## **Diatom flora in three Springs of Golestan Province**

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#### Abstract

Springs are highly important habitats for biodiversity trend. Hence three springs in Golestan Province were selected for floristic study of diatom assemblages. Samples were collected seasonally from stony and sediment substrates. In total, 75 taxa belong to 38 genera were identified. Gomphonema and Nitzschia each with 7 species, Navicula with 6 species, Surirella with 5 species and Cymbella with 4 species were the most species-rich genera. Results revealed that Gol-e-Ramian Spring with 61 taxa had the highest species richness. Achnanthidium minutissimum, Cocconeis placentula, Fragilaria crotonensis, Gomphonema micropus, Meridion circulare, Nitzschia vermicularis, and Planothidium frequentissimum were the most abundant taxa. Most of the species identified in the present research have been observed within running water environments in Golestan province and other parts of Iran. Our study improved knowledge about thediatom communities of springs in Golestan province.

**Keywords**: Epilithon, Epipelon, Gol-e-Ramian Spring, Nilberg Spring, Seyyed Kalateh Spring.

## Introduction

Springs are unique aquatic habitats and can be defined as linkage between terrestrial and aquatic ecosystems, ground and surface waters. There is high habitat complexity and large number of different types of springs which allows them to be the important component of riverine landscape biodiversity. Springs are often very small, but they are numerous and thus, provide habitats for species that are rare elsewhere because of their sensitivity to anthropogenic (Cantonati et al., 2012b). Toxböck et al. (2017) considered springs as refugial habitats for Swiss freshwater microflora including diatoms. Sabater and Roca (1992) while studying diatom distribution in Pyrenean springs, noted that in comparison with other environments (e.g. streams and subaeria rocks), springs in the Pyrenees revealed a higher number of taxa with poorly-known distribution. Cantonati has extensive studies on spring diatoms

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(Cantonati, 1998; Cantonati and Lange-Bertalot, 2010; Cantonati et al., 2012a), however he believed that despite their importance for biodiversity and water quality, springs are much less studied than other aquatic ecosystems (Cantonati et al., 2012b). In Iran, although in recent years there has been an increased interest in diatom studies (Nejadsattari, 2005; Nejadsattari et al., 2007; Shams and Afsharzadeh, 2007; Soltanpour Gargari et al., 2011; Kheiri et al., 2019; Pourheydar Khoshkrudi et al., 2014; Panahy Mirzahasanlou et al., 2018), but there is a data lacks in current literature about diatom flora of springs. There are many springs in the south part of Golestan province. Regarding the importance of springs in biodiversity trend, three springs (Gol-e-Ramian, Seyved Kalateh, and Nilberg) in the southern part of Ramian city selected for this study.

#### **Material and Methods**

Ramian county is almost located in middle of Golestan province which is 75 Kilometer away from Gorgan (Capital of Province). There is green nature and types of streams and springs in this country. In this study, we selected three springs (Fig. 1). All three springs are situated in south part of Ramian city. (1) Gol-e-Ramian spring is a karstic spring along the road of Ramian to Alang. This spring is in the form of natural pool with length of 90 meters, width of 80 meters, and a depth of 44 to 80 meters. It is formed on a river. (2) Seyyed Kalateh Spring is located in a valley next to Seyyed Kalateh village. It is about 6 km away from the Ramian City; and (3) Nilberg Spring is located in 4 km away from Ramian city and is situated in the middle of beautiful forest. To investigate springs hydrochemistry, water samples together with stony substrates were seasonally collected from each spring. Some parameters such as electrical conductivity (EC), pH, Temperature (T) and dissolved oxygen (DO) were directly measured at the site. The rest of physicochemical parameters including calcium, magnesium, sodium, potassium, bicarbonate, sulfate, chloride, nitrate, phosphate, silica, total hardness was measured at the water chemistry laboratory of Gonbad Kavous University.

Benthic diatoms were examined seasonally during 2018 and 2019. Epilithic samples were collected from stony substrates by brushing using a toothbrush; epipelic samples were collected by gently scooping up the top layer of sediment. Then, samples were fixed with 4% formalin. Preparation for microscopic analysis was done by acid digestion method (Taylor et al., 2007). Permanent slides were prepared by mounting the cleaned diatom valves in Canada Balsam and analyzed using light microscope. Identifications were performed using Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b); Krammer, 2000; Lange-Bertalot, 2001; Krammer, 2002; Krammer, 2003; Bahls, 2006; Bishop et al., 2017; Jüttner et al., 2018. In each slide 300-400 valves were counted to estimate the relative abundances.

## Results

Preliminary hydrochemistry results showed calcium and bicarbonate ions are

the most abundant cation and anions in the water of all three springs, respectively (Table 1). Hence,  $Ca-HCO_3$  is a dominate type

of springs water. This is because limestone is most probably the reservoir of the springs in study area.



Fig. 1. Location of the study sites.

**Table 1.** Physicochemical parameters of three springs. Here, all parameters except EC, pH, and T are in mg/l. pH is unitless; the unites of T and EC are  $^{\circ}$ C and  $\mu$ s/cm3, respectively.

Paremeters	Gol-e-Ramin	Seyyed Kalateh	Nilberg
Ca <sup>2+</sup>	251	201	191
$Mg^{2+}$	56	53	74.5
$\mathrm{K}^+$	19.94	38.445	27.605
$Na^+$	24.26	45.145	47.955
HCO <sub>3</sub>	293	284	265
SO4 <sup>2-</sup>	42	38	45
Cl-	23	20	27.5
NO <sub>3</sub> -	0.46	1.775	1.685
PO4 <sup>3-</sup>	0.109	0.0995	0.0975
$SiO_2$	0.165	0.525	0.49
DO	5.695	6.635	7.505
TH	307	254	265.5
TDS	296	243.5	264
EC	607.5	500.5	542
pН	6.32	6.485	6.24
Т	22.25	16.2	14.7

Taxa	Gol-e-	Seyyed	Nilberg
Achnanthidium minutissimum (Kutzing) Czarnecki	+	+	+
Achnanthidium sp.	+	-	-
Amphipleura pellucida (Kützing) Kützing	+	+	-
Amphora pediculus (Kützing) Grunow	+	+	+
Amphora copulata (Kutzing) Schoeman & REM.	+	-	- <u>-</u>
Aulacoseira granulata (Ehrenberg) Simonsen	+	-	
Bacillaria Paxillifera (O.F.Muller) T. Marsson	+	+	+
Caloneis budensis (Grunow) Krammer	+	3 <del>4</del>	-
Caloneis sp.	+	12	8 <b>-</b> 8
Cocconeis pediculus Ehrenberg	20	+	220
Cocconets placentula Enrenberg	-	+	+
Cycolena menegrinnana Kulzing Cymatooleura elliotica (Brébisson) W. Smith	1	-	+
<i>Cymbella affinis</i> Kutzing	+	+	+
Cymbella compacta Østrup	+	+	+
Cymbella cymbiformis C. Agardh	·+	+	+
Cymbella tumida (Brébisson) Van Heurek	21	+	120
Cymbopleura amphicephala (Någeli ex Kützing)	+	+	-
Combonleura citrus (1.P. Carter & Bailey-Watts)	+	+	+
Krammer		50)) (1))	1949
Denticula Kuetzingii Grunow	-	+	+
Diatoma moniliformis (Kützing) DM. Williams	÷	+	+
Diatoma vulgaris Bory de Saint-Vincent	-	+	2 <b>.</b>
Diploneis calcilacustris Lange-Bertalot & A.Fuhrmann	+	+	+
Diploneis Krammeri Lange-Bertalot & E. Reichardt	+	5	+
Diptonets Separanda Lange-Bertalot	+	+	1.0
Encyononsis minuta Krammer & F. Reichardt	+	+	+
Eunotia sp.	+	-	1927
Fallacia subhamulata (Grunow) D.G.Mann	+	+	+
Fragilaria crotonensis Kitton	+	-	-
Fragilaria recapitellata Lange-Bertalot & Metzelltir.	+	+	+
Frustulia vulgaris (Thwaites) De Toni	+	+	+
Gomphonema acuminatum Ehrenberg	+	÷	5 <b>.</b>
Gomphonema of numihum C Agardh	+	-	-
Gomphonema micropus Kützing	+	+	+
Gomphonema olivaceum (Hornemann) Brébisson	+	+	-
Gomphonema parvulum (Kützing) Kützing	+	+	+
Gomphonema sp.	+	+	+
Gyrosigma attenuatum (Kutzing) Rabenhorst		+	+
Gyrosigma scalproides (Rabenhorst) Cleve	+	+	+
Hant-schia amphiorys (Ehrenberg) Grunow	+	+	+
Luticola goeppertiana (Bleisch)D.G.Mann ex J.Rarick	+	+	
Luticola ventricosa (Kützing) D.G.Mann	+	+	+
Melosira varians C.Agardh	+	-	1 <del></del> )
Meridion circulare (Greville) C.Agardh	÷.	+	+
Neidium binode (Ehrenberg) Hustedt	2	+	+
Navicula broetzii Lange-Bertalot & E.Reichardt	+	+	+
Navicula capitaloradiata H.Germin ex Gasse	+	+	+
Navicula costellata Kutzing	+	+	+
Navicula trivialis Lange-Bertalot	+	+	+
Navicula tripunctata (O.F.Muller) Bory	+	+	+
Nitzschia dissipata (Kützing) Rabenhorst	#	+	1
Nitzschia heufleriana Grunow	+	+	+
Nitzschia inconspicua Grunow	-	5	+
Nitzschia nalea (Kützing) W Smith	+	Ŧ	-
Nitzschia sigmoidea (Nitzsch) W Smith	+	+	+
Nitzschia vermicularis (Kützing) Hantzsch	+	+	+
Pinnularia brebissonii (Kützing) Rabenhorst	+	32	+
Pinnularia graciloides var. triundulata (Fontell) Krammer	2	-	+
Planothidium freguentissimum (Lange-Bertalot) Lange-	+	+	+
Bertalot	a.	83	
Rhonalodia aibha (Ebrophere) O Müller	+	+	( <b>-</b> 0)
Stouroneis smithii Grupow	+	-	-+
Surirella angusta Kutzing	+	+	+
Surirella brebissonii Krammer & Lange-Bertalot	+	+	+
Surirella librile (Ehrenberg) Ehrenberg	+	+	+
Surirella minuta Brebisson	+	+	+
Surirella tenera W.Gregory	+	7	-
Tryptionella apiculata W.Gregory	+	+	+
Librari gulna (Nitzsch) Compere	+	-	-+
CHARTER GRAND (INIZACII) COMPCIE	1 m	200 million (1990)	

Table	2.	Taxa	identified	in	three	springs	of	Golestan	
** *									

Totally 75 taxa belong to 38 genera were identified in this study (Table 2 and Plate I-III) among them 40 taxa were common between three springs. Most of the taxa (71 taxa) belonged to class Bacillariophyceae; Coscinodiscophyceae and Mediophyceae each had 2 species. Gomphonema and Nitzschia each with 7 species were the most species rich genera; Navicula with 6 species, Surirella with 5 species and Cymbella with 4 species were in the next order (Fig. 2). Gol-E-Ramian Spring with 61 taxa had the highest species richness; following by Seyyed Kalate Spring with 55 taxa and Nilberg Spring with 51 taxa. The genus Gomphonema was the most species rich genus in the Gol-E-Ramian Spring, but in Seyyed Kalate Spring Navicula with 6 species and in Nilberg Spring both Navicula and Nitzschia each with 6 species had the highest number of species.

The most abundant taxa in Gol-E-Ramian Spring were *Ulnaria ulna* (maximum relative abundance 74.14%), *Fragilaria crotonensis*  (36.06%), Nitzschia vermicularis (34.98%), Achnanthidium minutissimum (26.76%), Planothidium frequentissimum (23.58%); in Seyyed Kalate Spring were Planothidium frequentissimum (52.13%), Gomphonema micropus (38.9%), Achnanthidium nanum (35.16%), Meridion circulare (30.06%), Surirella minuta (15.01%), Cocconeis placentula (23.44%); and in Nilberg Spring were Cocconeis placentula (41.06%), Planothidium frequentissimum (40.06%), Navicula rostellata (18.85%), Achnanthidium minutissimum (13.17%), Halamphora montana (13.17).

### Discussion

Similar to early floristic studies (e.g., Soltanpour-Gargari et al., 2011; Pourheydar Khoshkrudi et al., 2014; Panahy-Mirzahasanlou et al., 2018; Kheiri et al., 2019) the genera *Gomphonema*, *Nitzschia*, *Navicula* were found as the species rich genera in our study.





Fig. 2. Number of taxa in each genus in three springs.



**Plate I.** 1: Discostella stelligera. 2: Aulacoseira granulate. 3: Melosira varians. 4: Diatoma moniliformis. 5: Diatoma vulgaris. 6,7: Meridion circulare. 8: Fragilaria recapitallata. 9, 10: Fragilaria crotonensis. 11: Eunotia sp. 12: Cocconeis placentula. 13: Gomphonema acuminatum. 14: Gomphonema affine. 15: Gomphonema micropus. 16: Gomphonema parvulum. 17: Gomphonema cf. pumilum. Bar: 10 μm.



**Plate II.** 18: Navicula capitatoradiata. 19: Navicula rostellata. 20: Navicula tripunctata. 21: Navicula trivialis. 22: Navicula broetzii, 23: Navicula cryptotenella. 24: Fallacia sp. 25: Luticola ventricosa. 26: Luticola goeppertiana. 27: Frustulia vulgaris. 28: Neidium binode. 29: Stauroneis smithii. 30: Caloneis sp. 31: Pinnularia graciloides var. triundulata. 32: Pinnularia brebissonii. 33: Diploneis Krammeri. 34: Diploneis Separanda. 35: Diploneis calcilacustris. 36: Cymbella affinis. 37, 38: Cymbella compacta. 39: Cymbella tumida. 40: Cymbopleura amphicephala. 41: Cymbopleura citrus. 42: Halamphora montana. 43: Amphora copulata. Bar: 10 μm.



**Plate III.** 44: Cymbella cymbiformis. 45: Gyrosigma sp. 46: Gyrosigma scalproides. 47: Nitzschia inconspicua. 48: Nitzschia liebethruthii. 49: Nitzschia palea. 50: Nitzschia dissipata. 51: Nitzschia heufleriana. 52: Nitzschia sigmoidea. 53: Amphipleura pellucida. 54: Cymatopleura elliptica. 55: Surirella librile. 56: Surirella tenera. 57: Surirella minuta. 58: Surirella angusta. 59: Surirella brebissonii. 60: Hantzschia amphioxys.61: Tryblionella apiculate. 62: Tryblionella calida. 63: Rhopalodia gibba. Bar: 10 μm.

40 taxa were observed in all three springs; however, 13 taxa were observed only in Gol-E-Ramina Spring. This may relate to hydrology and chemistry of this spring. The occurrence of planktonic species such as Aulacoseira granulata, Discotella stelligera, Fragilaria crotonensis in Gol-E-Ramian Spring is explainable too, due to the width and depth of the spring. Also, only three species comprising Cymbella tumida, Diatoma vulgaris and N. Liebethruthii found in Seyved Kalate Spring and three species Cymatopleura elliptica, Nitzschia inconspicua, Pinnularia graciloides var. triundulata in Nilberg Spring. The rest of the species were common between two springs.

Results revealed that most of the species identified in the present research were observed also by other researchers in running water environments in Golestan province (Dadgar, 2016; Bayani, 2018; Lakzaie et al., 2018), and only a limited number of the species exclusively found in our research. For example, species of Amphipleura pellucida, Diploneis calcilacustris, D, separanda, D. Krammeri, Discostella stelligera, Fragilaria crotonensis, Halamphora bicapitat, Luticola ventricosa, Pinnularia graciloides var. triundulata, Rhopalodia gibba, Stauroneis smithii were identified in this research, however some of them were reported in other parts of Iran (Kheiri et al., 2019). Cantonati et al. (2012a) reported Planothidium frequentissiumum and Meridion circulare as characteristic for carbonate springs; Amphora pediculus and *Cocconeis placentula* as indicator of carbonate spring types. Moga et al. (2015) also reported *Meridion circulare* as crenophilous (mainly living in springs).

Finally, our study improved the knowledge on diatom communities of springs in Golestan province and provided fine observations of diatom taxa.

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