

Taxonomic Diversity and Biological Water Quality Assessment of Rivers Bhagirathi and Yamuna at Gangotri and Yamunotri Using Benthic Macroinvertebrates

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Abstract Two pristine holy rivers of India, Bhagirathi and Yamuna, originate from Gangotri and Yamunotri glaciers and are sacred places for all the pilgrims of Char Dham of Uttarakhand state, India. Various anthropogenic activities at these places may have a deleterious impact on water quality and river ecosystems. Thus, bio-assessment of River Bhagirathi and Yamuna at Gangotri and Yamunotri respectively at different locations was carried out using benthic macroinvertebrates as bio-indicators and the findings of study are presented. The research was conducted in the month of May, 2022 and the study revealed the presence of various taxonomic families such as Heptageniidae, Baetidae (order: Ephemeroptera), Perlidae, Perlolidae (order: Plecoptera) Blepharoceridae, Chironomidae (Order: Diptera) which are sensitive or resistant to the pollution. The proportion of Ephemeroptera and Plecoptera to the total number of organisms gives a decent picture of water quality at these locations of rivers. These groups are sensitive to pollution, prefer an unpolluted environment for survival, and thus indicate low organic contamination. Further, Jaccard similarity index was calculated to relate the biodiversity among sites.

The saprobic scores of Gangotri and Yamunotri indicates ‘Unpolluted to very lightly polluted’ and ‘Lightly polluted’ biological water quality class as per the criteria developed by Central Pollution Control Board.

Keywords Benthic macroinvertebrates · Saprobic score · Diversity · Jaccard coefficient

Introduction

River ecosystems have a high ecological importance (Nguyen et al. 2018) and the wellbeing of these ecosystems is crucial for human societies (Dickens et al. 2018). A variety of anthropogenic pressures have a great impact on freshwater ecosystems and affect freshwater availability (Allan 2004; Best and Darby 2020). In light of this, it is essential to periodically assess the water quality of various rivers. Aquatic species in comparison to environmental variables have been found more effective in ecological studies because the aquatic community, specifically, benthic macroinvertebrates indicates the health of streams and blends morphological and functional aspects (Bonada et al. 2006; He et al. 2020).

Freshwater ecosystems depend on benthic macroinvertebrates as a means of transferring energy from lower-level autotrophs to higher-level heterotrophs. Being less mobile, sensitive to contamination, and having such a life cycle that makes them good sensors of change in the aquatic environment (Smith et al. 1999; Shah and Shah 2013). Benthic macroinvertebrates are valuable bio-indicators because they are able to adapt to physical, chemical, and biological stimuli in their habitat during the entirety of their aquatic life cycle. Measures of community abundance reveal the amount of organic matter in the water body

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and when organic matter contamination rises, pollution-sensitive species are replaced by pollution-tolerant ones. In order to quantitatively assess macroinvertebrate species replacement, a number of bio-indices/biotic scoring systems have been developed in various countries. Such indices are used to evaluate the biological water quality of lotic systems. Trent biotic index, created by the Trent River Board in 1960, served as the foundation for the majority of contemporary biotic indices and scores. Due to certain limitations, biotic indices/scoring systems were revised and modified, leading to the development of systems like the Chandler scoring method and Belgian biotic index, etc.

In 1976, the Biological Monitoring Working Party (BMWP) created the most improved version of the biotic scoring system to overcome all prior constraints, and is one of the most popular bio-assessment indices. Since 1980, the regulatory authorities of the UK have utilized the BMWP scoring system as the foundation for classifying the status of rivers' invertebrate populations (Metcalf 1989). This index assigns sensitivity scores from 1 to 10 to each macroinvertebrate taxon according to their responses to oxygen deficits caused by organic pollution.

The BMWP scores for each taxon need to be standardized for each ecological region since the different geographical, ecological, and anthropogenic conditions promote diverse taxonomic compositions (Paisley et al. 2013). Therefore, to calibrate the BMWP scoring system for Indian Rivers, the Indo-Dutch project was carried out from 1985 to 1994 in a joint collaboration with the Indian Department of Environment and Forests and the Netherlands Department of International Cooperation (CPCB Newsletter, 1999). Based on the findings of the project, a Biological Water Quality Classification (BWQC) was established by the CPCB (Zwart and Trivedi 1995). Later, this system was reviewed and revised in 2020 as shown in Table 2.

Numerous perennial rivers originate in the Himalayas. The Bhagirathi River arises from the snout (Gaumukh) of the Gangotri glacier, situated in the Western Himalayas. River Yamuna rises from the Yamunotri Glacier in the Western Himalayas. Being the holy sites of the 'Char Dham Pilgrimage' Gangotri and Yamunotri have great religious significance. From April to November each year, a substantial number of people participate in both of these pilgrimages.

The current research aimed to evaluate the biological water quality of the Bhagirathi and Yamuna Rivers in the Himalayan region of Gangotri and Yamunotri. Benthic macroinvertebrates and water samples were collected for the examination of biological and biochemical characteristics of river water in the month of May 2022. The saprobic score was calculated using the modified BMWP score chart and the biological water quality class was defined as per BWQC provided by the Central Pollution Control Board, Delhi.

Materials and Methods

Study Area

The research was conducted on river Bhagirathi at Gangotri and river Yamuna at Yamunotri. The location coordinates are provided in Table 1.

River Bhagirathi at Gangotri

The sacred shrine of Gangotri is a small hamlet, centered around the Gangotri temple in the Uttarkashi District, Uttarakhand, at an altitude of 3048 m. The sampling location was situated approximately 350 m upstream of the Gangotri temple (30.993587 N, 78.945849 E) (Fig. 1). The width of the River Bhagirathi was estimated to be 60 m and the riverbed primarily consisted of rocks, cobbles, pebbles, gravels, and sand.

River Yamuna at Yamunotri

Yamunotri, a sacred site, is situated in the western Garhwal Himalayas at an altitude of 3293 m and is around 140 kms north of Uttarkashi District (30.998921 N, 78.461917 E). The sampling point was at the start of the Yamunotri trek and 4.5 kms downstream from the Yamunotri Temple (Fig. 2). The width of River Yamuna was around 20 m, and the riverbed contains stones, cobbles, pebbles, gravel, and silt.

Sample Collection and Preservation

Benthic Macroinvertebrates Sampling

Benthic macro-invertebrates sampling was conducted once in May, 2022, using procedure no.10500 B, APHA, 2017. The sampling devices were adopted according to the nature of the substratum. The substrate percentage of each sampling site was estimated visually and was dominated by boulders, cobbles, pebbles, gravel, sand, and silt. Accordingly, benthic macro-invertebrates were collected by brushing off stones or using soft forceps pins (Fig. 3). As per the CPCB field protocol, it was ensured that at least 50 no. of organisms must be collected from site. The sampled organisms were preserved in a 250 mL wide-mouth polyethylene bottle containing

Table 1 Global positioning system (GPS) location of sampling sites

S. no	Location	Latitude	Longitude
1	Gangotri	30.993587 N	78.945849 E
2	Yamunotri	30.998921 N	78.461917 E

Fig. 1 Geographical map of sampling point at Gangotri

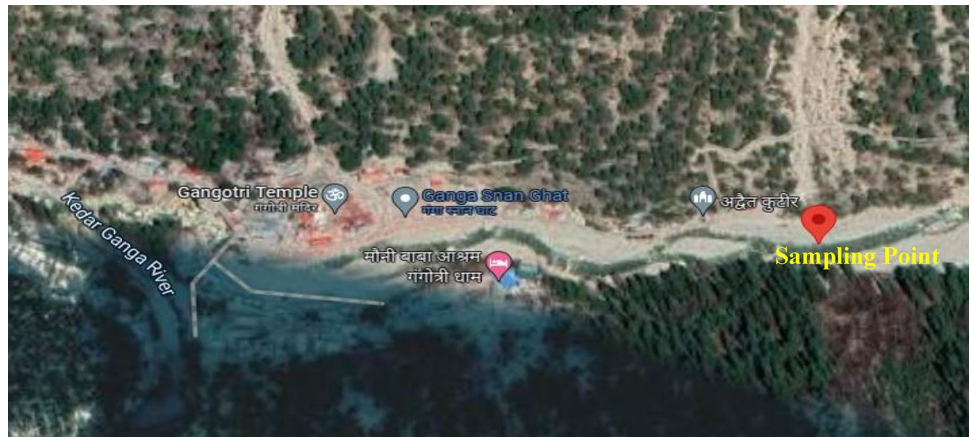


Fig. 2 Geographical map of sampling point at Yamunotri

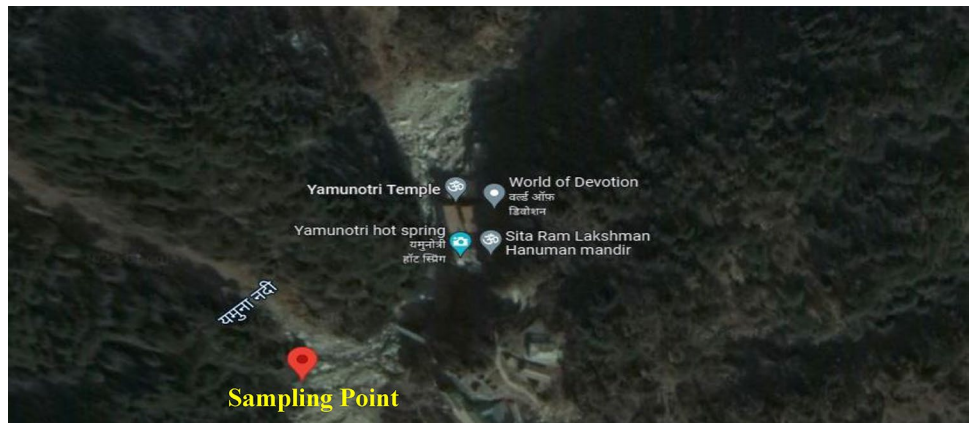


Fig. 3 Sample collection at **a** Gangotri and **b** Yamunotri



4% formalin solution and transported to the laboratory for identification.

Water Sample for Biochemical Variables

The river water samples were collected as described in the Standard Methods for Examination of Water and Waste Water, 23rd Edition of American Public Health Association

Table 2 Biological water quality criteria by CPCB

Saprobic score	Biological water quality class (BWQC)
7.0 or more	Unpolluted to very lightly polluted
5.0–6.9	Lightly polluted
3.0–4.9	Moderately polluted
1.1–2.9	Heavily polluted
1.0	Severely polluted

(APHA 2017) in 2000 ml and 500 ml narrow mouth polyethylene bottles to study biochemical parameters which were preserved in ice and ammonia samples were preserved with conc. H₂SO₄.

Sample Analysis

Identification of Benthic Macroinvertebrates

The benthic macroinvertebrates fauna was identified at the family level using a stereo-zoom microscope (Leica M80). Order Ephemeroptera, Plecoptera, and Diptera, taxa were identified using reference keys (Zwart and Trivedi 1995; Jessup et al. 2003; Graf et al. 2006; Akolkar et al. 2017).

Water Sample Analysis for Biochemical Parameters

Water temperature and Dissolved Oxygen (DO) were recorded in the field using a calibrated thermometer and a DO meter (HI98193-HANNA Instruments) respectively. Critical parameters like pH, Electrical Conductivity (EC), Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Ammonia, and Nitrate were analyzed using methods described in the Standard Methods for Examination of Water and Waste Water, 23rd Edition of American Public Health Association (APHA 2017).

Data Analysis

Jaccard's Index (J)

It is used to compare biodiversity levels among locations in terms of richness and not abundance. The formula is as given below:

$$J = 100 * \{Rc / (R1 + R2)\}$$

where Rc is the number of common families encountered between sites; (R1) = Total no. of families encountered at Gangotri and (R2) = Total no. of families encountered at Yamunotri (Jaccard 1908).

Saprobic Score

The saprobic score was calculated after the identification of benthic macroinvertebrates. This method involves a quantitative inventory of the presence of macro-invertebrate benthic fauna up to the family level of taxonomic precision. All the families of different order are classified on a score scale of 1–10 according to their preference for saprobity level. The families which are most sensitive to pollution are getting a score of 9 and 10 while the most pollution-tolerant families were getting a score of 1 and 2. The other intermediately sensitive families are placed in between the scoring scales of 3–8.

$$\text{Saprobic score} = \frac{\sum \text{BMWP score}}{\sum \text{Number of families encountered}}$$

Biological Water Quality Assessment

Biological water quality was determined using Biological Water Quality Class (BWQC) criteria which is based on the range of Saprobic Score values of taxonomic groups of benthic macro-invertebrate families with respect to water quality. The water quality is classified into five different classes from unpolluted to very lightly polluted class to severely polluted class with a corresponding Saprobic Score range from 1 to 10. Table 2.

Results and Discussion

The present study was carried out to evaluate the water quality of River Bhagirathi and Yamuna at Gangotri and Yamunotri respectively. Biological water quality was assessed based on data on aquatic macroinvertebrate families' composition and abundance while biochemical characteristics were based on the pH, water temperature, COD, BOD, DO, nitrate, and ammonia. The saprobity level of collected benthic macroinvertebrates toward organic pollutants was used to generate the saprobic score to define the biological water quality class.

According to the River Continuum Concept (RCC), a variation in biochemical characteristics along the river length leads to the functional difference in the benthic macroinvertebrate communities (Vannote et al. 1980). The nature of the stream bed (substrate type) is a major controlling factor among the various physical factors in the distribution of benthic macroinvertebrates. The availability of the substratum may restrict or increase the macroinvertebrate abilities to adhere, cling, build cases, lay eggs and protect from predators and water currents. (Minshall 1984). Figure 4 shows the

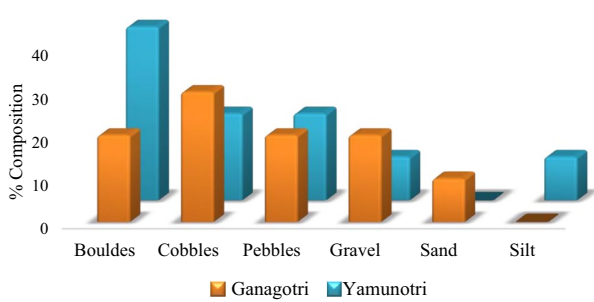


Fig. 4 Substratum composition (%) at Gangotri and Yamunotri

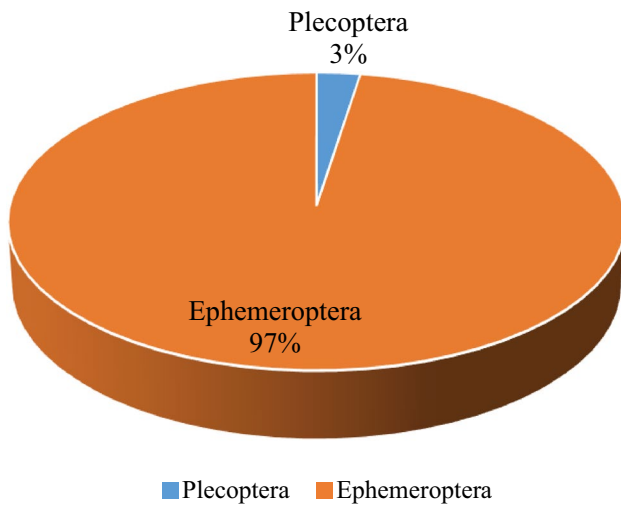
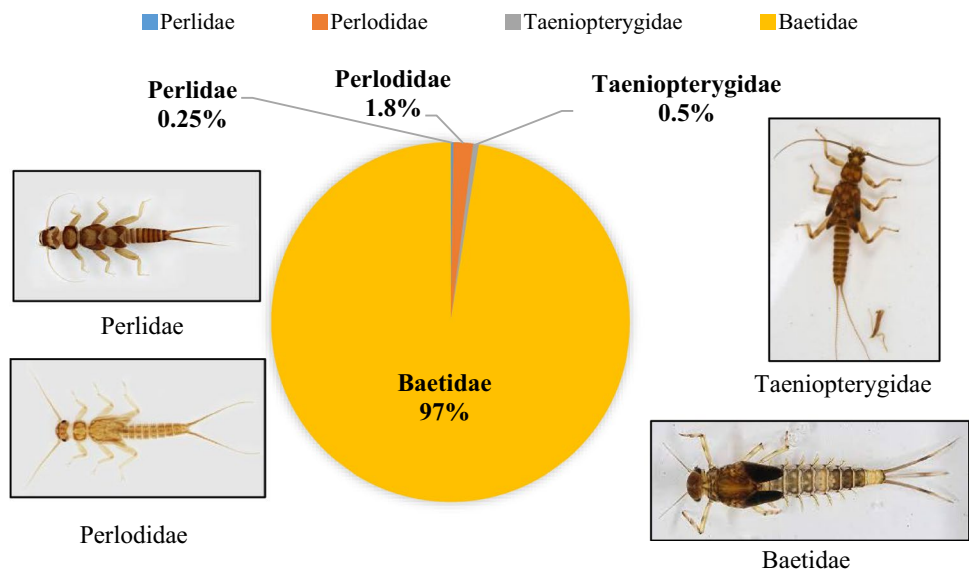


Fig. 5 % abundance of benthic macroinvertebrates' orders at Gangotri

Fig. 6 % abundance of benthic macroinvertebrates' families at Gangotri



substratum composition at Gangotri and Yamunotri locations. Boulders, cobbles, and pebbles were the main constituents of the substratum of riverbeds. At Gangotri, the riverbed contained approx. 20% boulders, 30% cobbles, 20% pebbles, 20% gravel, and very little sand 10%. Whereas at Yamunotri, the riverbed consisted mainly of approx. 40% boulders, 20% Cobbles, 20% pebbles, 10% gravels, and 10% silt. Such type of substratum provides a habitat for stoneflies and Mayflies larvae. Mayflies inhabit all aquatic habitats except for marine environments, polluted and underground waters.

At Gangotri, taxonomic orders that predominated were Ephemeroptera (97%) and Plecoptera (3%) (Fig. 5). Baetidae was the dominant family (97.5%) that were collected at this location, followed by Perlidae 0.25%, Perlodidae 1.75%, and Taeniopterygidae 0.5% dominance (Fig. 6). Research studies show that the sensitivity of Baetidae family differs on the generic level and many genera of this family have been described in clean or low organic matter environments (Kubendran et al. 2017). However, the Baetidae family has been given BMWP score of 4 which indicates that this group is moderately tolerant towards organic pollution. Nagell (1980) reported that Baetidae, *Cloeonipterum* (European) can survive relatively in low oxygen concentrations for a short period of time. Several species of Baetidae have been reported to move into a particular microhabitat for oxygen (Brittain and Nagell 1981).

A score-based assessment method, “HKHbios” was developed to evaluate the Ecological Status of Rivers in the Hindu Kush-Himalayan Region. In this method, different weightage scores were assigned to various genera of the Baetidae family for lowland and mountain regions. *Baetiella* and *Platybaetis* have been assigned a score of 8 for both mountain and lowland regions. Similarly, *Baetis* is given a

score of 8 and *Cleoninae* has been given a score of 5 for the lowland region. This shows that the Baetidae family must be scored according to the abundance of its different genera in a particular region (Hartmann and Moog 2010). Kubendran et al. have reported the presence of different genera of the Baetidae family in both the polluted and unpolluted reaches of the Rivers of the Southern Western Ghats of India. The genera *Cloeon bimaculatum* and *Procloeon regularum* were tolerant to organic pollution. Other genera such as *Cleon*, *Nigrobaetis*, *Labiobaetis*, *Indobaetis*, and *Baetis* sp. were described as a very sensitive genus of the Baetidae family (Kubendran et al. 2017). In order to confirm and record the presence of a specific genus of the Baetidae family in the

Himalayan region, the identification was extended up to the genus level. The genus was identified as *Labiobaetis* sp.

At Yamunotri, macroinvertebrate diversity was observed to be more in comparison to Gangotri. Ephemeroptera (96%), Diptera (3%), and Plecoptera (1%) were shown to be the three taxonomic orders that predominated at this location (Fig. 7). Heptageniidae family (90%) belonging to the order Ephemeroptera was in abundance, other families observed were Baetidae (6%), Chironomidae (2%), Perlodidae (1%) and Blepharoceridae (1%) were abundant of the total population (Fig. 8). Ephemeroptera larvae usually occur everywhere in water bodies, ranging from slow to fast flowing water and fresh to brackish water (Alhejoj et al. 2014). Both Gangotri and Yamunotri were found to be suitable for the Ephemeroptera order, which is the most important group of insects due to their sensitivity and abundance in a wide variety of habitats (Nerbonne and Vondracek 2001). The comparison in BMWP scores of taxonomic families encountered at both locations is described in Table 3.

The term “*Saprobia*” means the dependence of an organism on decomposing organic substances as a food source. The detailed calculation of the saprobic score is shown in Table 4. The saprobic scores calculated for Gangotri and Yamunotri were 8.5 and 6.2 respectively. This indicates the ‘None to very lightly polluted’ biological water quality class at Gangotri and the ‘lightly polluted’ biological water quality class at Yamunotri as presented in Table 5. Anthropogenic activities like open defecation and bathing were witnessed at the sampling site of Yamunotri which is clearly associated with the presence of tolerant species of Diptera order families in River Yamuna at Yamunotri. This resulted in a low saprobic score at Yamunotri as compared to Gangotri. The abundance of pollution-sensitive taxa (Ephemeroptera and Plecoptera) at both sampling sites indicated good water quality.

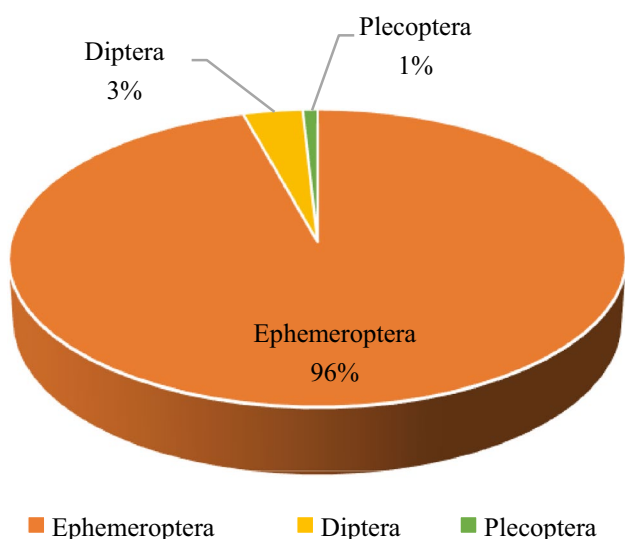


Fig. 7 % abundance of benthic macroinvertebrates' orders at Yamunotri

Fig. 8 % abundance of benthic macroinvertebrates' families at Yamunotri

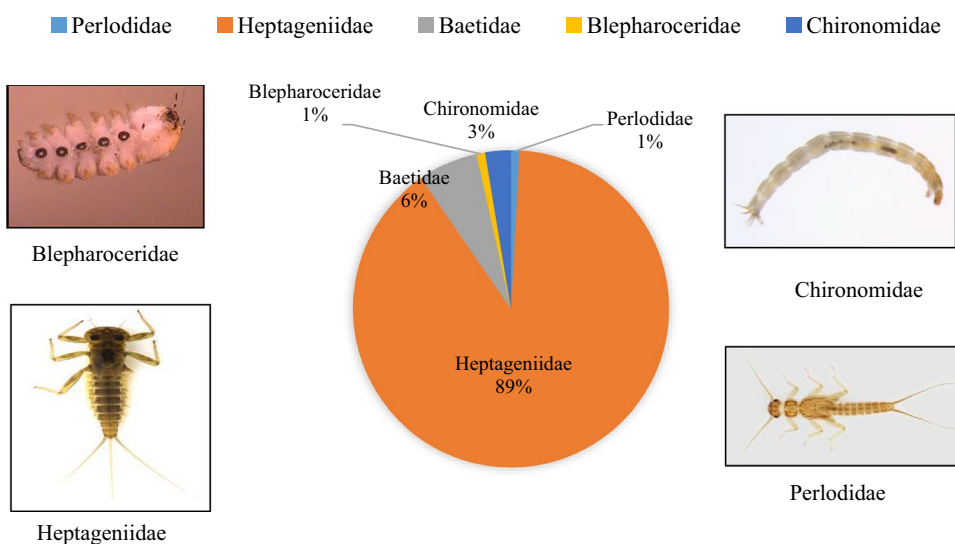


Table 3 Comparison of taxonomic families

Taxonomic families	Locations		BMWP score	Sensitive/tolerant
	Gangotri	Yamunotri		
Perlidae	+	–	10	Sensitive
Perlodidae	+	+	10	Sensitive
Taenaopterigidae	+	–	10	Sensitive
Heptageniidae	–	+	10	Sensitive
Blepharoceridae	–	+	5	Moderate
Baetidae	+	+	4	Moderate
Chironomidae	–	+	2	Tolerant

Table 4 Calculation of saprobic score

Taxonomical group	Taxonomical families	Mark encountered families (within families also mark abundance as—A, B, C, D, E)*	Total families	BMWP Score	Score Multiplied
<i>Location: River Bhagirathi, Gangotri</i>					
Plecoptera	Perlidae	A	3	3*10	30
	Perlodidae	B			
	Taenaopterigidae	B			
Ephemeroptera	Baetidae	E	1	1*4	4
Grand total families encountered and grand total multiplied score			4		34
<i>Location: River Yamuna, Yamunotri</i>					
Plecoptera	Perlodidae	B	1	1*10	10
Ephemeroptera	Heptageniidae	E	1	1*10	10
Ephemeroptera	Baetidae	C	1	1*4	4
Diptera	Blepharoceridae	B	1	1*5	5
Diptera	Chironomidae	B	1	1*2	2
Grand total families encountered and grand total multiplied score			5		31

Saprobic score is calculated by dividing total families and multiplied score

*Abundance scale of families: A = single (one individual), B = scarce (2–10 individuals), C = common (10–50 individuals), D = abundant (50–100 individuals), E = excessive (more than 100 individuals)

#The score is assigned as per BMWP score system designed by CPCB

Table 5 Biological water quality of rivers Bhagirathi and Yamuna

Biological water quality	Gangotri	Yamunotri
Saprobic score	8.5	6.2
Biological water quality class	A	B
Water quality	Very good	Good

In order to relate the diversity of benthic fauna at Gangotri and Yamunotri, Jaccard's index was calculated. It showed that there is a 22.22% similarity between them in Table 6. Both places shared the Perlodidae and Baetidae families. Due to the existence of silt and more organic contamination at the site of Yamunotri, the Jaccard coefficient revealed less similarity among the sampling locations in terms of the biodiversity of aquatic families.

Alterations in biochemical variables have a substantial impact on the distribution, periodicity, quantitative

Table 6 Jaccard's similarity index values

Locations	Distance (kms)	Jaccard similarity index (%)
Gangotri and Yamunotri	227	22.22

and qualitative composition of biota (Sharma et al. 2016). Hence, various biochemical parameters (pH, temperature, conductivity, nitrates, DO, COD, BOD) were analyzed (Table 7). The biochemical data provides only the momentary account of water quality, i.e., the quality that prevails at a particular time of monitoring. On the contrary, biological monitoring has a much longer dimension and represents long term environmental conditions (Akolkar et al. 2017).

The pH of Rivers Bhagirathi and Yamuna was 7.1 and 7.5 respectively, indicating that Yamuna river water was near neutral. Courtney and Clements (1998) studied the response

Table 7 Comparison of physio-chemical parameters

Parameters	Gangotri	Yamunotri
pH	7.1	7.5
Temperature (°C)	5.0	5.5
Electrical conductivity (µmho/cm)	111	137
Dissolved oxygen (mg/l)	8.7	8.4
BOD (mg/l)	<1.0	<1.0
COD (mg/l)	10.0	<5.0
Ammonia (mg/l)	<0.2	<0.2
Nitrate (mg/l)	0.3	0.4

of pH on benthic macroinvertebrate communities and found that communities responded to variations in pH levels. In another study, Bernard (1985) showed that the Ephemeroptera group had a survival rate of more than 95% when held in near-neutral water pH for 24 h. Similarly, the same effect of pH has been also reported by Fjellheim and Raddum (1990).

The temperature at Gangotri and Yamunotri were observed to be low i.e. 5 °C and 5.5 °C respectively (Table 7). Imtiyaz et al. (2013) reported that the Ephemeroptera group is positively correlated with temperature. Changes in water temperature can eliminate necessary life-cycle thresholds, cause the extinction of macroinvertebrates, and can impact on fecundity (Sartori and Brittain 2015).

Electrical Conductivity at Gangotri and Yamunotri river water was recorded at 111 µmho/cm and 137 µmho/cm respectively (Table 6). The effect of salinity is one approach to investigating toxicity on aquatic fauna in terms of electrical conductivity. This approach is advantageous because the sources of salinity and other pollutants result in greater covariance with electrical conductivity (Williams and Williams 1991; Bacher and Garnham 1992).

Chemical characteristics of both the locations exhibit almost similar water quality except for COD values near Gangotri. Intermittent rain conditions were prevailing at the time of sampling in Yamunotri which may be one of the conditions for dilution in various chemical parameters.

At Gangotri, COD and Nitrate were observed to be 10 mg/l and 0.3 mg/l respectively, whereas at Yamunotri, COD was measured below the detection limit (5 mg/l) and Nitrate was found to be 0.4 mg/l (Table 7). For both the sampling locations BOD and Ammonia were measured below the detection limits (1 mg/l and 0.2 mg/l) respectively. A slight variation in DO was observed at Gangotri (8.7 mg/l) and Yamunotri (8.4 mg/l). Bate and Sam (2019), reported that all macroinvertebrate classes had a positive correlation with dissolved oxygen (DO). Ephemeroptera, Plecoptera, Trichoptera, and Odonata (EPTO) orders are influenced by DO and have a significant positive correlation (Tampo et al. 2021). Ephemeroptera, Plecoptera, Trichoptera, and Odonata (EPTO) groups are distinguished as indicator taxa with

water quality parameters which is sensitive to the decrease in DO and to the increase of BOD, COD, TSS, and NH₄ (Tampo et al. 2021).

Conclusion

The present research shows that, both the sampling sites have similar benthic macroinvertebrate communities except for the diptera group and provide a suitable habitat for the growth of pollution-sensitive families. Ephemeroptera (97%) and Plecoptera (3%) were the prevalent orders at Gangotri, while Ephemeroptera (96%), Diptera (3%), and Plecoptera (1%) were the dominant orders at Yamunotri.

Taxonomic families such as Perlidae, Perlodidae, Taeniopterygidae and Heptagenidae with BMWP scores of 10 were present at Gangotri and Yamunotri. These pollution-sensitive families indicate good water quality that correlates with higher concentrations of DO and low amounts of BOD, COD, Nitrate, and Ammonia at these sites.

The pollution-tolerant family, Chironomidae (BMWP score 2) which indicates existence of biodegradable organic matter at Yamunotri. The presence of human and animal excreta act as a food source for aquatic insects of tolerant groups and one of the major contributing factors of organic contamination at Yamunotri.

Jaccard's index was calculated to compare the taxonomic richness across sampling sites which revealed less similarity in macroinvertebrate diversity. This might be due to the differences in substratum composition and availability of diverse food resources.

Water quality of both locations' falls under the 'A' class category of the CPCB-developed Designated Best Use Water Quality Criteria, which is based on physicochemical characteristics. Class 'A' signifies drinking water sources without conventional treatment but after disinfection. While as per BWQC, the biological water quality of Gangotri and Yamunotri falls under I and II class i.e., 'Unpolluted to very lightly polluted' and 'Lightly polluted' biological water quality class respectively. Physical and chemical constituents fluctuate with environmental conditions such as seasonal variation, changes in flow etc. and thus, provide a short term information of pollution. While bio-monitoring detects the long term impact of different stressors on biological health of ecosystem.

This study presents the most recent status of the variety of aquatic insects and offers a database that will be helpful for future research. Further, research may be extended to study the pollution sensitivity of different genus of Baetidae to revise the sensitivity score. As a future prospect, research may be carried out to study the seasonal variation in biodiversity and water quality of rivers in the Himalayan regions.

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Author Contributions YY and SR contributed for sample collection. JS and SR processed the experimental data, performed the analysis, drafted the manuscript and designed the figures. HJ and YY reviewed and edited the manuscript. VKS encouraged to investigate the research findings and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

Declarations

Conflict of interest The authors declare that they have no competing interests.

Consent for Publication All the authors of the manuscript have unanimously agreed and given their consent to publish this article to your journal.

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