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Research Article

Evaluation of freshwater springs utilizing benthic macroinvertebrates as key bioindicators in the Panchadewal Binayak Municipality, Middle Karnali Watershed, Nepal

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Abstract
In the mountainous regions of Nepal, springs are the primary source of freshwater for rural communities and also serve as unique habitats for endemic and threatened aquatic species, particularly macroinvertebrates that are excellent bioindicators of water quality. Therefore, a comprehensive assessment of benthic macroinvertebrates is imperative for species conservation before they face extinction. This study in Panchadewal Binayak Municipality focused on evaluating the community structure of Benthic Macroinvertebrates and identifying key environmental factors influencing them. A total of 25 springs and 4 streams were selected, all currently in use or with potential for future use. Altogether, 439 benthic macroinvertebrate individuals belonging to 32 families were observed, revealing high richness in Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa. Most springs were categorized as class II, indicating moderate pollution. Several environmental variables such as turbidity, dissolved oxygen, chloride, nitrate, sodium, iron, and potassium, significantly influenced the community structure. Human activities such as bathing, clothes washing, littering, livestock watering, and agricultural runoff were identified as major stressors. Although none of the springs were classified as critically degraded, the risk of deterioration remains high. This study emphasizes the importance of springs as habitats for a diverse group of benthic macroinvertebrates, providing valuable information for conservationists and resource managers. Informed decisions can now be made to protect these habitats and the associated services they provide in the Panchadewal Binayak Municipality.

Keywords: Benthic macroinvertebrates; environmental variables; stressor; water quality.

Introduction
Springs play a crucial role in sustaining the rural population of the Hindu Kush Himalayan (HKH) region (Matheswaran et al., 2019). The escalating human influence and the growing demand for drinking water intensify the issue of spring groundwater pollution, particularly in the Middle Mountains of the HKH region including Nepal (Lamichhane et al., 2020). Anthropogenic activities, such as catchment degradation, alterations in land use, and the expansion of human settlement and road networks, have disrupted hillslope hydrology in Nepal's middle mountains (Adhikari et al., 2021). Non-point source pollution from human waste and intensive agricultural practices, including the use of mineral fertilizers and pesticides, as well as the increasing number of cattle in dairy farming, contribute significantly to the decline in water quality (Merz et al., 2003). Furthermore, during the monsoon season, the washing of pollutants, including human waste, into water sources exacerbates health risks. Climate change further accelerates this process, leading to the degradation and drying of springs (Cartwright et al., 2020). This has a direct impact on water scarcity in Nepal's middle mountains, contributing to a situation where people are compelled to consume whatever water is available without the knowledge of its quality (Adhikari et al., 2021).

Conversely, these springs function as distinctive habitats, providing a conducive environment for a varied array of aquatic organisms (Dumnicka et al., 2013). Housing more than one-third of the local freshwater biodiversity, these habitats host a multitude of endangered, rare, and endemic species (Živić et al., 2022; Cantonati et al., 2012). The presence of complex habitats and the connectivity between aquatic and terrestrial ecosystems contribute to the presence of abundant, diverse, and specialized macroinvertebrate communities in springs (Staudacher & Füreder, 2007). Further, springs play a pivotal role as ecohydrological refuges, especially in the context of climate change, offering a habitat that fosters the thriving of numerous species (Cartwright et al., 2020). Therefore, the assessment of springs is crucial for the protection and conservation of aquatic species and its habitat. To adequately assess freshwater ecosystem, relying solely on physicochemical parameters is inadequate, as these parameters offer information only at the time of sampling. A comprehensive evaluation necessitates a biological assessment, involving the examination of aquatic life within the water body (Chamia & Kutuny, 2022). The presence and condition of aquatic life serve as indicators of the cumulative impacts of stressors like chemicals, temperature changes, and nutrient concentration, providing insights into the effects of these stressors (Chamia & Kutuny, 2022). Among the various indicators of water quality, aquatic macroinvertebrates are widely recognized as one of the

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most reliable (Shah et al., 2011; Shah & Shah, 2013). These organisms predominantly inhabit water throughout their life stages (Tachamo-Shah et al., 2020). The composition of macroinvertebrate species can mirror various anthropogenic impacts, including acidification, organic pollution, habitat alteration, and disturbances (Hartmann et al., 2010). Ranging from highly tolerant to sensitive, these organisms demonstrate diverse pollution tolerances (Shah et al., 2011; Shah & Shah, 2013). Due to their sensitivity to hydrochemical changes, flow patterns, habitat alterations, and other environmental factors, macroinvertebrates are well-suited as indicators for monitoring and assessing springs. Given their significant life spent in water, the presence, abundance, and diversity of macroinvertebrates can serve as reliable predictors of water quality and habitat conditions (Chamia & Kutuny, 2022).

In the middle Karnali watershed, springs serve as the backbone of rural communities, yet they face challenges from both natural such as soil erosion and landslides and human induced activities such as road construction, agricultural intensification and alteration in land use. Conducting assessments is crucial for their conservation and sustainable management. The current study provides a comprehensive examination of the aquatic biodiversity and ecological health of springs in the Panchadewal Binayak Municipality, Nepal. Furthermore, it investigates the factors contributing to the decline of these spring's status and identifies those that are particularly critical.

Materials and Methods

Study Area

The research was conducted in Panchadewal Binayak Municipality, situated in the Achham district of the Sudurpaschim Province in Nepal, nestled within the lesser Himalayas (see Figure 1). It is bordered by the Kalikot district of the Karnali province to the east, Kamalbazar to the west, Ramaroshan to the north, and Dailekh district to the south. The total population of Panchadewal Binayak Municipality is 26,088 [11,893 males (45.6%) and 14,195 females (54.4%)], and it covers a geographical area of 147.75 square kilometers (57.05 square miles). The municipality is divided into nine wards, with a total of 5,412 households. Among these households, 650 use tap or piped water within their homes, while 3,152 rely on water sources situated outside (CBS, 2021). Furthermore, 37 households utilize covered wells, 136 use uncovered wells, 1,314 access spout water, 121 rely on rivers or streams, and the remaining households depend on other sources for their drinking water needs. The literacy rate in the municipality is recorded at 69.8%, with a male literacy rate of 79.8% and a female literacy rate of 61.8% (CBS, 2021). The study area exhibits diverse geological formations, including the Kalikot formation, Ulleri formation, Raimata formation, and Kushma formation (ICIMOD, 2020).

Methods

Focus Group Discussion (FGD) and Key Informant Interview (KII)

Details regarding the number, names, and locations of water sources, along with other pertinent components, were obtained through Focus Group Discussions (FGD) and Key Informant Interviews (KII) conducted at the ward level. Engaging with local villagers, historical data concerning the water sources were collected, and the flow of these sources was measured using standard methods.

Direct Observations

Information on the stressing factors such as farming systems, land-use types, and degradation activity variables was collected from the sites through direct observation of their surroundings and also through survey. A specific form was employed for the systematic collection of data concerning the stress factors in the vicinity of the springs.

Aquatic Macroinvertebrates

The aquatic macroinvertebrates was assessed in 25 springs and 4 streams of Panchadewal Binayak Municipality during the post-monsoon season of 2022. Among the 25 springs, two were depression type. At each spring, a macroinvertebrates sample was collected following a multi-habitat sampling procedure. Samples were collected using a circular hand net of 500 μm mesh size. The samples were processed in the field to avoid any large materials and then preserved in 90% ethanol. For sorting and identification at the lowest possible taxonomic resolution, the samples were transported to the Central Department of Environmental Science laboratory. The macroinvertebrates were identified referring to the Rivers Handbook (Tachamo-Shah et al., 2020).

Environmental Variables

Water Quality

Physicochemical parameters such as water temperature, pH, Electrical Conductivity (EC), Total Dissolved Solids (TDS), alkalinity, dissolved oxygen concentration, total hardness, and chloride were measured on site. A water sample was collected from each spring in an HDPE bottle for the measurement of ammonia, iron, sodium, nitrate, phosphate, potassium and sulfate in the laboratory. These were measured using the standard methods (Table 1; APHA, 2017).

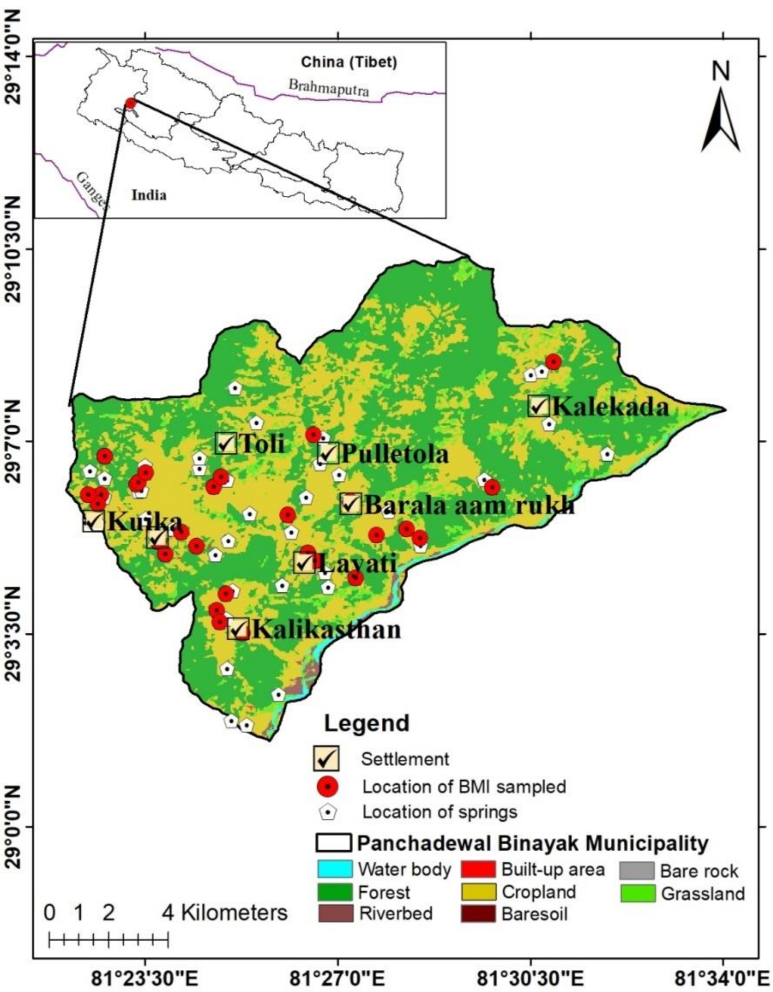

Figure 1 Panchadewal Binayak Municipality and selected springs for macroinvertebrates assessment are shown. The inset map shows the location of the study area in Nepal.

Table 1 Standard methods used for water quality test (APHA, 2017)

SN	Parameters	Method	Reagent/ Instrument
1	Temperature	Electrometric	Hanna Multipurpose (Hanna Combo Hi98129)
2	pH	Electrometric	Hanna Multipurpose (Hanna Combo Hi98129)
3	Electrical Conductivity (EC)	Electrometric	Hanna Multipurpose (Hanna Combo Hi98129)
4	Total Dissolved Solid (TDS)	Electrometric	Hanna Multipurpose (Hanna Combo Hi98129)
5	Dissolved Oxygen (DO)	Electrochemical	YSI–DO Meter Pro20
6	Total Hardness (as $CaCO_3$)	Tritrimetry	Titration with EDTA
7	Alkalinity	Tritrimetry	Titration with HCl
8	Chloride	Argentometric	Silver nitrate (AgNO3), potassiumchromate
9	Ammonia	Indophenol Blue Method	Spectrophotometer
10	Iron	Phenanthroline Method	Spectrophotometer
11	Sodium	Flame photometric	Flame photometer
12	Nitrate	Ultraviolent Spectrophotometric screening	Spectrophotometer
13	Phosphate	Spectrophotometer	Ammonium Molybdate, Stannous chloride
14	Potassium	Phenate method (Flame photometer)	Flame photometer
15	Sulfate	Turbidimetric	Spectrophotometer

Discharge

The volumetric method was used to measure the discharge of spring water from the fracture or foliation springs (Tiwari et al., 2020). For the depression spring, the water level drop method was used. A known volume of water was extracted from the spring cavity by putting a ruler inserted at the center of the springs. The rise of the water level at regular intervals was observed and the time taken by the springs to reach the original level was noted. The final discharge of the spring of both fracture and depression springs was calculated by applying the following formula:

$$Q = V/T$$

Where, V is the volume of water filled, measured in liters. T is the time required to fill the desired water volume, measured in seconds.

Data Analysis

The percentages of Ephemeroptera, Plecoptera, and Trichoptera were computed individually in MS Excel, along with taxonomic richness and total abundance. In R Studio Version 4.1.3, the diversity function in vegan package was employed to calculate the Shannon-Wiener index, Simpson's diversity index, Pielou's evenness index, and Margalef's index (refer to Table 2). Explorations of multivariate relationships in macro-invertebrate data were conducted using the Vegan package in RStudio.

Table 2 Calculation methods of metrics

Metrics	Calculation
Taxa richness	Total number of present taxa at a site
Total abundance	Total number of individuals at a site
Percentage of EPT abundance	(Sum of Ephemeroptera, Plecoptera, and Trichoptera individuals in a site/ Total abundance in a site) *100
Shannon-Wiener index	diversity() function in R
Simpson's diversity index	diversity() function in R
Pielou's evenness index	H/ln(S)
Margalef's index	(S-1)/lnN

The GRSbios Index was applied for running-type springs (Tachamo-Shah et al., 2020), while NLBI was employed for stagnant-type springs (Tachamo-Shah et al., 2011). To evaluate the springs' status, a decision support table was utilized, facilitating the identification of springs that were critically degraded. This decision support table incorporates various factors such as flow, sensitive BMI measures, the Biotic Index, Settlement, and Land-use. These factors are categorized, and a corresponding score is assigned to represent their conditions, with a score of 3 indicating good condition and a score of 1 indicating poor condition. The determination of each spring's status involves summing up their scores and comparing the total scores with the values provided in Table 3.

Table 3 Spring status from the total score

Score	Description	Class
13-18	Very good	I
7-12	Good	II
≤6	Poor	III

Correspondence Analysis

The association between environmental variables and BMI assemblages was assessed using Canonical Correspondence Analysis (CCA) with the cca function in the vegan package within R Studio Version 4.1.3. The choice between Redundancy Analysis (RDA) and Canonical Correspondence Analysis (CCA) was determined through Detrended Correspondence Analysis (DCA). Since the axis length exceeded 2.5, CCA was deemed appropriate. Before conducting CCA, log transformation was applied to the BMI abundance data to address potential statistical errors related to rare or very common species. Variance inflation factors (VIF) were computed for each constraint (variable), and variables with VIF>10 were eliminated from the analysis due to collinearity with other variables. Subsequently, the backward elimination method was employed using the ordistep function to remove variables until only significant ones remained.

Results and Discussion

A total of 439 individuals from 32 families belonging to 9 orders/classes were recorded in the studied springs. The number of individuals per spring ranged from 3 to 56 individuals. The Chironomidae had the highest number of recorded individuals (116), followed by Hydropsychidae (61), Lepidostomatidae (41), Baetidae (39), and Uenoidae (38). In Panchadewal Binayak Municipality, Chironomidae emerged as the most prevalent taxon across all habitat types, aligning with Orendt (2000) assertion that Chironomidae displays the highest prevalence among all taxonomic groups in springs. In line with this, Myers and Resh (2002) also noted that springs commonly exhibit a high diversity of Chironomids. They furthermore observed a widespread distribution of Plecoptera, similar to the finding of this study. Dipterans, belonging to various families, possess the ability to coexist and exploit all available energy sources while colonizing diverse micro-habitats within the spring. However, the dipteran fauna of springs in Nepal remains relatively underexplored, and detailed investigations often reveal intriguing observations with significant zoogeographic implications (Cantonati et al., 2006). In a study by Pokorny et al. (2012), Leptophlebiidae family were identified as the most abundant family in stream sites characterized by higher pH levels and water temperature. However, the findings from the present study contradict this statement.

Among the total taxa identified, 15 families (47%) (refer to Table 4) belonged to EPT taxa. EPT taxa constituted 54.67% of the overall individuals, signifying a high abundance compared to other taxa in Panchadewal Binayak Municipality.

At the family level, richness at individual springs ranged from 2 to 11 taxa. Trichoptera had the highest number of individuals, followed by Diptera, Ephemeroptera, and Plecoptera. Conversely, Hemiptera had the lowest number of individuals. The occurrence of Ephemeroptera, Plecoptera, and Trichoptera (EPT), particularly the dominance of the Ephemeroptera order with diverse families, signifies a lower level of pollution, as these taxa are known for their sensitivity to environmental stress. A study by Maiolini et al. (2011) noted a significant abundance of Heptageniidae, a family within the Ephemeroptera order, in various springs, a finding in line with the present study. Conversely, the infrequent presence of other Ephemeroptera families corresponds with the findings reported by Bottová et al. (2013).

Table 4 Taxa recorded at the springs of Panchadewal Binayak Municipality

Order/Class	Family				
Hemiptera	Mesoveliidae				
Tricladida	Dugesiidae				
Plecoptera	Perlidae	Perlodidae			
Clitellata	Megascolecidae	Salifidae	Tubificidae		
Coleoptera	Dytiscidae	Elmidae	Psephenidae		
Diptera	Chironomidae	Limoniidae	Pediciidae	Tabanidae	
Odonata	Coenagrionidae	Epiophlebiidae	Euphaeidae	Gomphidae	
Ephemeroptera	Ameletidae	Baetidae	Ephemerellidae	Heptageniidae	
Trichoptera	Apataniidae	Glossosomatidae	Goeridae	Hydropsychidae	Hydroptilidae
	Lepidostomatidae	Limnephilidae	Philopotamidae	Psychomyiidae	Uenoidae

Margalef's index varied from 0.36 to 2.91, while Pielou's evenness index varied from 0.34 to 1, indicating differences in species diversity and evenness among the studied springs. The springs in Panchadewal Binayak Municipality exhibited a biotic index ranging from 4.00 to 7.31. Based on the biotic index, 24.14% springs were classified as undisturbed, 27.59% as good, 34.48% as fair, and 13.79% as poor (Figs. 2 and 3).

The springs exhibited a wide range of water quality, from non-polluted to heavily polluted. This variation supports both pollution-tolerant and pollution-intolerant species. Springs with minimal human disturbance had good water quality, whereas those heavily impacted by human activities showed poor water quality. The decline in water quality not only adversely affects aquatic life but also poses risks to human health and the overall health of the aquatic ecosystem. Factors contributing to the deterioration include human activities like the deposition of organic waste and runoff from agricultural fields. In polluted water with elevated concentrations of organic materials and nutrients, pollution-tolerant organisms tend to dominate, while pollution-sensitive organisms struggle to survive.

Physicochemical parameter values obtained from the spring water samples are within the acceptable limits, except pH for one spring site, as per the World Health Organization (WHO) guidelines. The highest values of the measured parameters were found in the sprigs near the settlement, littering areas, near the agricultural land and the springs near to road access.

Various environmental factors explained the variations in BMI assemblages (Figure 4). BMI families such as Elmidae, Dugesiidae, Megascolecidae, and Limoniidae exhibited a positive correlation with dissolved oxygen (DO), suggesting a higher likelihood of occurrence in areas with elevated DO levels. While they were negatively correlated with nitrate, and iron. Chloride, sodium, turbidity, nitrate, and iron were all correlated with Chironomidae species, which were highly abundant. This indicates that they are more likely to inhabit at higher chloride, sodium, turbidity, nitrate, and iron level. Among all, iron was the most significant parameter responsible for varying abundance of taxa across the different springs. The proportion explained by CCA1 and CCA2 is 0.404% and 0.0.327% respectively.

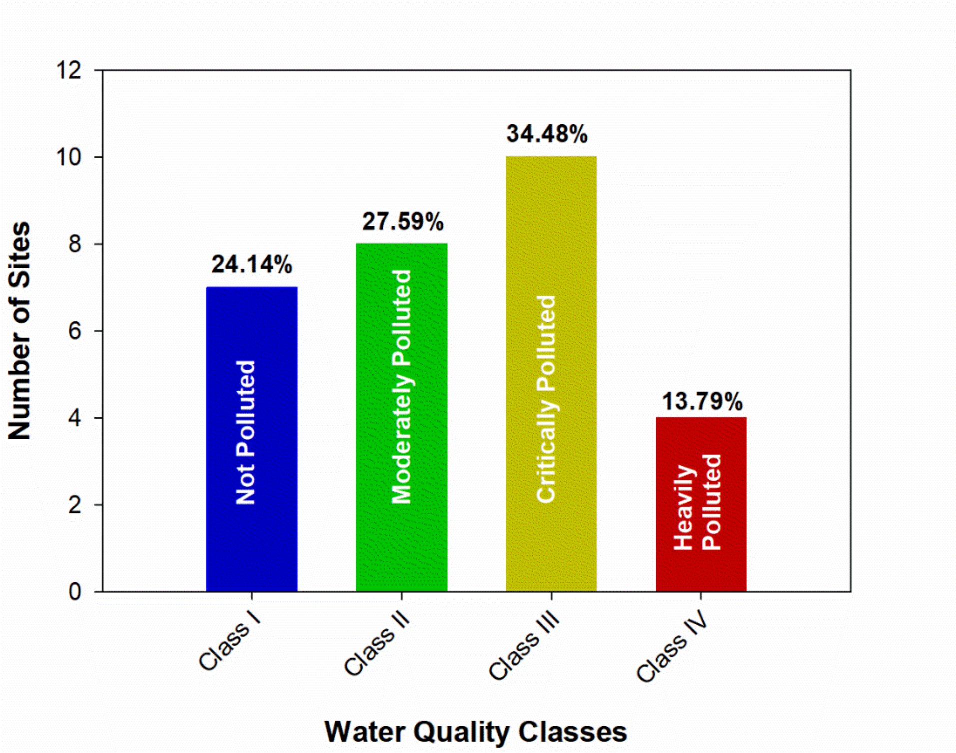

Figure 2 Percentage of springs' status based on the biotic index [Undisturbed - Not polluted; Good - Moderately polluted; Fair - Critically polluted; Poor - Heavily polluted]

Table 5 Physicochemical parameters in comparison with WHO standards

SN	Parameters	Range	Mean± SD	WHO (2017)
1	Temperature (°C)	17.6- 22.2	19.70±1.09	-
2	pH	6.8- 8.7	7.43±0.37	6.5 -8.5
3	Electrical Conductivity (EC) (µS/cm)	32- 342	122.00±74.00	1500
4	Total Dissolved Solids (TDS) (mg/L)	16- 171	60.70±37.07	1000
5	Dissolved Oxygen (DO) (mg/L)	6.18- 8.1	7.03±0.49	5
6	Total hardness as $CaCO_3$ (mg/L)	28- 388	163.62±120.51	500
7	Alkalinity (mg/L)	35- 160	67.41±33.51	-
8	Chloride (mg/L)	4.26- 31.24	10.37±7.03	250
9	Ammonia	0.06- 0.22	0.09±0.04	1.5
10	Iron (ppm)	0.018- 0.062	0.03±0.01	0.3
11	Sodium (ppm)	4.8- 26.1	14.08±8.18	-
12	Nitrate	0.041- 0.055	0.05±0.00	50
13	Phosphate	0.169- 1.240	0.61±0.26	-
14	Turbidity (NTU)	4.41- 12.46	1.58±2.22	5
15	Potassium (mg/L)	4.1- 11.9	6.58±2.61	-
16	Sulphate (mg/L)	1.42- 0.07	75.43±18.97	250
17	Discharge (L/sec)	0.33- 0.045	0.13±0.18	-

According to Lindegaard (1995), the limited richness or absence of species in springs can be attributed to the low levels of oxygen, a result consistent with the findings of this study. Conversely, the present study did not establish any significant correlations between the presence or abundance of species and pH values or conductivity, similar to Orendt (2000) study. This lack of correlation could potentially be linked to the limited number of springs studied, diminishing the level of statistical significance. Erman and Erman (1995), in their study, identified a positive relationship between species richness and alkalinity, leading to the conclusion that alkalinity can serve as an indicator of spring permanence. However, the current investigation of the examined springs yielded contrasting results regarding the association with alkalinity. In a separate study by Dumnicka et al. (2013), it was noted that springs with lower discharge had a lower number of taxa, while those with higher discharge exhibited a greater diversity of fauna. This finding aligns with the outcomes of the present study.

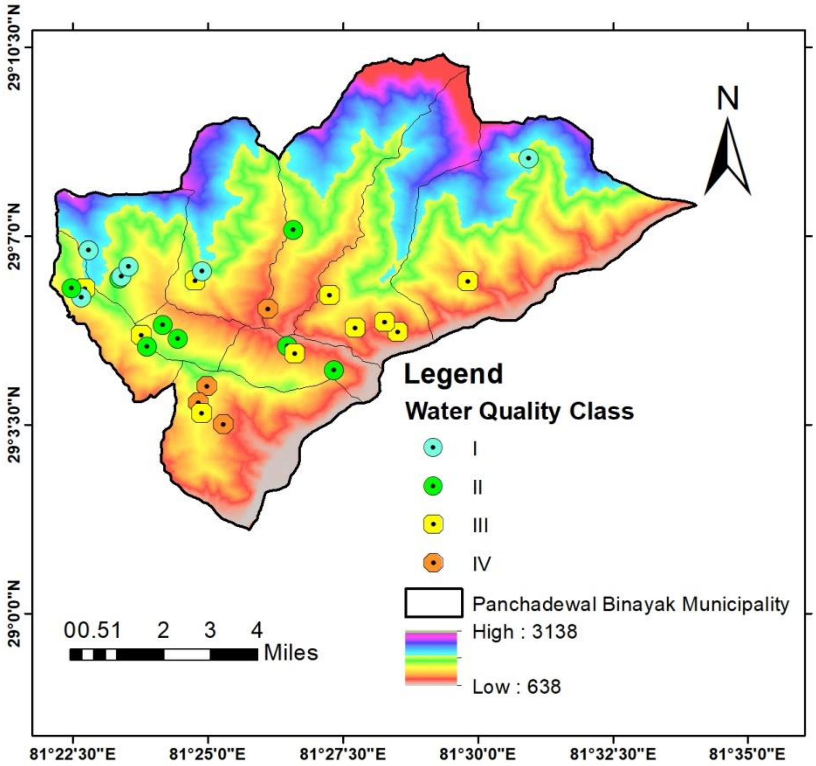

Figure 3 The water quality class of studied springs represented by distinct colors.

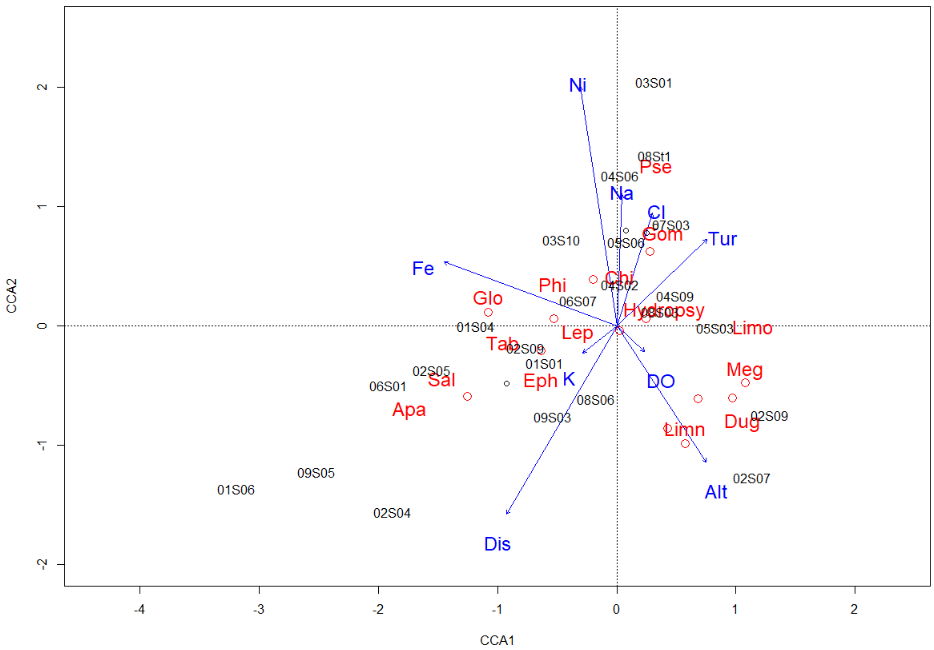

Figure 4 Canonical Correspondence Analysis plot of the log-transformed benthic macroinvertebrates abundance data constrained by environmental parameters. Where macroinvertebrate families are abbreviated as Ame. Ameletidae, Apa. Apataniidae, Bae. Baetidae, Chi. Chironomidae, Coe. Coenagrionidae, Dug. Dugesiidae, Dyt. Dytiscidae, Elm. Elmidae, Eph. Ephemerillidae, Epi. Epiophlebiidae, Eup. Euphaeidae, Glo. Glossosomatidae, Goe. Goeridae, Gom. Gomphidae, Hep. Heptagenidae, Hydropsy. Hydropsychidae, Hydropti. Hydroptilidae, Lep. Lepidostomatidae, Limn. Limnephilidae, Limo. Limonidae, Meg. Megascolecidae, Mes. Mesovellidae, Ped. Pediciidae, Per. Perlidae, Per1. Perlodidae, Phi. Philopotamidae, Pse. Psephenidae, Psy. Psychomyiidae, Sal. Salifidae, Tab. Tabanidae, Tub. Tubificidae, Uen. Uenoidae. Environmental variables are abbreviated as DO. Dissolved Oxygen, Tur. Turbidity, Cl. Chloride, Ni. Nitrate, Fe. Iron, Na. Sodium, K. Potassium, Dis. Discharge, Alt. Altitude.

Primary stressors identified in proximity to the springs included activities such as bathing/washing clothes, littering, waste from nearby settlements, livestock grazing near the spring source, and the presence of chemical fertilizers from adjacent agricultural fields. Additionally, social, religious, or recreational sites near the spring source were observed as stressors (Fig. 5). Among these, bathing or washing clothes was the most observed stressor at the majority of springs, followed by littering by visitors and the nearby settlement. Only a few springs were found to have nearby social, religious, or recreational sites as stressors.

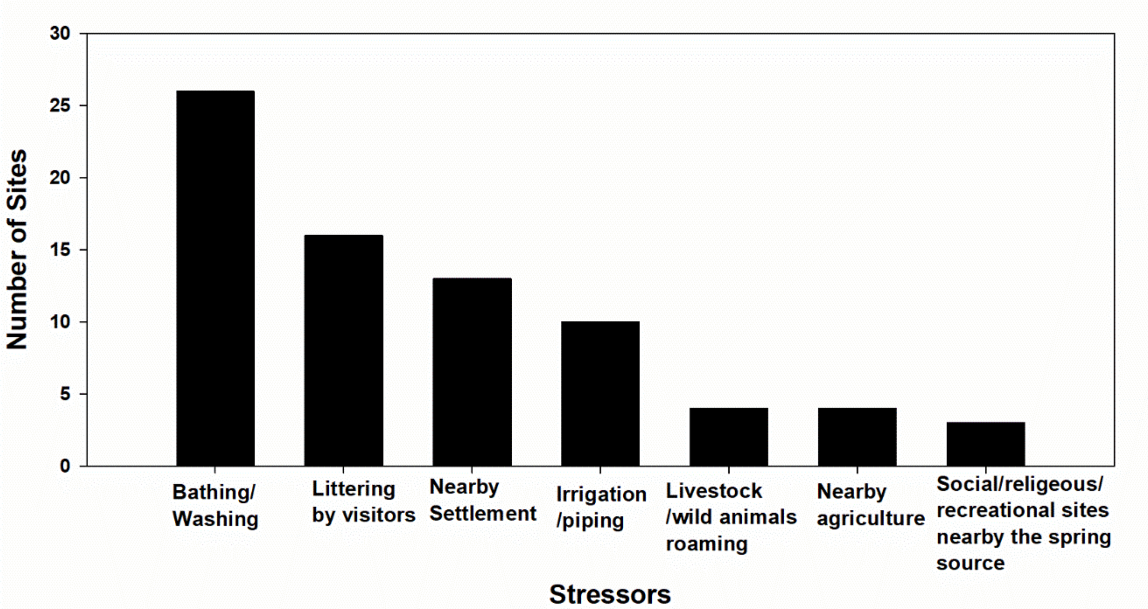

Figure 5 Stressing factors recorded at the springs in the Panchadewal Binayak Municipality.

Human activities, such as the expansion of settlement areas, agricultural runoff, and excessive groundwater extraction, have negatively impacted many springs, leading to the degradation of their habitats and biodiversity. The degradation of spring habitats poses a threat to species uniquely adapted to these environments, and the loss of springs could result in the extinction of these specially adapted species. To ensure the protection of endangered fauna dependent on springs, it is essential to have a comprehensive understanding of the ecological factors related to these species, as emphasized by Orendt (2000). Many springs and natural water bodies have been altered, diverted, or channeled into underground pipes, emphasizing the importance of prioritizing the conservation of these water bodies to safeguard the spring communities they host. These water bodies play a critical role as the last refuges for distinctive benthic fauna in this heavily affected region (Orendt, 2000). Springs, serving as habitats for specific species, should be a focal point in biodiversity conservation efforts.

Using the established assessment protocol for spring status and identifying critically degraded springs, it was found that 7% of the springs in Panchadewal Binayak Municipality are in very good condition (class 1), while 93% are in good condition (class 2). The findings of present study contradict those of Chauhan et al. (2023), which reported that springs are degrading at an alarming rate. Chauhan et al.'s study was based on people's perceptions of declining springs. However, perception and actual findings differ. Currently, none of the springs in the municipality are in critically degraded condition. Nevertheless, certain springs are at a critical juncture and face a risk of further deterioration.

Conclusions

The prevalence of Ephemeroptera, Plecoptera, and Trichoptera taxa indicated good water quality in the springs. Classification of springs based on the biotic index revealed that the majority fell within the I-II water quality classes, signifying an overall water quality ranging from excellent to good. Spatial variations in macroinvertebrate distribution were noted, attributed to fluctuations in environmental parameters such as turbidity, dissolved oxygen, chloride, nitrate, sodium, iron, and potassium. Human activities, including bathing/washing, littering by visitors, nearby settlements, irrigation via spring pipes, livestock roaming and grazing near the spring source, as well as chemical fertilizers from nearby agricultural land, had discernible impacts on the springs. While none of the springs were found to be in a critically degraded state, there is a high likelihood of such deterioration in the near future. Therefore, there is an imperative need to develop effective strategies for the preservation of spring sources, ensuring the proper management of their biodiversity, and maintaining the quality of spring water for long-term and sustainable utilization. Additionally, the study provides crucial baseline data for future comparisons and for the formulation of water use management plans.

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Conflict of Interests: The authors declare no conflict of interests.

Data Availability Statement: The data that support the finding of this study are available from the corresponding author, upon reasonable request.

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