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Research Article

DIVERSITY OF DIATOMS IN THE RIVERS OF BUNDELKHAND PLATEAU: A MULTIVARIATE APPROACH FOR FLORAL PATTERNS

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ABSTRACT
This study examines the diatom flora of the rivers of Bundelkhand Plateau in Central Highland Eco region. The diatoms were studied by examining Naphrax diatom mounts prepared from samples obtained by scraping 3x3 cm. from cobbles at the 11 sampling stations situated between latitude 23º30’ to 26º N and longitude 78º30’ to 82º30’ E along the rivers Ken, Paisuni and Tons. Two hundred ninety three diatom species belonging to 48 genera were recorded. The taxonomic richness, and diversity indices were analyzed by using PAST Software. Among the pennate diatoms, the species-rich genera were *Navicula* (32) and *Cymbella* (31). The cluster analysis reveals that each river has its own characteristic flora owing to which there were separate clusters even within major cluster for each river. The flora of Paisuni was more dissimilar than the rivers Ken and Tons which could be attributed to major differences in length of the rivers, the stream-orders and the land use practices in their basins. Ordination and Cluster analyses indicate prevalence of ‘intra basin’ rather than the ‘inter basin’ gradients of abundance. The proximate factors seem to govern the assemblages rather than eco regional properties (latitude, altitude). The present study will be useful to understand the regional patterns of diatom diversity and formulate reference conditions in these and other rivers of this region.

Keywords: *Diatom flora, Bundelkhand Plateau, Paisuni, Cluster Analysis*

INTRODUCTION
Diatoms are primary producers in freshwater ecosystems and their rapid response to environmental change diatoms have long been used to assess ecological conditions (Rimet, 2012). Diatoms, the unicellular siliceous yellow-brown algae, are one of the most successful groups distributed universally in all types of aquatic environment. In the present situation, diatoms have gained considerable popularity throughout the world as a tool to provide an integrated reflection of water quality, and in support of management decisions for rivers and streams, particularly in the last two decades (Resende *et al.,* 2010). With respect to India, the pioneering investigations on the diatom flora came from Ehrenberg (1854), Dickie (1882) and Carter (1926). Diatom flora has been studied scantily in India. Most studies are from southern parts of the Peninsular India, its islands Andaman and Nicobar and some from Himalaya region (Dickie, 1882; Carter, 1926; Gosh and Gaur, 1991; Rout and Gaur, 1994; Ormerod *et al.*, 1994; Ormerod *et al.*, 1996; Jüttner *et al.*, 1996; Rothfritz *et al.*, 1997; Jüttner *et al.*, 1998; Gandhi, 1998; Nautiyal and Nautiyal, 1999 a, b; Jüttner and Cox, 2001; Khan, 2002 and Nautiyal *et al.*, 2004 a, b).
The Central Highlands subdivision of the major physiographic region the Peninsular Plateau is synonymous with the 6th bio-geographic region- Deccan Peninsula. The Central Highlands alongside Chota Nagpur are the northern-most biotic provinces of this bio geographic region (Source: wiienvis.nic.in/database/htmlpages/ bioprovincemap.htm WII, 2000). Central Highlands is an important region for the freshwater biodiversity of hill streams of the Indian subcontinent. This eco region is a vital junction in the mountain streams and rivers. In view of above, a study was performed to determine the diversity indices and diatom abundance patterns among the three Central Highlands Rivers of Bundelkhand Plateau; Ken, Paisuni and Tons. Principal Component Analysis (PCA) was used to determine the characteristic taxa at each station. It is a pioneer investigation of this nature in central

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highland Rivers and will significantly contribute to the knowledge of phycological information of Central India.

MATERIALS AND METHODS

Study Area

The Central Highlands consist of the Malwa, Bundelkhand, and Chota Nagpur Plateaus that form the northern subdivision of the ancient (Gondwana) triangular shaped tableland- the Peninsular Plateau. The Vindhya Ranges give rise to the tributaries of the Yamuna (Chambal, Kali-Sindh, Parbati, Betwa, Dhasan, Ken, Paisuni) and Ganga (Tons, Son) Rivers that flow northwards. The Ken, Paisuni and Tons drainages in the northeast are the subject of this study. The Ken and Tons are high stream order rivers, while the Paisuni is a lower order stream. These rivers are important for irrigation, domestic supply and power generation including proposed river linking projects on the Indian Peninsula (NWDA, 2006). The rivers also have high religious significance. These drainages lie within 24 to 26°N latitude, 79 to 82°E longitude and altitude 360 to 72 m above sea level from source to confluence. Locations sampled on the Ken are labeled K1 to K4, Paisuni P1 to P4 and Tons T1 to T4 (Figure 1). The physiography, climate, and vegetation have been described earlier (Nautiyal and Mishra, 2012).

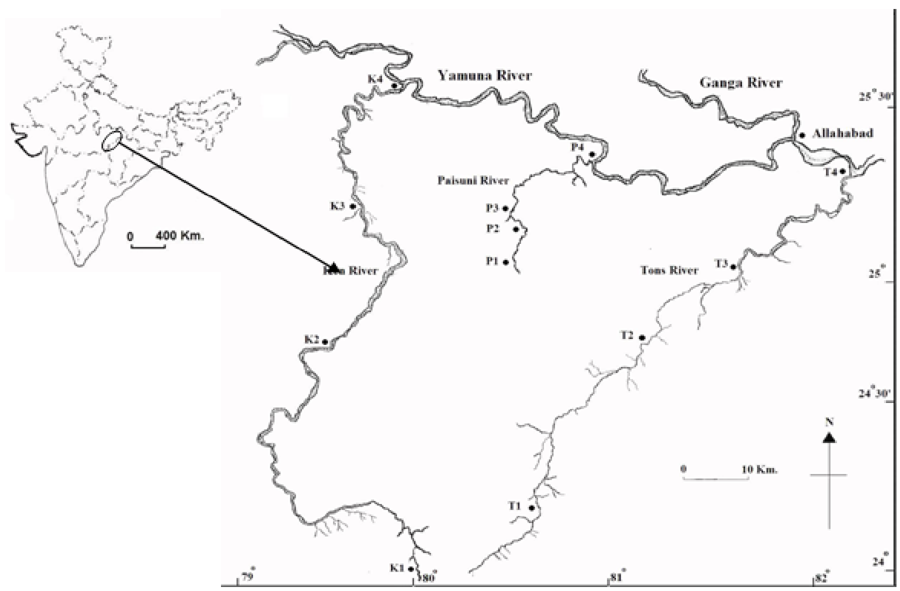

Figure 1: Location of the Study Area in Central Highlands in India

The rivers (Ken, Paisuni and Tons) Selected for Study, Sampling the Stations along their Course from Origin to Confluence. The Paisuni and Tons have Similar Length. The Distances among the Basins Increase from Upper to Lower Section. The Stations Selected to Represent each Section are as follows; Upper Section (K1, P1, T1), M- Middle Section (K2, P2, T2), L- Lower Section (K3, P3, T3), Mo- Mouth Section (K4, P4, T4). Acronyms: K- Ken, P- Paisuni, T- Tons

Physico-chemical analysis of river water was done by using an onsite water monitoring probe (Horiba U51). Diatom samples were collected by scraping the cobble surface with a brush to dislodge diatoms from crevices and minute cavities from an area of 3 x 3 cm^2. The scrapings from each cobble were collected in a petri-dish and transferred to storage vials. Samples were preserved in 4% formaldehyde solution for further processing.

Preparation of Diatom Mounts

The preserved samples were first cleaned with double distilled water to remove traces of formaldehyde. Samples were digested with hydrochloric acid. The treated samples were washed repeatedly with double distilled water to remove all traces of acid. Samples were then cleaned with hydrogen per oxide and distilled water. The processed material was mounted in Naphrax for preparing the permanent slides from each sample. Each slide was examined under a BX-40 Trinocular Olympus microscope (x15 wide field eyepiece) fitted with Universal condenser and PLANAPO x100 oil immersion objective under bright field using appropriate filters to identify and count the species.

Identifications were made according to standard literature (Schmidt, 1914-1954; Hustedt, 1931-1959; Krammer and Lange-Bertalot, 1986-1991, 1999, 2004; Gandhi, 1998; Lange-Bertalot and Krammer, 2002; Metzeltin and Lange-Bertalot, 2002; Krammer, 2003; Lange-Bertalot *et al.*, 2003; Werum and Lange-Bertalot, 2004; Metzeltin *et al.*, 2005). The permanent mounts have been adequately stored at the Aquatic Biodiversity Unit, Department of Zoology, H. N. B. Garhwal University, Srinagar where preparation of the slides and their microscopic examination was undertaken.

In each sample 500-600 valves were counted for computing relative abundance, measures of diversity within and among rivers using PAST v.2.03 (Hammer *et al.*, 2001). The cluster analysis was based on the raw data, i.e. presence of diatom flora in each river/streams and Principal Component Analysis (PCA) was applied to determine the characteristic taxa among the streams based on assemblages of diatom >10%.

RESULTS AND DISCUSSION

Results

The range of water temperature (WT) and pH varies in the rivers Ken (16-27ºC; 7.0-7.5), Paisuni (15.5-33ºC; 7.0-7.7) and Tons (17-31.4ºC; 7.0-7.8). Similarly, the range value of current velocity (CV) varies in the Ken (1.0-60cm s^{-1}), Paisuni (0.0-30.9cm s^{-1}) and Tons (0.1-50 cm s^{-1}). The WT and pH increases downstream of the rivers and current velocity decreases. Substrate particle size decreases gradually from the upper to lower plateau section but abruptly changes to small sediments in the mouth section, which lies in the Gangetic Plains (Table 1).

The diatom flora belonged to three sub orders (one centric, two pennate) and nine families, two of the former (Thalassiosiraceae, Meloseriaceae) and seven of the latter (Fragilariaceae, Eunotiaceae, Achnanthaceae, Naviculaceae, Epithemiaceae, Bacillariaceae and Surirellaceae). In the Vindhya Rivers centric diatoms were represented by Thalassiosiraceae comprising *Aulacoseira* (1 spp.) and *Cyclotella* (2 spp.). The Suborder Araphidineae represented by family Fragilariaceae comprised 5 genera and twenty eight taxa. *Synedra* accounted for nineteen from these rivers (Table 3). The Suborder Raphidineae was represented by six families; Eunotiaeae, Achnantheacae, Naviculaceae, Epithemiaceae, Bacillariaceae and Surirelliaceae. Eunotiaceae was represented by six taxa of *Eunotia*. Achnantheacae comprised twenty six taxa with four genera (*Achnanthidium*, *Achnanthes*, *Planothidium*, *Cocconeis*). Naviculaceae was represented by one hundred eighty three taxa of 30 genera. A bulk of the Naviculaceae flora belonged to five genera; *Navicula* (32 taxa), *Cymbella* (31 taxa), *Gomphonema* (15 taxa), *Cymbopleura* (14 taxa) and *Amphora* (12 taxa). Epithemiaceae was comprised two genera and three species; *Epithemia* and *Rhopalodia* with one and two species. Bacillariaceae consisted of three genera (*Bacillaria*, *Denticula*, *Nitzschia*) and twenty two taxa. *Nitzschia* (32) accounted for major share of species in this family. Surirellaceae was represented by a genus *Surirella* of 9 taxa (Table 3).

Two hundred ninety three (290 pennate and 3 centric forms) diatom species belonging to 48 genera were recorded. The taxonomic richness in the River Ken, Tons and Paisuni was 205, 202, and 211 respectively (Table 2). Shannon diversity Index (H') was 5.01, 5.07, 5.02, Evenness (E) 0.31, 0.23, 0.33 and Margalef species richness (d') 31.8, 31.5, 32.8 for Ken, Tons and Paisuni respectively (Table 2).

The cluster analysis revealed two broad groups among the streams with respect to taxonomic composition observed in them. The first group comprised Paisuni, while the second cluster included two subgroups the Ken and Tons. The sub clusters explained the grouping of streams with similar flora. There is weak similarity among the Ken, Paisuni and Tons, Paisuni while the Ken and Tons basins are distantly located but they were showing similarity due to similar order streams (Figure 2). Principal component analysis revealed that all the plateau rivers associated with their characteristics taxa in different axis. Along the right upper axis Ac, Ncr Gp associated with Tons at T4 where as Ams, Na, Nv associated with T2. Along the right lower axis Nc, Nca, Cd, Ct were associated distantly with P1, P2, P3. On the left upper axis Ce, Nl Ct was associated distantly with T3. On the left lower axis of ordination diagram Nr, Mg, Ca, Su, Al were very distantly associated with all the station of Ken River (Figure 3).

Table 1: Physico-Chemical Variables, Richness, Diversity and Evenness in the Central Highlands Rivers

Section/ Station	A (masl)	DS (Km.)	LU	Sub.	WT (^{O}C)	CV (cm^{-1})	pH
Shahnagar (K1)	365	ca.10	Ag-V	R;B;C;P	16-31.1	1-50	7.0-7.5
Panna (K2)	200	142.5	F-Ag	B;R;C;P ;G	16.5-22	10-60	7.0-7.2
Banda (K3)	95	267.5	Ag-C	C ;P ;G; B ; S; Si	17-24.5	2.0-12	7.2-7.5
Chilla (K4)	86	340	Ag	Cl,-Si	20.5-27	1.0	7.2-7.5
Anusuya (P1)	180	10	F	R; B; C; P	21-22.5	6.2- 23.2	7.0-7.3
Chitrakut (P2)	135	26	Ag-T	Cl; Si; B; P-G	16.5-21.5	0 - 30.9	7.3-7.6
Purwa (P3)	131	42	Ag	Si; C; C-P; B; S; P-G; Cl	15.5-17	2.81-30.2	7.4-7.7
Rajapur (P4)	80	100	Ag	Si; C-P; Cl; S; P-G	28.5-33	10.3-20.6	7.4-7.6
Maihar (T1)	326	Ca.56	Ag	B; C; P; G; Si	17-31.4	1-50	7.0-7.6
Satna (T2)	290	98	Ag-V	C;P; P-G; S; Si	17.5-28	1.6-4.8	7.4-7.6
Chakghat (T3)	94	232.5	Ag-T	R; B-P; C, P-G; Si	17-27	1.5-15	7.4-7.6
Meja (T4)	72	305	Ag	Cl-Si	17-24	<0.1	7.5-7.8

A- Altitude, DS - Distance from Source, LU- Landuse, Sub- Substratum, WT- Water Temperature, CV- Current Velocity, R-Rock, B- Boulder, C- Cobble, P- Pebble, G-Gravel, Cl-Clay, S-Sand, Si-Silt, Ag- Agriculture, C- City, F- Forest, T- Town, V- Village

Table 2: Measures of Species Diversity Indices in the Ken (K), Paisuni (P) and Tons (T) Rivers by Application of Various Diversity Indices

	K	P	T
Taxa_S	205	202	211
Dominance_D	0.010	0.009	0.010
Shannon_H	5.01	5.02	5.07
Simpson_1-D	0.98	0.99	0.98
Evenness_e^H/S	0.31	0.33	0.23
Menhinick	8.4	8.8	8.3
Margalef	31.8	32.8	31.5
Equitability_J	1.049	1.053	1.069
Fisher_alpha	112.1	119.9	107.5
Berger-Parker	0.020	0.023	0.0172

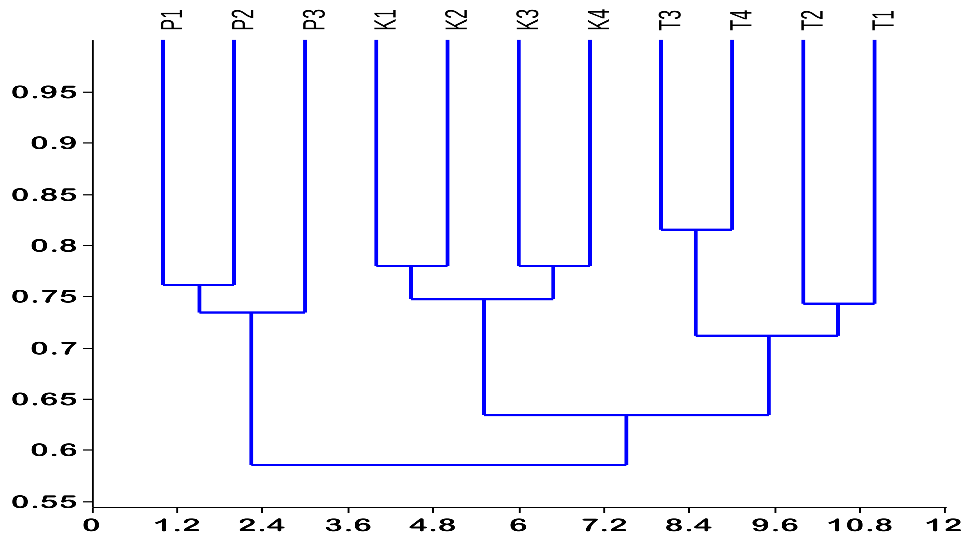

Figure 2: Cluster Analysis Based on Benthic Diatom Flora for the Stations in Above Rivers
Sites along river Ken (K1 K2 K3 K4), Paisuni (P1 P2 P3 P4) and Tons (T1 T2 T3 T4)

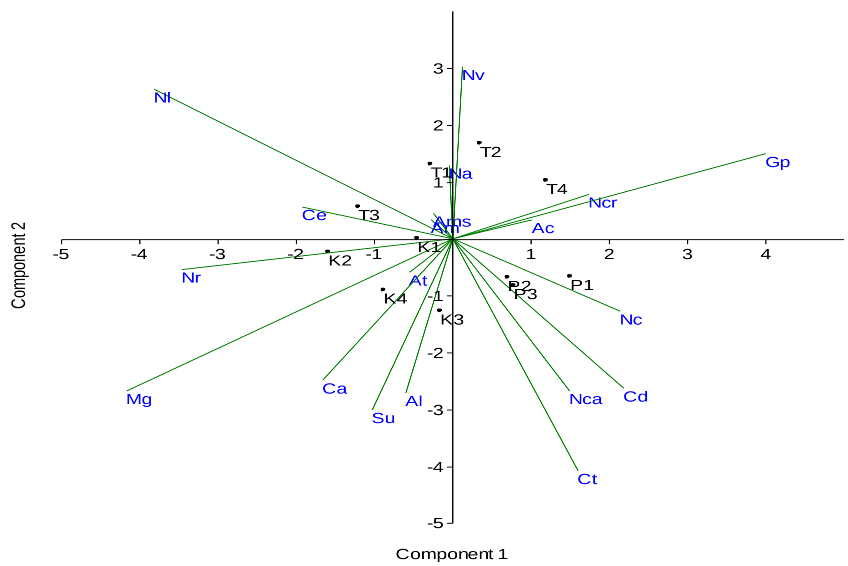

Figure 3: PCA Biplot to Identify the Characteristic Taxa at the Each Station of Central Highlands Rivers
Ken, Paisuni and Tons. Acronyms: Am=*A. minutissimum*, Ac=*A. Chitrakootense*, Al=*A. Linearis*, Ncr=*Navicula Cryptotenella*, Su=*Synedra Ulna*, Cd=*Cymbella Diversa*, At=*Amphora Twentiana*, Nca=*Navicula Capitatoradiata*, Ct=*Cymbella Tumida*, Ca=*Cymbella Australica*, Na=*Navicula Antonii*, Ce=*Cymbella Excise*, Nr=*Navicula Rostellata*, Nl=*Nitzschia Linearis*, Gp=*Gomphonema Parvulum*, Mg=*Melosira Granulate*, Nc=*Navicula Caterva*, Nv=*Navicula Viridula*

Table 3: Spatial Variations in the Number of Species Occurring in Various Genera Recorded from the Vindhyan Rivers: Ken (K), Paisuni (P) and Tons (T)

GENERA	K	P	T
THALASSIOSIRACEAE			
1. Aulacoseira Thwaites	1	1	
2. *Cyclotella* Kutzing	1	1	2
FRAGILARIACEAE			
3. *Diatoma* Bory	2	1	4
4. *Fragilaria* Lyngbye	1	1	2
5.*Staurosira* Williams & Round	1	2	1
6. *Synedra* Ehrenberg	13	14	15
7. *Tabellaria* Ehrenberg	1		1
EUNOTIACEAE			
8. *Eunotia* Ehrenberg	3	4	4
ACHNANTHACEAE			
9. *Achnanthes* Bory	1	1	
10. *Achnanthidium* Kützing	8	11	10
11. *Planothidium* Round and Bukhtiyarova	3	5	3
12. *Cocconeis* Ehrenberg	6	3	3
NAVICULACEAE			
13. *Amphipleura* Kützing		1	
14. *Amphora* Ehrenberg ex Kützing	10	8	8
15. *Anomoeoneis* Pfitzer		1	1
16. *Brachysira* Kützing	1	2	2
17. *Caloneis* Cleve	5	3	5
18. *Cymbella* Agardh	21	22	20
19. *Cymbopleura* Krammer	12	6	10
20. *Encyonema* Kützing	4	4	4
21. *Diploneis* Cleve	5	5	4
22. *Frustulia* Rabenhorst	1		
23. *Gomphocymbelopsis* Krammer	1	1	

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24. *Gyrosigma* Hassall	2	2	3
25. *Gomphonema* Ehrenberg	11	10	12
26. *Mastogloia* Thwaites	1		
27. *Navicula* Bory	30	32	31
28. *Adlafia* Moser, Lange-Bertalot and Metzeltin	1	1	2
29. *Aneumastus* Mann and Stickle		2	
30. *Craticula* Grunow	4	3	3
31. *Diadesmis* Kützing	1	2	1
32. *Fallacia* Stickle et Mann	1	1	2
33. *Geissleria* Lange-Bertalot et Metzeltin	1	1	1
GENERA	**K**	**P**	**T**
34. *Hippodonta* LB, Witkowski and Metzeltin	1		2
35. *Luticola* Mann	6	3	7
36.*Placoneis* Mereschkowsky	2	1	2
37. *Sellaphora* Lange-Bertalot et Metzeltin	5	5	5
38. *Neidium* Pfitzer	2	4	2
39. *Pinnularia* Ehrenberg	3	7	3
40. *Scoliopleura* Grunow		1	
41. *Stauroneis* Ehrenberg	2		
EPITHEMIACEAE			
42. *Epithemia* Kützing			1
43. *Rhopalodia* O. Muller		1	2
BACILLARIACEAE			
44. *Bacillaria* Gmelin			1
45. *Denticula* Kützing	1	1	1
46. *Hantzschia* Grunow	1	1	
47. *Nitzschia* Hassall	21	20	25
SURIRELLACEAE			
48. *Surirella* Turpin	8	7	5
TOTAL	205	202	211

Discussion

The centric diatoms comprise just 1% of the total flora (3 taxa). The araphids accounted for 10.5% (38 spp.) and the raphids (mono and biraphids) accounted for forty two genera with two hundred fifty eight taxa (80%) while monoraphids accounted for 8% (26 spp.). The Central highland rivers are dominated by biraphids.

In Himalayan rivers also the biraphid content is known to be high (Nautiyal and Nautiyal 1999 b, Nautiyal *et al.*, 2004b). The Peninsular or other parts of India including the mountainous zones like the Western Ghats (Krishnamurthy, 1954) are equally rich in biraphid flora.

The major share in floristic composition was family Naviculaceae (62%), Bacillariaceae (12%) and Fragilariaceae (9.5%) from Central Highland**.** *Navicula* 32, *Cymbella* 31, *Gomphonema* 15 and *Cymbopleura* 14 were species rich genera in family Naviculaceae, *Synedra* 19 in family Fragilariaceae and *Nitzschia* 32 in family Bacillariaceae. The Vindhya was richer in *Navicula* sensustricto, *Nitzschia* and *Cymbella sensu stricto,* which was similar to that reported in Europe and Asia (Atazadeh *et al.*, 2007; Kupe *et al.*, 2008; Pongpan & Yuwadee, 2011).

The indices of taxa richness (d) and taxa diversity (H, E) indicate high diversity in these plateau rivers, as compared with other regions of India 2.6 to 4.1in the Himalaya and Alps (Cantonati *et al.*, 2001), 1.8 (E 0.6) to 3.5 (1.04) in the Mandakini basin, West Himalaya, 1.6 to 4.1 in northeast Himalaya (Rout and Gaur, 1994), 1.3 to 1.7 Betwa (Agarwal, 2009).

In fact, higher diversity in the Vindhya may be due to the moderate nutrient levels generated by agriculture as also observed in Nepal (Jüttner *et al.*, 1996). Extensive agriculture is comparable to moderate amount of disturbances, one of the causes for higher diversity (Begon *et al.*, 1990).

With respect to taxa diversity and evenness, the Paisuni was followed by Tons and Ken. Though the number of taxa was higher in the Tons, the Paisuni was more diverse because of its pristine condition compared to the impacted Tons and Ken, attributable to the forested headwaters and organic enrichment due to religious activities between Anusuya (P1) and Chitrakoot (P2). Kawecka & Olech (2004) recorded 270 taxa from Finnish Lapland, which was lower than Central Highland eco region. Thus, richness of Central Highland rivers is higher than for temperate region and rivers; 165 taxa from Estonian River (Vilbaste, 2001), 72 taxa from Coastal Oregon Streams (Naymik *et al.*, 2005) and 98 and 94 taxa respectively from Vit and Osum, the Bulgarian rivers (Stancheva *et al.*, 2007).

The use of multivariate tool cluster analysis to examine the extent of similarity among the rivers of one region revealed that each river had unique elements in the flora sufficient enough to isolate one river from another.

In order of their confluence with the Yamuna and the Ganga, the Ken is followed by the Paisuni and the Tons. Though, the river Paisuni is located in between Ken and Tons, but the cluster of Paisuni was clearly separated from that of the Tons and Ken, indicating that its flora was sufficiently different from both the rivers.

In contrast there was enough similarity between the Ken and Tons such that they formed one major cluster, even though these rivers lie far away. Still there were some unique floral elements in either of them such that the Ken and Tons formed separate sub-clusters.

Probably, in each of them some diverse elements have evolved over years of isolation that impart uniqueness to their flora.

Certain level of similarity among the Ken and Tons can be attributed to the fact that these rivers are of similar character (identical geographical and climatic conditions-draining the Bundelkhand Plateau), both are 350-400 km in length, with extensive agriculture on their banks all along their length, except in case of the Ken, some part of which passes through the Panna National Park. The Paisuni on the other hand is a short river, one-third the length of the Ken or Tons.

Considerable part of its headwaters flows through forested area while the lower part experiences agriculture. Also its stream-order is lower than the Ken and Tons.This supports the alternate hypothesis of Corkum (1990) that even adjacent basins can be dissimilar despite identical climatic and physiographic conditions and distant localities can be similar, the prime hypothesis being that the association of the rivers of one zone can be explained through 'biome dependency hypothesis' (Ross, 1963; Corkum, 1989). This hypothesis predicts that similar assemblages of diatoms are most likely to occur at sites along river, if the drainage basin occupies the same biome. It seems that the dissimilarity of abundant fauna in the rivers of two different eco region is influenced by geographical factors i.e., terrain, latitude and soil type. The reasons for this need further investigation.

Conclusion

The Central Highlands eco region supports high richness and diversity of diatoms in the Bundelkhand Plateau Rivers. There is no definite spatial trend in the species diversity and richness. The cluster analysis reveals that each river has its own characteristic flora owing to which there were separate clusters even within major cluster for each river. The flora of Paisuni was more dissimilar to the Ken and Tons which can be attributed to major differences in length of the rivers and hence the stream-orders and the land use practices in their basins. In case of the Ken and Tons that formed separate sub cluster for each river, in each of them some diverse elements must have evolved over years of isolation that imparts uniqueness to their flora.

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