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# Epilithic diatoms as biological water quality indicators–A study in three geographically isolated hill streams in India

**Shyam Sundar Paul, Bipul Mallik, Manjushree Mandal, Biswajit Biswas, Sanoyaz Sekh and Neera Sen Sarkar***

Phycology Section, Department of Botany, University of Kalyani, Kalyani-741 235, India

*Corresponding Author E-mail: neerashen@yahoo.co.in

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**Abstract**

Epilithic diatoms from three geographically isolated hill streams of Central and Eastern India were studied and analysed to find their efficacy in determining difference in ecological conditions of aquatic systems. Three sampling sites (site-1, site-2 and site-3) shared commonness of being hill streams of forests with difference in source points. 34 diatom species were identified with species-richness of 17 at site 1, 10 at site 2 and 19 at site 3. Two sets of hypotheses – null (H01, H02 and H03) and alternative (HA1, HA2 and HA3) were framed. Null hypotheses were rejected in favour of alternative hypotheses. Diversity t-tests yielded significant 't' values: at $\alpha$<0.0001 and <0.002, implying differences within the sampling sites. Furthermore, Mann-Whitney, Kruskal-Wallis and Wilcoxon paired-sample tests were performed to test the alternative hypotheses, of which Kruskal-Wallis yielded significant difference between the sample medians with $x^2$=5.457 at p=0.03801, and Wilcoxon paired-sample test yielded significant differences between sampling site pairs at $\alpha$(2)=0.05 and 0.10. Thus significant differences could be established based only on diversity profile study of the epilithic diatoms.

**Key words**

Bioassessment, Diversity profiles, Epilithic diatoms, Hill streams

## Introduction

Diatoms are a fascinating group of algae found in almost every wet habitat. Epilithic diatoms are one of the many habitat diversity types that diatom habitats can be classified into and refer to those forms which are found attached to rock/stone/pebble surfaces. Such habitats are found in different regions like mountains regions, flowing waters, as belts of vegetation on rocks along tidal coasts. Nevertheless, only tidal coast habitat has received extensive attention from researchers as compared to other habitats. In fact, assessment of global distribution of diatom communities in aquatic ecosystems is on average poor because there are major gaps in the accumulated knowledge on diatom diversity. Such a major gap in knowledge exists majorly around mountain chains and hill streams (Cantonati *et al.,* 2001; Jüttner and Cox 2001; Ali *et al.,* 2003 and Nautiyal *et al.,* 2004).

Diatoms are known to be consistent and reliable indicators of the environment. There are a several large number of ecologically sensitive species, which are abundant in nearly all the habitats where water is at least occasionally present. Diatoms respond to environmental stress in a manner very similar to any other group of organisms used in bio monitoring. The most common response is in terms of changes in diversity profiles under different environmental conditions. These different environmental conditions may act as stress trigger and in certain cases may be obvious and identifiable because of the response of the species upon which such stressors act or because of an existing known correlation between the stress and its impact on the species under consideration as bio-monitors. In other situations, stress may be unknown or even unanticipated, but the bio monitor species will respond to such stresses in a way that gets recorded as a change in community composition. Thus, diatoms have long been used as an important criterion for

determining ecological integrity (Triest *et al.,* 2001; Stoermer and Smol 2004; Hering *et al.,* 2006 and Torrisi *et al.,* 2010).

Enumeration of epilithic forms in different hill/ mountain streams has been part of some interesting studies with varied objectives. Most of such studies were formulated with the objective of finding implications in assessment of ecological conditions of the respective hill streams and rivers. Today diatoms are increasingly being used to assess ecological conditions in streams and rivers around the world (Kelly and Yallop 2012; Lobo *et al.,* 2004; Wang *et al.,* 2005; Chessman *et al.,* 2007; Taylor *et al.,* 2007; Porter *et al.,* 2008). The study of Cantonati (2001) and Cantonati *et al.,* (2007) is a direct implication of using diatoms in assessment of stream ecosystems. The Himalayan streams of northern India and Nepal including Kumaon Himalayas, Nepalese middle hills, Alakananda-Ganga system in Garhwal, cold water rivers/ streams of Mandakini basin have received considerable attention in terms of studies on stream epilithic diatoms (Cantonati *et al.,* 2001; Jüttner and Cox 2001; Nautiyal *et al.,* 2004). Nautiyal and Verma (2009) described epilithic diatom flora of two biogeographic regions of India with diatom collections from rivers/ streams of the Himalayas and the Vindhya ranges. The results indicated low diversity in the Himalayan range as compared to the Vindhya range. High diversity values in the Vindhya range were attributed to extensive agriculture practices that prevail in the region. The study highlighted that primary factors (harshness, climatic variability) were more related to species diversity in the Himalayas, while secondary factors (spatial heterogeneity) played a pivotal role in the Vindhyas. Nautiyal and Verma (2009) also reported that the diatom flora in the rivers of Central Highlands of India or parts located between the Ganga and the Narmada are yet to receive attention as compared to the studies carried out in the Himalayas. The present study was undertaken with an objective to investigate, enumerate and statistically analyse the epilithic diatom assemblages of the selected sites and their role in understanding differences between the habitats without dwelling on physico-chemical variables.

## Materials and Methods

**Sampling sites : T**hree eastern Indian hill streams, selected for the study, were chosen from the mountain ranges of Eastern Himalayas and Central Highlands of India (Fig. 1). The sampling site at site-1 was the Upper Chel stream in Darjeeling district. The sampling site at site-2 was the Doodhdhara waterfall at Anuppur district (Amarkantak in Madhya Pradesh). The sampling site at site-3 was the Pradhanpat waterfall in Deoghar district (Sambalpur in Odisha).

Diatom samples on submerged stones and pebbles were collected from sampling sites during July 2012 to

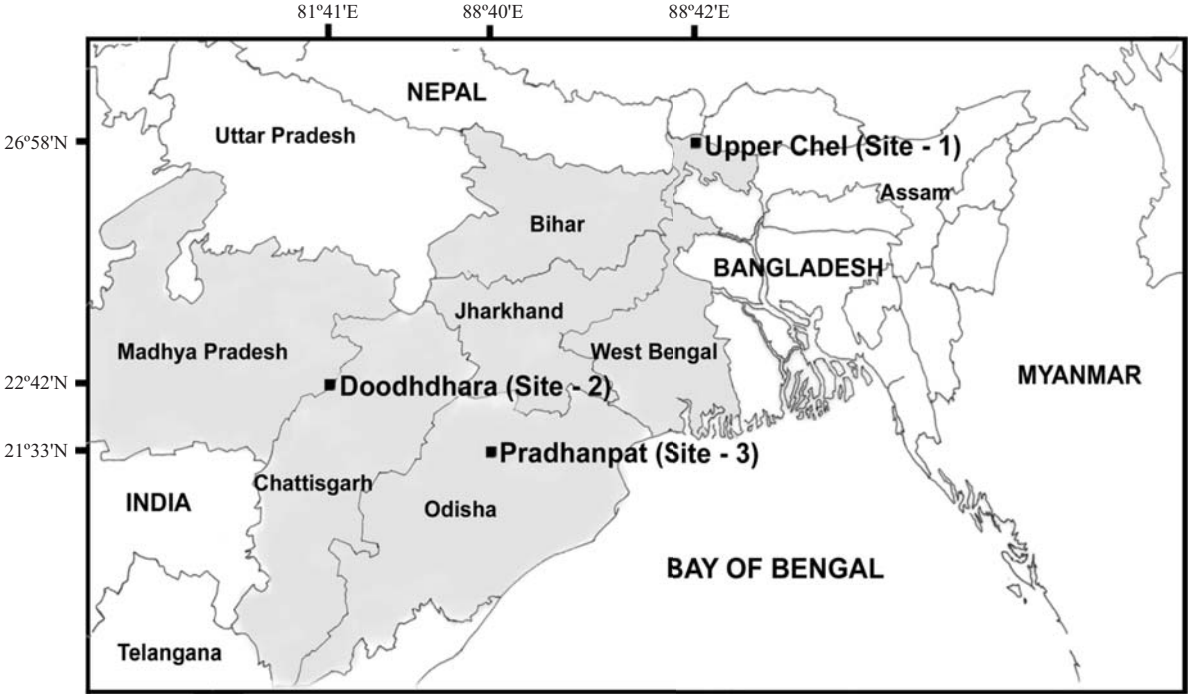


**Fig. 1 :** Sampling sites 1 - 3 in the states of West Bengal, Madhya Pradesh and Odisha

January 2013. From each site, 5 submerged stones/pebbles of approximately 8-10 cm diameter were selected randomly across the streams. Diatoms were scraped from these stones as subsamples by a tooth brush and fixed in Lugol's iodine. Subsequently, the subsamples were pooled into a composite sample for each site. These samples were centrifuged, followed by removal of supernatant. The pellets thus obtained were re-suspended in equal volumes of distilled water (15 ml) and were quantitatively analysed using drop count method. Measured sample drop of 60µl was found to suit the requirement of the study in each of the sample, in terms of general counting ease of the three sampling sites using a methodology similar to determining minimum quadrat size. Diatom sample (60µl) was taken on coverslips and dried over a hot plate and then carefully inverted on microscope slides having a drop of Naphrax (Brunel Microscopes Ltd. U.K.). The slides thus prepared were observed under Olympus Ch20*i* and Labomed LX-300 microscopes using oil immersion, whenever required. Minimum of 500 diatom frustules were studied for each site, for this 3 replicates of slides for each sampling site was studied. Standard manuals and journal publications were referred for the purpose of identification of the epilithic diatoms (Cox, 1996; Khalkho and Das, 2005; Jena *et al.,* 2006; Bellinger and Sigee, 2010; Round, 2009).

**Data analysis :** The obtained data was analysed to find if any comparable difference existed in the epilithic diatom diversity profiles of different sampling sites, which could reflect upon differences in the habitat characteristics of the sampling sites. Presence/absence data, species richness and frustule density were tabulated. Differences in morphological type profiles of each assemblage, similarity coefficients and diversity profiles were computed for the purpose using statistical softwares Minitab 16 and PAST. Two sets of hypotheses – null ($H_{01}$, $H_{02}$ and $H_{03}$) and alternative ($H_{A1}$, $H_{A2}$ and $H_{A3}$) were framed. The first set of null hypotheses $H_{01}$, $H_{02}$ and $H_{03}$ assume no significant difference between the diversity profiles of diatom assemblages between site-1 and site-2, site-2 and site-3 and site-1 and site-3 respectively. The second set of alternative hypotheses $H_{A1}$, $H_{A2}$ and $H_{A3}$ assume significant difference between the epilithic diatom diversity profiles of site-1 and site-2, site-2 and site-3 and site-1 and site-3, respectively. Diversity t tests for Shannon-Wiener Diversity Index and Simpson's Dominance Index were initially performed to statistically test the null hypotheses, followed by three non parametric tests – Mann-Whitney test (analogous to two-sample *t* test), Kruskal-Wallis test (as test of medians) and Wilcoxon paired-sample test (analogous to paired-sample *t* test) were performed to statistically test the alternative hypotheses.

## Results and Discussion

Thirty four diatom species were identified during the present study. A species richness of 17 was recorded at site-1 (Upper Chel), 10 at site-2 (Doodhdhara), and 19 at site-3 (Pradhanpat). Frustule count data (Table 1) showed average highest frustule density of 4488.8 cells $ml^{-1}$ at site-3; 3277.7 $cellsml^{-1}$ at site-2 and 4166.6 cells $ml^{-1}$ at site-1 At site-1 highest frustule density was recorded for *Fragilaria crotonensis; Surirella linearis* along with *Surirella minuta* dominated, in terms of frustule density, at site-2 and at site-3 maximum frustule density was recorded for *Gomphonema parvulum* (Fig. 2). Among these dominant forms, only *Fragilaria crotonensis* was also found at site-3, but *Surirella linearis, Surirella minuta* and *Gomphonema parvulum* were completely absent at other two sites.

The identified diatom species were classified according to their morphological types to find differences in terms of morphological group dominance (Table 2) and the same is represented in Fig. 3 as Box Plot Diagram with standard deviations for each type. Data was further analysed for diversity profiles of the sampling sites by employing different indices for calculating dominance, evenness and similarities. Abundance based (Simpson and Berger-Parker) and dominance based (Shannon-Wiener) diversity indices were generated along with evenness measure. The results highlighting the characteristics of individual sampling sites with regard to dominance, evenness and diversity is tabulated in Table 3.

A dendrogram (Fig. 4) was generated to find the ranking distance (Table 4) between the sets of data based on the computed values of different indices that define diversity profiles of the sampling sites. The ranking distance generated, yielded highest rank for the 1$^{st}$ replicate of sampling site-3. The ranks were generated with replicates of same sites together *i.e.,* with minimum ranking distance between themselves. So replicates of site-3 were followed by 3 replicates of site-1 with the replicates of site-2 placed with the lowest ranks of 1-3. The dendrogram (Fig. 4) emphasised the same, wherein it was seen that not only the 3 replicates of each sampling site clustered together but also site-3 clustered close to site-1 as compared to site-2 in terms of inter-dataset distance between the replicates of each sampling site. A greater affinity between site-1 and site-3 was thus inferred as initial finding.

Diversity t tests were performed between site-1 *vs.* site-2 site-1 *vs.* site-3 and site-2 *vs.* site-3 (Table 5). The results showed significant high difference between diversity profile values of site-1 and site-2 as between site-2 and site-3. Thus, two-tailed hypotheses $H_{01}$ and $H_{02}$ were rejected. The 't' values computed with respect to difference in diversity profiles of site-1 and site-3 implied lower level of significant difference in terms of Shannon-Wiener diversity parameter and no significant difference in terms of Simpson's dominance parameter. Thus, one-tailed hypothesis $H_{03}$ was

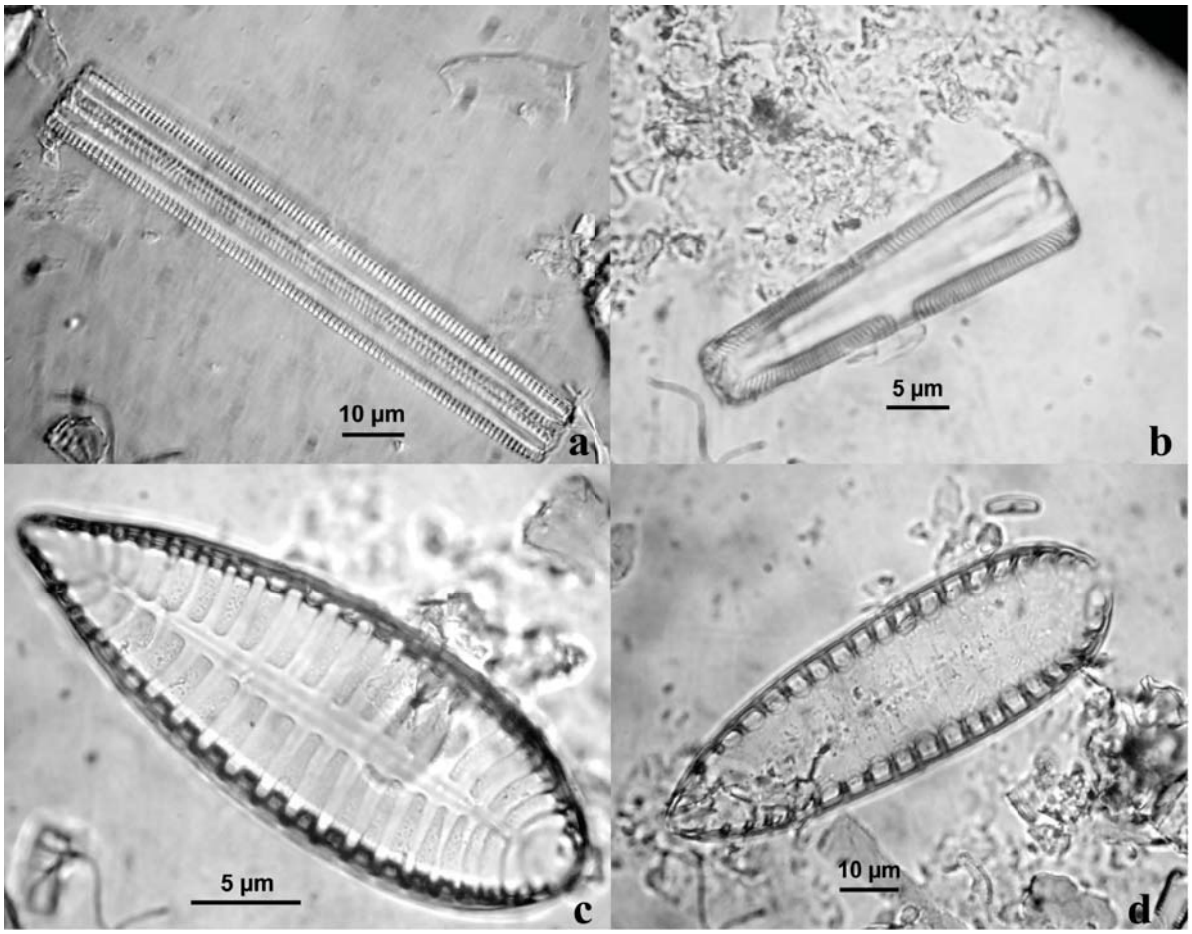


**Fig. 2 :** Diatom species dominant at the three sampling sites (a) Fragilaria crotonensis Kitton; (b) *Gomphonema parvulum* (Kutzing) Kutzing; (c) *Surirella minuta* Brebisson; (d) *Surirella linearis* W. Smith

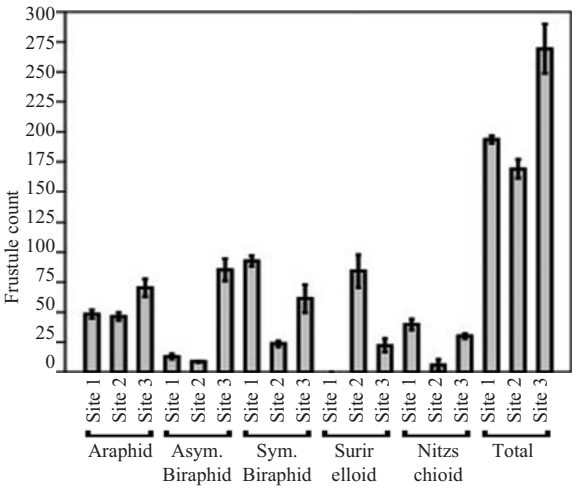


**Fig. 3 :** Box plot of frustule counts of different morphological types of diatoms at the three sampling sites

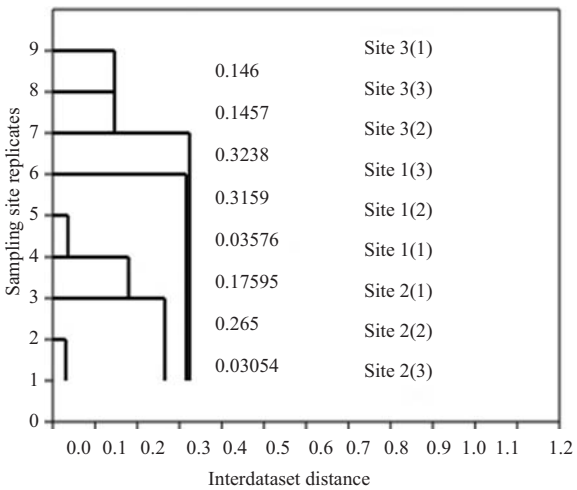


**Fig. 4 :** Dendrogram of ranking distance of diversity profiles at the three sampling sites

**Table 1 :** Frustule count data* of diatoms from three sampling sites

| Name of diatom | Site 1 | Site 2 | Site 3 |
|---|---|---|---|
| *Achnanthidium minutissimum* | 4.0±1.0 | — | 3.3±2.1 |
| *Amphora* sp. | — | 8.7±0.6 | — |
| *Caloneis silicula* | 4.0±1.0 | — | — |
| *Cymbella stuxbergii* | 4.7±1.0 | — | 10.3±3.2 |
| *Diatoma vulgare* var. *lineare* | — | 1.7±2.1 | 20.0±2.6 |
| *Diatoma* sp. | — | 29.7±4.0 | — |
| *Fragilaria capucina* | 4.3±0.6 | — | — |
| *Fragilaria crotonensis* | **29.2±0.5** | — | 3.6±1.5 |
| *Fragilaria virescens* | 4.2±0.0 | — | 15.0±2.6 |
| *Fragilaria* sp. | — | 15.0±2.6 | — |
| *Gomphonema angustatum* | 9.0±1.0 | — | 4.0±2.0 |
| *Gomphonema longiceps* var. *subclavata* | — | — | 12.0±1.0 |
| *Gomphonema parvulum* | — | — | **59.0±5.0** |
| *Hantzschia amphioxys* | — | 6.5±4.5 | 7.0±4.6 |
| *Navicula menisculus* | 4.0±0.0 | — | — |
| *Navicula placentula* | 22.5±1.0 | — | 19.0±7.5 |
| *Navicula spicula* | 12.7±2.9 | — | — |
| *Navicula viridis* | 23.0±1.7 | — | — |
| *Navicula* sp. | — | 1.6±2.1 | — |
| *Neidium iridis* | — | 3.5±1.7 | 14.3±3.7 |
| *Neidium* sp. | — | 20.0±3.0 | 15.6±4.2 |
| *Nitzschia acicularis* | 14.3±1.7 | — | 20.0±2.6 |
| *Nitzschia fruticosa* | 3.3±1.2 | — | — |
| *Nitzschia* sp. | 22.3±5.5 | — | — |
| *Pinnularia gibba* var. *mesogongyla* | — | — | 4.0±1.0 |
| *Pinnularia parvula* | 20.7±0.6 | — | 6.3±1.2 |
| *Pinnularia* sp. | 6.3±1.2 | — | — |
| *Surirella capronii* | — | — | 8.0±1.7 |
| *Surirella minuta* | — | **42.0±4.0** | — |
| *Surirella robusta* var. *splendid* | — | — | 14.3±4.1 |
| *Surirella linearis* | — | **42.3±11.9** | — |
| *Synedra acus* | — | — | 9.0±2.0 |
| *Synedra tabulata* | — | — | 19.3±6.5 |
| *Synedra vaucheriae* | 6.3±2.9 | — | — |
| **Species richness** | **17** | **10** | **19** |
| **Frustule density (cells ml$^{-1}$)** | **4166.6** | **3227.7** | **4488.8** |

**Values are mean±SD of frustule counts in 60µl of sample drops counted for a minimum of 500 frustules at each site; Site 1: Chel, Darjeeling; Site 2: Doodhdhara, Amarkantak; Site 3: Pradhanpat, Deoghar*

**Table 2 :** Morphological type counts of diatom frustules at the different sampling sites

| Morphological type | Site – 1* | | Site – 2* | | Site – 3* | |
|---|---|---|---|---|---|---|
| | Types | Count | Types | Count | Types | Count |
| Araphid | 5 | 145 | 3 | 139 | 6 | 211 |
| Asymmetrical biraphid | 2 | 39 | 1 | 26 | 3 | 256 |
| Symmetrical biraphid | 7 | 278 | 3 | 71 | 6 | 184 |
| Nitzschioid | 3 | 119 | 1 | 18 | 2 | 90 |
| Surirelloid | 0 | 0 | 2 | 253 | 2 | 67 |
| **Total** | **17** | **581** | **10** | **507** | **19** | **808** |

***** Total diatom frustule types and counts of 3 replicates of 60µl from each sampling site

rejected with respect to diversity index but accepted in terms of dominance index. For further analysis of epilithic diatoms from the sampling sites, alternative hypotheses were tested using non-parametric tests, since normal distribution of samples could not be established using normality tests.

Based on the results obtained in terms of total frustule count at three sampling sites, Mann-Whitney test was performed on all the combination pairs of three sampling sites (Table 6). Mann-Whitney test initially yielded a significant difference between the epilithic diatom assemblages of site-2 and site-3 in terms of raw p values (uncorrected significance), but further sequential analysis, Bonferroni significance on raw p values, followed by Bonferroni corrected p values and finally a check on critical Mann-Whitney U values did not yield any significant difference in terms of epilithic diatom assemblage within the sampling pairs. But, Kruskal-Wallis test performed for sample medians yielded significant difference between the sample medians with $\chi^2$=5.457 at p=0.03801. This incited to perform another univariate analysis of the sampling pairs using Wilcoxon paired-sample test (Table 7). The results obtained for Wilcoxon paired-sample test highlighted significant difference between all the three sampling sites with obtained values of W being > $W_{\alpha(2),34}$ at $\alpha(2) = 0.05$ for the sampling site pairs-site 1 and site 2; site 2 and site 3, but with W being > $W_{\alpha(2),34}$ at $\alpha(2) = 0.10$ for the sampling site pairs-site 1 and site 3. This infered more significant difference between the sampling site pairs-site 1 and site 2 and site 2 and site 3 as compared to significance in difference between site 1 and site 3. The results highlighted the higher sensitivity of Wilcoxon paired-sample test in detecting difference between population. Thus, null hypotheses $H_{01}$, $H_{02}$ and $H_{03}$ were rejected in favour of two-tailed alternative hypotheses $H_{A1}$, $H_{A2}$ and $H_{A3}$, thus establishing significant difference between three sampling sites based on epilithic diatom assemblages at each sampling site.

The results of Table 3 highlight the fact that no centric diatom was encountered in any of the three study sites, as such, species richness attributable to cyclotelloid and stephanodiscoid forms was nil in diatom assemblages at all three sites. Complete absence of eunotioid and epithemioid forms was also noticeable at all the sites.

Highest species richness was found at site 3, followed by site 1. Minimum species richness of three sites, in terms of total diatom forms identified, was found at site 2. This finding is corroborated by the observation that moderate disturbance is one of the cause for high species diversity. In the present study, site 2 was nearest to its point of origin at a distance of merely 8.4 km from the source of origin - Narmada Kund in Amarkantak and as such was least disturbed sampling site, relatively being free from anthropogenic pressures of agriculture and industries. This explains minimum species richness at this site. The other two sampling sites 1 and 3 were at considerable distance from their respective source of origin with the catchment areas under moderate anthropogenic pressures, explaining higher and near equal species richness of 17 and 19 at these two sampling sites. Naviculoid forms were found to dominate, in terms of species richness at all the three sampling sites followed by the araphid forms.

Frustules of *Fragilaria crotonensis* dominated site 1, in terms of frustule count data, with an average frustule count of 29.2. Similar to the present observation, *Fragilaria crotonensis* has frequently been designated as mesotrophic (Saros *et al.,* 2003, 2005; Rankovic *et al.,* 2006) and has been found to either dominate or be present with high relative abundance in systems where they occur (Vilbaste, 2001; Rankovic *et al.,* 2006). Dominance of species belonging to genera *Surirella* (*S. linearis* and *S. minuta*) was found in terms of frustule density at site 2. Site 2 being relatively near to the source of origin had lower pollution level as compared to distance that site 1 and site 3 were from their respective source of origin. Dominance of *Surirella* was reported in oligotrophic (Jasprica and Hafner, 2005) to slightly mesosaprobic conditions (Alfinito and Iberite, 2013). At site 3, highest frustule count data of 59.0 was found in case of *Gomphonema parvulum*. *G. parvulum* is a well documented species in terms of being an indicator of heavy industrial pollution (Moravcova *et al.,* 2013). The species has also been found to be tolerant to nutrient enrichment (Bellinger *et al.,* 2006; Schneck *et al.,* 2007; Tokatli and Dayioglu, 2011; Segura-Garcia *et al.,* 2012; Bohm *et al.,* 2013; Venkatachalapathy *et al.,* 2014).

Perceived differences existed in terms of geographical locations; distance of these hill streams from their respective sources of origin and effect of anthropogenic activities on nutrient enrichment of the same. Significant difference could be established between different sampling sites by Diversity t-test, based on diversity profile of each site. This difference was more pronounced in case of site 1 *vs.* site 2 and site 2 *vs*. site 3 and less pronounced in case of site 1 *vs.* site 3, which was consistent with the inherent qualities of sites as discussed before. Diversity profile of epilithic diatom assemblage at three sampling sites showed higher pronounced difference between the sites in terms of species richness (Margalef), diversity (Shannon-Wiener and Fisher's alpha) and dominance (Dominance and Berger-Parker). The results of Mann-Whitney test, performed on pairs of sampling sites, could have led to a Type II error as it was unable to detect any significant difference between the pairs of sampling sites, but further analysis using Wilcoxon paired-sample test yielded significant difference between the sites and the error could be avoided. This is corroborated by the

**Table 3 :** Diversity profiles of diatom frustules at the three sampling sites

| Indices of diversity profile | Site 1 | | | Site 2 | | | Site 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1(1) | 1(2) | 1(3) | 2(1) | 2(2) | 2(3) | 3(1) | 3(2) | 3(3) |
| Taxa # | 17 | 17 | 17 | 10 | 9 | 9 | 19 | 19 | 19 |
| Individuals # | 195 | 196 | 190 | 173 | 160 | 174 | 264 | 252 | 292 |
| Dominance | 0.095 | 0.093 | 0.089 | 0.169 | 0.181 | 0.207 | 0.084 | 0.102 | 0.089 |
| Simpson | 0.904 | 0.906 | 0.910 | 0.830 | 0.818 | 0.792 | 0.915 | 0.897 | 0.910 |
| Berger-Parker | 0.153 | 0.148 | 0.157 | 0.265 | 0.262 | 0.298 | 0.204 | 0.234 | 0.219 |
| Shannon-Wiener | 2.539 | 2.542 | 2.588 | 1.976 | 1.832 | 1.736 | 2.716 | 2.581 | 2.693 |
| Brillouin | 2.383 | 2.388 | 2.427 | 1.871 | 1.738 | 1.651 | 2.576 | 2.443 | 2.564 |
| Margalef | 3.034 | 3.031 | 3.049 | 1.746 | 1.576 | 1.551 | 3.228 | 3.255 | 3.171 |
| Evenness | 0.744 | 0.747 | 0.782 | 0.721 | 0.694 | 0.630 | 0.795 | 0.695 | 0.778 |
| Fisher's alpha | 4.478 | 4.470 | 4.518 | 2.31 | 2.062 | 2.013 | 4.695 | 4.766 | 4.548 |

**Table 4 :** Ranking distance between diversity profile data of sampling sites

| Ranking | Sampling site replicate | Distance | Cumulative distance | Std. deviation |
|---|---|---|---|---|
| 9 | Site 3(1) | | | |
| 8 | Site 3(3) | 0.1460 | 1.442 | 0.0728 |
| 7 | Site 3(2) | 0.1457 | 1.296 | 0.0822 |
| 6 | Site 1(3) | 0.3238 | 1.151 | 0.1547 |
| 5 | Site 1(2) | 0.3159 | 0.826 | 0.1076 |
| 4 | Site 1(1) | 0.0357 | 0.510 | 0.0214 |
| 3 | Site 2(1) | 0.1795 | 0.475 | 0.1988 |
| 2 | Site 2(2) | 0.2650 | 0.285 | 0.1729 |
| 1 | Site 2(3) | 0.0305 | 0.030 | 0.1146 |

**Table 5 :** Diversity t test between diversity profile data of sampling sites

| Sampling sites | Shannon-Wiener Diversity Index 'H' | | | | Simpson's Dominance Index 'D' | | | |
|---|---|---|---|---|---|---|---|---|
| | 1st Site 'H' | 2nd Site 'H' | df | t | 1st Site 'D' | 2nd Site 'D' | df | t |
| Site 1 *vs.* Site 2 | 2.566 | 1.880 | 1048.1 | 15.935** | 0.092 | 0.181 | 777.1 | 11.288** |
| Site 2 *vs.* Site 3 | 1.880 | 2.686 | 1095.5 | 19.382** | 0.181 | 0.089 | 989.14 | 10.662** |
| Site 1 *vs.* Site 3 | 2.566 | 2.686 | 1307.9 | 3.1093* | 0.092 | 0.089 | 1367.8 | 0.3471 |

Significance of 't' values: ** - $\alpha$<0.0001; * - $\alpha$<0.002

**Table 6 :** Mann-Whitney test on sample pairs of three sampling sites

| | Raw p values, uncorrected significance | | | Mann-Whitney U | | |
|---|---|---|---|---|---|---|
| | Site 1 | Site 2 | Site 3 | Site 1 | Site 2 | Site 3 |
| **Site 1** | | 0.0658 | 0.4068 | | 445.0 | 513.5 |
| **Site 2** | 0.0658 | | 0.0145* | 445.0 | | 398.0 |
| **Site 3** | 0.4068 | 0.0145* | | 513.5 | 398.0 | |

* significance generated by PAST ver3.02 software

**Table 7 :** Wilcoxon paired-sample test on paired-samples of three sampling sites

| | Raw p values, uncorrected significance | | | Wilcoxon 'W' | | |
|---|---|---|---|---|---|---|
| | Site 1 | Site 2 | Site 3 | Site 1 | Site 2 | Site 3 |
| **Site 1** | | 0.1298 | 0.3163 | | 190.0* | 201.5** |
| **Site 2** | 0.1298 | | 0.0324• | 190.0* | | 184.0* |
| **Site 3** | 0.3163 | 0.0324• | | 201.5** | 184.0* | |

•significance generated by PAST ver3.02 software; Significance of 'W' values: * - $\alpha(2) = 0.05$; ** - $\alpha(2) = 0.10$

observation that if data are paired use of Mann-Whitney test instead of Wilcoxon paired-sample test, may lead to commission of Type-II error, because of its inability to detect actual population differences (Zar, 2010).

Preliminary difference in aquatic system structures could be established by using only qualitative and quantitative data regarding diatom assemblage. The differences also reflect upon the integrity and well being of aquatic systems. Thus, diatoms prove to serve as excellent parameters for initial screening of water quality and ecological status of natural aquatic systems of hill streams under study. Further, inferences on water quality related parameters can be improved using chemical analyses to get a comprehensive report on the same.

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