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Research Article

Application and Efficacy of diatom diversity indices for water quality evaluation of Chambal River System

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Abstract

Species richness along environmental variables reveals a variety of patterns. In the present paper, we investigated the relationship between diatom communities and abiotic factors. About seven different species richness diversity indices were calculated for 27 sampling sites and Spearman's rank correlation was determined to reveal the relationship between environmental variables and species diversity index. In total, 131 different diatom taxa were identified during the study

Keywords: *diatoms, diversity indices, biotic indices, water quality, SIMPER.*

Introduction

Benthic algae are known as an important component of habitats in both marine and freshwater systems (Kingston et al. 1983; Gosh and Gaur 1991; Soininen 2004). In an aquatic environment, the study of algal community gives signals about the pollution (like changing pH, addition of oil, heavy metals, increase of organic matter, and chemical fertilizers) (Buragohain and Yasmin, 2014). The primary groups of algae in rivers are blue–green algae (Cyanobacteria), green algae (Chlorophyta), diatoms (Bacillariophyta), and red algae (Rhodophyta, Soininen 2004). Among these groups of algae, diatoms comprise the most common and diverse group in aquatic environments (Jones 1996). They are primary producers in river ecosystems and indicate the overall status of the ecosystem in which they occur (Hosmani, 2013). In comparison to other organisms, diatoms are a more suitable indicator due to their apparent ubiquity, short generation time, sensitivity to changes in nutrient levels, and vast assemblages (De la Rey, 2004).As microalgae, diatoms grow and reproduce more rapidly than large animals such as macroinvertebrates and fish, providing a potential for an early warning of environmental disturbances (Barbour et al. 1999) and indicate the health of an ecosystem (Jafari and Gunale, 2006). Diatoms respond sensitively to the physical, chemical, and biological variations in their ambient environment (Pan et al. 1996; Liu et al. 2013; Wang et al. 2014), as well as upper-level factors such as land use, geographic and climate changes (Potapova and Charles 2002; Soininen2007; Li et al. 2015b). Their response is possibly more sensitive than macrophytes and other algal groups (Schneider et al. 2012). Benthic diatom communities respond promptly to water quality changes caused by eutrophication and other types of pollution, such as urban, industrial, and agricultural discharges. The composition and diversity of the benthic diatom community are affected differently by changes in the physico–chemical characteristics of the water (Acs et al. 2004). Simultaneous use of physico-chemical and biological analysis is the best way to evaluate the ecological status of river water.

Diatom-based water quality monitoring has become a routine practice in many aquatic environments worldwide. The structure of periphytic diatom communities usually exhibits a strong dependence on many abiotic factors, especially those reflecting the ''biological quality'' of the water, a term that gathers many different aspects such as nutrient concentrations, habitat disturbances, or the presence of micropollutants. Hence, the abundance of many ''indicator'' taxa correlates with important limnological variables, this being the basis for the implementation of diatom indices for the diagnosis and surveillance of freshwater ecosystem health. In this context, many attempts have been carried out to assess the comparative performance of diatom-based methods (Blanco *et al.,* 2007). In general, two main groups of metrics using diatom communities have been historically proposed: autecological indices and diversity indices. The first ones are frequently based on the average of

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sensitivity values of the taxa present in the samples, weighted by their relative frequencies and their ecological amplitudes, making use of the niche requirements and habitat preferences of the individual species or higher taxonomic grouping (Ector &Rimet2005, de la Rey et al2008). On the other hand, the use of diversity measures for water quality assessment assumes that impairment (pollution, eutrophication, etc.) causes a decline in diversity, as the abundance of certain intolerant taxa decreases while tolerant species compete the others (Archibald1972, Patrick1973).

Biotic indices, such as diversity and evenness, have been used to monitor the impact of disturbance and pollution on streams and have been discussed by many authors (Stevenson 1984, Podani 1992, Stewart et al. 1999). While many researchers have reported that diversity decreases with pollution (Rott and Pfister 1988), some have stated that diversity values increase with pollution (Izsak et al. 2002), or that the relationship could depend on the type of pollution (Hillebrand and Sommer 2000, Juttner et al. 2003).

Diatom assemblages on substrates are well suited for water quality assessment (Patrick, 1977; Sabater *et al.,* 1988; Rott, 1991; Round, 1991; Rushforth& Brock, 1991; Dixit *et al.,* 1992; Prygiel & Coste, 1993; Rott *et al.,* 1998; Stevenson & Pan, 1999; Stewart et al, 1999; Hill *et al.,* 2000b). Indices of community structure (e.g., diversity, evenness, richness, similarity) have been used to monitor the impact of disturbance and pollution on streams, and are discussed by many researchers (e.g., Archibald, 1972; Patrick, 1973; Stevenson, 1984; Friedrich *et al.,* 1992; Podani, 1992; Ho &Peng, 1997; Stewart *et al.,* 1999; Hill *et al.,* 2000b). Descy (1979) estimated the degree of water pollution by calculating an index (diatomic index) based on sensitivity of benthic diatom species to pollution. Diatom indices were found to be correlated with organic pollution, ionic strength, and eutrophication (Prygiel & Coste, 1993; Kelly *et al.,* 1995).

Diversity indices are related to community structure (Rey et al 2006) and it consists mainly of three measures, namely: species richness, the evenness and a combined measure of many diversity indexes such as Fisher's alpha (Fisher et al, 1943), Shannon diversity (Shannon & Weaver, 1949), Simpson (Simpson, 1949),and many more. These diversity indices based on benthic diatom assemblages are regularly used in the study of water ecology. Diversity indexes are used to evaluate the impact of certain pollutants on aquatic systems (Cunningham et al., 2003; Gomez, 1999; Gracia-Criado et al 1999). Species richness is one of the major challenges of biological research and has received a considerable amount of attention from the last few decades. In the present study, diatom composition communities along environmental variables might provide key insights into the processes determining species richness along abiotic factors. Moreover, different diatom diversity index can be driven by different abiotic factors. Wang et al. 2017 for instance, found for stream diatom assemblages along elevational gradients in Asia and Europe that richness was mostly related to pH, while evenness was mostly explained by total phosphorus.

Diatoms are often the most important primary producers; diatom diversity can be influenced by a variety of environmental factors. Many studies showed that pH, conductivity, elevation, nitrate, sulphate, and total phosphorus are important environmental determinants of diatom richness (Cantonati et al. 2012, frankova et al 2009). Żelazna-Wieczorek et al 2011, studied that calcium ion concentration and nitrate concentrations can affect diatom species richness as shown for anthropogenically altered springs in Poland. Most of the studies revealed that environmental variables can only partly explain diatom diversity (Lukas taxbock et al, 2020). Environmental variables such as pH, conductivity, alkalinity, light availability, temperature, nitrate, and phosphorus can influence compositional change in diatom communities (Cantonati *et al.,* 2006 and 2012; Cantonati and Spitale, 2009; Teittinen et al 2017, Wang et al 2017)

The SIMPER i.e. similarity percentage (Clarke, 1993) is based on the disintegration of Bray-Curtis dissimilarity index. The SIMPER function performs pairwise of groups of sampling units and finds the contribution of each species to the average between-groups (Clarke and Gorley, 2006). Although the method is called "Similarity Percentages", it really studies dissimilarities instead of similarities (Clarke, 1993). Similarity Percentage analysis displays most important species for each pair of groups. These species contribute atleast 70% of the difference between groups. The most abundant species usually have highest variances, and they have high contributions even when they do not differ among groups.

The objective of this paper was: (Angeli, N. *et al.,* 2010) to study the relationship between biotic indices and environmental variables of the river Chambal; (Azrina, M. Z. *et al.,* 2006) to determine species richness and diversity by site and season; (Berger, W. H., & Parker, F. L. 1970) to examine the degree of similarity/dissimilarity among diatom assemblages within each group.

Materials and methods

Study area

Chambal River is the largest, 960 km long tributary of the Yamuna River which gradually drains into the Gangetic drainage system. It is a perennial river originating from Janapav hill of the Vindhyan range at 22° 27_ N and 75° 37_ E in Mhow, Madhya Pradesh of Central India. Chambal River makes its way through three large states of Madhya Pradesh, Rajasthan, and Uttar Pradesh. While thorn forests, undulating floodplains, gullies and ravines cover a major part of the river basin (Gopal& Srivastava 2008), evergreen riparian vegetation is reported to be completely absent, with sparse ground cover (Hussain, 1999).Rich in biodiversity, the Chambal river harbours globally threatened fauna including six critically endangered, 12 endangered, and 18 vulnerable species (IUCN 2025) including the Gangetic river dolphin (*Platanista gangetica gangetica*), Gharial (*Gavialis gangeticus*) and the red-crowned roofed turtle, (*Hardellathurjii*) (Nair and Chaitanya, 2013). The Government of India established the National Chambal Sanctuary (NCS) along the river between 24°55' to 26°50' N and 75°34' to 79°18'E to conserve the gharial and the unique Chambal ecosystem (Srivastava *et al.,* 2017).

Environmental variables

Samples were collected from 27 selected sites (Figure 1) along the Chambal River in 2023. At all sites temperature, pH, conductivity, turbidity, salinity, dissolved oxygen, and total dissolved solids were measured using multi-parameter probe (Horiba U-23). The analysis of nitrate (NO3), nitrite (NO2), orthophosphate (PO4), and silicate were conducted in the laboratory using a UV/VIS double beam spectrophotometer (UV-1700).

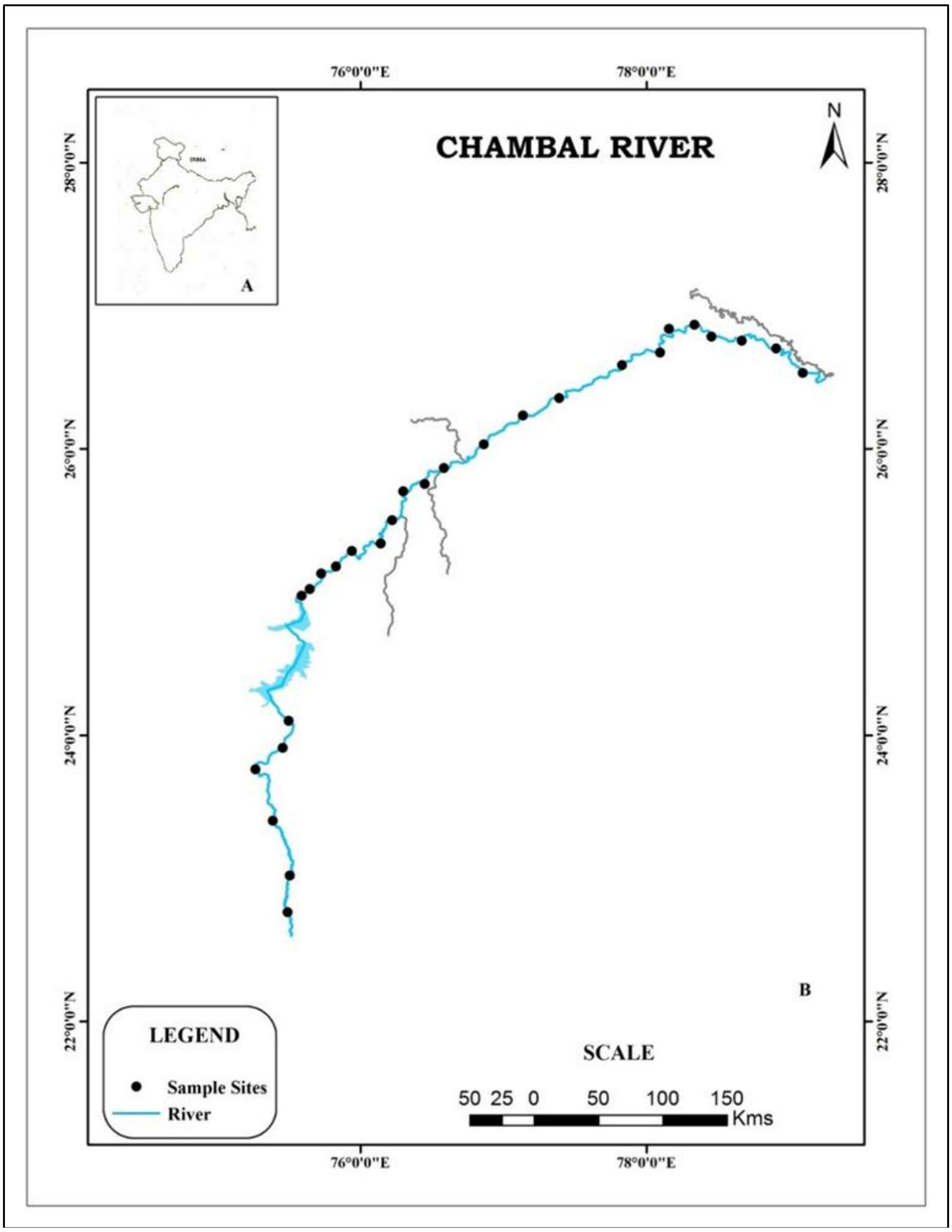

Figure1: A. Location of Chambal River in India, B. Location of the selected sites of the Chambal River

Diatom sampling and laboratory analysis

Diatoms were collected from all the 27 sampling sites along with the river water in both seasons. For the sampling of epilithic diatoms, five to ten cobbles or pebbles were randomly collected from each sampling site and diatoms were scraped off with a toothbrush following standard procedures (Kelly et al. 1998). Before sampling the epilithic surfaces, all substrata were gently shaken, and the resulting suspensions were pooled to form a single sample, which was then placed in a labeled plastic bottle. All diatom samples were homogenized and fixed with 4% formaldehyde. In the laboratory, diatom samples were cleaned with hot HCl and $KMnO_4$ to remove organic coatings (Hasle 1978) and Round et al. (1990). It has been found suitable for cleaning diatom samples collected in India (Karthick et al. 2010). Permanent slides were prepared using Naphrax (Brunel Microscopes Limited; Refractive index of 1.64)

Diversity Indices

The composition of diatom assemblages was examined by species richness (SR), the Fisher's alpha diversity index (Fisher et al, 1943), Shannon-Wiener diversity index (H′) (Shannon & Weaver, 1949), Simpson's index (Simpson, 1949), Margalef D (Margalef, 1958), Berger-Parker index (Berger and Parker 1970) and Evenness

(J′) (Pielou, 1975), calculated using OMNIDIA software (Lecointe, Coste & Prygiel, 1993).The same number of cells has been counted for the diversity indices (400 cells per slide). It is well known fact that there is a relationship between diversity indices and sample size (Seber, 1986; Lewins and Joanes, 1984).

Data analysis
Spearman's rank correlation was used to determine the relationship between diversity richness index and environmental variables using SPSS software (version 17). For all the species diversity indices, multiple regression analysis was also calculated to explain the observed variation in the diatom diversity indices. The R^2 and Adjusted R^2 values were used instead of the R values, as they are meticulous measures of the predictability of multiple regressions.

Multivariate analysis
Multivariate analysis was also performed to reveal the relation between environmental variables and diatom diversity indexes. For the calculation of various diversity indexes, we have used SDR (Species Diversity and Richness) software, PISCES conservation Ltd and following diversity indexes were measured as Fisher's alpha diversity, Shannon-Weiner index, Simpson's index, Margalef D, Berger-Parker, McIntosh D, Brillouin D, Evenness, Species Richness and Species Accumulation.

Similarity percentages (SIMPER) analysis using CAP software was calculated to trans evaluate the role of each species and to determine which individual species contributed most to the differences between the samples and to the similarities in species abundance (Clarke, 1993). SIMPER measures the percentage contribution of each species to average dissimilarity between groups (Clarke & Ainsworth, 1993).

Results

Environmental variables
The values of physical and chemical variables at all sampling sites in the present study are shown in Table 1 & 2

Table 1: The mean values of environmental variables of selected sites during winter 2023 data

	Temp	pH	EC	TURB	DO	TDS	COD	BOD	Nitrate	Nitrite	Phosphate	Silica
S1	27.59	8.43	0.822	103.33	6.13	0.526	16.69	4.63	2.92	1.72	0.450	22.33
S2	26.54	7.56	0.716	110.67	5.54	0.539	15.73	5.20	1.92	1.46	0.447	19.29
S3	28.03	8.34	0.815	126.33	6.29	0.425	16.51	4.82	2.82	2.07	0.383	21.20
S4	22.40	7.94	0.463	19.91	8.22	0.311	3.79	0.32	2.68	1.96	0.027	14.64
S5	22.41	7.97	0.465	20.81	8.29	0.314	3.49	0.40	2.01	0.99	0.030	12.52
S6	22.54	7.86	0.482	21.38	8.16	0.284	3.20	0.39	2.64	1.31	0.035	13.27
S7	22.01	7.65	0.303	7.31	6.43	0.203	4.59	0.46	0.45	0.28	0.281	10.75
S8	22.08	7.61	0.308	7.59	6.56	0.195	4.81	0.47	0.45	0.23	0.047	11.17
S9	23.05	7.72	0.315	7.71	6.62	0.201	4.19	0.50	0.53	0.28	0.043	10.66
S10	23.65	7.72	0.333	7.56	6.46	0.217	4.13	0.53	7.33	5.44	0.044	10.16
S11	24.13	6.96	0.222	6.91	7.17	0.392	4.06	0.53	7.04	5.03	0.049	9.93
S12	25.06	7.73	0.414	6.19	7.57	0.286	4.38	0.47	7.22	6.11	0.052	10.33
S13	22.32	7.86	0.732	93.72	6.36	0.476	68.35	18.08	0.38	0.31	0.677	10.86
S14	22.06	7.94	0.854	89.82	6.94	0.503	59.57	17.81	0.40	0.31	0.643	10.86
S15	23.30	8.54	0.792	99.16	7.07	0.527	57.54	18.08	0.45	0.21	0.560	10.86
S16	23.29	8.95	0.572	0.11	8.93	0.363	2.15	0.26	4.42	2.13	0.041	7.80
S17	23.27	8.96	0.577	0.13	9.02	0.367	2.10	0.24	3.76	2.47	0.037	6.59
S18	24.03	8.86	0.608	0.12	8.66	0.379	2.61	0.33	4.67	3.50	0.034	7.84
S19	21.10	8.11	0.576	12.27	8.69	0.369	2.19	0.29	2.30	0.76	0.038	8.43
S20	21.56	8.06	0.474	12.22	7.39	0.374	2.21	0.28	2.07	1.16	0.031	7.90
S21	21.42	8.70	0.542	11.58	7.72	0.425	2.55	0.29	2.24	1.18	0.044	8.64
S22	19.91	8.23	0.574	14.45	7.27	0.375	3.11	0.38	7.26	4.80	0.031	9.73
S23	18.92	8.06	0.624	14.83	7.14	0.391	3.41	0.38	7.12	6.32	0.031	9.75
S24	19.50	8.20	0.664	13.86	7.31	0.428	3.22	0.38	7.21	5.81	0.032	10.04
S25	17.24	8.19	0.623	18.53	8.22	0.380	2.08	0.15	2.23	1.56	0.045	11.04
S26	16.95	8.09	0.624	18.21	7.91	0.499	2.12	0.12	2.32	0.73	0.037	11.11

S27	20.14	7.42	0.717	16.87	8.92	0.536	2.30	0.17	2.10	1.10	0.041	11.20

Table 2: The mean values of environmental variables of selected sites during summer season 2023

	Temp	pH	EC	TURB	DO	TDS	SALT	COD	BOD	NO_3	NO2	PO_4	$Si0_2$
S1	38.4	8.96	0.789	52.5	5.67	0.656	0.4	12	10	0.2	0.18	0.09	0.9
S2	37.2	8.73	0.717	51.9	5.74	0.621	0.4	11.7	9.87	0.199	0.186	0.087	0.9
S3	37.9	8.88	0.765	52.3	5.55	0.634	0.4	11.9	10.3	0.203	0.199	0.089	0.9
S4	32.51	8.03	0.401	55.3	6.47	0.261	0.2	12	4	0.5	0.46	0.09	2.6
S5	32.56	8.08	0.402	54	6.65	0.268	0.2	11.5	4.5	0.55	0.43	0.09	2.8
S6	32.45	7.99	0.387	54.3	6.33	0.258	0.2	11.3	3.9	0.48	0.39	0.08	2.5
S7	31.83	8.5	0.307	17	8.81	0.199	0.1	12	4	0.2	0.19	0.09	2
S8	31.18	8.18	0.306	17.4	8.2	0.201	0.1	11.9	4.1	0.19	0.175	0.08	2.4
S9	31.21	8.2	0.306	17.6	8.15	0.203	0.1	12.1	4.3	0.23	0.228	0.07	2.5
S10	39.49	8.89	0.382	18.3	7.62	0.298	0.2	16	5	0.5	0.419	0.08	1
S11	38.7	8.65	0.372	17.9	7.55	0.218	0.1	15.8	5.1	0.52	0.467	0.085	0.99
S12	37.6	8.51	0.364	18.1	7.47	0.232	0.2	16.3	5.4	0.55	0.5	0.079	1.09
S13	32.35	8.74	0.837	49.7	7.54	0.536	0.4	39.8	12	0.2	0.188	0.1	0.9
S14	31.13	8.66	0.817	50.1	7.11	0.511	0.4	38.7	11.8	0.19	0.165	0.09	0.97
S15	32.05	8.53	0.825	48.6	7.23	0.542	0.4	37.3	12.4	0.22	0.2	0.1	1.3
S16	34.81	8.73	0.746	6.5	8.81	0.477	0.4	16	4	0.5	0.485	0.08	1.6
S17	34.11	8.64	0.716	6.1	8.87	0.465	0.3	15.7	3.99	0.54	0.53	0.09	1.9
S18	33.59	8.59	0.735	6.6	8.76	0.467	0.4	16.2	3.96	0.48	0.452	0.09	2.1
S19	35.79	9.32	0.718	17.6	7.28	0.46	0.3	12	4	0.14	0.133	0.09	1.9
S20	35.16	8.99	0.727	16.6	7.38	0.466	0.3	11.9	3.97	0.11	0.109	0.08	1.7
S21	35.53	8.78	0.746	17.9	7.46	0.51	0.3	11.8	4.14	0.17	0.14	0.09	2.2
S22	29.64	8.05	0.631	21.8	6.46	0.404	0.3	8	3	0.3	0.287	0.08	2.2
S23	29.63	8.04	0.631	21.5	6.41	0.402	0.3	7.99	2.97	0.33	0.311	0.08	2.31
S24	29.41	8.01	0.566	20.9	6.53	0.398	0.3	8.13	2.94	0.38	0.34	0.09	2.46
S25	37.87	7.78	0.692	12.8	7.03	0.443	0.3	17	5	0.5	0.45	0.09	2.2
S26	36.85	7.65	0.717	11.7	7.14	0.453	0.2	17.1	4.89	0.49	0.39	0.07	2.18
S27	37	7.8	0.7	12	7	0.4	0.2	16.4	4.97	0.51	0.44	0.08	2.3

Diversity Indices

A total of 131 taxa of benthic diatoms were reported from the study. Diatom composition varied in between sampling stations and among the allotted groups. The diatom codes used in the current study alongwith their names are given in Appendix I. Most dominant diatoms are given in Figure 2. Table 3 and Table 4represents the values of various diversity index during summer and winter seasons. During winter seasons, the maximum value for Fisher's alpha was 8.136 at S20 under SANT sites whereas minimum was found at S1 (3.249) under HVPL sites. According to the Rosenzweig, 1995, it is the only diversity index that reveals spatial and temporal distribution pattern of a species and has low sensitivity towards the sample size and is relatively insensitive to rare species (Kempton and Taylor, 1974; Magurran, 1988). Shannon-Wiener diversity index varies from 0 to 5. According to this index, values less than 1 characterize heavily polluted condition, and values in the range of 1 to 2 are characteristics of moderate polluted condition while the value above 3 signifies stable environmental conditions (Stub *et al.,* 1970; Mason, 1988). In the present study, Shannon Wiener index varied from a lowest of 1.8 at site S1 to a highest of 2.97 at S9 site. Simpson index varied from 3.87 at S13 to 12.61 at S9. Margalef index has no limit value and it shows a variation depending upon the number of species (Shah and Pandit 2013). Thus, it is used for comparison of the sites (Kocatas 1992) and takes only one component of diversity (species richness) into consideration reflecting sensitivity to sample size. The index was found to be highest at S20 (5.769) while lowest at S1 (2.271). Berger –Parker index was found to be low at S9 (0.161) and high at S14 (0.470).

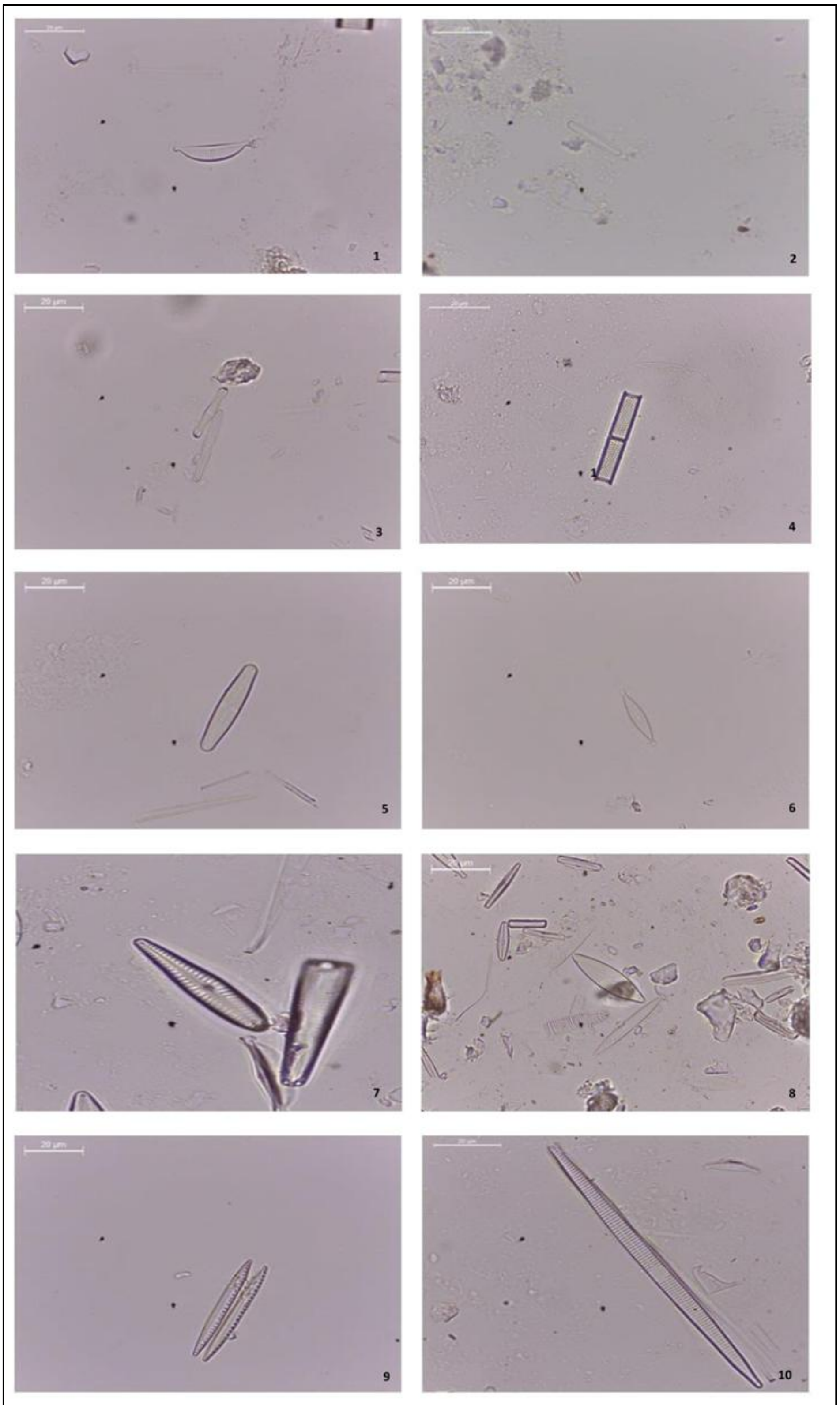

Figure 2. The most abundant taxa in the Chambal River basin1. *Amphora coffeaeformis*Kützing, 2. *Achnanthidiumpeterseni*Hustedt, 3. *Achnanthidium min. v. scotica.* 4. *Aulacoseiragranulata* Ehrenberg, 5. *Caloneisbeccariana*Grunow, 6. *Brachysiravitrea*Grunow, 7. *Gomphonemaangustum*Rabenhorst, 8. Naviculacataracta-rheni Lange- Bertalot, 9. *Nitzschiaamphibia*Grunow, 10. *Synedra ulna* Ehrenberg

Evenness index, however, varied from a minimum of 0.61 at site S14 to a maximum of 0.80 at site S9. The lowest species richness was found at S1 with a value of 25.87 whereas the highest richness occurred at three sites (S25 to S27) of SANT with the same value of 87.

During summer season, the index value of Fisher's alpha and Margalef D was found to be low at S2 and high at S7. Shannon index results showed the maximum value at S7 (3.29) while lowest at S24 (2.081) which comes under the SANT sites. Simpson index varied from 4.7 at S24 to 14.9 at S12. Berger –Parker index was found to be minimum at S12 (0.112) and maximum at S17 (0.425). Evenness was found to be highest at S7 while lowest at S24. Species richness was maximum at S27 and minimum at S1.

Table 3: Different diversity indices during summer season

Sites	**Fisher's alpha diversity**	**Shannon-Weiner**	**Simpson's index**	**Margalef D**	**Berger-Parker**	**Evenness**	**Species Richness**	**Species Accumulation**
S1	5.44	2.89	12.93	3.907	0.1933	0.6184	**29.03**	**30.7**
S2	**3.395**	2.677	13.37	**2.57**	0.1383	0.5728	47.83	45.8
S3	4.173	2.555	9.411	3.056	0.2395	0.5468	59.46	56.2
S13	5.599	2.662	9.981	4.058	0.2014	0.5697	95.23	94.4
S14	5.729	2.766	11.59	3.983	0.1654	0.592	96.73	96.1
S15	5.787	2.641	9.224	4.097	0.206	0.5651	97.73	97
S4	5.718	2.891	14.51	3.93	0.1559	0.6188	66.96	63.2
S5	5.948	2.632	8.56	4.117	0.2712	0.5632	72.45	67.7
S6	5.643	2.506	6.843	3.949	0.3381	0.5363	77.66	71.9
S7	**14.45**	**3.291**	13.27	**8.664**	0.2205	**0.7044**	82.64	75.3
S8	9.446	3.082	12.82	6.082	0.2089	0.6595	84.44	80
S9	7.935	2.9	9.608	5.302	0.2717	0.6205	88.24	84
S10	6.592	2.952	14.65	4.461	0.1316	0.6317	90.04	87.9
S11	4.031	2.669	12.45	2.991	0.1394	0.5711	93.14	90.8
S12	4.451	2.859	**14.9**	3.264	**0.1125**	0.6118	93.83	92.9
S16	8.038	2.44	5.935	5.339	0.3556	0.5221	99.03	97.8
S17	6.366	2.31	4.848	4.422	**0.4255**	0.4943	100.1	98.5
S18	6.568	2.489	5.834	4.547	0.382	0.5327	101.2	100.8
S19	10.49	2.867	11.23	6.689	0.1976	0.6134	102.3	101.5
S20	8.363	2.559	6.219	5.555	0.3585	0.5476	103.1	102.2
S21	5.568	2.587	8.266	3.962	0.2825	0.5537	103.9	103.5
S22	6.734	2.33	6.234	4.659	0.3222	0.4986	104.6	103.8
S23	6.131	2.192	4.828	4.326	0.3926	0.4691	105.3	104.6
S24	5.017	**2.081**	**4.719**	3.64	0.3973	0.4454	105.8	105.3
S25	6.319	2.373	7.699	4.403	0.2069	0.5079	106.5	105.6
S26	9.861	2.717	7.437	6.325	0.2876	0.5815	106.7	106.4
S27	6.712	2.804	12.09	4.65	0.1399	0.6002	**107**	**107**

Table 4: Different Diversity indices during winter season

Sites	**Fisher's alpha diversity**	**Shannon-Weiner**	**Simpson's index**	**Margalef D**	**Berger-Parker**	**Evenness**	**Species Richness**	**Species Accumulation**
S1	**3.249**	**1.867**	4.676	**2.271**	0.3937	0.7511	**25.87**	**24.6**
S2	5.867	2.269	6.407	3.761	0.3186	0.7454	40.05	38.2
S3	6.001	2.462	8.077	3.868	0.2632	0.7965	51.34	47.1
S13	4.35	1.917	**3.867**	3.178	0.4621	0.6297	82.71	81.5
S14	5.338	1.99	3.881	3.778	**0.4704**	**0.6181**	83.11	82.2
S15	5.517	2.099	4.237	3.897	0.4501	0.6444	83.71	82.5
S4	6.351	2.719	8.122	4.503	0.3071	0.7844	59.15	58.1
S5	6.682	2.785	7.882	4.724	0.3238	0.7897	67.74	63.5

S6	7.074	2.735	7.421	4.969	0.3319	0.7633	70.33	67.6
S7	6.624	2.836	11.37	4.778	0.1759	0.7915	73.03	70.4
S8	7.186	2.922	12.18	5.136	0.1652	0.7976	75.13	72.6
S9	7.537	**2.978**	**12.61**	5.362	**0.1613**	**0.8019**	76.43	75.5
S10	5.674	2.499	6.968	3.961	0.3267	0.7671	78.42	77.1
S11	7.721	2.764	8.493	5.358	0.2909	0.7544	80.72	78.7
S12	7.039	2.666	7.935	4.776	0.3035	0.7692	81.61	80.6
S16	3.349	1.992	4.904	2.645	0.304	0.6651	84.51	83.7
S17	6.728	2.832	10.87	4.898	0.1832	0.7784	85.2	84.7
S18	4.926	2.159	5.276	3.709	0.3103	0.6479	85.9	85.9
S19	3.595	2.223	7.236	2.838	0.2445	0.7193	86.2	86.1
S20	**8.136**	2.735	9.463	**5.769**	0.2435	0.7184	86.6	86.1
S21	4.752	2.285	7.104	3.623	0.261	0.6858	86.6	86.2
S22	4.196	2.229	7.086	3.246	0.246	0.6924	86.8	86.8
S23	4.81	2.316	7.559	3.652	0.234	0.6951	86.8	86.8
S24	4.944	2.259	7.048	3.751	0.2577	0.6708	86.8	86.8
S25	3.886	2.255	7.008	3.052	0.267	0.7094	**87**	**87**
S26	4.418	2.094	5.58	3.391	0.2828	0.6428	**87**	**87**
S27	5.297	2.259	6.712	3.989	0.2763	0.6577	**87**	**87**

Cluster analysis (complete linkage) was performed on diatom species diversity from the selected 27 sites. The dendrograms of the sampling sites based on relative abundance are given in Fig. 3 (during winter season). The formation of two major groups was observed: S1 to S15, all the moderately polluted to heavily polluted sites constituted group 1 while group 2 (S16 to S27) consisted of all the pristine sanctuary sites. Similarly, during the summer (figure 4) season two groups were formed. Group 1 constituted all the moderately and heavily polluted sites clustered with few sanctuary sites together such as S16 to S18 and S26. Group 2 constituted the other sanctuary sites together.

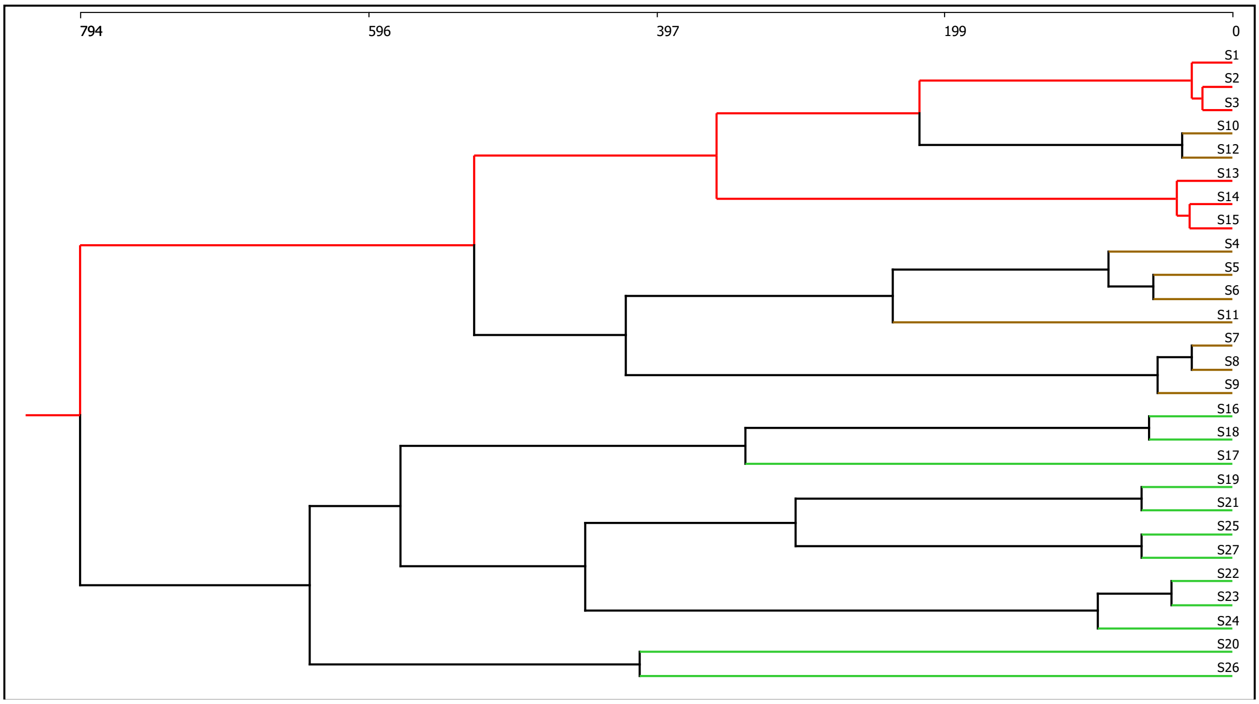

Figure 3: Results of cluster analysis (complete linkage) based on diatom species diversity sampled at 27 sampling stations from the Chambal River during winter season.

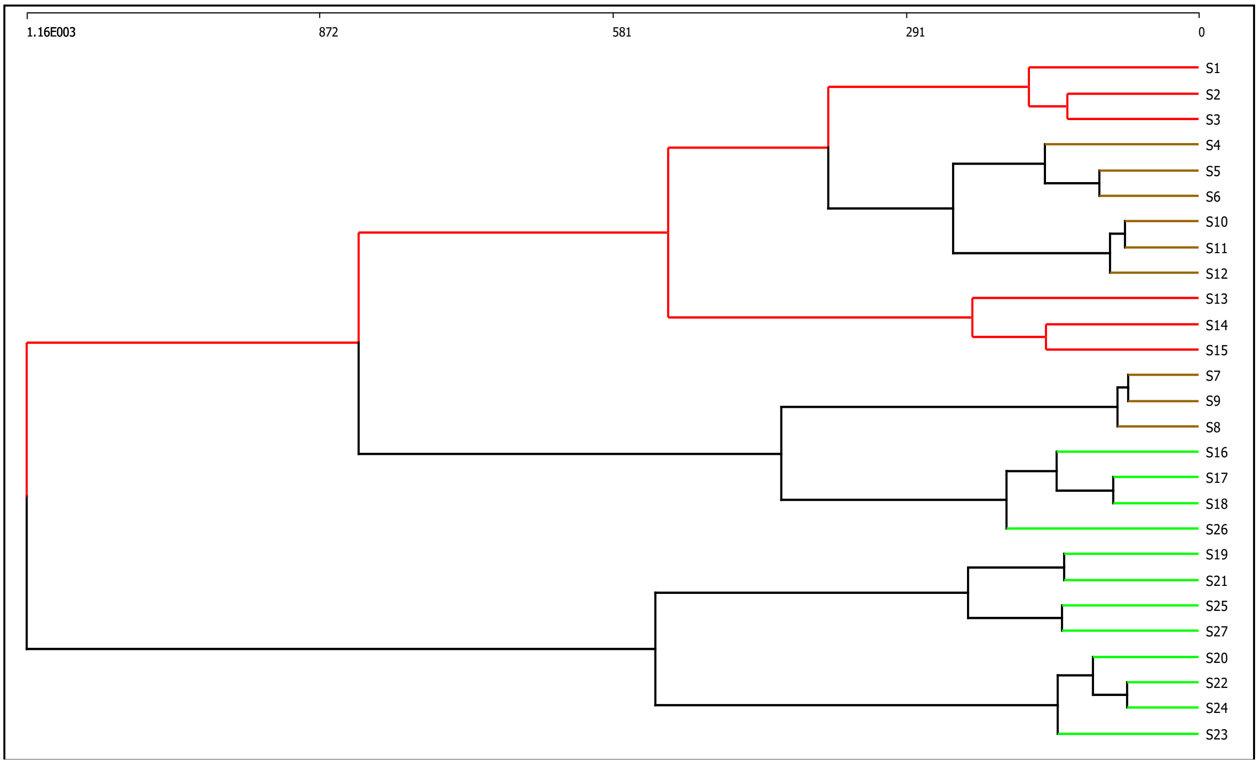

Figure 4: Results of cluster analysis (complete linkage) based on diatom species diversity sampled at 27 sampling stations from the Chambal River during summer season.

Relation between diversity indices and environmental variables

Spearman's rank correlation was calculated between Diatom diversity indices and environmental variables (Tables5 & 6) during both seasons. In the winter season, significant correlations ($p < 0.01$) were observed between most of the environmental variables and diatom indices. Environmental variables such as pH, conductivity, and total dissolved solids were negatively correlated with diversity indices such as Fisher's alpha, Shannon, Simpson and Margalef D. Positive correlations were observed only between the Berger-Parker index and environmental variables such as conductivity, turbidity, total dissolved solids, BOD, COD, and silica. Species richness was negatively correlated with temperature, BOD, COD, and silica content except DO, which was positively correlated. Summer season data showed that a few environmental variables were significantly correlated with diversity indices. Environmental variables such as turbidity and BOD were negatively correlated with Fisher's alpha and Margalef D indices, while positive correlations were observed with Shannon, Simpson, and species evenness.

Table 5: Spearman's rank correlation between various diversity indices and environmental variables during Jan 2023.

	Fisher's □ Index	Shannon Index	Simpson's Index	Margalef D Index	Berger-Parker Index	Evenness	Species Richness
Temp	0.308	0.104	-0.228	-0.318	-0.298	0.366	**-0.744****
pH	**-0.517****	**-0.437***	-0.289	**-0.524****	0.009	-0.318	0.304
EC	**-0.572****	**-0.756****	**-0.716****	**-0.589****	**0.409***	**-0.615****	0.192
Turb	-0.198	-0.358	**-0.389***	-0.254	**0.477***	-0.159	-0.276
DO	-0.117	0.002	0.047	-0.046	-0.121	-0.181	**0.506****
TDS	**-0.459***	**-0.686****	**-0.678****	**-0.479***	**0.405***	**-0.686****	0.257
COD	0.208	-0.033	-0.129	0.143	**0.401***	0.153	**-0.668****
BOD	0.219	-0.035	-0.159	0.138	**0.447***	0.138	**-0.656****
NO_3	-0.155	-0.021	0.053	-0.167	-0.076	0.056	0.183
NO_2	-0.103	-0.005	0.034	-0.147	-0.015	0.034	0.133
PO_4	-0.074	-0.277	-0.333	-0.119	0.344	-0.090	-0.337
SiO_2	0.086	-0.012	-0.106	0.044	**0.386***	0.243	**-0.555****

Table6: Spearman's rank correlation between various diversity indices and environmental variables during May 2023.

	Fisher's Index	Shannon Index	Simpson's Index	Margalef D Index	Berger-Parker Index	Evenness	Species Richness
Temperature	-0.242	0.208	**0.399***	-0.242	**-0.501****	0.208	-0.157
pH	-0.125	0.178	0.226	-0.089	-0.207	0.178	-0.319
EC	-0.180	-0.311	-0.261	-0.130	0.091	-0.311	0.168
Turbidity	**-0.617****	0.093	0.313	**-0.650****	-0.321	0.093	**-0.601****
DO	**0.477***	0.211	0.004	**0.512****	0.106	0.211	0.141
TDS	-0.289	-0.319	-0.203	-0.245	0.050	-0.319	0.045
COD	0.110	0.283	0.256	0.128	**-0.398***	0.283	0.178
BOD	**-0.401***	**0.419***	**0.599****	**-0.381***	**-0.723****	**0.419***	-0.348
Nitrate	-0.130	-0.190	-0.047	-0.165	-0.047	-0.190	0.080
Nitrite	-0.105	-0.257	-0.118	-0.139	0.040	-0.257	0.106
Phosphate	-0.272	-0.122	-0.021	-0.244	-0.038	-0.122	-0.110
Silica	0.328	-0.122	-0.328	0.294	**0.422***	-0.122	0.237
SALT	-0.310	**-0.456***	-0.302	-0.271	0.138	**-0.456***	0.059

Regression analysis was also performed with diatom diversity indices and environmental parameters (Table 7). The Adjusted R^2 value for Fisher's alpha index was 0.480. This shows that approximately 48% of the variation in the diatom species diversity could be attributes to the measured environmental variables. An environmental variable that contributes significantly ($p<0.05$) was nitrate. The Shannon index showed 74% of the variation and nitrate and nitrite were the significant contributors in the regression model. Diversity indices such as Simpson and Berger-Parker showed variation of 83.6% and 72.7% respectively. Species richness showed 90% of the variation with silica as most significant contributor.

Table 7: Regression summary for the diversity indices

	Adjusted R^2	R^2 value
Fisher's alpha diversity	0.480425	0.740212
Shannon-Weiner	0.740382	0.870191
Simpson's index	0.727118	0.863559
Berger-Parker	0.8361379	0.9180689
Evenness	0.805635	0.902818
Species Richness	0.903319	0.951659

SIMPER was calculated using CAP software. During winter season (Table 8 & 9), average similarity within groups for HVPL sites was 42.80%. The results indicate that *Nitzschiaamphibia* is the species that contributes the most to the within group similarities at HVPL sites followed by *Naviculacryptotenella* (19.3%), *Nitzschiaacicularis* (13.2%) and *Achnanthidiumminutissimum* (7.9%) while the species with the least contribution was *Cyclotellameneghiniana*. The average similarities for MDPL and SANT were 51.10% and 57.55% respectively. The greatest contribution to the similarities for MDPL and SANT sites was provided by *Achnanthidiumminutissimum* (40.95%) and *Brachysiravitrea* (41.8%) respectively. Similarly, during summer season the same species contributed most to the within group similarities at HVPL, MDPL and SANT sites with slightly different similarity percentage within groups (HVPL 37.25%, MDPL 44.7% and SANT 49.85%).

Table 8: SIMPER analysis for the comparison within sites during winter season using CAP software

HVPL	Average Similarity	42.8038		
Name	Average Abundance	Average Similarity	% Contribution	Cumulative %
NAMP	133.167	9.12661	21.322	21.322
NCTE	35.8333	8.27859	19.3408	40.6628
NACI	36.6667	5.68657	13.2852	53.948
ADMI	17	3.42041	7.99091	61.9389
CSTE	14.3333	2.88639	6.74331	68.6822
GEXL	41.6667	2.66667	6.22998	74.9122

BVIT	12.3333	2.53422	5.92055	80.8327
AUGR	7.66667	1.66627	3.89282	84.7255
ATWE	10	1.42372	3.32615	88.0517
CMEN	11.8333	1.015	2.37129	90.423
MDPL	Average Similarity	51.1039		
Name	Average Abundance	Average Similarity	% Contribution	Cumulative %
ADMI	279.667	20.93	40.9559	40.9559
STAB	110.333	3.27079	6.40028	47.3562
NAMP	56.1111	3.16655	6.1963	53.5525
CSTE	37	2.668	5.22073	58.7732
GANG	72.4444	1.672	3.27176	62.0449
SRUM	24	1.63918	3.20754	65.2525
NCTE	21.6667	1.38991	2.71978	67.9723
GEXL	19.6667	1.29331	2.53074	70.503
CVUL	27.5556	1.11116	2.17432	72.6773
NACI	23.7778	1.04559	2.04601	74.7233
CMTZ	26.3333	1.04217	2.03931	76.7626
ATWE	20.2222	0.934456	1.82854	78.5912
CMEN	33.4444	0.909521	1.77975	80.3709
AUGR	14.8889	0.906321	1.77349	82.1444
NOBT	15.8889	0.780275	1.52684	83.6713
BVIT	14.8889	0.698537	1.3669	85.0382
SULN	21.3333	0.650402	1.2727	86.3109
FCRT	28.1111	0.624004	1.22105	87.5319
APET	26.4444	0.620282	1.21377	88.7457
NCTV	14.8889	0.597852	1.16987	89.9156
CPED	33.3333	0.556696	1.08934	91.0049
SANT	Average Similarity	57.5532		
Name	Average Abundance	Average Similarity	% Contribution	Cumulative %
BVIT	436.667	24.0599	41.8046	41.8046
ADMI	184.917	7.10386	12.3431	54.1477
SRUM	144	5.72563	9.94842	64.0962
NCTE	164.25	4.68793	8.1454	72.2416
CBEC	73.1667	2.95017	5.126	77.3676
NACU	111.833	2.92896	5.08913	82.4567
NACI	113.75	2.86174	4.97235	87.429
SULN	60.3333	1.09332	1.89967	89.3287
NSTR	43.5833	1.01666	1.76647	91.0952

Table 9: SIMPER analysis for the comparison within sites during summer season using CAP software

HVPL	**Average Similarity**	**37.2596**		
	Average Abundance	Average Similarity	% Contribution	Cumulative %
NAMP	92.1667	8.01009	21.498	21.498
CMEN	75.1667	4.62323	12.4081	33.9062
NPAL	33.3333	3.09464	8.30561	42.2118
STAB	56.6667	2.90781	7.80418	50.0159
AUGR	35	2.74739	7.37362	57.3896
AVEN	18.8333	1.86644	5.00927	62.3988
ADMI	19.8333	1.59479	4.28021	66.6791
SRUM	19.3333	1.54675	4.15127	70.8303

NCTE	19.1667	1.45746	3.91163	74.742
CPED	14.1667	1.26762	3.40214	78.1441
NCTV	21	0.864003	2.31887	80.463
CSTE	14.5	0.840532	2.25588	82.7188
FCRT	15.3333	0.826671	2.21868	84.9375
SULN	10	0.814452	2.18588	87.1234
ESOR	15.5	0.773528	2.07605	89.1994
GEXL	13.5	0.751313	2.01643	91.2159
MDPL	**Average Similarity**	**44.7068**		
	Average Abundance	Average Similarity	% Contribution	Cumulative %
ADMI	131.111	15.301	34.2252	34.2252
BVIT	38.4444	4.20678	9.4097	43.6349
APET	23.7778	1.64137	3.6714	47.3063
NACI	24.4444	1.58192	3.53843	50.8448
SRUM	13	1.5254	3.412	54.2568
AUGR	20.5556	1.46199	3.27017	57.5269
NCTE	15.4444	1.31066	2.93168	60.4586
GANG	14.8889	1.24807	2.79167	63.2503
NAMP	14.5556	1.24558	2.7861	66.0364
SULN	14.4444	1.21799	2.72439	68.7608
FCRT	23.1111	1.07587	2.40649	71.1673
CPED	29	1.06575	2.38387	73.5511
NCTV	16.7778	1.04105	2.32861	75.8797
CPLI	20.2222	0.976182	2.18352	78.0632
CSTE	14	0.971132	2.17222	80.2355
CVUL	9.22222	0.909711	2.03484	82.2703
NACU	21	0.886531	1.98299	84.2533
ADMJ	13.3333	0.709067	1.58604	85.8393
GEXL	10.5556	0.665127	1.48775	87.3271
CMEN	9.88889	0.65701	1.4696	88.7967
STAB	8.44444	0.537918	1.20321	89.9999
NPAL	11.2222	0.439001	0.981954	90.9818
SANT	**Average Similarity**	**49.8563**		
	Average Abundance	Average Similarity	% Contribution	Cumulative %
BVIT	163.583	13.2954	26.6675	26.6675
ADMI	142.917	12.1401	24.3501	51.0176
NCTE	74.25	6.60879	13.2557	64.2733
APET	51.1667	2.95821	5.93348	70.2067
ADMJ	23.5	1.53974	3.08836	73.2951
CBEC	16.9167	1.16448	2.33567	75.6308
NACU	21.1667	1.10389	2.21415	77.8449
NSTR	30.6667	1.05784	2.12178	79.9667
DKUE	15.9167	0.975652	1.95693	81.9236
NCTV	11.0833	0.807096	1.61885	83.5425
NCTT	23.1667	0.799184	1.60298	85.1455
SULN	8.41667	0.587767	1.17892	86.3244
AAEQ	7.66667	0.521272	1.04555	87.3699
NIFR	8.66667	0.51053	1.024	88.3939
ACMG	16.8333	0.506438	1.0158	89.4097
NCPL	11.1667	0.482814	0.968412	90.3781

According to the SIMPER analysis, the average dissimilarity between HVPL and MDPL was 78.43% for the winter season. The greatest contribution to the differences was provided by *Achnanthidiumminutissimum*

(22.8%) and *Nitzschiaamphibia* (10.7%) (Table 10). For the comparison between HVPL and SANT, the average dissimilarity between groups was about 89.18%. The greatest dissimilarity is generated by *Brachysiravitrea* (23.2%) and *Achnanthidiumminutissimum* (9.5%) while the species responsible for least contribution were *Naviculacataracta-rheni* (0.8%), *Cymbopleurarupicola* (0.7%). The average dissimilarity between MDPL with SANT was 77.68% with highest contributed species of *Brachysiravitrea* (19.31%). For the summer season, the mean dissimilarities between groups when comparing HVPL with MDPL (73.03%), HVPL with SANT (83.7%) and MDPL with SANT (64.86%) as shown in Table 11.

Table 10: SIMPER analysis for the comparison between sites during winter season using CAP software

HVPL With MDPL		Average Dissimilarity	78.4395		
	HVPL	MDPL			
Name	Average Abundance	Average Abundance	Average Dissimilarity	% Contribution	Cumulative %
ADMI	17	279.667	17.9072	22.8294	22.8294
NAMP	133.167	56.1111	8.41043	10.7222	33.5516
STAB	2.66667	110.333	6.28694	8.01503	41.5666
GANG	3.83333	72.4444	3.71309	4.7337	46.3003
GEXL	41.6667	19.6667	2.72897	3.47908	49.7794
CMEN	11.8333	33.4444	1.89749	2.41905	52.1984
SRUM	0.833333	24	1.88401	2.40186	54.6003
APET	1.16667	26.4444	1.86653	2.37958	56.9799
CSTE	14.3333	37	1.84963	2.35803	59.3379
NCTE	35.8333	21.6667	1.82898	2.33171	61.6696
NACI	36.6667	23.7778	1.74192	2.22071	63.8903
CPED	0.5	33.3333	1.6938	2.15937	66.0497
CVUL	0	27.5556	1.60003	2.03983	68.0895
FCRT	1.33333	28.1111	1.4974	1.90899	69.9985
CMTZ	0.833333	26.3333	1.48205	1.88942	71.888
ADBI	0.666667	20.8889	1.47031	1.87446	73.7624
GSPH	0	25.8889	1.30561	1.66448	75.4269
ATWE	10	20.2222	1.21255	1.54585	76.9727
SULN	6.16667	21.3333	1.12387	1.43279	78.4055
ADEG	15	11.6667	1.03404	1.31827	79.7238
GANC	1	16.4444	1.00139	1.27664	81.0004
NOBT	0	15.8889	0.993271	1.26629	82.2667
NCTV	4	14.8889	0.942091	1.20104	83.4678
CPLI	1	16.8889	0.87191	1.11157	84.5793
BVIT	12.3333	14.8889	0.817041	1.04162	85.621
ACOF	0	15.2222	0.765086	0.975384	86.5963
NACU	0	7.22222	0.76316	0.972929	87.5693
NSYM	0.666667	6.44444	0.700951	0.89362	88.4629
DOCF	6.5	10.5556	0.635621	0.810334	89.2732
AUGR	7.66667	14.8889	0.616223	0.785603	90.0588
HVPL With SANT		Average Dissimilarity	89.1887		
	HVPL	SANT			
Name	Average Abundance	Average Abundance	Average Dissimilarity	% Contribution	Cumulative %
BVIT	12.3333	436.667	20.7078	23.218	23.218
ADMI	17	184.917	8.47801	9.50571	32.7237
SRUM	0.833333	144	7.05207	7.90691	40.6306

NCTE	35.8333	164.25	6.80274	7.62735	48.2579
NAMP	133.167	0	5.90239	6.61787	54.8758
NACU	0	111.833	5.45687	6.11834	60.9942
NACI	36.6667	113.75	5.13813	5.76097	66.7551
CBEC	0.5	73.1667	3.52693	3.95446	70.7096
SULN	6.16667	60.3333	2.75458	3.08849	73.7981
Cymbopleuramicrocephala	0	45.0833	2.2709	2.54618	76.3442
NSTR	0	43.5833	2.02269	2.26787	78.6121
GEXL	41.6667	0	1.84755	2.07151	80.6836
AMJA	0	35.4167	1.56404	1.75363	82.4373
SDSS	0.5	32.3333	1.4838	1.66366	84.1009
CVER	0.333333	29.1667	1.41537	1.58694	85.6879
NPBP	1	27.75	1.38593	1.55393	87.2418
APET	1.16667	21.25	0.921778	1.03351	88.2753
NCTT	0.5	15.8333	0.730357	0.81889	89.0942
AAEQ	0.5	16.25	0.723476	0.811174	89.9054
Cymbopleurarupicola	0	13.0833	0.693089	0.777104	90.6825
MDPL With SANT		Average Dissimilarity	77.6895		
	MDPL	SANT			
Name	Average Abundance	Average Abundance	Average Dissimilarity	% Contribution	Cumulative %
BVIT	14.8889	436.667	15.0047	19.3137	19.3137
NCTE	21.6667	164.25	5.24276	6.74835	26.0621
ADMI	279.667	184.917	5.01886	6.46015	32.5222
SRUM	24	144	4.27827	5.50688	38.0291
NACU	7.22222	111.833	3.89423	5.01256	43.0417
NACI	23.7778	113.75	3.83739	4.93939	47.981
STAB	110.333	1.83333	3.50165	4.50723	52.4883
CBEC	0.222222	73.1667	2.58928	3.33286	55.8211
GANG	72.4444	1.25	2.21832	2.85536	58.6765
SULN	21.3333	60.3333	2.10542	2.71005	61.3866
NAMP	56.1111	0	1.90121	2.44718	63.8337
Cymbopleuramicrocephala	0.666667	45.0833	1.63959	2.11044	65.9442
NSTR	0	43.5833	1.49712	1.92706	67.8712
APET	26.4444	21.25	1.4371	1.8498	69.721
AMJA	6.11111	35.4167	1.27834	1.64545	71.3665
CSTE	37	9.58333	1.0726	1.38062	72.7471
CVER	0	29.1667	1.03219	1.32861	74.0757
SDSS	4.88889	32.3333	1.01484	1.30628	75.382
CPED	33.3333	0.75	1.00756	1.29691	76.6789
CMEN	33.4444	7.33333	0.999812	1.28693	77.9658
NPBP	0	27.75	0.99817	1.28482	79.2506
FCRT	28.1111	6.5	0.860663	1.10782	80.3585
CVUL	27.5556	2.83333	0.852273	1.09702	81.4555
GSPH	25.8889	0.583333	0.789463	1.01618	82.4717
CMTZ	26.3333	5.16667	0.778762	1.0024	83.4741
ADBI	20.8889	0.333333	0.757433	0.974949	84.449
ATWE	20.2222	0.75	0.741	0.953797	85.4028
GEXL	19.6667	0	0.707087	0.910145	86.313
NCTT	5.66667	15.8333	0.672824	0.866042	87.179
NCTV	14.8889	10.75	0.597143	0.768628	87.9476

GANC	16.4444	1.25	0.543193	0.699184	88.6468
AAEQ	0.333333	16.25	0.541669	0.697223	89.344
NOBT	15.8889	0.833333	0.51179	0.658763	90.0028

Table 11: SIMPER analysis for the comparison between sites during summer season using CAP software

HVPL With MDPL	Average Dissimilarity	73.0354			
	HVPL	MDPL			
	Average Abundance	Average Abundance	Average Dissimilarity	% Contribution	Cumulative %
ADMI	19.8333	131.111	8.49913	11.637	11.637
NAMP	92.1667	14.5556	5.60029	7.66791	19.3049
CMEN	75.1667	9.88889	5.19545	7.11361	26.4185
STAB	56.6667	8.44444	4.59577	6.29252	32.711
BVIT	5.16667	38.4444	2.85785	3.91296	36.624
NACU	25.8333	21	2.50497	3.4298	40.0538
NPAL	33.3333	11.2222	2.47784	3.39266	43.4465
CPED	14.1667	29	2.26045	3.095	46.5415
AUGR	35	20.5556	2.25394	3.08609	49.6276
NACI	13.3333	24.4444	2.0225	2.76921	52.3968
APET	8	23.7778	1.83336	2.51024	54.907
FCRT	15.3333	23.1111	1.77169	2.42579	57.3328
NCTV	21	16.7778	1.66551	2.28042	59.6132
NIFR	16.6667	7.77778	1.52902	2.09354	61.7067
AVEN	18.8333	0	1.48484	2.03304	63.7398
CPLI	8	20.2222	1.47817	2.02391	65.7637
ATWE	17.5	5.22222	1.45799	1.99628	67.76
CSTE	14.5	14	1.30147	1.78196	69.5419
ADMJ	7.83333	13.3333	1.2399	1.69767	71.2396
SRUM	19.3333	13	1.20616	1.65148	72.8911
ESOR	15.5	0.222222	1.15185	1.57712	74.4682
NCTE	19.1667	15.4444	1.1478	1.57156	76.0398
GANG	3.83333	14.8889	1.08121	1.4804	77.5202
GEXL	13.5	10.5556	1.00664	1.37829	78.8985
CVUL	5.83333	9.22222	0.924632	1.26601	80.1645
ADEG	10.8333	5.11111	0.900163	1.2325	81.397
NCTT	8.33333	5.22222	0.888982	1.21719	82.6142
SULN	10	14.4444	0.823131	1.12703	83.7412
NPBP	4.33333	6.66667	0.747186	1.02305	84.7642
NROS	4.5	7	0.660702	0.904632	85.6689
NSTR	5.83333	1.66667	0.595829	0.815809	86.4847
NSYM	4.16667	5.77778	0.524815	0.718576	87.2033
AAEQ	5.5	0.555556	0.491204	0.672556	87.8758
SDSS	1.16667	6.22222	0.449822	0.615896	88.4917
CMTZ	1.16667	5.66667	0.415614	0.569058	89.0608
ACOF	0	5.33333	0.391268	0.535724	89.5965
SUSP	1.66667	4.44444	0.390883	0.535196	90.1317
HVPL With SANT	Average Dissimilarity	83.7035			
	HVPL	SANT			
	Average Abundance	Average Abundance	Average Dissimilarity	% Contribution	Cumulative %

BVIT	5.16667	163.583	11.1712	13.3461	13.3461
ADMI	19.8333	142.917	8.84741	10.5699	23.916
NAMP	92.1667	0.583333	6.11527	7.30587	31.2219
CMEN	75.1667	1.25	4.98113	5.95092	37.1728
NCTE	19.1667	74.25	4.16469	4.97552	42.1483
STAB	56.6667	2.16667	4.14639	4.95366	47.102
APET	8	51.1667	3.47163	4.14753	51.2495
NPAL	33.3333	5.41667	2.38312	2.8471	54.0966
AUGR	35	4.41667	2.30843	2.75787	56.8545
NACU	25.8333	21.1667	2.21953	2.65166	59.5062
NSTR	5.83333	30.6667	2.18701	2.61281	62.119
NCTT	8.33333	23.1667	1.80335	2.15444	64.2734
ADMJ	7.83333	23.5	1.53308	1.83155	66.105
NIFR	16.6667	8.66667	1.41239	1.68737	67.7923
AVEN	18.8333	0.166667	1.3295	1.58834	69.3807
NCTV	21	11.0833	1.29996	1.55306	70.9337
ACMG	0	16.8333	1.22377	1.46202	72.3958
SRUM	19.3333	7	1.21377	1.45008	73.8458
ATWE	17.5	0.916667	1.21143	1.44728	75.2931
CBEC	1.66667	16.9167	1.12953	1.34944	76.6426
CSTE	14.5	1.58333	1.10032	1.31454	77.9571
NACI	13.3333	4.91667	1.08761	1.29936	79.2565
DKUE	2.66667	15.9167	1.05426	1.25952	80.516
ESOR	15.5	0.166667	1.0434	1.24654	81.7625
FCRT	15.3333	1.91667	0.995812	1.18969	82.9522
CPED	14.1667	2	0.87929	1.05048	84.0027
GEXL	13.5	1.41667	0.845515	1.01013	85.0128
NCPL	0	11.1667	0.796492	0.951563	85.9644
AAEQ	5.5	7.66667	0.68583	0.819356	86.7838
SDSS	1.16667	10.1667	0.658687	0.786929	87.5707
NDEN	0	8.91667	0.64288	0.768044	88.3387
CVUL	5.83333	5.16667	0.640761	0.765512	89.1042
ADEG	10.8333	0	0.638776	0.763141	89.8674
NPBP	4.33333	7.5	0.615356	0.735161	90.6025
MDPL With SANT	Average Dissimilarity	64.8689			
	MDPL	SANT			
	Average Abundance	Average Abundance	Average Dissimilarity	% Contribution	Cumulative %
BVIT	38.4444	163.583	9.25949	14.2742	14.2742
ADMI	131.111	142.917	6.23755	9.61563	23.8898
NCTE	15.4444	74.25	4.40711	6.79387	30.6837
APET	23.7778	51.1667	3.41877	5.27028	35.9539
NSTR	1.66667	30.6667	2.2124	3.41057	39.3645
NACU	21	21.1667	1.95767	3.01789	42.3824
CPED	29	2	1.8667	2.87765	45.2601
NACI	24.4444	4.91667	1.78598	2.75321	48.0133
NCTT	5.22222	23.1667	1.65393	2.54965	50.5629
ADMJ	13.3333	23.5	1.54538	2.38232	52.9452
FCRT	23.1111	1.91667	1.49946	2.31152	55.2568
AUGR	20.5556	4.41667	1.36107	2.09819	57.3549
CPLI	20.2222	1.41667	1.30939	2.01851	59.3735

ACMG	2.55556	16.8333	1.29533	1.99684	61.3703
NCTV	16.7778	11.0833	1.14243	1.76114	63.1314
DKUE	0.444444	15.9167	1.12701	1.73736	64.8688
CBEC	3.44444	16.9167	1.08092	1.66631	66.5351
NAMP	14.5556	0.583333	1.06774	1.646	68.1811
CSTE	14	1.58333	0.981271	1.5127	69.6938
GANG	14.8889	4.41667	0.979186	1.50948	71.2033
NIFR	7.77778	8.66667	0.863456	1.33108	72.5344
NPAL	11.2222	5.41667	0.860519	1.32655	73.8609
NPBP	6.66667	7.5	0.824442	1.27094	75.1319
NCPL	1	11.1667	0.806115	1.24268	76.3745
SULN	14.4444	8.41667	0.782361	1.20607	77.5806
GEXL	10.5556	1.41667	0.770521	1.18781	78.7684
SDSS	6.22222	10.1667	0.767047	1.18246	79.9509
CMEN	9.88889	1.25	0.696076	1.07305	81.0239
NDEN	2	8.91667	0.654103	1.00835	82.0323
SRUM	13	7	0.643596	0.99215	83.0244
STAB	8.44444	2.16667	0.604873	0.932455	83.9569
AMSC	3.33333	8.33333	0.598899	0.923247	84.8801
NROS	7	3.16667	0.553633	0.853465	85.7336
CVUL	9.22222	5.16667	0.55225	0.851333	86.5849
AAEQ	0.555556	7.66667	0.534305	0.823669	87.4086
NSYM	5.77778	1.91667	0.435738	0.671722	88.0803
ATWE	5.22222	0.916667	0.399987	0.616609	88.6969
CMTZ	5.66667	2.41667	0.396345	0.610994	89.3079
ADEG	5.11111	0	0.37918	0.584533	89.8924
ACOF	5.33333	0	0.363452	0.560288	90.4527

Discussion

Present study revealed the relationship between diatom diversity indices and environmental variables. Most of the diversity indices were negatively significant correlated with many environmental variables such as conductivity, total dissolved solids, pH, BOD, COD, and turbidity during winter season except Berger-Parker index which was positively significant correlated with these environmental variables. In the present study, results showed that values of diversity indices were found to be low at heavily polluted sites and high at moderately polluted sites and sanctuary sites except few indices such as Shannon, Simpson and evenness showed lowest values at S24 site during summer season. The reason for lower values could be unavailability of good diatom samples, which indicates eutrophic environment (Kelly *et al.,* 1998). An interpretation might be that the commonly known environmental variables, such as conductivity, pH, flow velocity, and temperature, may select the dominating diatom species on the different substrates, but these variables may not be so important for rare species (Lennon *et al.,* 2011).

Water conductivity has been detected as environmental determinant of diatom richness and community composition by various other authors in different habitats (Vyverman *et al.,* 2007, Potapova *et al.,* 2005), in Alpine springs (Cantonati et al 2012), and in carbonate, low-altitude springs (Angeli *et al.,* 2010, Wotjal & Sobczyk, 2012). Many researchers also found that diatom species has been reported to be associated with waters of relatively high values of abiotic factors especially conductivity and is known to organic pollution and heavy metal pollution (Round 1991; Leland, 1995; Biggs and Kilroy, 2000; Potapova and Charles, 2003; Duong *et al.,* 2006) and increased level may be accompanied by high up dissolved nutrients in streams (Leland 1995; Walker and Pan, 2006). Studies also showed that conductivity has been found to best explain diatom distribution (Reed 1998; Shinneman et al. 2009; Pestryakova et al. 2012; Reed et al. 2012) and found the significant role in determining the composition and diversity of microbial communities in aquatic ecosystem (Hemraj et al. 2017; Stenger-Kovács et al 2013; Toman et al 2014). Pestryakov et al 2018 mentioned that a good conductivity indicator taxon in one region may not be so reliable elsewhere.

pH is also one of the most important factors that serve as an index of the pollution. The Chambal River was slightly alkaline to alkaline (range from 7.42 – 8.96). The higher value of pH during the summer season may be due to increased photosynthetic activity as they demand more CO2 than quantities furnished by respiration and decomposition (Singh *et al.,* 2011). According to the present study, pH showed a significant negative correlation with diversity indices. The relationship between diatoms and pH is strong because pH exerts a direct physiological stress on diatoms (Gensemer, 1991) and strongly influences other water chemistry variables (Stumm & Morgen, 1981). Bere & Tundisi, 2010 showed that diatom assemblages have been distributed continuously along a gradient of pH (TerBraak & Van dam 1989; Weilhoefer and Pan, 2008).

A diatom's need for silica depends on its habitat and the physiological condition of its cell (Round et al. 1990). Studies showed that diatoms absorb considerable quantities of silica (Lund 1950; Wang & Evan 1969). In the present study, silica content showed a positively significant correlation with the Berger-Parker index during both seasons.

Table: Diatom Codes and species names

Code	**Species name**
AAEQ	*Amphora aequalis*Krammer
ACMG	*Achnanthidiumminutissimum* (Kütz.) Czarnecki var. *gracillima* (Meist.)Bukhtiyarova
ACOF	*Amphora coffeaeformis*Kützing
ADBI	*Achnanthidiumbiasolettiana*Grunow
ADEG	*Achnanthidiumexiguum*Grunow
ADMI	*Achnanthidiumminutissimum*Kützing
ADMJ	*Achnanthidium min. v. jackii*Rabenhorst
AMJA	*Achnanthidium min. v. jackii*Rabenhorst
AMSC	*Achnanthidium min. v .scotica*
APET	*Achnanthidiumpetersenii*Hustedt
ATWE	*Amphora twentiana*Krammer
AUGR	*Aulacoseiragranulata* Ehrenberg
AVEN	*Amphora veneta*Kützing
BVIT	*Brachysiravitrea*Grunow
CBEC	*Caloneisbeccariana*Grunow
CMEN	*Cyclotellameneghiniana*Kützing
CMTZ	*Cymbellametzeltinii*Krammer
CPED	*Cocconeispediculus*Ehrenberg
CPLI	*Cocconeisplacentula v. Lineata* Ehrenberg
CSTE	*Cyclotellastelligera*Grunow
CVUL	*Cymbellavulgata*Krammer
DKUE	*Denticulakuetzingii*Grunow
DCOF	*Diadesmisconfervacea*Kützing
ESOR	*Epithemiasorex*Kützing
FCRT	*Fragilariacrotonensis*Kitton
GANC	*Gomphocymbelopsisancyli*(Grunow) Hustedt
GANG	*Gomphonemaangustum*Rabenhorst
GEXL	*Gomphonemaexilissimum* Lange- Bertalot
GSPH	*Gomphonemasphaerophorum* Ehrenberg
NACI	*Nitzschiaacicularis*Kützing
NACU	*Nitzschiaacuta*Hantzsch
NAMP	*Nitzschiaamphibia*Grunow
NCPL	*Nitzschiacapitellata*Hustedt
NCTE	*Naviculacryptotenella* Lange- Bertalot
NCTT	Naviculacataracta-rheni Lange- Bertalot
NCTV	*Naviculacaterva*Hohn&Hellerman
NDEN	*Nitzschiadenticula*Grunow in Cleve &Grunow
NIFR	*Nitzschiafrustulum*(Kützing)Grunow

NOBT	*Nitzschiaobtusa* W. Smith
NPAL	*Nitzschiapalea* (Kützing) W. Smith
NPBP	*Naviculaparabryophila* Lange- Bertalot
NROS	*Navicularostellata* (Kützing) Cleve
NSTR	*Naviculastroemii*Hustedt
NSYM	*Naviculasymmetrica* Patrick
SDSS	*Sellaphoradensistriata* Lange- Bertalot&Metzel
SRUM	*Synedrarumpens*Kützing
STAB	*Synedratabulata*Kützing
SULN	*Synedra ulna* Ehrenberg
SUSP	*Synedra ulna* Ehrenberg

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