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Research Article

ASSESSMENT OF RIVER WATER QUALITY USING BENTHIC MACROINVERTEBRATES AS BIOINDICATORS OF HANUMANTE RIVER, KATHMANDU VALLEY, NEPAL

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ABSTRACT

Benthic macroinvertebrates play a significant role in assessing the water quality of aquatic ecosystems. This study was conducted to assess the river water quality of the Hanumante River situated at Kathmandu Valley, Nepal using benthic macroinvertebrates as bioindicators. Altogether seventeen sites were monitored following a globally recognized multi-habitat sampling along the Hanumante River from Muhanpokhari to Narephant (Jadibuti) using a standard hand net with a frame width of 25 × 25 cm^2, metallic frame with 500 µm mesh size. The collected macroinvertebrates were identified at the family level. A total of 10 orders, 33 families, and 4419 total individuals of macroinvertebrates were observed. Among all, the family Chironomidae (red) under order Diptera were the highest in number. The results showed that upstream sites were characterized by high taxa richness, abundance, and presence of pollution-sensitive taxa such as Ephemeroptera, Plecoptera, and Trichoptera, while Chironomidae, Tubificidae, and Simuliidae dominated downstream. The obtained results reflected that the water quality of the river in the upstream sites was comparatively better compared to the downstream. The water quality was deteriorating due to increasing human disturbances in the river, which demands the regular monitoring of water quality and aquatic biodiversity in the river.

Keywords: Benthic macroinvertebrates, bioindicators, ecological health, Hanumante River

INTRODUCTION

River biota are specialized to live with the flow conditions, and the river ecosystem has its own naturally balanced ecological health that continuously changes its physical state and has a high degree of spatial and temporal heterogeneity (Yadav *et al.*, 2015). The river has both, natural and social attributes each contributing to the overall health of water bodies (Rai *et al.*, 2019). In recent decades population growth coupled with an elevated standard of living have greatly contributed to the increasing demand for clean water (Sakhare & Kamble, 2014). Indiscriminate dumping of industrial and household wastes into streams and rivers is one of the main problems for freshwater deterioration and is now becoming a global problem at both temporal and spatial scales (Cock *et al.*, 2021). In addition to this severe river pollution has increased due to the discharge of organic wastes human excreta, sewage, polyethylene, municipal garbage, and toxic discharge from the factories (Sakhare & Kamble, 2014). The use of bio-indicators allows for the assessment of the natural state of a particular region and the determination of the extent of anthropogenic disturbances or environmental changes, such as pollution levels and ecosystem stress (Khatri & Tyagi, 2015). Among all others, benthic macroinvertebrates (BMI) are ideal biological indicators because they are found abundantly in water and long-lived compared to other organisms, possess varying tolerance to perturbations in streams, and are much more cost-effective (Wallace & Webster, 1996). The small animals like insects, mollusks, arachnids, and annelids that are retained on a 0.25 mm mesh net which do not have a backbone but can be seen with the naked eye are generally referred to as macroinvertebrates and are mostly found in rivers, lakes, streams, wetlands and other water bodies (Water and Rivers Commission, 2001). The use of such aquatic organisms to assess the water quality is not a new approach, it has been widely used since a long time ago (Cairns & Pratt, 1993) and biomonitoring of aquatic bodies is now well established with such an approach throughout the world. From the molecular to ecosystem level, macroinvertebrates have been used to evaluate the effects of various stressors including anthropogenic disturbances. (Rosenberg & Resh, 1993). Thus, to assess the ecological health of an aquatic environment, naturally occurring bio-indicators are commonly used (Parmar *et al.*, 2016). The application of bioindicators is widely applied to predict the natural state or the level/degree of a certain region (Khatri & Tyagi, 2015). Bioindicators can also detect changes in the environment due to the presence of pollutants which can affect the composition, structure and the biodiversity as a whole (Holt & Miller, 2011).

Many metrics, including total richness (the total number of species, genera, and families), or the richness of particular groups of taxa, particularly Ephemeroptera, Plecoptera, and Trichoptera (also known as EPT), are diagnostic of various environmental impacts (Carter & Resh, 2013; Lenat, 1988). The composition of different tolerant species like Chironomidae and intolerant (like EPT) taxonomic groupings has also been used to assess both natural and anthropogenic causes, in addition to richness measurements. These measures are frequently combined to create a multi-metric index (MMI) (Carter *et al.*, 2017). Each taxon of benthic macroinvertebrates is assigned a tolerance score, which serves as a biotic measure or index for evaluating the ecological river quality based on pollution tolerance gradients (Tachamo Shah & Shah, 2013). In nature, there are certain factors that govern the presence of bioindicators in the environment such as the transmission of light, water, temperature, and suspended solids that act on many spatiotemporal scales (Parmar *et al.*, 2016). Additionally, physicochemical parameters, hydrological regimes, land use patterns, habitat types, sediment properties, biotic interaction, etc. are a few of these variables (Rai *et al.*, 2019). This complex interaction makes it difficult to characterize the individual effects of each factor (Rempel *et al.*, 2000). Their relative contributions have barely been quantified (Peeters *et al.*, 2004) and despite their recognition, there is a dearth of knowledge on the relative contributions of these factors in influencing the benthic macroinvertebrate assemblages.

The index for biological assessment of water quality is an integrated assessment by monitoring habitat conditions, water quality, and organisms living in the water. The principle is that the summation of the quality of both habitat and water can reflect the community structure of the organisms (Tachamo Shah *et al.*, 2011). Hanumante River is important from a cultural, religious, ecological, and economic perspective to the residents of Bhaktapur and the surrounding area. But the river has now essentially become less suitable for any use. Thus, the present study has evaluated the current water quality status and level of pollution in the Hanumante River using benthic macroinvertebrates as bio-indicators.

MATERIALS AND METHODS

Study Area

The present study was carried out in Hanumante River, one of the tributaries of the Bagmati River (Fig. 1) which originates from Mahadev Pokhari at Nagarkot (Mahabharat Hill) and passes through Bhaktapur and Thimi municipalities (Sada, 2012). The river has a total catchment area of 143 km^2 and the elevation ranges from 1300 to 2191 masl. The river is nearly dry in certain places prior to the monsoon while becoming wide and speedily flowing during the monsoon with water levels up to two to five meters (Sada, 2012). The average river width decreased from 6 m in 1964 to 2 m in recent times (Kindermann *et al.*, 2020). Due to the significant increase in urban built-up areas and the sharp decline in forested and agricultural areas between 1988 and 2015, the land use pattern of Bhaktapur district has undergone significant change (Kindermann *et al.*, 2020). The Hanumante River is well known for its ecological, cultural, and religious significance among Hindus, and is particularly vulnerable to the impacts of urbanization, such as increased pollution, habitat degradation, and altered water flow patterns, making its continuous quality assessment crucial for maintaining its ecological functions and safeguarding its cultural heritage.

Sampling Sites

To minimize potential bias, we adopted a systematic sampling approach for sample collection. A total of seventeen sites were selected along the Hanumante River [from Muhanpokhari to Narephant (Jadibuti)] with samples collected at regular intervals of 1 km. The details of all the sampling sites along with their habitat type are presented in Table 1.

Sampling Approach

Multi-Habitat sampling, a globally recognized, standardized sampling method for biomonitoring (Moog, 2007), was adopted for the collection of samples from the Hanumante River. This is the representative sampling of all major habitats that can only be applied at wade-able river sections. At all sampling sites, 20 sub-sample units were taken within a 100 m river stretch using a hand net of 25 cm × 25 cm metallic frame with a mesh size of 500 µm. Overall, each benthic sample covered an area of 1.25 m^2. The net was placed against water flow and habitat/substrates were rigorously scrolled for a minute from a maximum depth of 5 cm. The drifted macroinvertebrates then passed through the sampling net and finally transferred to a sampling container. The sample was collected in a white tray and large cobbles, stone, wood, leaves, and litter were washed up and removed by placing the entire collected sample in a bucket. Lastly, the collected sample was kept in a vial with the addition of a standard preservative (99% ethanol). The samples were labeled well, and further analysis was carried out in the Central Department of Environmental Science. Laboratory In the laboratory, the samples were washed, sieved, sorted, and identified to family level based on available literature (Dudgeon, 1999; Nesemann *et al.*, 2007, 2011; Tachamo Shah *et al.*, 2020) with the help of a stereo microscope.

Data Analysis

Diversity indices such as Shannon diversity (Shannon & Weaver, 1949), Simpson diversity (Simpson, 1949), Pielou's evenness (Pielou, 1966) and Margalef's index (Margalef, 1958) were calculated. Total taxa richness, total abundance, Ephemeroptera, Plecoptera, and Trichoptera (EPT) richness and abundance and sensitive measures: sensitive and tolerant taxa richness were also determined. The water quality classification of the Hanumante River was carried out based on biotic index value, a taxa on sensitive score to pollution or disturbances in a river. The score list includes sensitive scores for 158 macroinvertebrates taxa of family, subfamily and genus. The biotic score is the modified version of Nepalese's Biotic Score (NEPBIOS, Sharma, 1996) and Ganga River System Biotic Score (GRSBIOS, Nesemann, 2006). The equation for the biotic index is presented below.

$$\text{Biotic index} = \frac{\sum_{i=1}^{n} \text{TSS (i)}}{n}$$

Where, TSS, is the taxa-sensitive score of taxa i and n is the total number of taxa scored

To determine the river quality class of a site with respect to mountain rivers, the obtained biotic index value for each site was compared with the transformation scales (Table 2; Tachamo Shah *et al.*, 2020). The obtained data were analyzed using PASW Statistic 26 and ArcGIS 10.8.

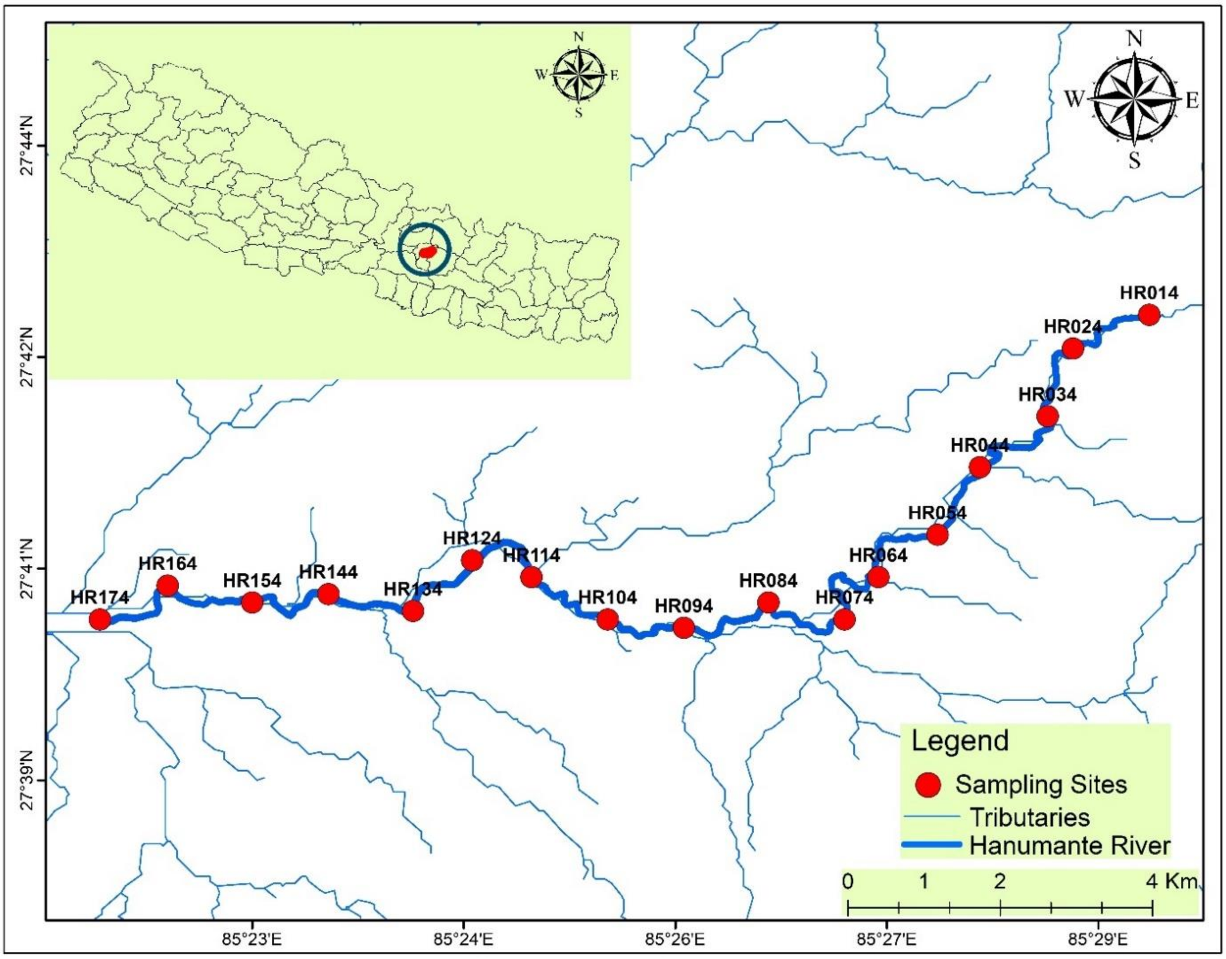

Figure 1. Map showing the study area along with sampling sites

Table 1. Geographic locations and descriptions of the sampling sites

Site Code	Latitude	Longitude	Elevation (m)	Description of the sampling sites
Muhan Pokhari (HR014)	27.70510556	85.48116667	1415	Site rich in vegetation diversity, both banks of the site with feeder road. Site dominant with boulders, pebbles and sand.
Tathali (HR024)	27.70106389	85.47223056	1360	Bamboo dominant in the site, agricultural land on left side of the site. Site dominant with boulders, pebbles, sand and mud.
Tathali (HR034)	27.69259722	85.46896111	1293	Agricultural land on both sides of the site. Site dominant with pebbles, sand and silt. Human disturbances low.
Tathali (HR044)	27.68678056	85.46128889	1274	Agricultural land on both sides of the site. Vegetation abundance high with dense crown cover. Site dominant with pebbles, sand and silt, and abundant leaf litter.
Tathali (HR054)	27.67925556	85.45578056	1314	Site near to the feeder road, human disturbance moderate. Site dominant with pebbles, sand and clay.
Tathali (HR064)	27.67444444	85.44850833	1273	Site near to feeder road, human disturbance moderate. Site dominant with pebbles, sand and clay.
Liwali (HR074)	27.66949444	85.44493333	1311	Left side of the site near the human settlement and the right-side open space, human disturbance moderate. Site dominant with pebbles, sand and clay.
Hanuman Ghat (HR084)	27.67090556	85.43584722	1302	Both sides of the site disturbed by the human activities. Site with crematoria activities. Site dominant with sand, silt and clay.
Near to Suryabinayak (HR094)	27.66833056	85.42630556	1289	Site disturbed by human activities. Right side of the site with agricultural land and the left side with

				human settlement. Site dominant with sand, silt and clay.
Near Sainik School **(**HR104)	27.66894722	85.41665000	1292	Site disturbed by human activities, both banks of the site with agricultural land. Open dumping of wastes near the site. Site dominant with sand, clay and silt.
Tinkune (HR114)	27.67366944	85.40810556	1304	Human settlement observed on the right side of the site, with left side open space, vegetation absent. Site dominant with sand, silt and clay.
Between Radheradhe and Tinkune **(**HR124)	27.67583056	85.40145833	1237	Human disturbances high. Open dumping of wastes, vegetation absent. Dominant proportion of silt and clay.
Near Radhe Radhe **(**HR134)	27.67026944	85.39351944	1246	A tributary mixed at this site. Open dumping of wastes, vegetation absent. Proportion of silt and clay high.
Between Thimi and Chardobato (HR144)	27.67187222	85.38363056	1246	Human disturbances high, mixing of sewerage systems. Both sides of the site with of the road - also known as Hanumante Corridor. Site dominant with sand and clay.
Near Kaushaltar (HR154)	27.67120556	85.37458889	1241	High proportion of sand, silt and clay. Household and commercial wastes observed.
Confluence of Hanumante and Godawari Khola (HR164)	27.67298611	85.36503611	1252	Open dumping of wastes, vegetation absent. Silt and clay in high proportion.
Near Confluence of Hanumante and Manohara (HR174)	27.66855278	85.35703056	1242	Open dumping of wastes, vegetation absent. Site with high proportion of silt and clay.

*****(Code used for sites: HR=Hanumante River, 01= sampling point, and 4= sampling month of year i.e., winter)

Table 2. Transformation scale for river quality classification

Biotic index for Mountain	Biotic index for Lowland Tarai	River quality class	Status	Color band
7.00-10.00	6.50-10.00	I	High	Blue
5.51-6.99	5.00-6.49	II	Good	Green
4.51-5.50	4.00-4.99	III	Fair	Yellow
3.51-4.50	2.50-3.99	IV	Poor	Orange
1.00-3.50	1.01-2.49	V	Bad	Red

(Tachamo Shah *et al.*, 2020)

RESULTS AND DISCUSSION

Distribution of Benthic Macroinvertebrates

In the present study, a total of 10 Orders, 33 Families, and 4419 individuals of benthic macroinvertebrates were found in the Hanumante River (Table 3). Among all orders, the order Diptera has the highest total number of individuals followed by orders Ephemeroptera, Trichoptera, Clitellata, Coleoptera, and Odonata. Orders like Mollusca, Megaloptera, and Plecoptera have a moderate number of individuals whereas Hemiptera has the least number of individuals. Similarly, the family Chironomidae (red) was found in the highest number followed by Baitidae, Hydropsychidae, Tubificidae, Chironomidae (not red), Simuliidae, and Goeridae. Families like Polycentropopidae, Glossomatidae, Lepidostomatidae, Lymnaeidae, Aphelocheiridae, Libellulidae, and Rhyacophilidae were recorded in the very least number whereas the remaining families have few to moderate numbers of individuals (Table 3). The availability of freshwater is changing due to human influence (Rodell *et al.*, 2018). Among all freshwater environments, streams and rivers are most influenced and threatened by a range of anthropogenic stresses (Allan, 2004). Aquatic macroinvertebrates are an important part of river components that are most sensitive to anthropogenic pressure (Agboola *et al.*, 2020; Ko *et al.*, 2020). The use of macroinvertebrates response to determine the change in aquatic ecosystem is a universally recognized method and has been widely used to monitor ecosystem integrity and decision-making process (Edegbene *et al.*, 2021; Lallébila *et al.*, 2015). The results of the present study show that the Family Chironomidae were abundant in almost all sampling sites which belong to order Diptera. The human disturbances were found to be high with the disposal of household waste, agricultural effluent, industrial effluent, and urban runoff in most of the sampling sites of the Hanumante River. Worldwide, Chironomidae has been widely used for monitoring the changes in freshwater environments (Nicacio & Juen, 2015) mainly because their distribution responds rapidly to the variation in air temperature, oxygen concentration, salinity, nutrients, pollution, etc. (Velle *et al.*, 2010; Walker *et al.*, 1991). Chironomidae is the representative of a wide range of pollution tolerance taxa (Ruffer, 2006). Chironomidae are also considered the most diverse and abundant group that is found almost in all streams (Yule & Sen, 2004). The presence of Chironomidae indicates that the water is polluted, i.e., inappropriate for any domestic purpose (Pradhan, 2005). According to Padmanabha and Belagali (2007), water pollution can cause a decline in the richness and abundance of the Ephemeropteran population. On the other hand, EPT richness and composition are affected by the availability of suitable habitats. However, the present study found moderate pollution-sensitive Ephemeropteran and Trichoptera family, Baetidae and Hydropsychidae had their highest densities. The present observation corroborates with the findings of Berisa *et al.*, (2019) and Buss *et al.* (2002) who reported that Baetidae and Hydropsychidae are tolerant to the adverse conditions that cause an increment in its population in relation to other taxa.

Species Richness and Abundance

The taxa richness in the studied sites of the Hanumante River ranged from 2-17. The higher taxa richness was found in site HR024 (17) followed by site HR034 (14), HR054 (10), HR064 (9), HR014 (7), and HR044 (6). The site HR094 (2) has the least species richness among all sites. The result indicates that the species richness was found to be high upstream of the Hanumante River (Fig. 2). Generally, higher species richness and abundance could be found in natural pristine rivers (Barbour *et al.*, 1999). A range of environmental variables such as hydrology, nutrient concentration, temperature, dissolved oxygen, pH, etc. affects taxa richness and diversity (Zhuang, 2016). According to Norris (1993), the overall trend of reduced species richness and shift from sensitive to tolerant species is an indication of multiple stressors. As the river enters the urban region the species richness was found to be decreasing which can be seen with decreased species richness value from site HR074 to HR174 that falls under the core urban region of Bhaktapur. Factors like geographical factors such as available species pool and dispersal pattern, biotic factors such as competition, predation, pathogens, parasites, etc., and abiotic factors also affect the richness of macroinvertebrate species (Brown *et al.*, 2007). A decrease in species richness in response to disturbance is reported by a previous study (Barbour *et al.*, 1996). In the case of total abundance, higher abundance was found along upstream sites of the river such as HR054 followed by HR024, HR064, HR034, HR044, HR074, HR084, HR104, HR134, HR174, HR014, HR154, HR114, HR094, HR144, HR164 and HR124 (Fig. 3). Direct discharge of untreated domestic and industrials wastes along with wastewater discharge are the main reasons responsible for degraded river water quality (Haack & Rafter, 2006). The abundance and distribution of species are highly dependent on water chemistry variables or trophic status (Brodersen *et al.*, 1998). Thus, various species can sufficiently represent the water quality of a stream or river. The presence of more species richness and total abundance shows less pollution upstream than downstream sites of the Hanumante River.

EPT Composition and Sensitive Measures

EPT are mainly considered a significant water quality indicator due to their sensitive nature to water pollution. Their presence in the water indicates a healthy river. In this study, higher EPT taxa were found in sites HR014, HR024, and HR054. Sites HR034, HR044, HR064, HR124, and HR154 have only Ephemeroptera and Trichoptera taxa richness whereas sites HR074 and HR084 have only Ephemeroptera richness. Similarly, sites HR094 to HR174 except sites HR124 and HR154 have only Trichoptera richness. In the case of total abundance, higher abundance was found in site HR054 and least abundance was found in site HR104 (Fig. 5). From the study, it can be ascertained that moving towards the downstream of the river, EPT taxa richness was found to be decreasing (Fig. 4). In general, Oligochaetes, Dipterans, and Gastropods are more common in a polluted river, on the other hand, Ephemeropterons, Trichopterons, Plecopterons, and Odonates are found in pure water (Dhakal, 2006). In the present study, pollutant-tolerant organisms were found to be more dominant since order Diptera have high density. The occurrence of a few Plecoptera and Ephemeroptera shows a higher degree of pollution downstream than upstream part of the river. Reduced richness and abundance of Ephemeroptera from site HR084 to HR174 except for HR124 and HR154 and Plecoptera from site HR024 to HR174 except HR054 can be associated with stressors; increase in agricultural activities, waste dumping, and industrial wastes (Berisa *et al.*, 2019). These results correspond to the previous study (Berisa *et al.*, 2019), which also reported having a lower diversity of benthic macroinvertebrates at polluted sites of the water bodies. Taxa having a tolerance score equal to or greater than 7 are categorized as sensitive taxa. Such taxa are very sensitive to pollution and any disturbances in the natural habitat and their numbers decline with increasing pollution either in the lake or river environment. Taxa that are tolerant to moderate pollution have tolerance scores between 4 and 6. These taxa are adaptive to nutrient enrichment and perturbation. Similarly, tolerant taxa have pollution tolerance scores between 1 and 3. These taxa can survive in highly deteriorated environments even with lower oxygen concentration than 4 mg/L (Tachamo Shah *et al.*, 2020). Comparatively, the richness of all sensitive, moderate and tolerant species was found to be high in the upstream of the river. The taxa richness found to be declined at, the downstream sites of the river due to clumped urbanization and hence increase in river pollution. Overall moderate taxa were found to be high in number (Fig. 6).

Table 3. Distribution and average density of Benthic macroinvertebrates at the study sites of Hanumante River (ind./m²)

Order/Class	**Family**	**Density (ind./m²)**																
		HR -014	**HR-024**	**HR-034**	**HR-044**	**HR-054**	**HR-064**	**HR-074**	**HR-084**	**HR-094**	**HR-104**	**HR-114**	**HR-124**	**HR-134**	**HR-144**	**HR -154**	**HR -164**	**HR -174**
Diptera	Tipulidae	3.2	2.4	0	0	0.8	0	0	0	0	0	0	0	0	0	0	0	0
	Tabanidae	3.2	0.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Chironomidae (not red)	0	117.6	68.8	0	0	8.8	0	0	0	4	0	0	0	0	23.2	5.6	34.4
	Chironomidae (red)	0	13.4	67.2	176.8	268.8	24	168	82.4	34.4	0	0	12.8	8.8	1.6	0	0	0
	Simuliidae	0	147.2	83.2	0.8	0	0	0	0	0	1.6	0	0	0	0	0	0	0
	Ceratopogonidae	0	0	0	0	0	0	0	0	0	0.8	2.4	0	4.8	2.4	0	0	0
	Muscidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15.2	13.6	31.2
Ephemeroptera	Baetidae	59.2	155.2	128	12	128.8	14.8	5.6	0	0	0	0	0	0	0	18.4	0	0
	Neoephemeridae	0	1.6	0	0.8	0	2.4	0	0	0	0	0	0	0	0	0	0	0
	Heptageniidae (Iron sp.)	0	4.8	13.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Ephemerellidae	0	0	4.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Ephemeridae	0	0	6.4	0	4.8	0	0	0	0	0	0	0	0	0	0	0	0
	Ameletidae	0	0	0	0	4.8	1.4	2.4	1.6	0	0	0	0.8	0	0	0	0	0
Megaloptera	Corydalidae	1.6	1.6	1.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Plecoptera	Perlidae	1.6	1.6	0	0	1.6	0	0	0	0	0	0	0	0	0	0	0	0
Trichoptera	Hydropsychidae	2.4	13.6	69.6	4.8	265.6	22.4	0	0	0	3.2	0	0	0	0	0	0	0
	Glossomatidae	0.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Lepidostomatidae	0	0.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Leptoceridae	0	22.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Rhyacophilidae	0	2.4	0.8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Limnephilidae	0	0	0	0	0	0.8	0	0	0	0	0	0	0	0	0	0	0
	Goeridae	0	0	0	0	0	0	0	0	4.8	0	2	6.4	0	29.6	14.4	1.4	16
	Polycentropopidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0.8	0	0	0
Coleoptera	Hydrophilidae	0	0.8	0.8	0	0	0	0	0	0	0	42.4	0	0	0	0	0	0
Odonata	Macromiidae	0	13.6	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Gomphidae	0	0.8	23.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Libellulidae	0	0	0	0.8	0	0	0	0	0	0	0	0	0	0	0	0	0
Clitellata	Salifidae	0	0	0.8	0	5.6	0	0	7.2	0	0	1.6	0	0	0	0	0	0
	Tubificidae	0	0	0	0	0	2	4	78.4	0	84	0	7.2	0	0	0	0	0
	Megascolecidae	0	0	0	0	0	0	13.6	0	0	0	0	0	0.8	0	0	0	0
Mollusca	Physidae	0	0	0	0	4	6.4	0	0.8	0	0	0	0	0	0	0	0	0
	Lymnaeidae	0	0	0	0	0.8	0	0	0	0	0	0	0	0	0	0	0	0
Hemiptera	Aphelocheiridae	0	0	0	0	0	0	0	0	0	0	0	1.6	0	0	0	0	0
	Total density (ind./m²)	**70.4**	**480.8**	**468**	**191.2**	**682.4**	**39.2**	**193.6**	**168.8**	**39.2**	**92**	**46.4**	**27.2**	**4**	**32.8**	**71.2**	**19.2**	**81.6**

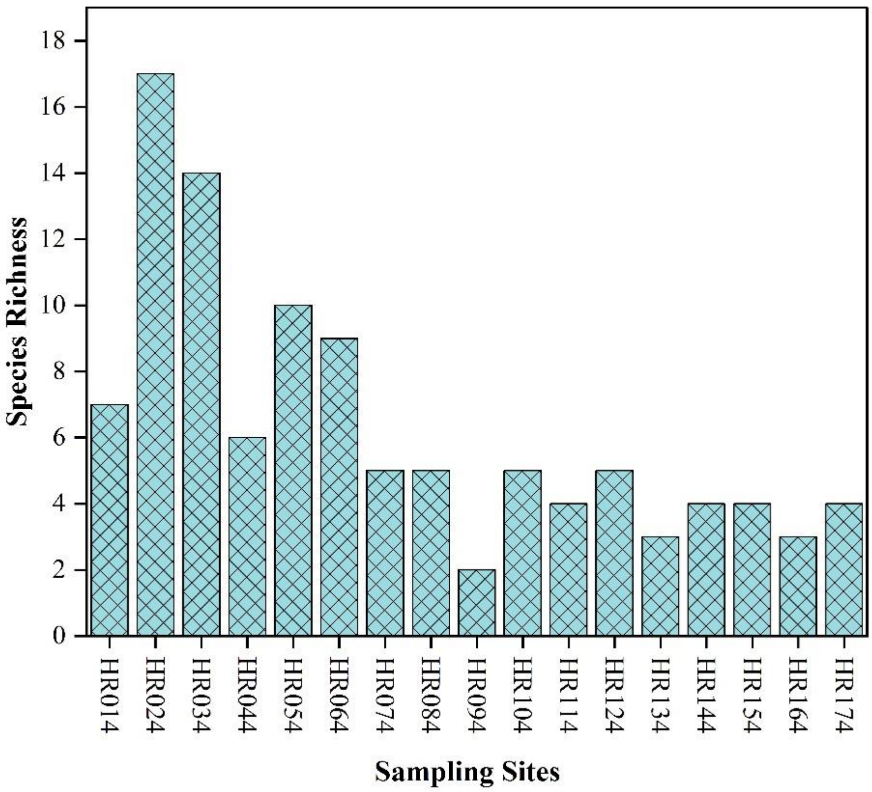

Figure 2. Species richness of BMI at the study sites of the Hanumante River

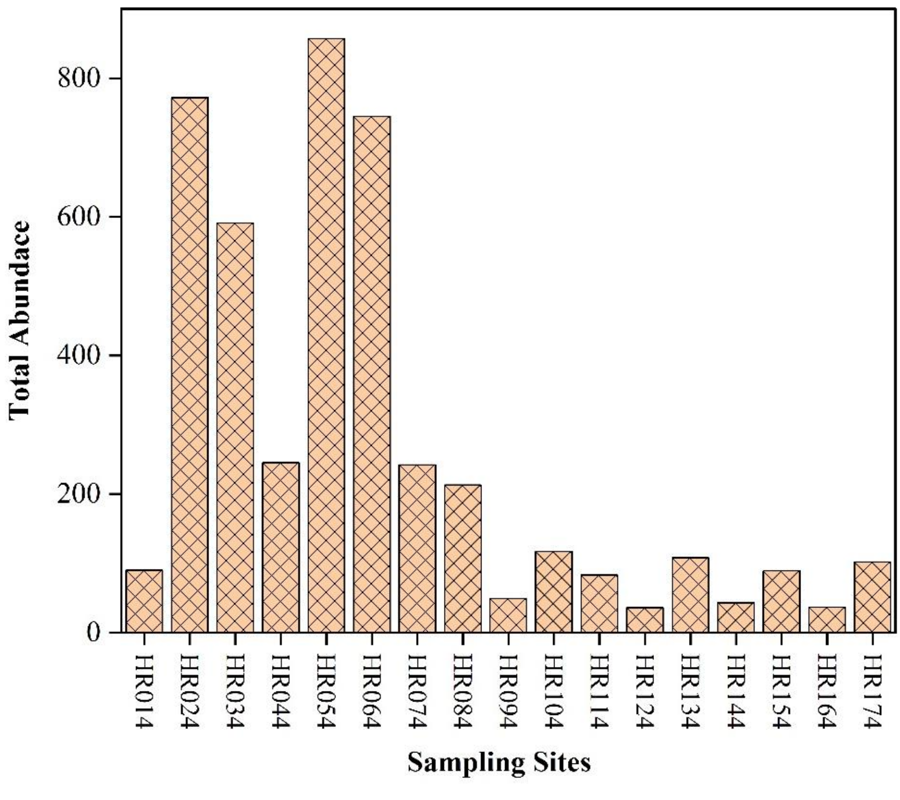

Figure 3. Total abundance of BMI at the study sites of the Hanumante River

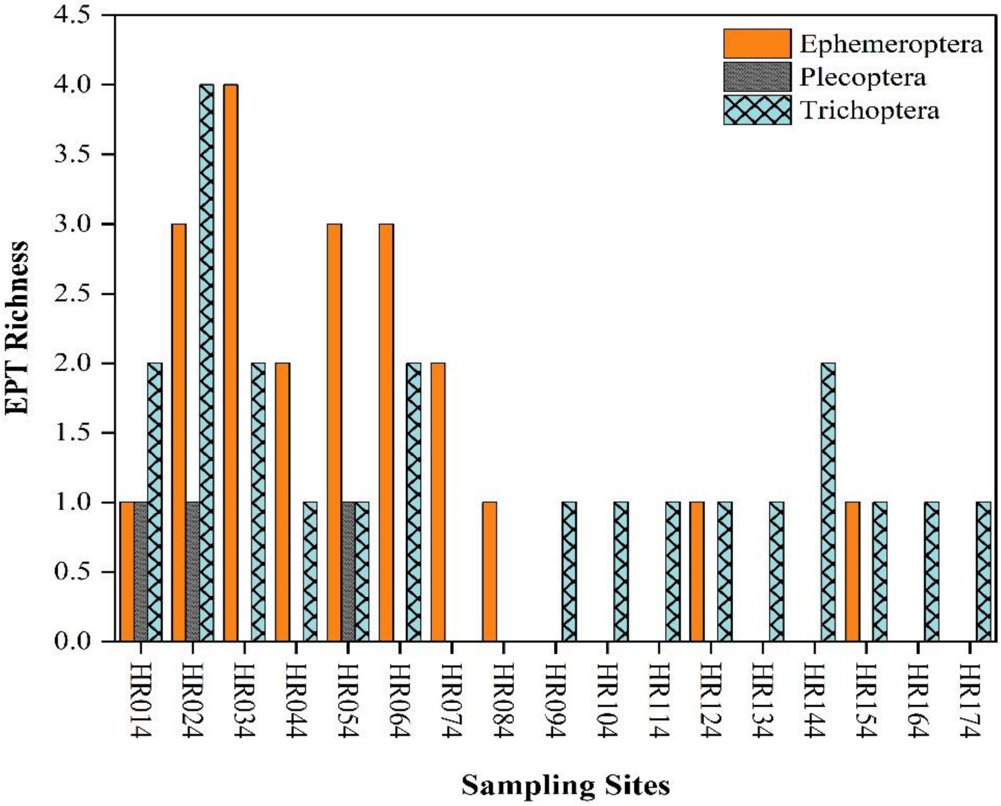

Figure 4. EPT richness at the study sites of the Hanumante River

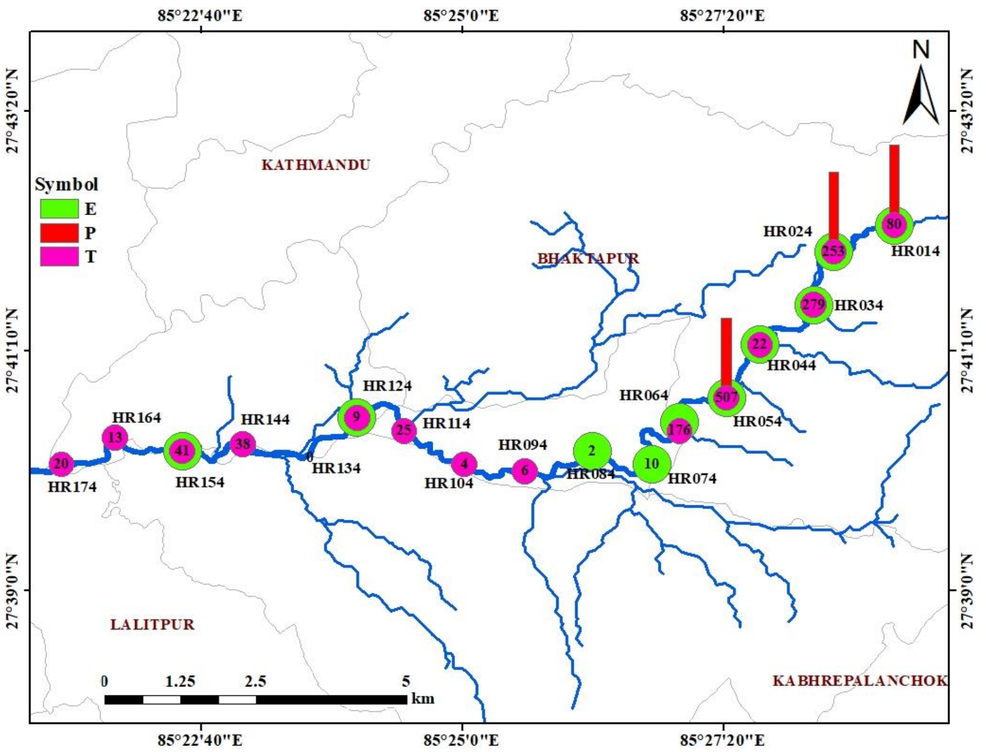

Figure 5. Total abundance of EPT at the study sites of the Hanumante River

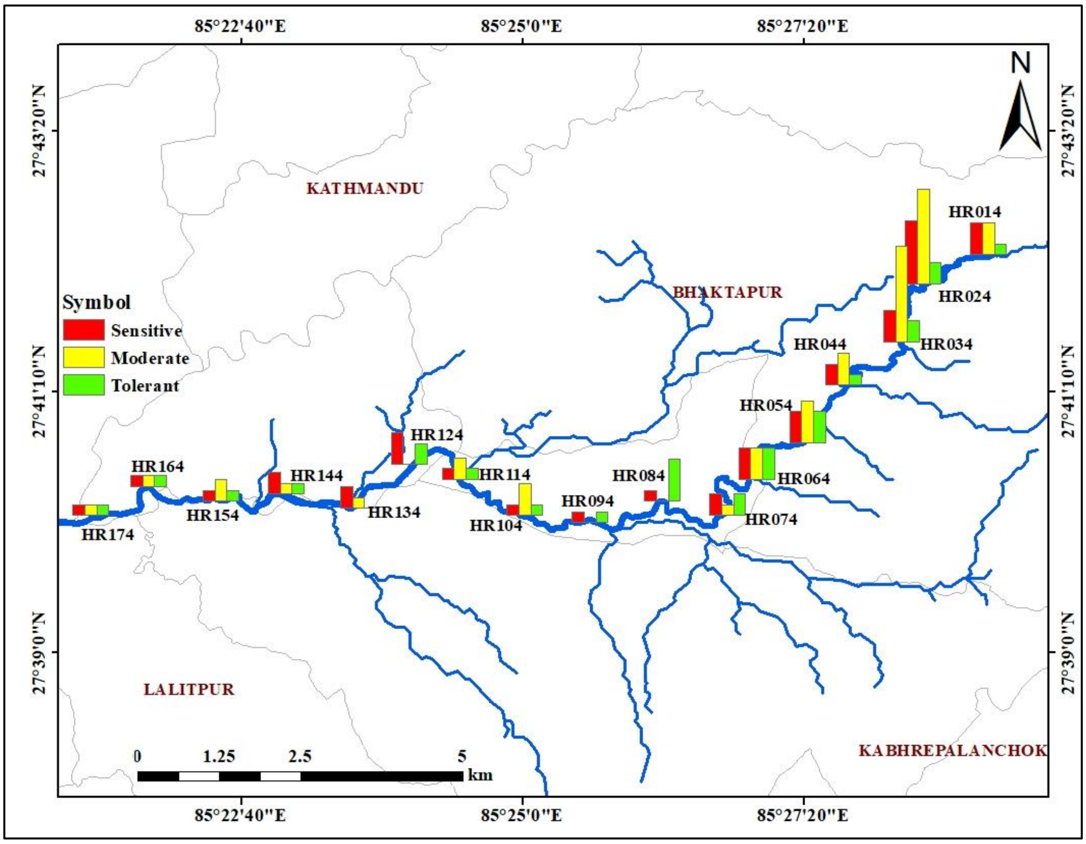

Figure 6. The richness of sensitive, moderate, and tolerant taxa at the study sites of the Hanumante River

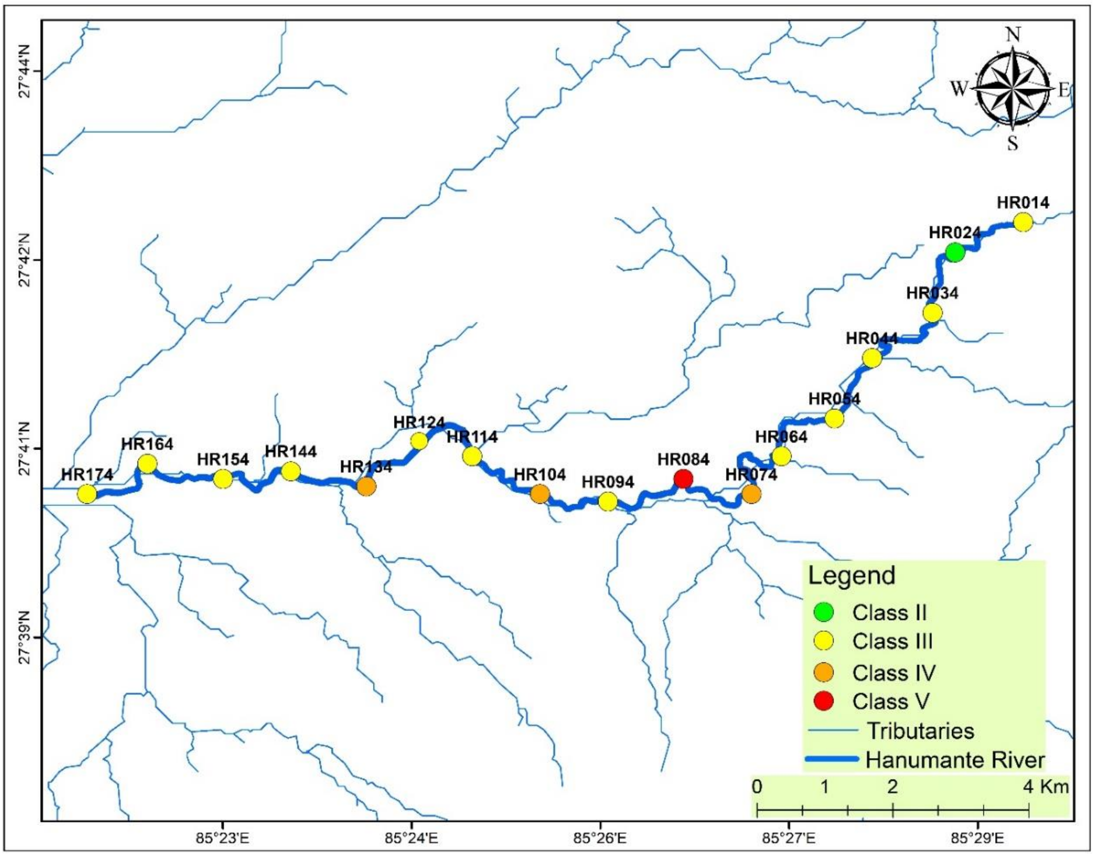

Figure 7. River quality map of study sites of the Hanumante River

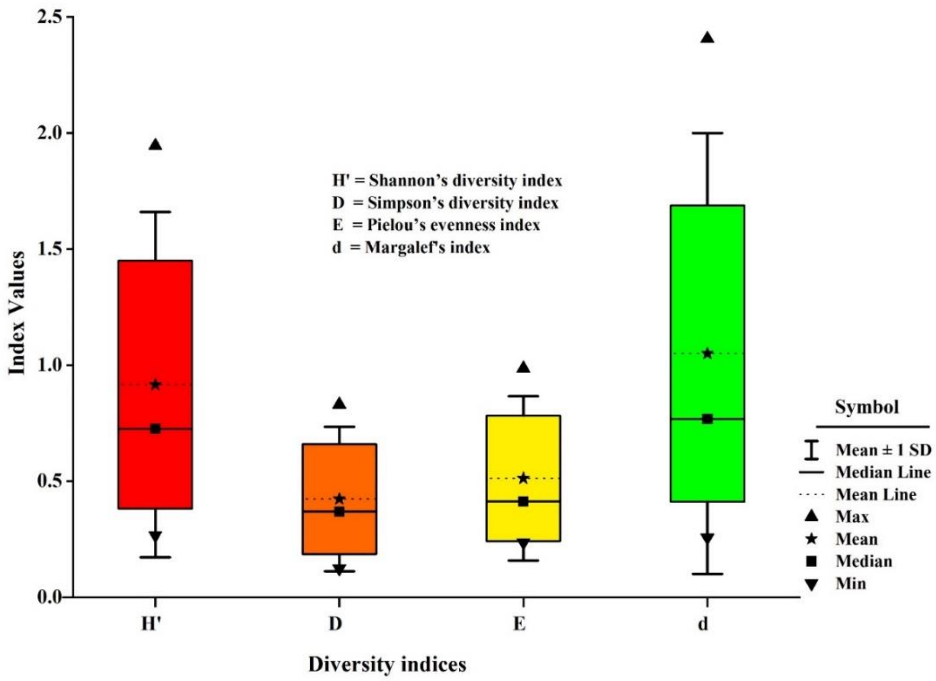

Figure 8. Box plot illustrating diversity indices (Shannon's diversity index (H'), Simpson's diversity index (D), Pielou's Evenness index (E), and Margalef's index (d) of BMI at the study sites of Hanumante River

River Water Quality

The ecological water quality class of the river Hanumante was determined by using the biotic index (BI) and obtained the average value of 4.91 which falls under fair water quality with water quality class III. The site-specific value of BI shows that site HR024 has good water quality, sites HR074, HR104, and HR134 have poor water quality, and site HR084 has bad water quality. The remaining sites have fair water quality (Fig. 7). We observed that only site HR08 has bad water quality. As both sides of the river were disturbed by human activities, religious discards were found. Additionally, the site was also used for doing crematoria activities. The disturbances in the natural flow regimes or changes in parameters can alter the habitat availability of BMI. Similarly, such disturbances can disrupt the life cycle, structure, and well-being of aquatic ecosystems. The availability of macroinvertebrates is also affected by flow rate. Low flow rates first affect the availability, diversity, and suitability of macrohabitats by altering their depth and water flow velocity (Poff, 2018).

The water quality class of the Hanumante River downstream indicates that downstream water quality of the river was deteriorating. The stressful factors for the deterioration of river water quality are agricultural effluent, industrial effluent, and sewage; waste dumping, washing, etc. Essentially, reducing water velocity increases fine sediment deposition and reduces food supply which in turn affects the composition of the substrate, its suitability for macroinvertebrates, and its ability to take up food (Wood & Armitage, 1997). Hence, lowering the water level very often results in lower habitat, and a decrease in biodiversity as well as species composition (Dewson *et al.*, 2007). Low-order streams were more individualistic, probably because of a greater influence of local (terrestrial) environmental conditions (Minshall *et al.*, 1985). Similarly, benthic macroinvertebrates in nature are affected by various concurrently occurring environmental factors operating at multiple spatiotemporal scales. Some of these factors include physicochemical parameters, hydrological regimes, land use patterns, habitat type, sediment characteristics, biotic interaction, etc. This complex interaction makes it difficult to characterize the individual effects of each factor (Rai *et al.*, 2019). Thus, understanding their response to these factors is key to assessing freshwater quality.

Diversity Indices

Diversity indices help to explain the richness, abundance, variation in communities, commonness, and rarity of species. Considering the various changes in the river biota, these diversity indices will help to better understand the community structure of benthic organisms in Hanumante River In this study, four different diversity indices were calculated (Fig. 8) Simpson's and Shannon's index accounts for both abundance and evenness of the species present, Pielou's index refers to how close in number of each species in an environment whereas Margalef index simply helps to determine the species richness (Magurran, 2004). Fig. 8 depicts the overall average values of diversity indices at the study sites of the Hanumante River. The results showed the average and standard deviation values of Shannon diversity index (0.96 ± 0.49), Simpson's diversity index (0.49 ± 0.25), Pielou's Evenness index (0.58 ± 0.25), and Margalef's index (0.97 ± 0.57). The maximum value of the Shannon diversity index of all sites was found to be 1.95 whereas 0.83, 0.98, and 2.40 for Simpson's diversity index, Pielou's Evenness index, and Margalef's index respectively. The Shannon Diversity Index is the most preferred diversity index among all other diversity indices. The index value usually ranges between 1-5, with a value lower than one being as highly polluted, 1-3 as slightly polluted, and a value higher than 4 indicating that the water is not contaminated (Wilhm & Dorris, 1968). In the present study, the maximum value of the Shannon diversity index was found to be 1.95 which indicates the river is slightly polluted. Simpson's diversity index accounts for both richness as well as for abundance within a community. The index value ranges between zero to one. A value close to one indicates infinite diversity and 0 indicates no diversity (Magurran, 2004). Pielou's evenness index is derived from the Shannon Diversity Index, where values of two evenness indices lie between 0-1. Zero represents less variation and a value close to or above one represents higher variation within communities or individuals are distributed equally (Pielou, 1966). The obtained value of Pielou's evenness index in the present study revealed that aquatic macroinvertebrate individuals are distributed equally at the study sites of the Hanumante River. Margalef's diversity index is the most sensitive diversity index that is used to assess the structural changes in benthic macroinvertebrate communities. Margalef's index is nearly similar to that of family richness and biotic indices, which might indicate that Margalef's index is more affected by changes in the number of species (Abdel Gawad, 2019). This index is most commonly used for the comparison of sites, since it has no limit value because it simply shows variation depending upon number of individuals in the communities (Magurran, 2004).

CONCLUSIONS

A total of 10 Orders, 33 Families, and 4419 individuals of the macroinvertebrates were observed, with Order Diptera as the most abundant. The study revealed that the pollution-sensitive families such as Ephemeroptera, Plecoptera, and Trichoptera are abundant at the upstream sites of the river, while pollution-tolerant families such as Chironomidae (red), Tubificidae and Simuliidae are dominating in downstream areas. The absence of Plecoptera from site Tathali (HR054) indicates the shifting status of the river from a non-polluted to a polluted state. Total species richness, abundance, and sensitive measures revealed that the upstream site was characterized by the sensitive species while moderate and tolerant species dominated towards the downstream. The results of the biotic index calculation revealed that only one site has good water quality and most of the sites have fair water quality. The distribution pattern, richness, and abundance of several macroinvertebrates which correspond to the water quality of the river at each studied site suggested macroinvertebrates could be used as potential indicators for biomonitoring.

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AUTHOR CONTRIBUTIONS

M.P. Uprety and G.K. Raut conducted fieldwork, and laboratory activities and prepared the draft manuscript; S. Adhikari and M.P. Awasthi performed laboratory activities, data analysis, and interpretation of results. R.P. Sapkota contributed to the research design, analysis, review, and finalization of the manuscript. All the authors have equally contributed their time to make this research work publishable.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the first author and corresponding author, upon reasonable request.

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