreme thermal and chemical conditions (Wachnicka et al. 2011).Thermophilic algae have long attracted the attention of ecologists because of their unique adaptations to harsh environments (Sompong et al. 2005; Owen et al. 2008). Diatoms represented in Qowah by 23 species that is the highest diversity of Bacillariophyta recorded so far in Saudi Arabia thermal spring. New species were found only in this spring, again flood seeding might be the principal factor controlling such diversity. Generally, Diatoms present in high abundance and in 80% of the collected samples. Indeed, diatoms are an interesting component of thermal spring. This may indicate that diatoms have an essential biological role in the development and stability of the thermal spring ecosystem. Indeed, diatoms are the most frequent species present in Saudi Arabia thermal springs based on this study. *Achnanthes* genus is the highest frequent followed by *Diploneis* and *Navicula*. Only four species of diatoms were common. Four species of diatoms *Cocconeis pinnata* W.Gregory ex Greville, *Cocconeis scutellum* Ehrenberg, *Hantzschia* amphioxys (Ehrenberg) Grunow, and *Melosira varians* C.Agard. were only recorded in Qowah and are the only rare diatoms species.

However, high abundance of diatoms species may be due to its physiological adaption to the high concentration of micronutrients present in the water. Indeed, high silica and potassium are the governing factors for the high diversity of diatoms in this site. In their natural environment, phytoplankton communities are confronted with a variety of local and global changes including sunlight level, increasing temperature, acidity and nutrient concentrations. Algal communities may respond differently to these changes but in order to survive and to be successful these organisms need to acclimate and eventually adapt adequately (Caperon and Meyer 1972; Margalef, 1978; Parmesan, 2006; Massie et al. 2010; McLaughlin, 2012)

Qowah is one of the high temperature thermal spring like Khobah. Measurements were recorded during the winter season for these small sources. However, during spring measurements of all sources were carried out and average values are provided in and compared with the flood water measurements to role out that thermal water changed the property of the flood water. The obvious difference is clear between thermal water from spring source in both seasons and the flood water. Flood water is close to the fresh water characteristics (very low ions concentrations). Only, pH appears to be alkaline that may indicate high carbonate content in this wadi bed. The conductivity of thermal water is of moderate values compared to other springs. Temperature decreased followed by the decrease in conductivity values.

Chemical analysis of the hot water in Qowah spring. Potassium, silica, Molybdenum and Sulfate concentration values are the highest values recorded in Qowah spring with respect to all hot springs. Nickel concentration shows the lowest level in Qowah. Indeed, it is expected that silica and potassium will help support a dense population of diatoms that is obvious during spring. Diatoms represent one of the largest and most diverse groups of phytoplankton. In contrast to other phytoplankton groups, most diatoms are unique because they have a requirement for silicon, which is taken up as orthosilicic acid (Wischmeyer et al. 2003; Martin-Jézéquel et al. 2000). The temperature did not have a significant effect on the silica pattern or features of diatoms Javaheri (2015).

In Khobah spring Bacillariophyta as usual present in high diversity and frequency during the spring season. Therefore, high temperature spring is favorable for the growth of Diatoms. Indeed, diatoms are known to adapt for high temperature than other groups. Khobah is one of the attractive spring in Gizan area. However, the remodeled site for recreational activity drains its waste to the natural site. It is also possible to speculate that Diatoms are resistant to pollution. Most government bio assessment programs that examine diatom community structure employ a version of a pollution tolerance index (PTI), similar to the Hilsenhoff Biotic Index for invertebrates (Barbour et al. 1999). These indices rate diatom taxa by their sensitivities to increased environmental degradation, using diatom communities as a measure of environmental health. Barbour et al. (1999).

Temperature decreased in the spring season in all site measured in Khobah springs except the final Lake that store thermal water. The obvious reason is due to the presence of flood during winter that effectively decreased the temperature of the thermal water after its discharge from the source. In the spring season, due to the absence of cool flood water, thermal spring water was able to keep its temperature above 43ºC. The original value of the pH from the spring source is slightly alkaline. However, pH starts to increase from 7.15 to 9.06 during the spring season.

The average temperature of Aridah springs is between 45ºC and 57ºC. Conductivity is similar to each other which may indicate that these springs share the same thermal water source. In fact, it was observed that water flow in old heavily used spring start to decrease at the beginning until it completely stopped and new spring start to form in a close by the site. pH values are slightly alkaline during the winter season. However, in spring season most pH values increased to more alkaline values especially in Aridah 2, 4. Calcium, zinc, molybdenum and copper are among the lowest level compared to all analyzed hot springs in Gizan. Potassium and sulfur are within the average high of all springs. Nickel values in Aridah springs within the average middle compared to the other springs. Indeed, Aridah spring is clearly marked with pollution signs as a result of visitor’s activities and low management effort for protections. However, Bacillariophyta is present with 19 species. These species are mostly concentrated in Spring 3 and 4, they show more abundance during the spring season. Van Dam (1994) have assigned indicator values to diatom taxa based on broad regional surveys. These values are specific to environmental parameters such as pH, salinity, and trophic status.

No significant changes in temperature during the sampled seasons in Labibah Hot Springs. Ions concentration are high, pH changed from slightly acidic during winter to slightly alkaline during the spring season. The pH changes might be the factor that causes the increase in the biological composition of this spring. Change in turbidity is mostly due to the remodeling effect. Where, remolding provide more stable margin and the wall that minimize turbidity. In the spring season, four water bodies recorded nearby Labibah. There was no rain or flood. To understand these water bodies, physical parameters were measured in order to understand their nature. It was surprising to recorded high variations of temperature, conductivity, pH and salinity. These variations may support the speculation that these are the starter of new springs in this location. Bacillariophyta are present in low diversity compared to other spring. However, seasonal changes in Bacillariophyta diversity is almost absent. Therefore, changes caused due to seasonal variations are not the governing factors that control the diversity and abundance of Diatoms in Labibah. However, slightly seasonal variation in species composition of Bacillariophyta is observed. Indeed, remodeling has no effect on Bacillariophyta.

The temperature in Tharban is higher in the spring than that of the canal. pH value is higher than other springs that are may be due to the high activity of the visitors related to recreational activities. This is considerable high interference that may play a key factor in the physical, chemical and biological characteristics of Tharban spring. In Tharaban. Again, the limited number of Bacillariophyta provides additional evidence that human interference is a limiting factor for high diversity and causes a decrease in the diversity of all photoautotrophic groups inhibiting thermal springs. Bacillariophyta abundance is higher in spring compared to the winter season.

Moderate temperature, low salinity and slightly alkaline water are found in Bin Malik thermal spring. No significant seasonal differences in the water parameters observed. Pressure measurements in this spring show low values due to the location in the high elevated area. The flow rate of water in this spring is among the highest spring that is enough to support large recreational site. Chemical compositions are generally in the average range compared with other analyzed springs. Only, magnesium shows the second highest level among all springs. However, copper is the lowest concentration among all hot springs analyzed. Sulfate deposited is obvious throughout the path of hot water. Zinc and molybdenum are higher that may exert some physiological pressure on the biotic components of this spring. In addition to the high level of aluminum and nickel that are not required for algae growth.

However, diatoms are dominant by 19 species present in abundant quantities. However, high abundance of diatoms species may be due to its physiological adaption to the high concentration of micronutrients present in the water. Bahls, 2013; Seckbach and Kociolek (2011) used the following autecological criteria to categorize the tolerance of diatoms: nutrients, organics, salts, temperature, toxics, substrate stability, and suspended solids. Obscure diatom taxa were assigned an average tolerance value by genus. Diatoms are usually found in high abundance on rocky and cobble substrates; however, they are also known to inhabit epiphytic and epipsammic communities.

In Gizan sites *Cocconeis pinnata*; *Cocconeis scutellum; Diploneis interrupta* (Kutz.) Cleve; *Gomphonema angustatum* (Kutz.) Rabenh. *Hantzschia* sp; *Melosira* sp; *Navicula cincta* (Ehr.) Kutz; and *Navicula radiosa* Kutz were abundanant species. This study is supported the result by different authors revealed that diatom distribution is distinguished by environmental characteristics that limit species distribution and the scope is restricted by the capabilities of species (Suphan,2004,2009; Inthasotti, 2006; Chayakorn et al. 2018) The specific arrangement of diatoms can also broaden according to the relevant environmental factors (Kristiansen,1996). Diatoms are important organisms present at the beginning of the food chain in aquatic ecosystems. In fact, diatoms can grow in extreme habitats such as hot springs that have high temperatures and differing physicochemical characteristics from other ecosystems. The study of diatoms in such habitats may lead to the discovery of potent diatoms that can be used in various heat-tolerant applications (Pruetiworanan et al., 2017). Such as diatoms that can be grown at high temperatures and this would allow researchers to make use of enormous un-used tracks of desert land for biofuel production (Gendy and El-Temtamy, 2013). Therefore, it is important to study the biodiversity database of hot spring diatoms.

Conclusion:

This analysis will help to understand the distribution of diatoms species and their frequency rate that simplify field search for certain species. Indeed, 23 species of diatoms are easy to pick them from the thermal springs. However, rare species might easily be sampled due to their presence in only one spring (Qowah). Therefore, Qowah may be considered as a reservoir of rare species. This finding is critically important because rare thermal species may need some protection and care. Accordingly, Qowah protection will help protect these rare species. Indeed, it is recommended to generate certain regulations to create a protection site in Qowah thermal spring.

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.

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