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Investigation of Diatom Communities in Highlands of Western Himalayas: Geographical and Ecological Patterns

Ajay Singh Rana[1*], Priyanka Verma[2], Priyanka Mittal[1], Meenakshi Mahajan[3]
[1]Research Scholar, Department of Forensic Science, Chandigarh University, Gharuan Punjab
[2]Assossiate Professor, Department of Forensic Science, Chandigarh University, Gharuan Punjab
[3]Deputy Director, Regional Forensic Science Laboratory, Northern Range Dharamshala
*Corresponding Author: Research Scholar, Department of Forensic Science, Chandigarh University, Gharuan Punjab, email-ajayrana9185@gmail.com

ABSTRACT

Diatom assemblages were investigated and compared from highlands of Peer Panjal ranges of western Himalayas, India. Samples were collected from surface sediments and stones in 15 sites located in 3 different locations during 2019. A total of 40 taxa (78 species) were reported in the study. Multivariate analyses of diatoms assemblages and physico-chemical variables showed significant difference of species composition within each site, which suggests that regional and climatic factors plays important role in species distribution. Abundance data and common occurrence of diatoms species showed that species like *Achnanthidium, Amphora, Encyonema, Discostella, Gomphonema, Halamphora, Hantzschia, Odontidium, Pinnularia and Planothidium* were most abundant while some species were seen restricted to particular locations. Conductivity, pH and temperature were the most significant environment variables in the study. The results of our study also highlight the homogeneous communities in these highlands suggesting dominance of pioneer species. Future taxonomic insights based on ecological characterization and comparison would be helpful for predictions for environmental change due to human interference and climate change.

Keywords: Highlands, Biodiversity, Bioindicators, Multivariate analyses, Light Microscope

1. Introduction

Altitude lakes are least impacted by human interference thus can be used to understand that how these biological communities are impacted by natural factors. Littoral benthic diatom communities can be used as ecological indicators to study the local or regional varying factors of such ecosystems (Feret *et al,* 2017; Rimet & Druart, 2018). Diatoms nutrient relationships study on the continental-scale can be used to develop metrics and further be refined up to regional scale studies (Potapova and Charles, 2007). Diatoms are important members of food chain in freshwater ecosystems and thus high taxonomic and functional diversity support is important for protection against ongoing climatic changes and anthropogenic impacts (Viktoria *et al,* 2021). Lakes with homogeneous communities along their shoreline are dominated by pioneer species adapted for strong physical disturbances while the lakes with heterogeneous communities are

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dominated by high profile diatoms adapted to compete for light and nutrients (Rimet *et al*, 2019). Diatoms and other microbes found at higher altitudes in glacier ice provide useful information about their dispersed and microbial biogeography (Fritz *et al*, 2015) and thus can be used for the evaluation of springs and high altitude mountain brooks (Battegazzore *et al*, 2004). Information on temporal and spatial distribution of epiphytic diatoms communities is rare in shallow lakes (Riato & Leira, 2020).

Changes in diatoms assemblages showed the aquatic regimes of sites which get changes in the aquatic states through time and mesohabitats (Novais *et al,* 2020) and can also be used as bioindicators for stressor response information (Kireta *et al*, 2012). Diatoms can also be used to study Forensic ecology (Wiltshire, 2019) and in Forensic diatomology for diagnosis of drowning deaths (Rana and Manhas, 2018; Rana and Verma, 2019). Diatom Index of Biotic integrity (DIBI) is one of the most useful limnological variables which can be used in correlation with stressors (Hill and Kurtenbach, 2009). Diatoms can be used as sentinels of environment change but the studies conducted on diatoms community distribution patterns at high latitude and altitude streams are limited (Teittinen *et al*, 2016). Diatoms can also be used for the monitoring of eutrophication in rivers and lakes including past changes by paleolimnological methods using recent assemblages (Kelly and Whitton, 1995).

In many freshwater systems diatoms communities is often dominated over algal communities and their populations is mainly impacted by variety of environmental variables (Ruhland *et al*, 2015). Diatoms are considered pioneer taxa and also dispersed rapidly and regarded as early colonizer i.e. *Achnanthidium minitissimum*. The diatoms species shows specific environmental preferences (Van Dam *et al,* 1994) and thus ecological guilds developed by the diatoms species provide useful information about the ecosystems and their environmental preferences (Peszek *et al*, 2021). Warming and anthrogenic disturbances if increased from threshold then it lead to severe ecological consequences of terrestrial ecosystems (Wischnewski *et al*, 2011). High mountain lakes host low diversity assemblages and poorly-structured trophic networks that respond rapidly to environmental change (Sienkiewicz and Gasiorowski, 2018). Moreover high proportion of diatoms species of threat categories in red list can be found in high ecological-integrity springs and lakes (Cantonati *et al*, 2021). Physicochemical conditions like alkanity-pH, total phosphorous and true colour gradients act as indicators for epilithic diatoms assemblages in calcifying lakes (Kennedy and Buckley, 2021).

Himalayan streams are important natural resources some of which are under threats due to pollution and hydrological changes (Juttner *et al*, 2010). Medium altitudes in Himalayas are vulnerable due to increase in pressure on water resources due to growth of population, agriculture and industrial development and higher altitudes are also impacted due to mountain tourism (Schreirer, 2005). Himalayas are rich in biodiversity even with diatom flora (Radhakrishnan, 2020). Taxonomic investigations including larger survey on Himalayan lakes

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over wide range of for acid base status can provide specific information to monitor environmental changes in the region (Simkhada, 2006).

The main objectives of our study are: (i) to document the diatoms communities at higher altitudes; (ii) to study the structuring parameters like altitude and physico-chemical variables which act as main drivers for these communities; (iii) to generate diatomological profile of diatoms for forensic and medico legal purpose.

2. Materials and Methods

2.1 Study Area and sampling strategy

The present study was conducted in three higher altitudes located in Peer Panjal range of Himachal Pradesh, India during the year september 2019 (Fig-1). The selected sites were Sach Pass glacier (33˚00'228" N, 76˚14'231" E) at an altitude of 4313 meters, Manimahesh lake (32˚23'42" N, 76˚38'14" E) at an altitude 4088 meters and Padri pass (32˚91'096" N, 75˚80'260"E) at an altitude of 3000 meters. The study areas are not used for agriculture or areas inhabitants. The specific areas are pilgrims' sites and tourists' destinations which open during the specific period of the year.

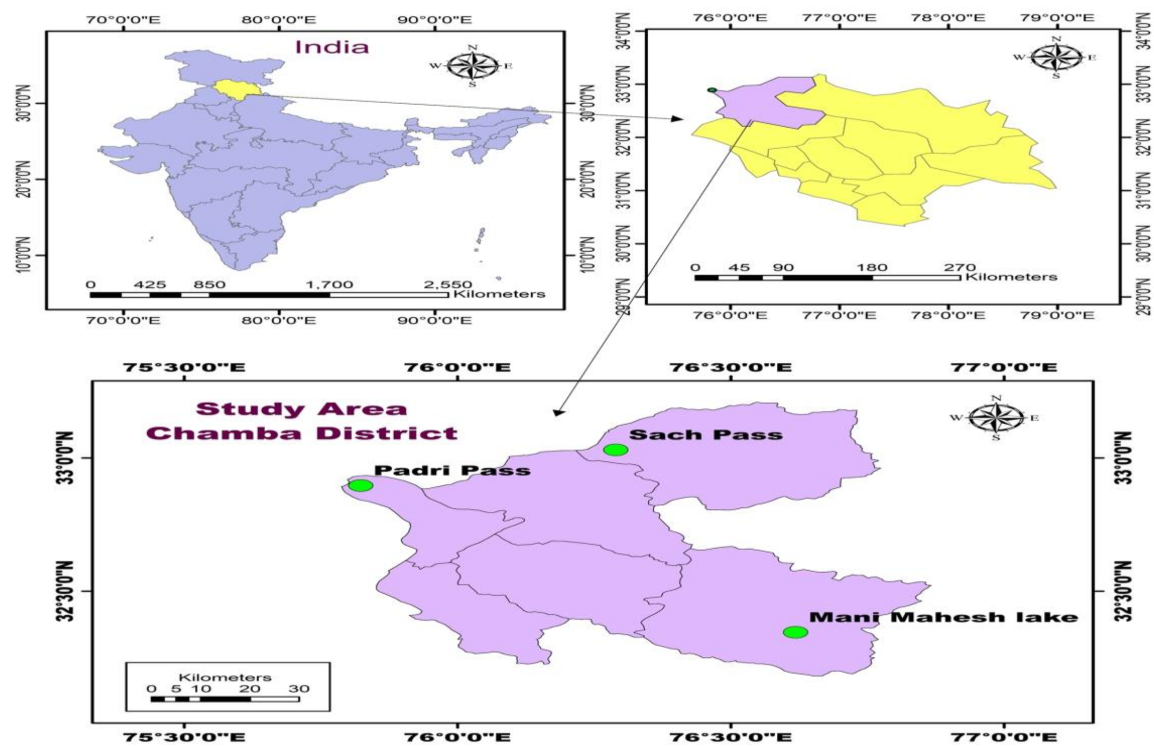

Figure-1: Site map showing sampling sites surveyed in the study

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2.2 Sampling and Laboratory procedures

Benthic diatoms were sampled according to the standard protocol (Kelly *et al*, 1998; Lai *et al* 2019) in september 2019. At each location of study five different sites were selected. At least five stones and surface sediments from each site submerged in water having biofilms were selected and then their upper surface was scraped with tooth brush, transferred in tarson plastic containers and fixed with 70% ethanol. Water temperature (T^0 C) was measured by using digital thermometer (Multi- Thermometer ST-9283) and Electrolytic conductivity (µS cm^{-1}), pH, and TDS (Total Dissolved Solids) were measured by using Cyberscan pH meter. Potassium (K), sodium (Na), calcium (Ca) were measured by using Flame photometer and copper (Cu) was measured by using Atomic Absorption Spectrophotometer (AAS). Diatoms samples (50 ml) were treated by oxidation process with H_2O_2 (30% v/v) and HCl (37% v/v) for several days to remove organic matter and centrifuged at 4500 rpm and then after centrifugation the pellet was transferred on a microscopic slide, air dried and mounts in DPX by using cover slips. Diatoms observations and counts were performed by using Leica DM 3000 LED microscope at 1000X magnification equipped with phase contrast and micrometric scale. The cleaned diatoms samples were also analyzed by scanning electron microscope (SEM, JEOL IT 500) coated with gold coated and images were captured using high vacuum conditions, at a voltage of 15kV, probe current of 15-30µA and working distance (WD) of 10-12 mm. Diatoms species were identified by the photomicrographs of diatoms taken and identified at the lowest taxonomic level by using taxonomic resources (Krammer, 1997a, 1997b; Krammer & Lange-Bertalot, 1986; Krammer & Lange-Bertalot, 1988; Levkov, 2009; INSTAAR 2020; Diatom flora of Britain and Ireland, 2020; ADIAC 2020; Bahls and Luna, 2018).

2.3 Statistical analysis and Data processing

The main structuring parameters of higher altitudes were calculated from the biological, chemical and environmental data which was at first standardized and then subjected for statistical analysis. Species richness and density at various locations was calculated as per standard procedures (Barbour *et al.*, 1999). Diversity indices (Shannon index, Evenness index, Berger Parker index, Simpson Index and Analysis of similarity)**,** ordinations (Principal Component analysis (PCA), Principal coordinate analysis (PCoA), Correspondence analysis (CA) and Canonical Correspondence analysis (CCA), clustering analysis (k-means clustering) and Analysis of Variance (ANOVA) were calculated with PAST 4.03 software (Hammer *et al*, 2001). Diatom count data was converted to relative abundances for the statistical analyses.

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3. Results

3.1 Physiochemical parameter

The results of physico-chemical variables are presented in Table-1 & Fig-2. Physico-chemical variables in Sach Pass, Manimahesh lake and Padri Pass were: temperature of water (^{0}C) 13.8-16.2, 13.3-13.9 and 16.4-17.9, ANOVA p<0.05, f-ratio 28.87; electrical conductivity (µS cm^{-1}**)** 45-115, 25.1-847 and 230-405, ANOVA p>0.05, f-ratio 3.11; pH 5.58-6.85, 3.74-5.92 and 5.45-7.31, ANOVA p<0.05, f-ratio 4.55; TDS (ppm) 0.03-0.13, 0.16-0.21 and 0.1-0.18, ANOVA p>0.05, f-ratio 1.028; K (ppm) 2.7-5.6, 10.4-24.2 and 2.7-5.7, ANOVA p<0.05, f-ratio 14.18; Na (ppm) 0.1-4.7, 3.6-4.8, 2.51-3.8, ANOVA p<0.05, f-ratio 7.592; Ca (ppm) 20.5-21.3, 21.4-22.7, 16.3-21.3, ANOVA p<0.05, f-ratio 10.49; Cu (ppm) 0.074-0.010, 0.73-0.98 and 0.34-0.67, ANOVA p<0.05, f-ratio 84.0172. Significant difference (ANOVA p<0.05) was observed for pH, Temperature, K, Na, Ca and Cu. No significant difference (ANOVA p>0.05) was observed for conductivity and TDS. Similarly the variations of environmental variables with range, mean and standard variations are shown in table-2.

Sample code	**pH**	**EC** µS cm^{-1}	**Temperature ^{0}C**	**TDS (ppm)**	**K (ppm)**	**Na (ppm)**	**Ca (ppm)**	**Cu (ppm)**
SA001	6.85	111.00	13.8	0.13	3.70	1.00	20.80	0.105
SA002	5.58	100.00	14.5	0.03	2.70	4.70	21.30	0.090
SA003	6.70	45.00	15.9	0.13	3.60	0.10	20.50	0.077
SA004	6.45	115.00	16.2	0.13	5.60	0.20	20.50	0.074
SA005	6.35	84.00	15.6	0.10	3.00	0.30	21.10	0.079
MA001	3.95	824.00	13.3	0.19	10.70	4.80	22.70	0.966
MA002	5.92	497.00	13.9	0.21	24.20	4.20	21.70	0.989
MA003	5.65	77.30	13.4	0.16	14.30	3.90	22.20	0.887
MA004	5.49	25.10	13.7	0.16	11.00	3.60	21.40	0.887
MA005	3.74	847.00	13.8	0.17	10.40	4.50	21.80	0.737
PA001	6.72	234.20	17.1	0.12	2.70	2.90	21.30	0.357
PA002	7.31	345.60	16.5	0.16	3.70	2.80	19.50	0.345
PA003	5.78	230.00	17.9	0.18	4.00	2.70	17.60	0.560
PA004	5.53	405.00	16.4	0.10	5.70	2.51	18.70	0.678
PA005	5.45	390.51	16.9	0.14	4.50	3.80	16.30	0.523

Table-1: Represents the physico-chemical variables observed at three locations, sample code SA001-SA005 represents the sampling sites of Sach Pass Glacier, MA001-MA005 of Manimahesh Lake and PA001-PA005 of Padri Pass

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Site	pH Min-Max Mean±Std	EC µS cm^{-1} Min-Max Mean±Std	Temperature ^{0}C Min-Max Mean±Std	TDS (ppm) Min-Max Mean±Std	K (ppm) Min-Max Mean±Std	Na (ppm) Min-Max Mean±Std	Ca (ppm) Min-Max Mean±Std	Cu (ppm) Min-Max Mean±Std
Sach Pass	5.58-6.85 6.38±0.49	45-115 91±28.38	13.8-16.2 15.26±1.012	0.03-0.13 0.104±0.04	2.70-5.60 3.72±1.130	0.10-4.70 1.26±1.95	20.50-21.30 20.84±0.357	0.074-0.105 0.085±0.0127
Manimahesh Lake	3.74-5.92 4.85±1.023	25.10-847 458±393.4	13.3-13.9 13.62±0.258	0.16-0.21 0.178±0.02	10.4-24.2 14.12±5.85	3.60-4.80 4.2±0.474	21.40-22.70 21.96±0.50	0.737-0.989 0.89±0.098
Padri Pass	5.45-7.31 6.15±0.818	230-405 321±84.12	16.4-17.9 16.96±0.59	0.10-0.18 0.14±0.031	2.7-5.7 4.12±1.10	2.51-3.80 2.94±0.50	16.30-21.30 18.68±1.89	0.345-0.678 0.492±0.141

Table-2: Range, Mean±Standard Deviation of Physico-Chemical variables observed at three sites

3.2 Diatoms Assemblages

In our study a total of 78 diatoms taxa were identified on the various sampling sites located in Pir Panjal range of western Himalayas (Table-3). 45 diatoms taxa were found in Sach Pass, 43 taxa in Padri pass and 28 taxa were found in Manimahesh Lake. The diatoms communities were mainly dominated by *Achnanthidium, Amphora, Encyonema, Discostella, Gomphonema, Halamphora, Hantzschia, Odontidium, Pinnularia and Planothidium. Achnanthidium minutissimum* (Kützing) Czarnecki is most abundance species with its average abundance of 13%. Likewise other diatoms species which were found most abundant were *Amphora copulata (Kutz.)* Schoeman and R.E.M. Archibald (2.8%), *Encyonema minutum* (Hilse) DG Mann (2.33%), *Discostella stelligera* (Cleve & Grunow) Houk & Klee (3.5%), *Gomphonema angustum* C. Agardh (2.5), *Gomphonema lagenula* Kutz (2.33%), *Gomphonema parvulum* Kützing (3.66%), *Halamphora veneta* (Kutzing) Levkov (3.16%), *Hantzschia amphioxys* (Ehrenberg) Grunow (3.33%), *Hantzschia elongata* (Hantzsch) Grunow (2.83%), *Odontidium mesodon* Kutzing (2.83%), *Pinnularia borealis* Ehrenb (2.33%). *Pinnularia viridis* (Nitzsch) Ehrenberg (2.83%) and *Planothidium lanceolatum* (Brebisson ex Kutzing) Bukhtiyayarova (2.83 %).

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Table-3: Represents the diatoms taxa observed in three sites

S.No.	Name of species with Authority	Sach Pass	Padri Pass	Manimahesh Lake
1.	*Achnanthes coarctata* (Brébisson ex W. Smith) Grunow	X		
2.	*Achnanthes exigua* Grunow		X	
3.	*Achnanthes lanceolata* (Brébisson ex Kützing) Grunow		X	
4.	*Achnanthidium minutissimum* (Kützing) Czarnecki	X	X	X
5.	*Amphora copulata (Kutz.)* Schoeman and R.E.M.Archibald		X	X
6.	*Aulacoseira granulata* (Ehrenb.) Simonson	X		
7.	*Caloneis silicula* (Ehrenberg) Cleve			X
8.	*Cocconeis placentula* Ehrenberg	X		
9.	*Craticula cuspidata* (Kutzing) DG Mann		X	
10.	*Cyclotella ocellata* Pantocsek		X	
11.	*Cymbella aspera* (Ehrenberg) Cleve		X	
12.	*Cymbella tumida* (Brébisson) van Heurck	X	X	
13.	*Cymbella neocistula* Krammer	X	X	
14.	*Cyclotella meneghiniana* Kützing	X		
15.	*Cymbopleura naviculiformis* (Auerswald) Krammer	X	X	
16.	*Denticula tenuis* Kutzing	X		
17.	*Diatoma vulgaris* Bory	X	X	
18.	*Discostella stelligera* (Cleve & Grunow) Houk & Klee	X	X	
19.	*Encyonopsis krammeri*	X		
20.	*Eunotia arcus* Ehrenberg	X		
21.	*Eunotia montuosa* Furey, Lowe and Johansen		X	X

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22.	*Eunotia pectinalis* (Kutzing) Rabenhorst		X	X
23.	*Eunotia tenella* (Grunow) Hustedt			X
24.	*Encyonema minutum* (Hilse) DG Mann	X	X	X
25.	*Encyonema gracile* Rabenhorst		X	
26.	*Fragilaria capucina* (Kützing) Lange-Bertalot		X	
27.	*Fragilaria elliptica* (Schumann)	X		
28.	*Fragilaria vaucheriae* (Kützing) J.B. Peterson	X	X	
29.	*Fragilaria arcus* (Ehrenberg) Cleve	X		
30.	*Fragilaria tenera* (WM Smith) Lange-Bertalo	X		
31.	*Gyrosigma acuminatum* (Kutzing) Rabenhorst		X	
32.	*Gyrosigma scalproides* (Rabenhorst) Cleve		X	
33.	*Gomphonema angustum* C. Agardh		X	X
34.	*Gomphonema minutum* (Agardh) Agardh	X		
35.	*Gomphonema lagenula* Kutz	X	X	
36.	*Gomphonema parvulum* Kützing		X	X
37.	*Gomphonema truncatum* Ehrenberg		X	
38.	*Gomphonema pumilum* (Grunow) E.Reichardt & Lange-Bertalot	X		
39.	*Gomphonema acuminatum* Ehrenberg	X	X	
40.	*Grunowia tabellaria* Grunow (Rabenhorst)	X		
41.	*Halamphora veneta* (Kutzing) Levkov			X
42.	*Hantzschia amphioxys* (Ehrenberg) Grunow	X	X	X
43.	*Hantzschia elongata* (Hantzsch) Grunow			X
44.	*Hantzschia hyperborea* (Grunow) Lange-Bertalot			X

45.	*Luticola goeppertiana* (Bleisch) D.G. Mann	X		
46.	*Luticola mutica* (Kutzing) D.G. Mann			X
47.	*Luticola ventricosa* (Kutzing) D.G. Mann			X
48.	*Melosira varians* C Agardh	X		
49.	*Meridon circulare* (Greville) C. Agardh	X	X	
50.	*Nitzschia linearis var. subtilis* (Grunow) Hustedt		X	
51.	*Nitzschia palea* (Kützing) W.Smith	X	X	X
52.	*Nitzschia dissipata* (Kutz) Rabenh.	X	X	X
53.	*Nitzschia clausii* Hantzsch	X		
54.	*Navicula captatoradiata* Germain	X		
55.	*Navicula caterva* Hohn & Hellermann	X	X	
56.	*Navicula cryptocephala* Kutzing		X	
57.	*Neidium affine* (Ehrenberg) Pfitzer	X	X	
58.	*Neidium productum* (W. Smith) Cleve		X	X
59.	*Odontidium mesodon* Kutzing	X	X	X
60.	*Pinnularia borealis* Ehrenb.		X	X
61.	*Pinnularia divergens* W. Smith		X	
62.	*Pinnularia nodosa* (Ehrenberg) W. Smith			X
63.	*Pinnularia undula* (Schumann) Krammer			X
64.	*Pinnularia viridis* (Nitzsch) Ehrenberg	X	X	X
65.	*Pinnularia species*	X		
66.	*Pinnularia subbrevistriata* Krammer			X
67.	*Planothidium lanceolatum* (Brebisson ex Kutzing) Bukhtiyayarova	X	X	X

68.	*Psammothidium helveticum* (Hustedt) Bukhtiyayarova & Round	X		
69.	*Reimeria sinuate* (Gregory) Kociolek & Stoermer	X	X	
70.	*Sellaphora bacillum* (Ehrenb.) D.G. Mann	X		
71.	*Sellaphora nigri* (De Not.) Wetzel and Ector	X		
72.	*Surirella angustata* Kutzing	X	X	X
73.	*Surirella tenera* W. Gregory			X
74.	*Stauroseira construens* (Ehrenberg)	X		
75.	*Staurosirella pinnata* (Ehrenberg) Willian Spomg		X	
76.	*Synedra inaequalis* H. Kobayasi	X		
77.	*Tabularia* Kutzing	X		X
78.	*Ulnaria ulna* (Nitzsch) Ehrenberg		X	

3.3 Diatoms diversity and correlation among physico-chemical characteristics

Higher values of diversity indexes in Sach Pass, Manimahesh lake and Padri Pass were: Shannon Weiner index 3.441, 3.454 and 3.088; Simpson index 0.9543, 0.9537 and 0.9455; Evenness 0.6937, 0.7352 and 0.7837; Dominance 0.0457, 0.0463 and 0.0545; Berger-Parker index 0.13, 0.15 and 0.11 (Table-4). Higher values of diversity indexes indicated healthy status of the sites while lower values indicated unhealthy and polluted status of sites. Lower values of diatoms diversity indexes in Manimahesh Lake as compared to Sach Pass and Padri Pass indicated the polluted status which also decrease in the species diversity.

Significantly negative but weak correlation was observed between temperature of water and Shannon (-0.333, $p<0.01$) and Simpson index (-0.173, $p<0.01$). Shannon and Simpson index showed positive correlation with pH (0.332, 0.157), conductivity (0.282, 0.369), TDS (0.526, 0.446), K (0.447, 0.363), Na (0.674, 0.603), Ca (0.804, 0.746) and Cu (0.827, 0.875) at p level of 0.01. Evenness and Dominance shows negative but significant correlation with conductivity (-0.714, -0.369), Na (-0.228. -0.603), Ca (-0.412, -0.746) and Cu (-0.996, -0.875) at the p level of 0.05. Positive but weak correlation was observed between pH (0.164, 0.162), temperature (0.164, 0.168), TDS (0.448, 0.446) and K (0.460, 0.363) at the p level of 0.05. Berger parker index shows negative but significant correlation with conductivity (-0.204), TDS (-0.866) and K (-0.817) at the p level of 0.05. Berger parker index shows significant and positive correlation

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with pH (0.497), temperature (0.496), Na (0.943), Ca (0.989) and Cu (0.463 at the p level of 0.05.

Name of sites	Shannon-Weiner Index	Simpson index	Evenness	Dominance	Berger-parker index
Sach Pass	3.441	0.9543	0.6937	0.0457	0.13
Padri Pass	3.454	0.9537	0.7352	0.0463	0.15
Manimahesh Lake	3.088	0.9455	0.7837	0.0545	0.11

Table-4: Represents various diversity indexes of diatoms assemblages at three sites

3.4 Characterization of sites by ordination methods

Sampling sites were classified by various ordination methods viz. Principal Component Analysis (PCA), Principal coordinate analysis (PCoA), Correspondence analysis (CA) and Canonical Correspondence analysis (CCA) and clustering analysis (k-means clustering).

3.4.1 Correspondence Analysis

The four axes in the dataset showed physico-chemical gradients (Figure-3). The first axis (85.486%) showed strong correlation with temperature, calcium, TDS, sodium and thermal conductivity. The second axis (11.491%) showed a strong correlation with copper and potassium. The third axis (2.393%) showed a strong correlation with pH.

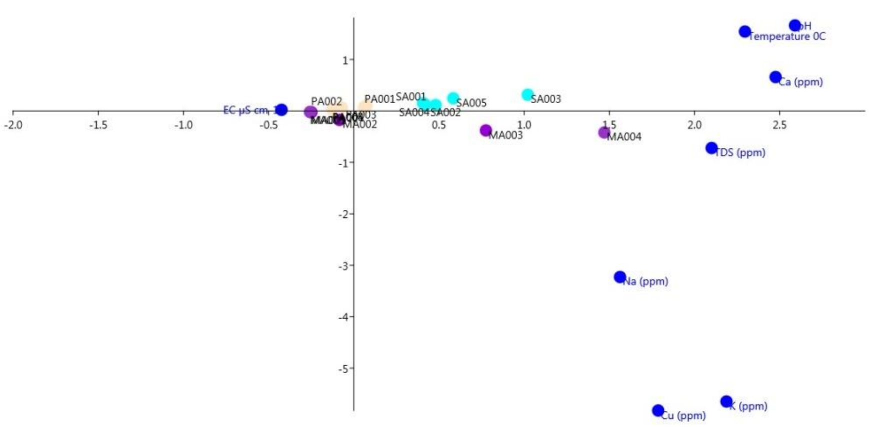

Figure-3: Plot of the Correspondence Analysis (CA) of Physico-Chemical variables observed at three sites

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3.4.2 Principal Component Analysis (PCA)

The first two components of principle component analysis showed 97.3% of the total variance in the dataset (Figure-4). The graph of PCA revealed temperature, pH, TDS, and thermal conductivity were correlated with the first axis, and Ca, Cu, K and K were correlated with the second axis.

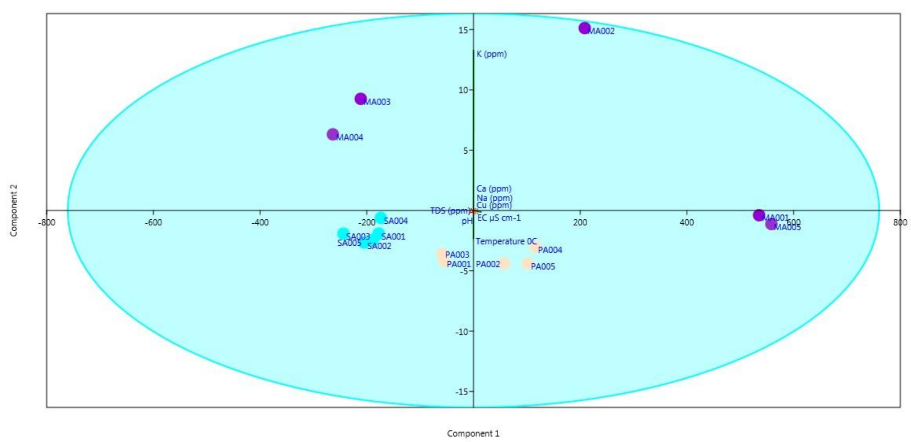

Figure-4: Plot of the Principal Component Analysis (PCA) performed on Physico-Chemical variables observed at three sites.

3.4.3 Principal coordinate analysis (PCoA) The four coordinates of principle coordinate exhibited 99.9% of the total variation (Figure-5). The first coordinate (63.40%) separated Manimahesh lake with Padri Pass. Second (19.78%), third (6.22%), and fourth (3.35%) separated Sach pass and Manimahesh lake from Padri Pass.

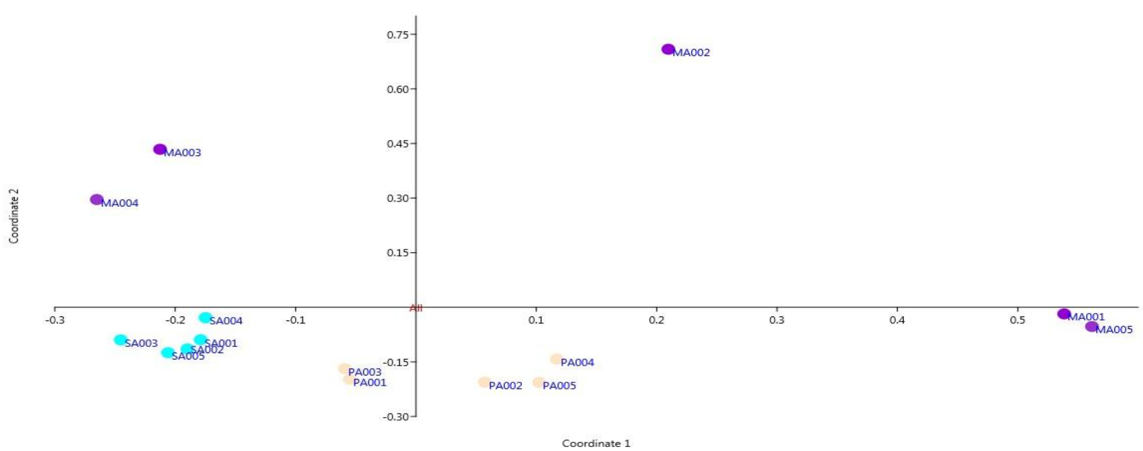

Figure-5: Plot of Principal coordinate analysis (PCoA) performed on Physico-Chemical variables observed at three sites.

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3.4.4 Canonical Correspondence analysis (CCA) CCA showed a strong association of diatom diversity with K, thermal conductivity, TDS, Cu, and Na along the first axis (eigen value=0.194) and with temperature, pH, Ca, and altitude along the second axis (Figure-6). Both the axis accounted for 77.25% of the variance. Diatoms species viz. *Achnanthidium minutissimum*, *Nitzschia palea*, *Nitzschia dissipata*, *Hantzschia amphioxys*, *Suirella angustata*, *and Encyonema minutum* present on the right side of the axis were associated with K, thermal conductivity, TDS, Cu, Na and temperature that corresponds to Manimahesh Lake and Padri Pass . While *Odontidium mesodon and Pinnularia viridis* present on the left side of the axis showed preference to altitude, Ca and pH that corresponds to Sach Pass.

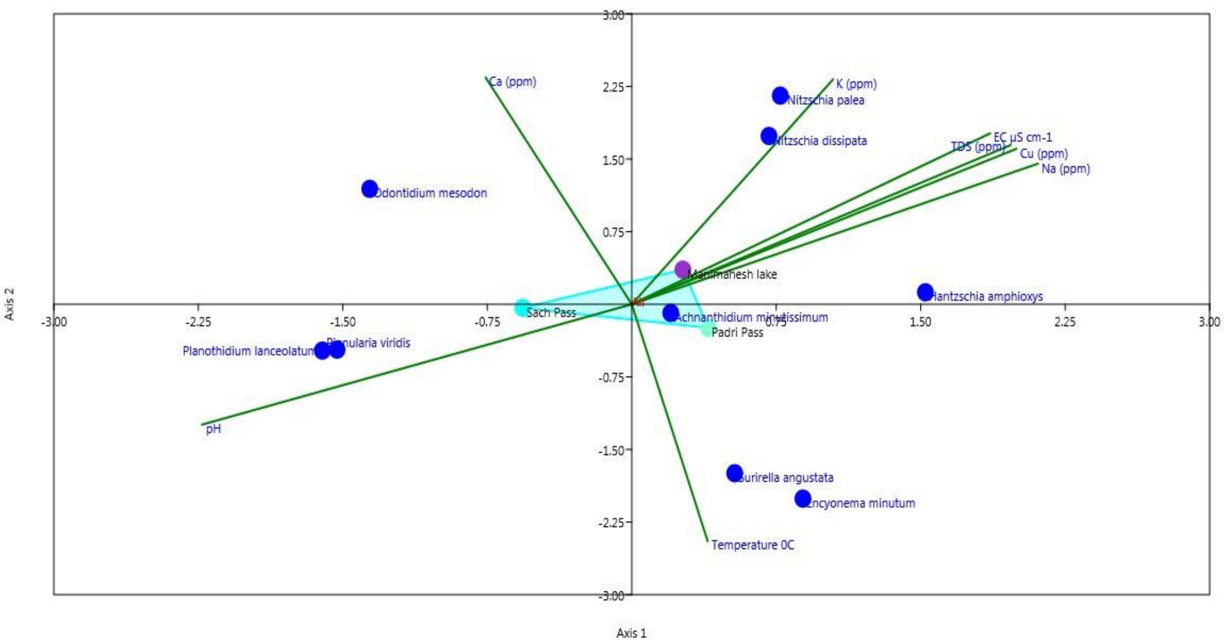

Figure-6: Canonical Correspondence analysis (CCA) triplots showing the distribution of sites along the axes based on a relationship between Physico-Chemical variables and diatoms taxa

3.4.5 Clustering analysis (Hierarchical clustering)

The sampling sites were classified into different clusters by hierarchical clustering for sorting the data based on diatoms diversity and sampling sites (Figure-7 & 8). In hierarchical clustering of diatoms, the species are cluster-based on abundance which shows the species on the right side are highly abundant in the samples and on the left side shows a lower abundance of the species. Similarly, the second figure of cluster analysis the sampling sites of Padri Pass and Sach Pass are closely related in the cluster based on physicochemical variables and diatoms abundance while Manimahesh Lake lies in a different cluster indicated that this is weakly related.

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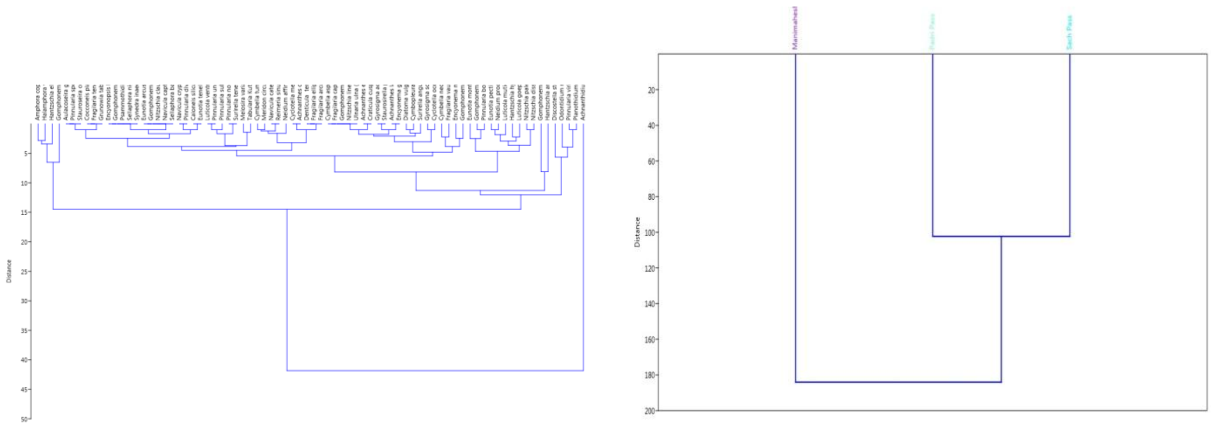

Figure-7 & 8: Hierarchical clustering of Diatoms assemblages based on abundance and locations observed at three sites.

4. Discussion

The present study conducted in highlands of western Himalayas showed that diatoms diversity and composition varied significantly with respect to environmental conditions such as water chemistry, temperature and character of habitat. The water quality in remote mountains are least affected due to direct inputs of pollutants however Manimahesh Lake is periodically affected as this lake is a important pilgrimage site and thus visited by number of piligrims during Manimahesh Yatra in the august month of the year. Similarly the Sach Pass and Padri pass are also got impacted by tourists and local inhabitants. Biodiversity and composition of benthic diatoms assemblages can be influenced by the level of protection of lakes and ponds which maintains higher ecological status and functional diversity than non protected ones (Viktoria *et al*, 2021). The sites having naturalized catchments and low human interference are mainly dominated by tychoplanktic diatoms which are generally found in higher altitudes (Griffiths *et al*, 2021). In our study the sampling sites are located in highlands within range from 3000 m to 4313 m having diatoms taxa ranged from 28-45. A study conducted by Compere (1983) in western Himalayas showed that there is sharp decrease of diatoms taxa in highlands localities above 3000 m.

In our study conductivity at Sach Pass (4313 m) ranges from 45-115 µS cm^{-1}, Manimahesh lake (4088 m) 25.10-847 µS cm^{-1} and Padri Pass (3000 m) 230-405 µS cm^{-1}. Loffler (1969) reported that specific conductivity is higher in low drainage lakes and also decreases with increase in altitude while Lacoul & Freedman (2005) in their study showed that there is linear decline of macrophytes with respect to species richness and diversity with increase in altitude. Yang *et al* (2003) studied interference models from eastern Tibetan plateau for diatoms based conductivity and water level inference models and showed that conductivity explained 98% of variation due to high covariance among two variables. Studies conducted on diatom assemblages in springs with high conductivity and lower temperature showed higher diversity of diatoms assemblages (Beauger *et al*, 2017; Angel *et al*, 2018; Lai *et al*, 2019). Yu *et al*, 2019 in their study showed

that spatial distribution of diatoms communities is mainly influenced by temperature, water depth, and grain size based on canonical correspondence analysis. Epilithic diatoms communities in calcifying lakes also show responses to pH confirming their effectiveness as indicators to physiological conditions (Kennedy & Buckley, 2021). The study of diatoms assemblages in these diverse habitats provided evidence that many species are restricted to specific locations. Despite diversity of habitats and a wide range of chemical conditions the diatoms reported in three different locations was low i.e. Sach Pass (45), Padri Pass (43) and Manimahesh lake (28). The sites also shows low diversity H' and Evenness E' and are dominated by only one or two species which have relative abundance >10%. *Achnanthidium minutissimum* (Kützing) Czarnecki with average abundance of 13% was reported to be most abundant species of diatoms in our study. Similarly Simkhada & Juttner (2006) reported *Achnanthidium minutissimum* (Kützing) Czarnecki was characteristics of sites with least impacted by agriculture and with high Ca concentrations while Ruwer *et al*, (2021) in their study showed that relative abundance of A. minutissimum increases with lower temperature, higher turbidity and pH based on generalized linear models. Michelutti *et al* (2003) in their study reported highest number of specific taxa on surface sediments than epiphyton. Cox (1988) in his study found that certain diatoms assemblages showed characteristics assemblages in different habitats from the genera of *Eunotia* and *Pinnularia* which reflects particular microhabitat conditions with respect to lower pH.
In our study Cu was reported to be negatively correlated with the diatoms diversity and higher concentration of Cu at sites have lower number of diatoms taxa i.e. in Sach Pass (0.074-0.105 ppm) have higher number of taxa while Manimahesh Lake (0.737-0.989 ppm) have reported lower number of taxa. Tolotti *et al*, 2019 also reported that high concentrations of metals like Cu and Zn causes lower diversity of diatoms and also shift from dominance to opportunistic species like *Achnanthidium minutissimum*, *Nitzschia palea* and *Fragilaria rumpens*. Accumulation of metals (Cu, Zn and Pb) also inhibited the growth, reduced cell number and also increases morphological abnormalities in diatoms (Pandey *et al*, 2014). In our study Ca was seen highly correlated however the correlation was seen weakened between respective fractions of variables indicating non equivalent effect of variables on the diatoms assemblages. Rivera-Rondon and Catalan (2020) in their study also showed that calcium, pH and ANC (acid neutralising capacity) were highly correlated with diatoms assemblages.

In the present study species richness, diversity and evenness were found significantly higher which might possible due to lower number of grazers in the higher altitude. Various studies (Czarnecki, 1979; Medlin 1980; Peterson *et al*, 1998) conducted on higher altitudes lakes also showed significant higher diversity of diatoms due to lack of removal of diatoms species due to grazing. Burkholder (1996) in his study showed that nature of chemical and microhabitat conditions are most important factors for the distribution of assemblages in different microhabitats.

The study of diatoms diversity in the highlands of western Himalayas resulted in identification of 78 taxa of diatoms. Similar diatoms studies and diversity were also investigated in Spain (Goma *et al*, 2005), Turkey (Solak *et al*, 2012), Britain and Ireland (Juttner *et al*, 2011) and Poland (Noga *et al*, 2016). Rothfritz *et al* (1997) and Juttner *et al* (2003) in their study showed that habitat character is most important for the distribution of diatoms in a particular habitat while studies conducted by various authors in many lakes (Hall & Smol, 1999; Battarbee *et al*, 1999; Potapova & Charles, 2003; Potapova & Charles, 2007; Clarke *et al*, 2005) advocated that chemical gradients are major determinants for the distribution of diatoms in freshwater habitats. Ponader *et al*, (2007) in their study showed that a nutrients concentration exhibits significant variations in the composition of diatoms species based on multivariate analysis.

5. Conclusion

Study of diatoms assemblages from three eco-regions of Peer Panjal range of Himachal Pradesh showed that they are sensitive indicators of environmental change due to changes in habitat character of the aquatic environment and the immediate surroundings. Data available on the distribution of diatoms in these highlands is very scarce. Developing of methods for assessing the environment status is required to assess the current status and to predict future environmental challenges. Generation of diatoms database of higher altitudes is also helpful for forensic diatomology and forensic ecology. The current study showed that diatoms assemblages can be used as indicators for water chemistry changes and generation of baseline data would be helpful for regular monitoring of environmental changes and better monitoring of these barren ecosystems. Further studies on large scale datasets with precise taxonomic identification on glaciers and lakes of highlands are required for better environmental management and conservations of highlands in western Himalayas.

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