Diatom-based ecologically water quality assessment of river Ganga in Western Himalayan region, India

Deepika Dimri
Doon University
Archana Sharma (archanasharma@doonuniversity.ac.in)
Doon University https://orcid.org/0000-0003-4977-3007

Research Article

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Abstract

Most of the diatom-based indices have not been widely tested on Ganga river in Himalayan region. Diatom samples were collected from epirhithron, metarhithron and hyporhithron zone of river Ganga and 12 physicochemical parameters (pH, electrical conductivity, total dissolved solids, total hardness, dissolved oxygen, biological oxygen demand, sodium, potassium, nitrate, phosphate, sulphate) were measured during the field and laboratory studies. According to the results of the physicochemical analysis, elevated nutrient concentration (SO_4^- and NO_3^-) in hyporhithron zone are due to organic pollution resulting from agriculture activities, discharge of municipal and industrial wastewater and urban clusters. In epirhithron zone, richest species were *Achnanthes* sp. (54) followed by *Nitzschia* sp. (46) and *Navicula* sp. (35). The metarhithron zone had dominant species of *Nitzschia* sp. (58) followed by *Diatoma vulgaris* (54) and *Navicula* sp. (47), while *Achnanthes* sp. (81) showed maximum number followed by *Nitzschia* sp. (66) and *Diatoma vulgaris* (57). Furthermore, Canonical Correspondence Analysis (CCA) identified five environmental variables (pH, SO_4^{-2}, NO_3^-, TDS, and EC) and their strong correlation with major diatom species. Shannon-Weiner diversity index, Simpson index and Margalef index showed moderately polluted sites under each ecological zone. Most of the applied diatom indices including, CEE, DESCY, IBD, IDSE, IDAP, IDP, IPS, SHE were effectively identified ecological status of Ganga river. This study suggests that the use of biotic indicators can provides useful information of the river health status and can be good indicators of Ganga river water quality. We recommend that attention can be paid towards use of biological indicators for water quality monitoring of river Ganga in Himalayan region in view of more detailed information on the use of foreign diatom indices.

1. Introduction

Latest developments in rivers monitoring have shifted from the evaluation of water quality parameters to the practice of biotic indicators, which are fast and cost-effective approach for the assessment of the effects of environmental pressures (Bere et al. 2014; Mangadze et al. 2019). Diatoms are commonly the most frequent and species-rich primary producers in mountain rivers (Rott et al. 2006).

Assessments using diatom-based indices can provide a holistic perspective and general indication of the ecological status of a river system (Mistri et al. 2008; Tan et al. 2015). Several diatom autecological indices of water pollution in rivers have been established and are extensively used worldwide. Due to the dearth of information regarding ecological preferences as well as tolerances of diatoms in developing nations, biological monitoring of streams and rivers has been assessed by foreign developed methods. In India, little research has been on the applicability and efficiency of foreign developed methods/indices for streams and rivers (Srivastava et al. 2017).

The glacier streams establish immensely rough environments for benthic algae, which results in the occurrence of a specific diatom species spectrum due to distinct factors including variations in discharge, turbulent flow, low temperatures, and greater ultraviolet radiation (Cantonati et al. 2001; Gesierich and Rott, 2012). The study on high mountain lotic system with reference to algal assemblages is quite recent. In various parts of mountainous regions across the world, several attempts have been made by researchers (Battegazzore et al. 2004; Goma et al. 2005; Cantonati and Spitale, 2009; Jüttner and Cox, 2000; Jüttner et al. 2011; Rimet et al. 2005; Rimet et al. 2007). However, for Himalayan regions, a detailed assessment of diatom diversity is scare, although studies on taxonomical and ecological studies were performed in some streams (Jüttner et al. 1996; Rothfritz et al. 1997; Jüttner et al. 2003; Li et al. 2007).

In Ganga River and its tributaries, the diatoms constitute about 70–90% of phytobenthos (Nautiyal 1996). Nevertheless, there is insufficient information related to the health of diatom assemblages, which has restricted their use as a bio-indicator for Ganga river. In upper Ganga basin, growing development activities and rising pollution stresses have been posing a major risk to the biodiversity and ecological sustainability of the Ganga river. The quality and quantity of water flow of Ganga river has significant effected, particularly during low flow period.

Few studies have been conducted on Ganga river and its tributaries in Himalayan region with respect to diatom composition by (Nautiyal et al. 1996; Nautiyal et al. 2014; Nautiyal et al. 2000; Matta et al. 2015; Matta, 2015; Malik et al. 2018; Singh et al. 2020). Therefore, present study fills the research gap by identifying the altitudinal and geographical distribution of benthic diatom assemblages in the Ganga river water with their related physical and chemical environmental variables. This study further investigates spatial and temporal variations of various diversity indices between monitored sites. The Canonical correspondence analysis (CCA) were also employed to assess the relation between documented diatom species and water quality variables to check the water quality status of river Ganga.

2. Material And Methods

2.1 Study area

The study area focused on upper Ganga basin in the Western Himalayan region between Gangotri to Haridwar, covering an altitudinal range of 3073 to 261 meter (AMSL) (Fig. 1). The Bhagirathi river, which is traditionally considered as a source stream of Ganga river originates from Gaumukh (30°36' N; 79°04' E) at an elevation 4100 m above mean sea level (AMSL) and meets other tributary of river Ganga named as “Alaknanda” (originates from Satopanth Glacier) at Devprayag and known as mighty “Ganga river”. The catchment area of Bhagirathi river is 6921 Km^2 and Alaknanda river is 10,882 Km^2 (Kumar et al. 2021).

The water temperature of river water in the Himalayan segment is 4.3^o C – 19°C, which supports survival of various cold sensitive species (Tare et al. 2017). The water quality in upper segment is almost in pristine or near-pristine conditions comprising ultra-oligotrophic to oligotrophic conditions (Mathur and Kapoor 2015). The Bhagirathi and Ganga river travels in a North-South direction through three tectonic units as HHCS, Lesser Himalayas Series (LHS) and the Siwaliks at different altitudes before it enters to the plain region.

2.2 Categorization of study sites into three ecological zones

The ecological classification of upper Ganga basin is classified under rhithron zone of river as per the classification of rivers given by Ilies and Botosaneann (1963). We classified 19 study sites into three ecological zones. The sites were selected based on the topography of the area, altitudinal gradient, anthropogenic impacts and seasonal variations (Dimri et al. 2021). The detailed ecological classification of upper Ganga basin is depicted in the (Fig. 2).

2.3 Collection of water and diatom samples

The river water samples (one liter) were collected in triplicate from each study site from the center of the river (~ 0 to 5 cm below the surface). All the water samples were wide-mouth natural HDPE polypropylene bottles and transported to the lab using icebox and preserved at 4°C before further analysis. A total of 12 physical-chemical parameters: pH, Temperature, EC, TDS, DO, BOD, TH, SO_4^{-2}, Na^+, K^+, PO_4^{-3}, NO_3^- were analyzed and average value was determined for each site.

At each sampling site, diatom samples were randomly collected from 8–10 stones in riffle areas and diatoms were scraped off using toothbrushes following standard methods (Kelly et al. 1998). Diatom suspension was then preserved with formalin (5% final concentration) and decanted into a plastic sample bottle for further analysis.

Water and diatoms samples were collected from epirhithron, metarhithron and hyporhithron zones of upper Ganga basin during October-February 2019.

2.4 Analytical method for diatom samples

In the laboratory, diatoms samples were treated following the standard protocol of hot HCl and $KMnO_4$ to remove organic impurities (Hasle 1978; Round et al. 1990) (Fig. 3). This method is suitable for cleaning of diatom samples collected in India (Karthick et al. 2010). The cleaned solution was then air-dried onto cover slips and permanent slides were prepared using Napharax® medium. Using a light microscope at 1000x magnification (Zeiss, equipped with a Nikon camera) about 300–500 diatom valves were counted and were identified using the several taxonomic references: Krammer and Lange-Bertalot (1988); Lange-Bertalot (2001) and other relevant online literatures.

2.5 Diatom indices

Twelve diatom indices (Table 1), which have been widely employed for river monitoring were analyzed using OMNIDIA software 7 (Version 4.2) (Lecointe et al. 2003). Most of them (DESCY, IBD, IPS, TDI, TID) are based on the Zelinka and Marvan (1961) equation as follows,

$$ID = \frac{\sum {\rm A}j\ vj\ ij}{\sum Ajvi}$$

Where

Aj = relative abundance of the species j

vj = species indicator value ($1 \leq v \leq 3$)

ij = species sensitivity (Descy 1979)

2.6 Diversity Indices

The following ecological diversity indices for diatom species were undertaken for each monitored site.

Shannon Weiner's Index (Shannon 1948)

$$(\mathbf{H'}) = \sum_{I=1}^{s} \boldsymbol{pi}\,\mathbf{In}\,(\boldsymbol{pi})$$

Where, *pi* = Relative abundance of species 'i'

S = Total number of species

$$[p_i = \frac{ni}{N}]$$

Where, ni = number of individuals of species 'i', and N = total number of individuals of all species

Simpson's Dominance Index (Simpson 1949)

$$\mathbf{D} = \sum_{I=1}^{s} \boldsymbol{pi2}$$

Where, pi = Relative abundance of species 'i'

S = Total number of species

$$H' - \sum_{I=1}^{S} piInpi$$

Margalef Index

Specific diversity was calculated using Margalef's index (Margalef 1968) as follows,

$$D = \frac{\mathbf{S\text{-}1}}{\mathbf{InN}}$$

Where, S = Species number, N = Total number of individuals

3. Results And Discussion

3.1 Monthly and spatial variations in physical and chemical environmental variables

The physical and chemical parameters have a crucial role in monitoring of aquatic life, which results in the variations of diversity and community of aquatic life-forms (Fathi and Flower 2005). Monthly variation in average values and standard deviation of studied physical and chemical environmental parameters are presented in Table 2.

The pH did not exhibit any remarkable differences during all months. The average value of pH was highest at metarhithron (8.88±0.098) in the month of February and the lowest value was found at epirhithron (7.81±0.01) in October, signifying slightly alkaline property of Ganga river water. Water temperature is an important parameter in aquatic ecosystem, which controls the oxygen solubility and also affects the rate of metabolic activities of living organisms. Additionally, existence of various insoluble contaminants indicates elevated temperature in river water (Tyagi et al. 2020). The average values of temperature were ranged from (5.31±0.02 to16.69±0.34) during all months (Table 2). According to Paerl and Huisman (2008), diatoms favor cooling water temperature of (20-30 °C). The values of Electrical Conductivity (EC) in surface waters show variations depending on geological features and precipitation rate (Tepe and Boyd 2003). The average ranges of EC were in the range of (50.09±0.015 to 99.71±10) µS/cm, showed highest at hyporhithron during November and lowest at epirhithron in the month of February. Furthermore, average TDS values over the study period varied from (31.07±0.036) mg/L to (99.71±10) mg/L (Table 2). The EC and TDS were directly proportional to each other and this relationship has been documented in many literatures (Chatanga et al. 2019).

Dissolved Oxygen (DO) determines the ecological stability of water body and it depends on the concentration of dissolve salts, biological activities, and temperature in the water as well as the partial pressure of the atmospheric gas on the surface of water (Taş et al. 2010). The highest average DO value was found in epirhithron (12.29±0.01) mg/L in January, while the lowest average value was calculated for hyporhithron (8.6±0.043) mg/L in October. During all studied months, average maximum BOD was found (4.10±0.023) mg/L at hyporhithron in the month of October and minimum BOD was observed (2.8±0.01) mg/L at epirhithron site in the month of January. Average maximum and minimum values of hardness were measured at hyporhithron (73.06±6.33) during November and at epirhithron (34.62±0.025) mg/L February month, respectively. The concentrations of SO_4^- varied from (13.89±0.86) to (20.06±0.01) during all studied period. Ganga water samples showed a concentration range of Na between (1.21±0.24) and (3.14±0.11), while K had highest value (4.62±0.09) mg/L at hyporhithron during February and lowest range of (2.32±0.25) mg/L at metarhithron during October (Table 2).

The concentration of nutrients was comparatively lower in all studied sites. Phosphorus and nitrogen are regarded to be the most important limiting nutrients eutrophication in aquatic ecosystems. According to Geneviève and Neary (2006), PO_4^{-3} and NO_3^- can enter aquatic environments as runoff from human activities in urban and agricultural areas. The average highest and lowest values of PO_4^{-3} were in the range from (0.079±0.005) to (0.78±0.024) mg/L respectively (Table 2). The elevated concentration of NO_3^- (1.53±0.23) mg/L was observed at hyporhithron in February, while lowest value (0.0096±0.01) mg/L was observed at epirhithron in October. The amount of both nitrates and phosphates increased significantly in. hyporhithron zone. The elevated nutrient concentration in hyporhithron zone of upper Ganga basin might be due to organic pollution resulting from agriculture activities, discharge of municipal and industrial wastewater and urban clusters.

3.2 Diatom community composition

A total of 15 diatom species were recorded by light microscopy (LM) in epirhithron, metarhithron and hyporhithron, representing the families, Naviculaceae, Cocconeidaceae, Gomphonemataceae, Fragilariaceae, Bacillariophyceae, Sellaphoraceae, Stephanodiscaeae, Rhoicospheniaceae. Monthly variation in diatom assemblages showed most flourishing season as February and less flourishing season as October. In epirhithron zone, most abundant species were found to be *Achnanthes sp.* (54) followed *by Nitzschia sp.* (46) and *Navicula* sp. (35). The metarhithron zone had dominant species of *Nitzschia sp.* (58) followed by *Diatoma vulgaris* (54) and Navicula sp. (47), while *Achnanthes sp.* (81) showed maximum number followed *by Nitzschia sp.* (66) and *Diatoma vulgaris* (57). The less commonly species *(Sellaphora sp. and Stauroneis sp.)* were not observed in hyporhithron (Table 3). The same species predominantly present in all three ecological zones were *(Amphora sp. Achnanthes sp. Cocconeis placentula* Ehrenberg 1838, Cyclotella *meneghiniana* Kützing 1844, Diatoma vulgaris Bory 1824, *Gyrosigma sp. Gomphonema sp. Fragilaria sp. Navicula sp. Meridion circulare* (Greville) C.Agardh, 1831*, Nitzschia sp.* (Table 3). The result indicated remarkably similar diatom composition in upper Ganga basin with increasing number of diatom composition with respect to the rising water temperature and lower altitude. Jüttner et al. (1996), has documented *Navicula sp.* as the most dominant species in the streams flowing through forest area of Nepal Himalayas. Nautiyal et al. (2004) also reported similar observations with dominance of *Achnanthes sp.* in torrential high flowing water of Himalayan River "Alaknanda". Similarly, dominance of *Fragilaria sp.* and *Achnanthes sp.* at higher altitude, while *Navicula sp.* and *Nitzschia sp.* were observed at lower altitude in Nepalese Himalayan rivers in a study done by Ormerod et al. (1994). *Cyclotella sp.* has also been reported in the lakes of Jammu and Kashmir Himalayas (Khan 2002). Hence, present study indicates that species occurred in epirhithron had less diverse assemblage in water samples as compared to the metarhithron and hyporhithron. We can conclude that altitude and temperature can affect the number of diatom species, with maximum number of species occurred in rising water temperature. Further, we also conclude that stream velocity also played an important factor in composing diatom communities in the study area. For example, Species such as *Achnanthes* and *Fragilaria* better survive in high current velocity (Virtanen and Soininen 2012). The significant of velocity in shaping diatom communities has also been reported by researchers (Biggs and Kilroy 2000, Potapova and Charles 2002).

3.3 Diversity Indices

Composition of diatom diversity rapidly changes to the existed physical, chemical, and biological disturbances in aquatic body (Stevenson and Smol 2003). Diversity indices can perform a good indicator of the overall water pollution status. Sometimes, non-polluted waters are represented by high diversity, including large number of species and no individual species dominating in numbers over others (Thakur et al. 2013).

Species richness (Margalef index), species evenness/dominance (Simpson index), and a combination of richness and dominance (Shannon–Wiener index) are three bio-indices of species diversity, which are mainly derived from species counts (Thakur et al. 2013). These diversity indices are based on the number of species and their relative abundances, which states that higher the values of aforementioned diversity indices, higher the oligotrophic status of aquatic bodies.

The Shannon-Weiner diversity index was used as an indicator for water pollution in this study. Wilhm and Dorris (1968) proposed range of diversity index, (<1) for highly polluted water, (1–3) for moderately polluted water, and (>4) for unpolluted water. Furthermore, Staub et al. (1970) also suggested pollution scale referring to species diversity index. Range from (3 to 4.5) for slight pollution, (1 to 2) for moderate pollution and (0 to 1) for heavy pollution in water samples. In present study, Shannon diversity index ranged from (2.24) to (2.45) during all months (Table 4), where epirhithron had the highest average value (2.42), while average lowest value of Shannon diversity was observed in hyporhithron (2.34) (Figure 4). Therefore, all monitored sites under classified ecological zones were considered as moderately pollution sites as per Wilhm and Dorris (1968) based diversity index, and based on Staub et al. (1970) index, epirhithron, metarhithron, and hyporhithron zones were moderately polluted.

The range of Simpson index varies from 0 to 1, wherein values near 0 indicates less evenly distributed communities under pressure and values near 1 indicates most evenly distributed communities belonging to non-polluted samples (Thakur et al. 2013). Average lowest Simpson index was calculated in hyporhithron (0.88), while highest was calculated in epirhithron (0.90) (Figure 5). Obtained values of Simpson index in all monitored locations was close to 1, showing non-polluted monitored sites.

Margalef index describes the number of species to the total number of individuals. The decline in the range of Margalef index depicts the rise in the pollution level. Margalef index has advantage of having comparison of species richness corresponding to various study sites over the Simpson index (Shah and Pandit 2013). Regarding the Margalef index, epirhithron in the month of October had the highest value of (2.25), while hyporhithron in the month of February had minimum value of (1.87) (Table 4). According to the value of Margalef index, metarhithron and hyporhithron showed decline values in the month of December, January and February, thus, indicating pollution level.

3.4 Correlation between physico-chemical environmental variables and diatom

3.4.1 Canonical correspondence analysis (CCA)

Canonical Correspondence Analysis (CCA) was applied to analyze correlation between epilithic diatoms, environmental variables, and ecological zones. The length of the arrow indicates the influence of environmental variable, which indicates positive or negative correlations with axis. In the present study, two dimensional CCA map was drawn between the nine environmental parameters, dominant diatom species and three ecological zones (Figure 6). The first and second axis explained 93.54 % and 6.46 % of the variance respectively. Distribution of dominant diatom species was influenced by selected environmental factors (pH, SO_4^{-2}, NO_3^-, TDS, and EC). First quadrate contains species such as *Coconeis placentula* and *Fragilaria*, which are affected by low nutrient concentration (NO_3^- and PO_4^{-3}) and temperature. These species are highly influenced by environmental variables such as (pH, DO and SO_4^{-2}) corresponded to epirhithron zone. Here, we can relate to the finding which reported that *Coconeis placentula* is extremely distributed diatom taxa and found in nearly all freshwater sources, exhibiting neutral to alkaline pH range and moderately to less polluted environment (Bere et al. 2016). Similarly, preferred pH range for *Fragilaria* sp. is acidophilous to alkaliphilous (Van Dam et al. 1994) and it is found in the good or moderate water quality as defined by the Water Framework Directive (European Union 2000) (Kahlert et al. 2019). *Fragilaria* also grow in water having low nutrient content. It is noteworthy to mention that sites in epirhithron zone had alkaline water quality, low impact of organic pollution and moderately polluted water quality status according to our calculated value of Shannon-Weiner diversity index. In the second quadrant, species (*Gomphonema*, *Navicula*, *Nitzschia, Achanthes* and *Diatoma vulgaris)* are associated with high nutrient concentrations (NO_3^- and PO_4^{-3}). The second quadrant also showed effects of TDS and EC on these diatom species in downstream water of metarhithron and hyporhithron zones. *Gomphonema* sp. are prevalent in cold and high speed water with ultraoligotropic, nutrient rich, and organically contaminated water (Jiittner et al. 2000, Fore and Grafe 2002). They are also characterized by presence of neutral to slightly alkaline pH as well as low to high conductivity (Van Dam et al. 1994).

Achnanthes sp.does wellin rapid-moving water and in oligotraphentic, mesotraphentic and eutraphentic medium*.* *Navicula* sp. thrives in oligo and eutraphentic medium, while *Nitzschia* sp. favored in more mineralized water environment and it has tendency to be tolerant to manifold pollutants (Wang et al. 2005). The genus *Amphora* is also tolerant to eutrophication (Bellinger et al. 2006). Therefore, we can associate that positive correlation of *Achnanthes* sp.*Amphora sp. Diatoma vulgaris, Gomphonema* sp. *Nitzschia sp. and Navicula sp.* with high nutrient concentrations, EC and TDS inmetarhithron and hyporhithron zone could be an indicator of various pollutants in river water. However, more detailed studies are needed to understand the diatom based ecological assessment of Ganga river water.

3.5 Ecological status of river Ganga based on diatom indices

The limit values of water quality classes, and corresponding ecological and trophic status for diatom indices is given in Table 5.

Diatom index values were observed different at each ecological zone as per the percentage of species used in the calculation of indices. Throughout the study, IDG used 100 %, IPS 81 %, and TDI used 90.60 % of the species in index calculations, which included the greater amount of species diversity. DESCY, SHE, CEE, IBD, IDP, WAT, Sla, RottSI used 28.18 % of the species in index calculations. IDSE and RottTI used 18.78%, while IDAP used minimum number (9.39 %) of species. The calculated diatom index values and corresponding ecological status observed in each ecological zone is given in Table 6.

The Descy index values were ranged 18.3 in epirhithron, 18.8 in hyporhithron and 19.2 in metarhithron. According to Descy index the ecological status of Ganga river was high which reveals that the water quality belongs to the class (I) category and trophic level is Oligotrophic.

The TDI index evaluates the eutrophication level in aquatic system. In India, TDI is one of the very rare indices that have been applied and checked for water quality (Srivastava et al. 2017). The similar observation has been found between Europe and the Himalaya region in terms of TDI results (Jüttner et al. 2003). In present study, maximum value of TDI was calculated in hyporhithron (10.1) followed by epirhithron (8.7) and metarhithron (8.4). Therefore, according to TDI values, hyporhithron is considered as mesotrophic, which indicates “moderate” ecological status and corresponds to III water quality class.

IPS is one of the most frequently used diatom-based indices worldwide including European and non-European countries (Lavoie et al. 2009). In this study, highest value (15) was calculated in epirhithron and hyporhithron, while lowest (14.3) in metarhithron.

The highest IBD was found (16.1) in hyporhithron followed by 15.9 in epirhithron and metarhithron zone. The value of IDP was ranged (13.2) in epirhithron and metarhithron while (14.2) in hyporhithron. There was no significant difference was observed in the value of RottSI. It was recorded (13.3) in epirhithron and hyporhithron and (13) in metarhithron. On the other hand, IDAP and SHE showed a value of (15.2) and (13.7) respectively in all three zones. Based on the IPS, IBD, IDP, RottSI, IDAP and SHE diatomaceous indices, epirhithron metarhithron and hyporhithron zones in upper Ganga basin were evaluated as “good” ecological status and water quality belongs to classes (IV) with the trophic status being Oligo-mesotrophic.

WAT index was calculated (12.6) in epirhithron whereas (13) in metarhithron and hyporhithron. The epirhithron was characterized by a moderate ecological status, indicating mesotrophic, whereas metarhithron and hyporhithron had “good” ecological status with oligo mesotrophic based on the WAT index. Sla index was recorded (12.8) and (11.9) in epirhithron and metarhithron respectively whereas it was recorded (13.3) in hyporhithron. Sla index indicated “moderate” ecological status with Mesotrophic condition, belongs to III water quality class in epirhithron and metarhithron. However, Sla index indicated good ecological status with Oligo-mesotrophic condition, belongs to II water quality class.

The upper Ganga basin is very diverse in terms of topography, vegetation cover, settlements, human-activities and numerous other factors, which may affect the diatom assemblages. Majority of applied indices including, CEE, DESCY, IBD, IDSE, IDAP, IDP, IPS, SHE were successfully in identification of ecological status of Ganga river. These indices were found to be the reliable to assess the ecological status of river Ganga in Himalayan segment. Studies conducted in different streams worldwide revealed that IPS and IBD are suitable indices for water quality assessment (Prygiel et al. 2002, Deng et al. 2012).

However, two indices SLA and WAT were unable to distinguish between pristine (epirhithron) and contaminated (hyporhithron) sites, assigning “moderate” ecological status to epirhithron whereas “good” ecological status to hyporhithron. High ecological status was found in all ecological zones based on DESCY index, which were associated with relatively low nutrient content. The reason could be primarily because of the minimum percentage of taxa used by these indices for computation of index scores. According to Srivastava et al. (2017), IGD could be an effective tool for rapid biomonitoring as well as subsequently developing the databases with respect to ecological profiles of diatoms in India. It is noteworthy that IGD used 100% of species diversity and indicated “moderate” ecological status of epirhithron, metarhithron and hyporhithron in the present study.

We can conclude that further research can be done to evaluate ecological status of upper Ganga basin with respect to foreign diatom indices.

4. Conclusion

1. The study revealed the monthly differences in the diatom assemblages, with large number of species in February and lower in the October month in epirhithron, metarhithron and hyporhithron.
2. In the upper section (epirhithron), the order of species dominance was; *Achnanthes* > *Navicula* > *Nitzschia.* In the metarhithron, the order of species dominance was; *Nitzschia* > *Diatoma* > *Navicula*, while in the hyporhithron, species dominance was; *Achnanthes* > *Nitzschia* > *Navicula.*
3. Diatom communities in forested and pristine sites (epirhithron) were less diverse as compared to sites of high settlement region (metarhithron and hyporhithron).
4. It is noticeable from the study that *Achnanthes sp. was the* dominating diatom flora possessing maximum number of species in the upper (epirhithron) along with the lower segment (hyporhithron), while Nitzschia sp. dominated in the metarhithron.
5. Regarding Shannon-Weiner diversity index, water quality of river Ganga was considered as “moderately polluted”.
6. CCA bi-plot demonstrated that environmental variables such as nutrients, nutrients like phosphate and nitrate, TDS, and EC were positively correlated with species (*Gomphonema*, *Navicula*, *Nitzschia, Achanthes* and *Diatoma vulgaris)* zone, clearly indicating pollution level due to various human activities in hyporhithron.
7. Most of the abundant diatom floras were tolerant to higher nutrient concentration in metarhithron and hyporhithron zone that indicates contaminated water quality. CCA revealed that these diatoms were closely associated with the variables EC, TDS, NO_3^{-2}, and PO_4^{-3}.
8. Most of applied foreign diatom indices such as CEE, DESCY, IBD, IDSE, IDAP, IDP, IPS, SHE were significant in the evaluation of ecological status of Ganga river.
9. In the future, a detailed and long-term monitoring of diatom assemblages with altitudinal gradient along with the identification of major diatom indicator species for water quality monitoring of Ganga river in Himalayan region should be performed, which will be helpful to understand the diatom diversity pattern and relationship between diatom and environment variables.

Declarations

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2. Conflicts of Interest

I (Corresponding author) undersigned declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

I wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Dr. Archana Sharma
Assistant Professor
School of Environment and Natural Resources

Doon University Dehradun, India

archanasharma@doonuniversity.ac.in

3. Ethics Approval

I corresponding author confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing I confirm that all the authors have followed the regulations of our institutions concerning intellectual property.

Dr. Archana Sharma
Assistant Professor
School of Environment and Natural Resources

Doon University Dehradun, India

archanasharma@doonuniversity.ac.in

4. Consent to participate

I corresponding author on behalf of co-authors confirm that all the authors are consent to participate.

Dr. Archana Sharma
Assistant Professor
School of Environment and Natural Resources

Doon University Dehradun, India

archanasharma@doonuniversity.ac.in

5. Consent for Publications

I corresponding author on behalf of co-authors confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. I further confirm that all the authors are consent for publication.

Dr. Archana Sharma
Assistant Professor
School of Environment and Natural Resources

Doon University Dehradun, India

archanasharma@doonuniversity.ac.in

6. Availability of data and material

Data is available in the manuscript

7. Code availability

Not applicable

1. **Authors Contribution**

Deepika Dimri – Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Writing original draft,

Archana Sharma –Resources, Supervision,

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Tables

Table 1 Diatom-based indices with corresponding abbreviation and stressor type sensibility used in present study (Solak et al. 2020)

Abbreviation	Diatom indices	Stressor type sensibility
IDAP	Artois-Picardie Diatom Index (Prygiel and Coste 1996)	General pollution
IBD	Biological Diatom Index (Prygiel and Coste 2000)	General pollution
IDG	Generic Diatom Index (Coste and Ayphassorho 1991)	General pollution
IDP	Pampean Diatom Index (Gómez and Licursi 2001)	Organic pollution/eutrophication
IPS	Specific pollution sensitivity Index (Cemagref 1982)	General pollution
IDSE	Louis-Leclercq Diatomic Index	Saprobity
SHE	Schiefele and Schreiner's Index (Schiefele and Schreiner 1991)	Pollution/trophic status

Table 2 Monthly variation in average values (mg/L) and standard deviations of environmental variables in epirhithron, metarhithron and hyporhithron

Variables	Epirhithron					Metarhithron				
	Oct	Nov	Dec	Jan	Feb	Oct	Nov	Dec	Jan	Feb
*p*H	7.81±0.01	7.84±0.005	7.95±0.01	7.98±0.01	7.95±0.020	8.62±0.11	8.67±0.12	8.7±0.19	8.84±0.13	8.88±0.
Temp	8.6±0.1	7±0.005	6.27±0.06	5.18±0.072	5.31±0.020	13.49±1.8	12.05±1.2	11.02±1	9.78±0.11	9.96±0.
EC	55.07±0.01	57.81±0.005	54.1±0.12	51.65±0.02	50.09±0.015	72.14±3.6	74.90±2.4	71.78±2.9	62.17±0.2	60.72±0
TDS	36.38±0.25	38.14±0.005	36.3±0.01	32.25±0.01	31.07±0.036	47.68±2.4	50.05±3.1	47.07±2.0	42.43±0.8	41.89±0
DO	10.11±0.01	10.69±0.01	11.4±0.01	12.29±0.01	12.16±0.025	9.12±0.06	9.71±0.17	10.40±0.3	11.08±0.07	10.98±0
BOD	3.78±0.005	3.56±0.01	3.4±0.015	2.8±0.01	2.88±0.01	4.02±0.03	3.96±0.01	3.55±0.08	3.3±0.14	3.38±0.
TH	38.58±0.15	40.31±0.005	37.1±0.01	35.24±0.01	34.62±0.025	50.11±9.8	55.62±6.6	51.48±4.9	46.63±5.3	44.98±5
SO_4^{-2}	18.88±0.02	19.43±0.005	20±0.01	19.44±0.01	18.91±0.02	13.89±0.8	14.44±0.9	15.11±1	14.43±0.9	14.03±1
Na^+	1.29±0.015	1.57±0.01	1.76±0.01	2.12±0.02	2.22±0.015	1.21±0.24	1.41±0.20	1.65±0.35	2.01±0.15	2.15±0.
K^+	3.02±0.01	3.25±0.005	3.9±0.015	3.97±0.01	4±0.015	2.32±0.25	2.73±0.04	2.96±0.1	3.16±0.21	3.30±0.
PO_4^{-3}	0.079±0.005	0.082±0.005	0.092±0.01	0.098±0.001	0.09±0.0005	0.33±0.21	0.37±0.41	0.39±0.20	0.42±0.2	0.49±0.
NO_3^-	0.0096±0.01	0.0098±0.006	0.13±0.01	0.15±0.01	0.15±0.0057	0.68±0.04	0.71±0.2	0.78±0.05	0.803±0.05	0.83±0.

Table 3 Spatial and temporal variations in diatom species recorded in Ganga river water

Name of species	Epirhithron						Metarhithron						Hyporhithron					
	Oct	Nov	Dec	Jan	Feb	Average	Oct	Nov	Dec	Jan	Feb	Average	Oct	Nov	Dec	Jan	Feb	Average
Achanthes sp.	36	46	54	66	69	54	22	28	39	48	54	38	67	74	82	89	94	81
Coconeis placentula	16	19	21	28	31	23	9	12	30	34	39	24	12	15	20	25	28	20
Diatoma vulgaris	17	21	30	37	42	29	39	45	56	62	68	54	49	53	59	61	64	57
Gomphonema sp.	19	21	34	42	46	32	25	30	48	59	65	45	23	28	47	55	61	42
Navicula sp.	25	39	47	57	64	35	29	33	51	59	66	47	42	49	56	64	71	56
Nitzschia sp.	21	29	36	43	48	46	38	42	59	74	78	58	52	59	68	74	79	66
Rhoicosphenia sp.	9	8	12	17	23	13	5	8	13	16	19	12	3	6	12	15	21	11
Meridion circulare	12	9	10	15	19	13	8	10	14	19	23	14	29	32	44	56	60	44
Amphora sp.	11	11	14	18	21	15	11	13	21	30	35	22	13	15	19	33	38	23
Fragilaria sp.	16	21	29	37	43	29	27	32	47	56	61	44	7	10	14	17	19	13
Gyrosigma sp.	10	12	14	16	21	14	5	8	16	21	26	15	6	9	13	19	23	14
Cyclotella meneghiniana	7	10	14	18	22	14	4	5	8	12	18	9	5	8	12	16	19	12
S*ellaphora* sp.	6	8	7	9	13	8	-	-	-	-	-	-	-	-	-	-	-	-
Stauroneis sp.	-	-	-	-	-	-	2	4	7	11	15	7	-	-	-	-	-	-
Caloneis sp.	-	-	-	-	-	-	-	-	-	-	-	-	19	21	25	28	32	25

(-) Absent

Table 4 Monthly variations in diversity indices for the epilithic diatom community of epirhithron, metarhithron, and hyporhithron

	Epirhithron					Metarhithron					Hyporhithron				
	Oct	Nov	Dec	Jan	Feb	Oct	Nov	Dec	Jan	Feb	Oct	Nov	Dec	Jan	Feb
Simpson_1-D	0.90	0.89	0.90	0.90	0.91	0.88	0.88	0.89	0.9	0.90	0.87	0.88	0.89	0.89	0.90
Shannon_H	2.44	2.40	2.40	2.41	2.45	2.27	2.32	2.37	2.40	2.43	2.24	2.29	2.35	2.40	2.42
Margalef	2.25	2.16	2.07	2	1.95	2.21	2.14	1.99	1.93	1.89	2.07	2.02	1.95	1.90	1.87

Table 5 Limit values of water quality class, ecological and trophic state for diatom indices (Blue - High, Green - Good, Yellow - Moderate, Orange - Poor, Red - Bad) **(**Bytyçi et al. 2019)

Water Quality Class	Ecological state	IPS, CEE, IBD, IDG, DESCY, SLA, IDSE, IDAP, WAT, TDI, IDP, SHE	Trophic state
I	High	17–20	Oligotrophic
II	Good	13–16	Oligo-Mesotrophic
III	Moderate	9–12	Mesotrophic
IV	Poor	5–8	Eutrophic
V	Bad	1–4	Hypertrophic

Table 6 The diatom index values at sampling stations and corresponding ecological status

Diatom Index	Epirhithron		Metarhithron		Hyporhithron	
	Value	Ecological status	Value	Ecological status	Value	Ecological status
CEE	17.1	High	16.6	Moderate	17	High
DESCY	18.3	High	18.8	High	19.2	High
IBD	15.9	Good	15.9	Good	16.1	Good
IDG	11.9	Moderate	11.5	Moderate	12.5	Moderate
IDP	13.2	Good	13.2	Good	14.6	Good
IPS	15.1	Good	14.3	Good	15	Good
SHE	13.7	Good	13.7	Good	13.7	Good
TDI	8.7	Poor	8.4	Poor	10.1	Moderate
WAT	12.6	Moderate	13	Good	13.1	Good
Sla	12.8	Moderate	11.9	Moderate	13.3	Good
IDSE	13.4	Good	12.5	Moderate	14.3	Good
IDAP	15.2	Good	15.2	Good	15.2	Good

Figures

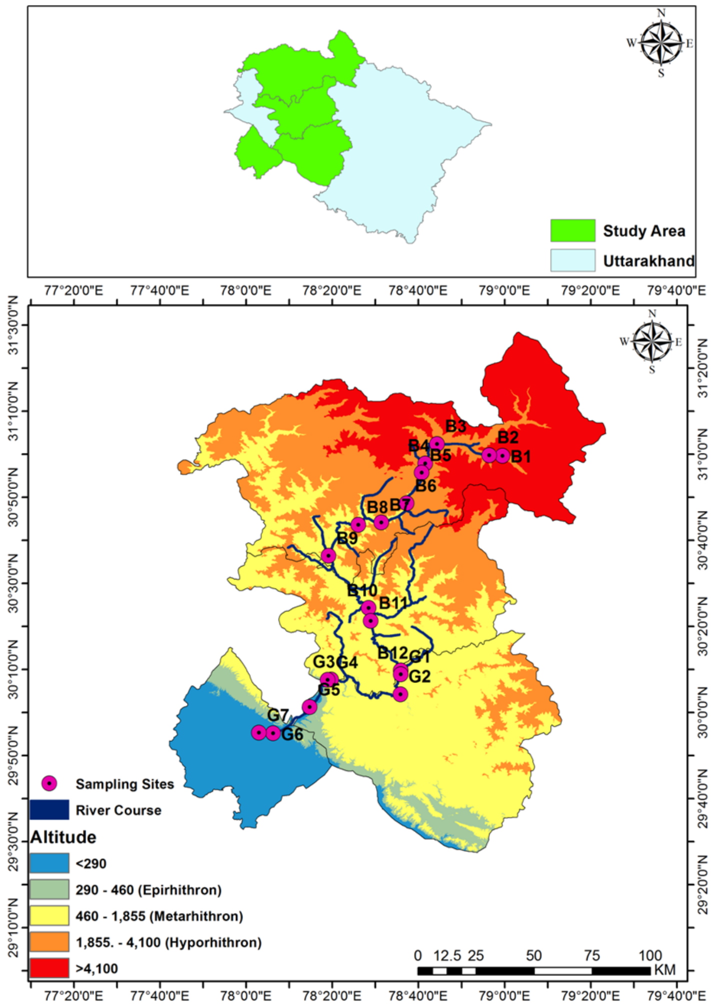

Figure 1

Sampling locations on Ganga river in Himalayan segment

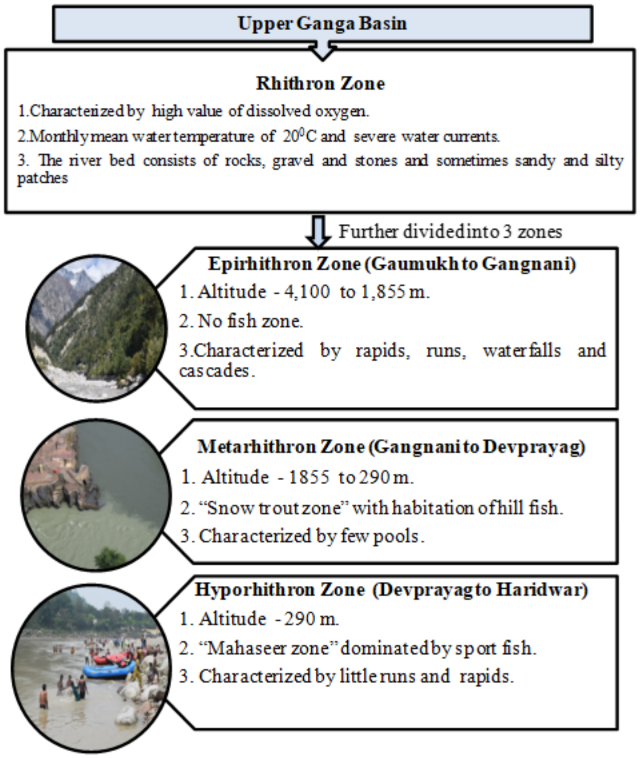

Figure 2

Ecological classification of upper Ganga basin

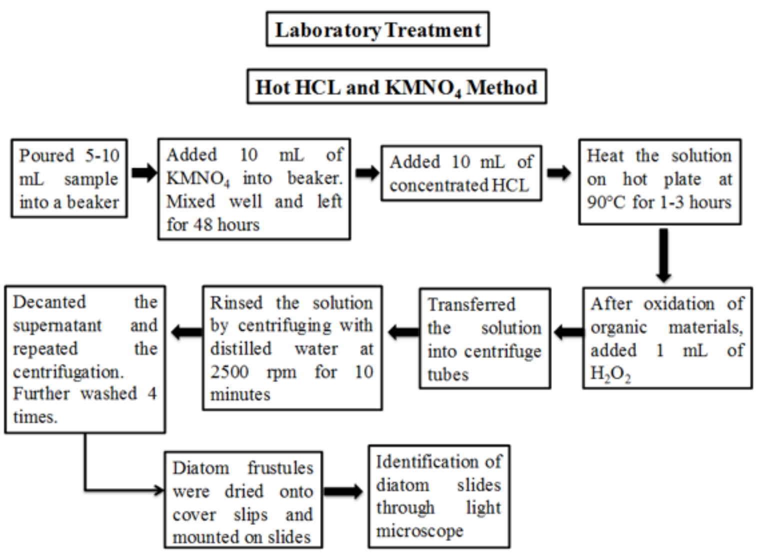

Figure 3

Methodology for diatom analysis in laboratory

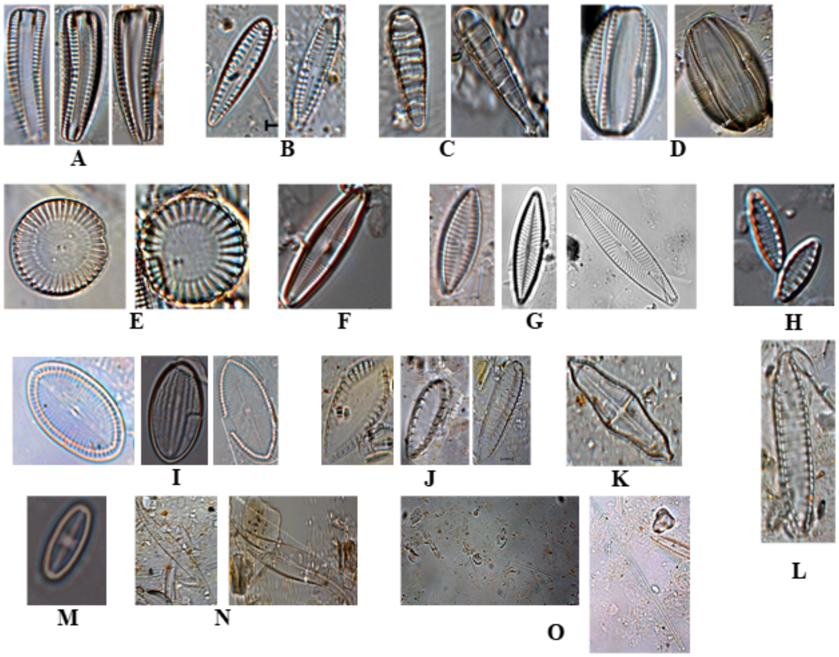

Figure 4

Dominant diatoms flora in the upper Ganga basin: A *Rhoicosphenia* sp., B *Gomphonema* sp., C *Meridion circulare* (Greville), D *Amphora* sp., E Cyclotella *meneghiniana* Kützing 1844., F *Caloneis* sp., G *Navicula* sp., H *Nitzschia* sp. I *Cocconeis placentula* Ehrenberg1838, J *Surirella* sp., K *Stauroneis sp., L* Diatoma vulgaris Bory 1824, M *Achanthes* sp., N *Gomphonema* sp., O *Fragilaria* sp.

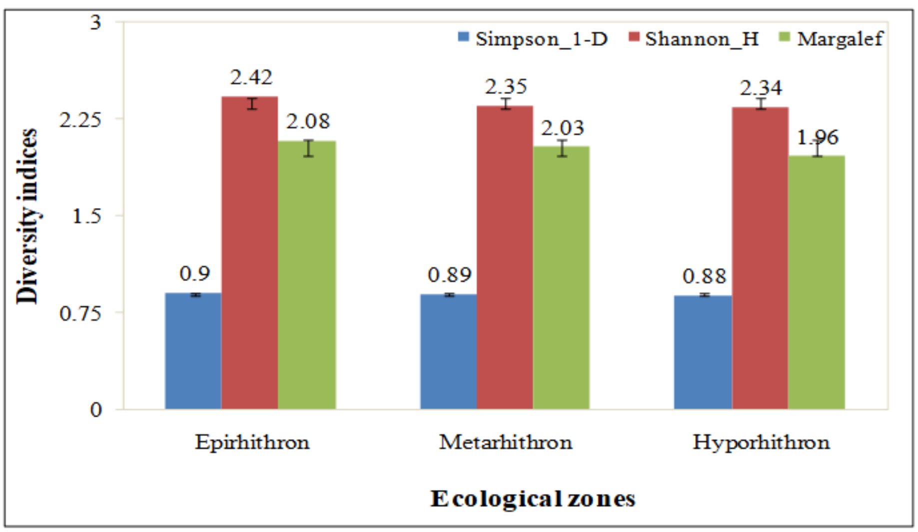

Figure 5

Average values of diversity indices in epirhithron, metarhithron and hyporhithron zone

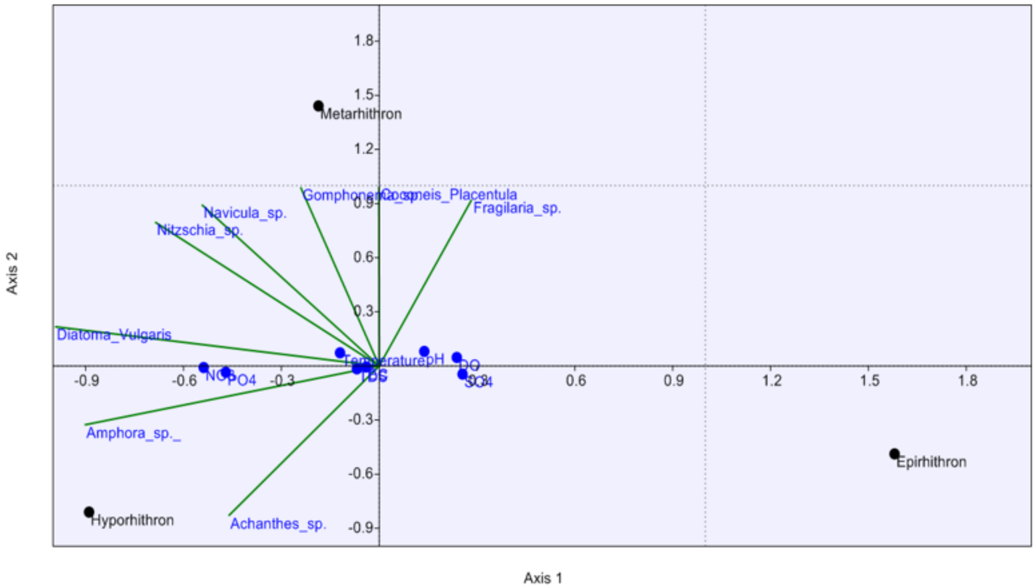

Figure 6

Canonical Correspondence Analysis (CCA) plot for indicating relationship among the environmental parameters and dominant diatom species