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ECOLOGICAL STUDY OF PERIPHYTON DIVERSITY IN CRENIC HABITAT OF SAHASHRADHARA (GARHWAL HIMALAYAS, INDIA)

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Abstract

A maiden attempt has been made to present a biodiversity of periphyton of the Sahashradhara springs, a group of one thousand springs and one of the most important clusters of limnocrenes and rheocrenes springs located in Doon Valley of Garhwal-Himalayas. The environmental monitoring of periphyton community of Sahashradhara springs was carried out for one complete annual cycle (October 2011-September 2012). Annual percentage composition of periphyton revealed that major contribution was represented by Bacillariophyceae (68-70%) followed by Chlorophyceae (26-28%) and Myxophyceae (3-5%). A total of 29 species of periphyton were recorded from different springs of Sahashradhara. A significant correlation between diversity of periphyton and environmental parameters- temperature, turbidity, conductivity, dissolved oxygen, free CO_2, alkalinity, FPOM and CPOM was observed. Regression analysis was performed between density of periphyton and physico-chemical variables, which revealed that temperature, conductivity, alkalinity, CO_2, TDS, CPOM and FPOM were found to have strong influence on the density and diversity of periphyton of Sahashradhara springs.

Key words: Periphyton, limnocrenes, rheocrenes, Sahashradhara springs, Doon Valley

Introduction

Springs in general possesses physico-chemicals stability, which make them special habitat compared to other freshwater habitat (Thienemann, 1922; Hynes, 1976) and thus, considered as hotspot for aquatic biodiversity (Williams and Williams, 1998; Cantonati *et al.*, 2006).Springs are one of the main components of the longitudinal zonation of lotic system, inspite of this; spring habitats have gained less attention than lakes and streams/rivers and are still understudied. Several research studies on biodiversity of springs have been conducted at the international level (Sabater and Roca, 1990, 1992; Williams, 1991; Roca and Baltanes, 1993; Zechmeister and Mucina, 1994; Botosaneaenu, 1998; Cantonati, 1998; Mezqueita *et al.*, 1999; Stoch, 2001; Di Sabtino *et al.*, 2003; Rosetti *et al.*, 2005; Cantonati *et al.*, 2006; Wojtal, 2006; Taxbock and Perisig, 2007; Bottazzi *et al.*, 2008; Cantonati and Spitale, 2009; Wojtal and Solak, 2009; Angeli *et al.*, 2010; Tomaselli *et al.*, 2011; Martin and Brunke, 2012; Abdelsalam and Tanida, 2013; Cantonati *et al.*, 2015).

A few scattered reports on some geological and limnological aspects of the springs of the Himalayas in India are available (Saha *et al.*, 1978; Qadri and Yousuf, 1979, 1988; Bhatt and Yousuf, 2002; Bhat and Pandit, 2011). Unfortunately, no work has been done so far on the in-depth study of biodiversity and physico-chemical stability of springs of Garhwal Himalayas. Therefore, the present study on the periphytonic community of springs of Sahashradhara of Garhwal Himalayas has been taken as an initiative. To the best of our knowledge, this will be the first extensive investigation on periphyton diversity from the spring habitats of Sahashradhara springs of Doon Valley of Garhwal Himalyas.

Materials and Methods

Sahashradhara (meaning cluster of thousand fold springs) is situated at 13 km away from the Dehradun city, the capital of Uttarakhand state, India. The Sahashradhara, a prominent place of tourists, is one of the most important clusters of helocrenes, limnocrenes and rheocrenes types of springs located in Doon Valley of Garhwal-Himalayas (Fig. 1).It lies on $30^{o}38'$N latitude and $78^{o}13'$E longitude. A total of five clusters of springs were selected as a representative subset (S_1, S_2, S_3, S_4 and S_5) of the area for study.

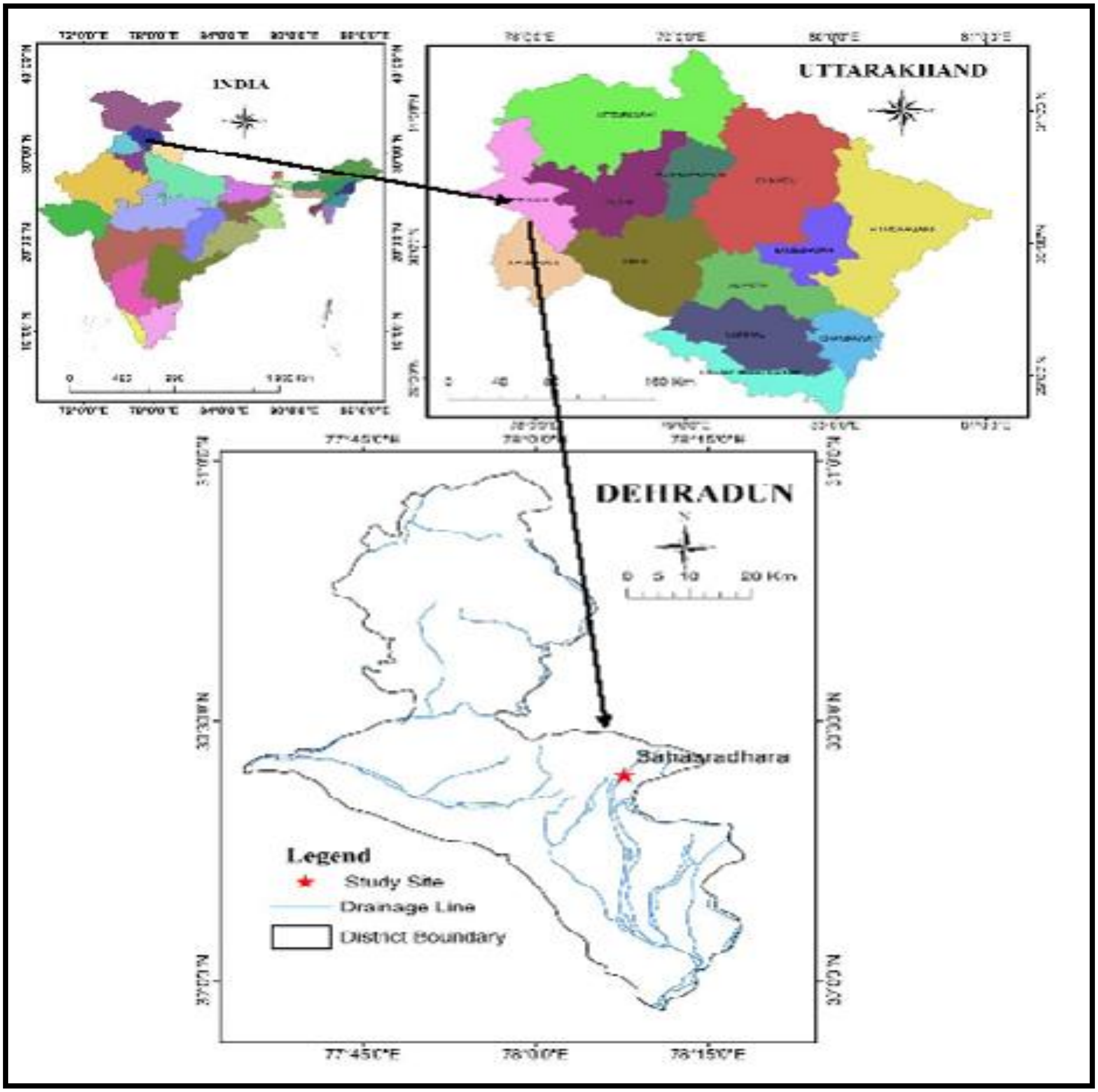

Figure 1. Location map of the study area; the Sahasharadhara springs

Regular monthly sampling for analyzing the hydrological attributes and biotic components of periphyton of the Sahashradhara springs was undertaken at each site (S_1, S_2S_3, S_4 and S_5) for one annual cycle (October2011- September 2012). Five replicates were obtained for each parameter and the results were integrated and recorded. Physico-chemical parameters of the Sahashradhara springs were determined following the standard methods outlined in Welch (1952), Wetzel and Likens (1991) and APHA (2005)

Collection of periphytonic community in three replicates was done by scratching 1 cm^2 of the substratum (bottom substratum). The scratched material was preserved in 4% formalin. Counting of periphyton was done using the Sedgwick-Rafter counting chambers after their identification with the help of standard taxonomic works of Fasset (1997), Fritsch (1945), Prescot (1962), Patric and Reimer (1966), Cleve-Euler (1968), Palmer (1968), Wetzel, (1979), Ward and Whipple (1992).

For the statistical analysis of the biological components, species diversity index ($\overline{H}$) was calculated using the Shannon and Wiener (1964).

$$\overline{H} = -\sum_{l-1}^{s}\left(\frac{ni}{N}\right)\log_2\left(\frac{ni}{N}\right)$$

Where, $\overline{H}$ = Shannon-Weiner index of diversity;
n_i = Total no of individual of a species;
N = Total no of individuals of all species.

In order to assess the effect of physical and chemical variables on periphyton density, Cannonical Correlation Analysis (CCA) was performed using PAleontological STatistics (PAST) version 3.06. To minimize the effect of rare species on the ordination, only species with $\leq$ 1% of the total periphyton density were ignored. The significance of environmental variables was determined using the Karl Pearson's correlation coefficient (r value).

To determine the influence of physico-chemical attributes on periphyton community, regression analysis was also performed between the density of periphyton and physico-chemical attributes. The total monthly density of all the macro-invertebrates from all the three sites was taken together, and means were used to perform regression. Regression analysis using XLSTAT 2015.1 was performed with each physico-chemical attribute one by one, and the results (R value, R^2, standard error, F value, intercept, X variable, t-stat and p-value) were pesented.

Results

1. *Physiographic attributes of spring water*

The Sahashradhara, cluster of thousands of springs is located at altitudes of 847 m to 889 m above m.s.l. (latitude 30°38'N; longitude 78° 13'E). The ecosystem of the Sahashradhara springs constitute of bottom substrate ranging from sand to big boulders. Silt and clay were totally absent in the springs. The substrate compositions were also different at (S_1, S_4 and S_5) but more or less similar at $S_2$and S_3 (Table 1)

TABLE 1. composition of bottom substrates of all the sampling sites (S_1-S_5) of the Sahashradhara springs

Name of substratum	S_1	S_2	S_3	S_4	S_5
Cobbles (64-256 mm)	14 %	48 %	45%	22%	28%
Pebbles (16-64 mm)	28%	33%	36%	17%	13%
Gravel (2-16 mm)	22%	14%	13%	33%	25%
Sand (<2mm)	36 %	5 %	6 %	28 %	34 %

2. Physico-chemical attributes of spring water

The physico- chemical parameters ($\overline{X}$ ± S.D.) recorded at all the five sites of the Sahashradhara springs are given in Table 2. Maximum air temperature was recorded in the month of July (26.46 ± 0.36^{o}C) and minimum in the month of December (15.88 ± 0.48^{o}C), while, the maximum water temperature was recorded in the month of July (22.76 ±0.51^{o}C) and minimum in the month of February (10.56 ± 0.79^{o}C). Water velocity was fairly same throughout the year with the highest value in the month of August (0.318 ±0.47 ms^{-1}) and lowest recorded in the month of October (0.186 ±0.70 ms^{-1}). Turbidity was found to be highest in the month of August (3.592 ±0.31 NTU). Dissolved oxygen was recorded highest in the month of December (8.21 ± 0.56 mg. l^{-1}) and minimum in the month of May (6.48 ± 0.38 mg. l^{-1}). Parameters like total dissolved solids (TDS), hardness, pH, alkalinity, chlorides, nitrates, phosphates showed an irregular trend with minor differences in their concentration in the Sahashradhara springs throughout the study (Table 2).

3. Springs classification based on environmental characteristics

Springs can be classified based on current velocity at spring mouth (Steinmann 1915 and Thienemann 1924), hydrology, geology, hydrochemistry, water temperature, biological assemblages, human use (Glazier 2009) and lithology (Sanders *et.al* 2010). The studied springs of Sahashradhara can be classified ecomorphologicaly as Helocrene (S_1andS_5), limnocrene (S_2, $S_{3)}$ and Rheocrene (S_4) following the Spitale *et al.* 2012 classification. Limnocrenes are pool springs with water filled depressions which lack noticeable water velocity. All the five clusters of springs studied can also be classified as hard water spring (Sanders *et al.*, 2010) as they contain high concentration of Ca^{2+} and HCO_3^-. Cluster analysis based on physico-chemical parameters performed on all the study sites revealed the formation of two major clusters (Figure 2).Cluster 1 comprised of springs of S_1 and $S_{5,}$ whereas cluster 2 comprises of springs of $S_{2,}$ $S_3$and $S_{4.}$

TABLE 2. Physico-chemical characteristics ($\overline{X}$ ± S.D.) of the aquatic environment of the Sahashradhara springs for the period October 2011-September 2012

Parameters	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
Ait temp (^{0}C)	22.62 ± 0.36	18.68 ± 0.30	15.88± 0.48	16.86 ± 0.32	18.1± 0.38	21.28± 0.21	22.64± 0.33	23.76± 0.68	26.42± 0.34	26.46± 0.36	24.18± 0.48	23.42± 0.55
Water temp. (^{0}C)	18.26 ± 0.70	15.46 ± 0.62	12.32 ± 0.83	12.56± 0.44	10.56± 0.79	17.18± 0.76	14.44 ± 0.13	16.28 ± 0.67	20.22± 0.73	22.76± 0.51	21.14± 0.47	20.06 ± 0.44
Water Velocity (m sec^{-1})	0.186 ± 0.70	0.226 ± 0.62	0.244 ± 0.83	0.228 ±0.44	0.198 ± 0.79	0.254 ± 0.76	0.292± 0.13	0.226 ± 0.67	0.24 ± 0.74	0.284 ± 0.51	0.318 ± 0.47	0.30 ± 0.44
Conductivity (µScm^{-1})	362 ± 27.30	357.8 ± 21.39	352.8 ± 14.55	333.8 ± 22.37	348.4 ± 15.32	343.4 ± 21.72	361.8 ± 19.84	367.4 ± 15.50	370.6 ± 38.17	417± 17.38	413.2± 11.67	401.5 ± 13.30
Alkalinity (mg.l^{-1})	153.6 ± 21.66	122.2 ± 13.08	151.8 ± 20.50	145.2 ± 10.80	168 ± 9.41	147 ± 21.32	145.2 ± 18.65	107.8 ± 41.64	117.4 ± 38.25	98.4 ± 32.91	118.2 ± 38.07	121.4 ± 33.09
TDS (mg l^{-1})	222.8 ± 13.33	182.2 ± 11.71	165.8 ± 14.31	138.2 ± 9.42	141.8 ± 6.26	163 ± 6.16	174.2 ± 7.66	185 ± 8.43	160.2 ± 8.67	275.6 ± 7.16	273.8 ± 5.69	222.2 ± 25.91
Free CO_2 (mg l^{-1})	0.434 ± 0.025	0.384 ± 0.018	0.356 ± 0.040	0.292 ± 0.016	0.294 ± 0.035	0.366 ± 0.018	0.488 ± 0.013	0.544 ± 0.018	0.686 ± 0.026	1.26 ± 0.043	0.988 ± 0.018	0.652 ± 0.091
Dissolved oxygen (mg.l^{-1})	7.63 ± 0.72	7.19 ± 0.13	8.21 ± 0.56	8.15 ± 0.47	7.21 ± 0.18	7.02 ± 0.19	7.12 ± 0.16	6.48 ± 0.38	6.62 ± 0.26	6.50 ± 0.28	6.94 ± 0.31	7.24 ± 0.15
pH	7.37 ± 0.19	7.36 ± 0.12	7.51 ± 0.06	7.72 ± 0.05	7.34 ± 0.12	7.61 ± 0.05	7.26 ± 0.06	7.37 ± 0.16	7.19 ± 0.05	7.44 ± 0.15	7.38 ± 0.16	7.41 ± 0.16
Turbidity (NTU)	1.97 ± 0.34	2.05 ± 0.46	1.74 ± 0.21	1.81 ± 0.17	1.76 ± 0.17	1.74 ± 0.14	0.98 ± 0.18	1.83 ± 0.09	2.74 ± 0.15	2.94 ± 0.42	3.592 ± 0.31	2.68 ± 0.19
Chlorides (mg l^{-1})	4.88 ± 0.73	5.76 ± 0.85	5.25 ± 0.92	3.79 ± 0.73	4.83 ± 0.56	5.94 ± 0.84	7.01 ± 1.08	7.42 ± 1.18	8.42 ±1.40	9.88 ± 1.98	4.94 ± 2.15	5.20 ± 1.18
Hardness(mg l^{-1})	263 ± 46.10	244.4 ± 43.07	247.2 ± 54.33	249.2 ± 70.15	236 ± 63.82	252.4 ± 59.80	259.2 ± 58.78	240.2 ± 30.63	245.4 ± 6.39	237.6 ± 10.64	243.2 ± 21.09	252 ± 24.36
Calcium(mg l^{-1})	194.8 ± 24.68	178 ± 29.43	185.2 ± 32.12	189.8 ± 40.75	188.6 ± 38.79	200.4 ± 35.76	209 ± 43.20	178.8 ± 19.87	178.8 ± 33.64	174.2 ± 25.77	182.4 ± 19.23	191.8 ± 25.96
Magnesium(mg l^{-1})	84.2 ± 10.78	79.6 ± 12.60	73.6 ± 13.37	68.4 ± 20.22	56.0 ± 15.46	61.2 ± 14.45	62.2 ± 3.77	73.8 ± 9.60	81.8 ± 4.49	77.4 ± 6.06	74.4 ± 5.32	77.0 ± 4.69
Nitrates (mg l^{-1})	0.083 ± 0.013	0.109 ± 0.009	0.103 ± 0.008	0.112 ± 0.008	0.304 ± 0.386	0.099 ± 0.014	0.097 ± 0.014	0.093 ± 0.010	0.080 ± 0.009	0.089 ± 0.196	0.090 ± 0.007	0.081 ± 0.010
Phosphates (mg l^{-1})	0.053 ± 0.003	0.048 ± 0.006	0.053 ± 0.004	0.051 ± 0.004	0.043 ± 0004	0.034 ± 0.007	0.043 ± 0.006	0.047 ± 0.005	0.051± 0.005	0.052 ± 0.004	0.043± 0.005	0.054 ± 0.006
CPOM (g l^{-1})	30.61 ± 6.87	13.71 ± 3.87	11.89 ± 3.39	7.27 ± 2.50	16.24 ± 4.51	20.51 ± 5.36	24.84 ± 5.53	26.44 ± 6.27	27.93 ± 5.73	31.32 ± 6.94	34.2 ± 7.39	27.72 ± 5.96
FPOM (g l^{-1})	18.93 ± 5.84	7.36 ± 2.90	7.18 ± 2.70	3.55 ± 1.94	10.11 ± 3.68	13.52 ± 3.80	16.78 ± 4.94	15.74 ± 4.34	16.18 ± 4.47	18.71 ± 4.42	20.49 ± 4.25	15.35± 4.20

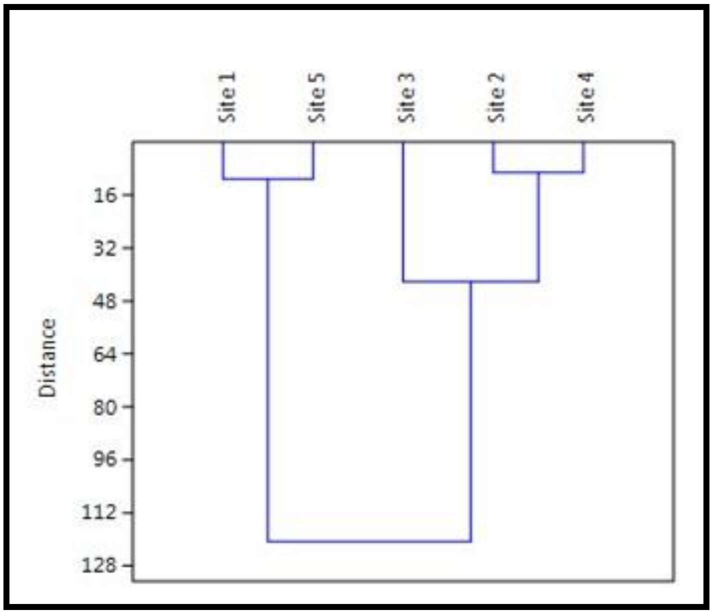

Figure 2. Dendrogram of the cluster formation of all five sites based on environmental variables

4. Density and diversity of periphyton

The mean monthly variations in the density of periphyton recorded for one annual cycle from all the five sites (S_1, S_2, S_3, S_4 and S_5) of the Sahashradhara springs have been presented in the Table 3. The overall mean density of the periphyton was found to be maximum in the month of February (1,534 ind. m^{-2}) and minimum in the month of July (36 ind. m^{-2}). Altogether 29 periphyton taxa were recorded from different springs at Sahashradhara (Table 4). Annual percentage composition of periphyton contributed by various taxa has revealed that the major contribution was made by Bacillariophyceae (68-70%) followed by Chlorophyceae (26-28%) and Myxophyceae (3-5%) Figure 3.

The Shannon Weiner diversity index was found to be maximum ($\overline{H}$ = 1.126) in March and minimum ($\overline{H}$ =0.879) in June during the period of observations (Table 5). Overall there was no significant difference in the annual mean ($\overline{H}$ = 1.012, 1.036, 0.998, 1.024, 1.045) of the Shannon-Weiner diversity index at all the five sampling sites (S_1, S_2, S_3, S_4 and S_5).

TABLE 3. Periphyton (mean value of all five sampling sites) of the aquatic environment of the Sahashradhara springs for the period October 2011-September 2012

Periphyton	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
Bacillariophyceae	473.8	569.4	650.8	917	1061.2	822.6	676.8	351.4	139.8	19	45.2	252
Chlorophyceae	198.8	234.6	284.6	337.2	403.6	356.2	212.4	124.6	37.2	17.2	46	139.2
Myxophyceae	9.8	25.8	27	65.8	74	62.2	35.8	31.4	4.4	0	0	3.2
Grand Total	682.4	829.8	962.4	1320	1538.8	1241	925	507.4	181.4	36.2	91.2	394.4

TABLE 4. List of periphyton density in all the five sites (S_1-S_5) of the Sahasradhara springs for the period October 2011-September 2012

	S_1	S_2	S_3	S_4	S_5
Bacillariophyceae					
Achnanthes minutissima Kützing, 1844	+++	++	++	++	+++
Amphora ovalis Kützing, 1844	+++	++	++	++	+++
Cocconeis placentula Ehrenberg 1838	++	++	++	++	++
Cymbella aequalis Fontell, 1917	++	+	+	+	+
Diatoma vulgaris Bory 1824	+++	++	++	++	+++
Fragilaria inflate, Pantocsek 1902	+++	+	+	+	+++
Frustulia rhomboides (Ehrenberg) De Toni 1891	+	+	+	+	+
Gomphonema geminate, (Lyngbye) C.Agardh 1824	+	+	+	+	+
Navicula radiosa Kützing 1844	++	++	++	++	+++
Nitzschia diversa Hustedt 1959	+++	+	+	+	+++
Nodularia moravica Hindák, Smarda & Komárek 2003	++	+	+	+	++
Pinnularia interrupta W.Smith, 1853	++	+	+	+	+++
Synedra ulna, (Nitzsch) Ehrenberg 1832	+++	+	+	+	+++
Tabellaria fenestrate (Lyngbye) Kützing 1844	+	+	+	+	++
Chlorophyceae					
Cladophora glomerata (Linnaeus) Kützing 1843	+++	+	+	++	++
Closterium longissima (Ehrenberg) Van Heurck 1885	+++	+	+	++	+++
Cosmarium granatum Fritsch 1921	++	++	+	+	+
Desmidium aptogonum Kützing 1849	++	++	+	+	++
Gonatozygon sp.	+	+	+	+	+
Hydrodictyon reticulatum (Linnaeus) Bory de Saint-Vincent 1824	+	+	+	+	+
Microspora sp.	++	++	+	+	++
Odegonium sp.	+	+	+	+	+
Spirogyra orientalis West & G.S.West 1907	+++	++	++	+++	+++
Ulothrix zonata (Weber & Mohr) Kützing 1843	++	+	+	+	++
Volvox sp.	+	++	++	+	+
Myxophyceae					
Anabaena ambigua C.B.Rao 1937	++	+	+	+	+
Chroococcus urgidus (Kützing) Nägeli, 1849	+	++	++	+	+
Oscillatoria tenuis , C.Agardh ex Gomont 1892	+	++	++	++	+
Phormidium lucidum Kützing ex Gomont 1892	++	+	+	+	+

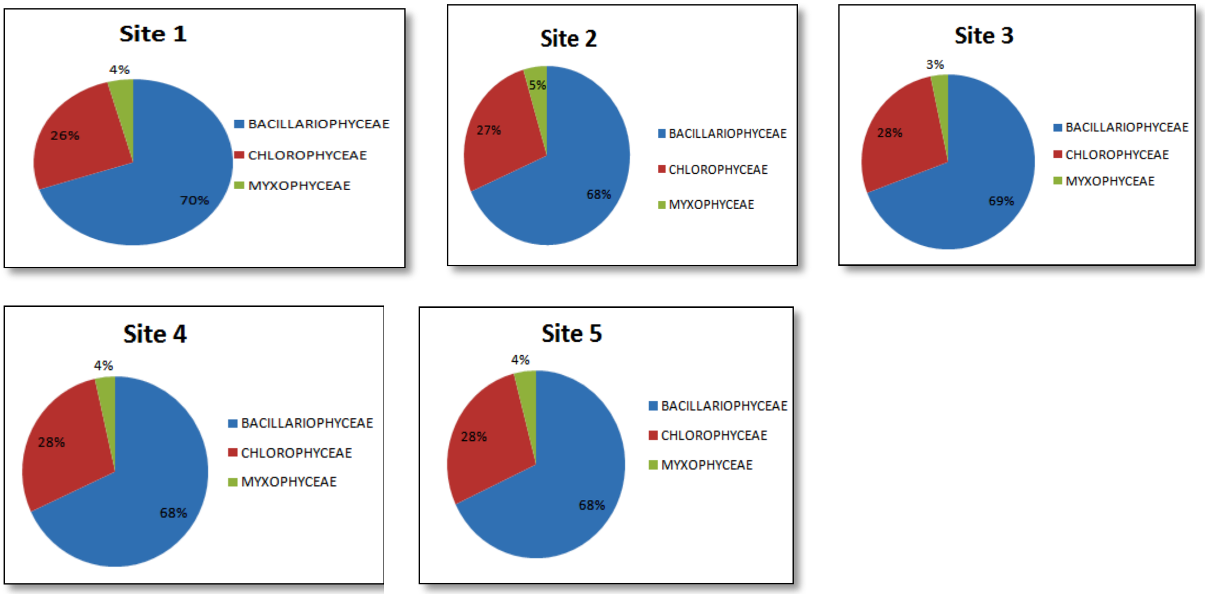

Fig 3: Density of periphyton community dwelling in the Sahashradhara springs for the period October 2011-September 2012

TABLE 5. Diversity index Shannon Wiener (1964) computed for periphyton dwelling in the Sahashradhara springs for the period October 2011-September 2012

	site 1	site 2	site3	site 4	site 5	average
Oct	0.999	0.995	0.925	0.949	0.915	0.957 ± 0.039
Nov	1.069	1.07	0.981	1.025	1.063	1.042 ± 0.039
Dec	1.047	1.076	1.002	1.047	1.053	1.045 ± 0.027
Jan	1.097	1.091	1.061	1.073	1.093	1.083 ± 0.015
Feb	1.092	1.104	1.075	1.083	1.094	1.090 ± 0.011
Mar	1.112	1.153	1.111	1.109	1.147	1.126 ± 0.022
Apr	0.978	1.003	0.967	1.026	1.012	0.997 ± 0.024
May	1.068	1.159	1.084	1.110	1.137	1.112 ± 0.037
Jun	0.789	0.881	0.833	0.901	0.991	0.879 ± 0.076
Jul	0.976	0.946	0.997	0.960	1.000	0.976 ± 0.023
Aug	1.480	1.00	0.989	0.980	0.994	1.089 ± 0.219
Sep	0.954	0.954	0.955	1.023	1.043	0.986 ± 0.044

5. *Relationship between physico-chemical attributes and the density of periphyton*

Correlation coefficient (r-values) calculated between physico-chemical environmental variables and periphyton dwelling in the Sahashradhara springs revealed that the air temperature, water temperature, turbidity, conductivity, dissolved oxygen, free carbon dioxide, alkalinity, CPOM and FPOM were found to be significantly correlated with density and diversity of periphyton dwelling in the Sahashradhara springs (Table 6). The periphyton density showed a negative relationship with the air temperature ($r = -0.82$, $p < 0.001$), water temperature ($r = -0.89$, $p < 0.001$), TDS ($r = - 0.79$, $p < 0.01$), free carbon dioxide ($r = - 0.86$, $p < 0.001$), conductivity ($r = - 0.87$, $p < 0.001$) and turbidity ($r = -0.77$, $p < 0.01$) Magnesium ($r = - 0.72$, $p < 0.01$), CPOM ($r = - 0.79$, $p < 0.01$), and FPOM ($r = - 0.71$, $p < 0.01$). Periphyton density showed a positive correlation with alkalinity ($r = 0.84$, $p < 0.001$), dissolved oxygen ($r = 0.58$, $p < 0.05$) and nitrate concentration ($r = 0.62$, $p < 0.05$).

TABLE 6 . Coefficient of correlation calculated between different physico-chemical parameters and periphyton community of the Sahashradhara springs for the period October 2011-September 2012

	AT	WT	WV	Co	Alk	TDS	$F.CO_2$	DO	pH	Tu	Cl	Ha	Ca	Mg	NO_3	PO_3	CPOM	FPOM
Mean density	-0.82	-0.89	-0.53	-0.87	0.84	-0.79	-0.86	0.58	0.45	-0.77	-0.59	0.14	0.52	-0.72	0.62	-0.39	-0.79	-0.71
$\overline{H}$	-0.60	-0.61	-0.26	-0.53	0.33	-0.43	-0.52	0.21	0.61	-0.50	-0.37	-0.22	0.17	-0.65	0.40	-0.50	-0.58	-0.53
Bacillariophyceae	-0.81	-0.90	-0.53	-0.88	0.85	-0.80	-0.86	0.57	0.43	-0.79	-0.57	0.15	0.53	-0.72	0.61	-0.38	-0.79	-0.71
Chlorophyceae	-0.85	-0.86	-0.51	-0.84	0.85	-0.73	-0.86	0.62	0.51	-0.71	-0.64	0.15	0.50	-0.68	0.59	-0.38	-0.79	-0.71
Myxophyceae	-0.68	-0.81	-0.45	-0.81	0.67	-0.78	-0.73	0.35	0.48	-0.68	-0.43	-0.07	0.40	-0.82	0.64	-0.54	-0.73	-0.65

AT: air temperature; WT: water temperature; WV: water velocity; Co: conductivity; Alk: alkalinity; TDS: total dissolved solids; F. CO_2: free carbon dioxide; DO: dissolved oxygen; pH: hydrogen ion concentration; Tu: turbidity; Cl: chlorides; Ha: Hardness; Ca: Calcium; Mg: Magnesium; NO_3: nitrates; PO_3: phosphates; CPOM: Coarse Particulate Organic Matter; FPOM: Fine Particulate Organic Matter; $\overline{H}$: Shannon Wiener diversity index

Canonical Correlation Analysis (CCA) was aimed to find the relationship between environmental variables and periphyton density. The CCA was performed between environmental variables of rheocrene (S_1 and S_5) and limnocrene ($S_{2,}$ and S_3) with their respective mean periphyton density. A total of 12 environmental variables and 17 periphyton species were selected. CCA performed between physico- chemical variables and periphyton density of helocrene springs revealed a that environmental variables like TDS, water velocity, hardness, turbidity along with conductivity and chloride concentration strongly influence the density of *Cymbellaa equalis*, *Navicula radiosa* , *Achnanthes minutissima* at axis 1(Figure 4). Eigen value of axis 1 (λ= 0.025) and axis 2 (0.015) together explained 66.16% relation between physico- chemical variables and periphyton density. On the other hand eigen value of axis 1 (λ= 0.020) and axis 2 (0.013) showed 58.67 % relation between physico- chemical variables and periphyton density for limnocrene springs. *Fragilaria inflate, Gonatozygon* sp. *and Cosmarium granatum* showed a strong relation with axis 2, which indicates the effect of pH, nitrate, alkanility and hardness (Figure 5).

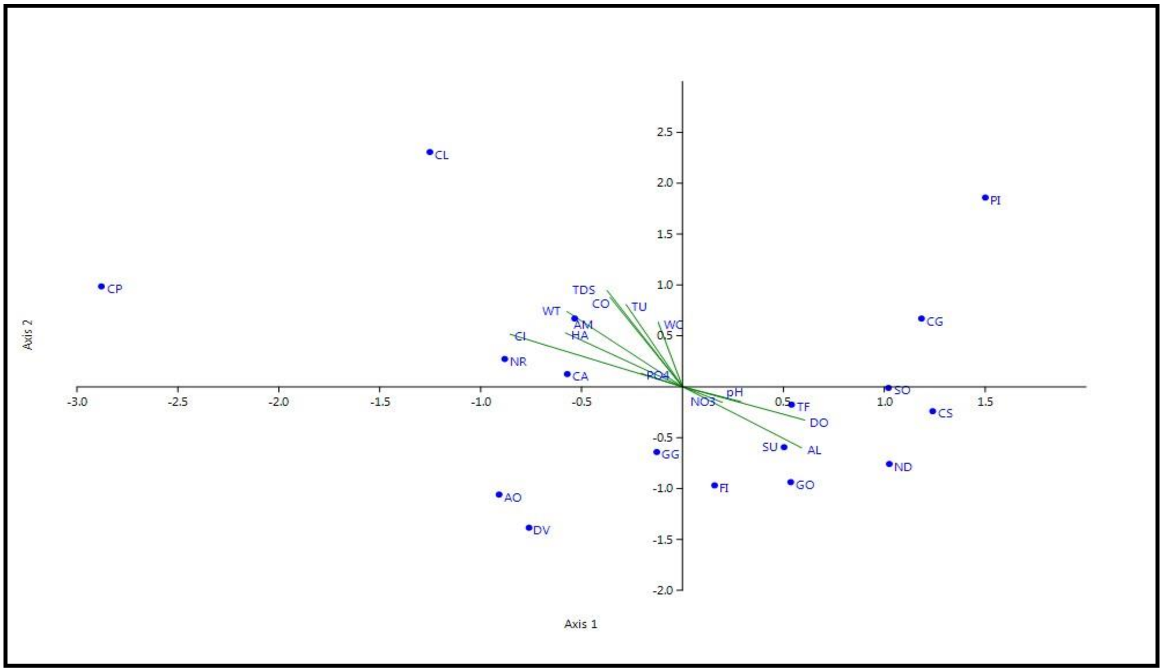

Fig 4. Canonical Correspondence Analysis (CCA) ordination of environmental variables and periphyton density for helocrene springs. *Achnanthes minutissima* (AM); *Amphora ovalis* (AO); *Cymbella aequalis* (CA); *Diatoma vulgaris* (DV);*Fragilaria inflate* (FI); *Gomphonema geminate* (GG); *Navicula radiosa* (NR); *Nitzschia diversa* (ND); *Pinnularia interrupta* (PI); *Synedra ulna,* (SU);*Tabellaria fenestrate* (TF); *Cladophora glomerata* (CG); *Closterium longissima* (CL); *Cosmarium granatum* (CS); *Gonatozygon* sp.(GO); *Microspora sp.*(MI); *Spirogyra orientalis* (SO); water temperature (WT); water velocity (WV); conductivity (CO); alkalinity (AL); total dissolved solids (TDS); dissolved oxygen (DO); hydrogen ion concentration (pH); turbidity (TU); chlorides (Cl); Hardness (HA); Nitrate (NO3)

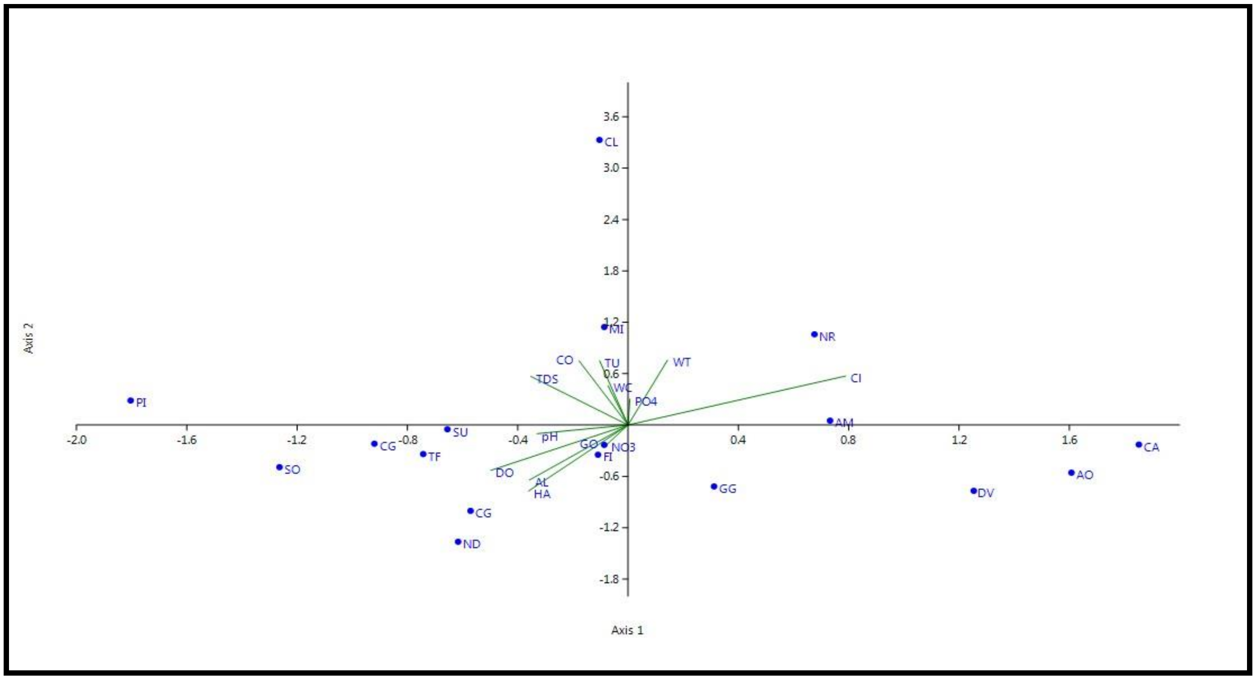

Fig 5. Canonical Correspondence Analysis (CCA) ordination of environmental variables and periphyton density for limnocrene springs. *Achnanthes minutissima* (AM); *Amphora ovalis* (AO); *Cymbella aequalis* (CA); *Diatoma vulgaris* (DV)*;Fragilaria inflate* (FI); *Gomphonema geminate* (GG); *Navicula radiosa* (NR); *Nitzschia diversa* (ND); *Pinnularia interrupta* (PI); *Synedra ulna,* (SU);*Tabellaria fenestrate* (TF); *Cladophora glomerata* (CG); *Closterium longissima* (CL); *Cosmarium granatum* (CS); *Gonatozygon* sp.(GO); *Microspora sp.*(MI); *Spirogyra orientalis* (SO); water temperature (WT); water velocity (WV); conductivity (CO); alkalinity (AL); total dissolved solids (TDS); dissolved oxygen (DO); hydrogen ion concentration (pH); turbidity (TU); chlorides (Cl); hardness (HA); nitrate (NO3); phosphates (PO4)

Discussion

The springs are unique in their characteristics; specific aquatic micro ecosystems; the contact zone of the above ground and underground of hydrosphere and refugia of rare and relict species of aquatic organisms (Takhteev *et al.*, 2010)

Maiolini *et al.* (2011) in their study of total of 90 springs in Trentino (South-eastern Alps, Italy) pointed out that each spring has its specific history and abiotic characteristics. They grouped these springs into seven different types and represented all the available lithologies in their study area. Based on eco-morphological conditions of study area, the Sahashradhara springs can be classified as – Helocrene, limnocrenes and rheocrenes.

Bhatt and Yousuf (2002) studied periphytonic community of seven springs of Kashmir and recorded a total of 50 taxa of periphytic algal community of which 33 belonged to Bacillariophyceae, nine to Chlorophyceae, five to Cyanophyceae, two to Chrysophyceae and one to Euglenophyceae Annual percentage composition of periphyton of Sahashradhara springs revealed that major contribution was made by Bacillariophyceae (68-70%) followed by Chlorophyceae (26-28%) and Myxophyceae (3-5%).

Periphyton species activity is restricted to certain temperature range. Temperature is recognized as a major driver for species distribution in springs (Brown and Hannah, 2008). Relationship between the mean density of periphyton and water temperature was found to be negatively correlated ($r = -0.89$, $p < 0.001$) in the present study on the Sahashradhara springs (Table 6). Maximum abundance of periphyton was found during winter season (November - February) in the Sahashradhara springs, which may be due to increased growth efficiency of periphyton during this period in addition to favourable physico-chemical attributes.

Density of periphyton was found to be decreasing from March to July. This may be due interaction of various physico-chemical parameters with periphyton. Water temperature was negatively correlated with the diversity index ($r = -0.61$, $p < 0.05$) in the Sahashradhara springs of the Doon valley. Hydrological factors particularly flow permanence, water chemistry and temperature are important ecological factors determining species distribution and community composition of periphyton (Cantonati *et al.*, 2012). The density of periphyton has a negative correlation with water velocity ($r = -0.53$, $p < 0.05$) under the present study on the Sahashradhara springs. Hence, the present study confirms that high water velocity during monsoon season in Sahashradhara springs influenced the periphyton density and diversity due to the unpredictable flow regime.

Among various environmental factors, pH and conductivity are considered to be the most important factors influencing diatom assemblages (Cantonati *et al.*, 1998; Frankova *et al.*, 2009). pH was found to be positively correlated with diversity indices of periphyton of the Sahashradhara springs ($r = 0.61$, $p < 0.05$), whereas conductivity was found to be negatively correlated ($r = -0.53$, $p < 0.05$).

When compared to structurally simple substrates, such as a sand and bedrock, the physical substrate types (leaves, gravel, wood and macrophytes) generally support more diversity (Angradi, 1996; Hawkins, 1984). This can be a good explanation for the high abundance and diversity of periphyton at sampling site S_5, which has high macrophyte growth.

The diversity of periphyton in the Sahashradhara springs at different sites was found to be in the order $S_5 > S_2 > S_4 > S_1 > S_3$. The annual mean periphyton diversity index was found to be highest ($\overline{H}$: 1.045) at S_5 and minimum ($\overline{H}$: 0.998) at S_3. This variation may be due to the variations in substrate composition as well as the physico-chemical attributes prevailing at all the sites.

The springs are excellent indicator of the ecological state of groundwater and atmospheric precipitation in contrast to other water bodies and water courses (lakes, rivers, streams, *etc*). The springs are much more stable in terms of hydrological and chemical conditions and are less exposed to occasional fluctuations. Ecosystem must be able to self-maintain or regulate and requires healthy community structure and sufficient integrity (Kay, 1991). The biotic and abiotic characteristics of the environment may be changed due to exposure to stress but ecosystem may still able to retain its homeostatic processes. The ecosystem of Sahashradhara springs undergoes homeostasis after spates and regulates the quality and quantity of aquatic plants and animal life.

Conclusion

Despite the relative homogeneity of the springs under investigation, periphyton diversity was found to be considerably high. The diversity variations were observed was not only between springs but also in different seasons. Difference in the physic-chemical parameters (water temperature, alkalinity, conductivity, turbidity, TDS etc.) may be considered as the reason behind variations in periphyton diversity in different types of springs in Sahashradhara. It is concluded on the basis of the present study on periphyton diversity of Sahashradhara springs that the members of the Bacillariophyceae thrive well in the Sahashradhara springs. Hence, they can be used as the most appropriate and efficient bio-indicators for assessing the health of this important aquatic ecosystem of Doon Valley of Garhwal Himalayas.

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