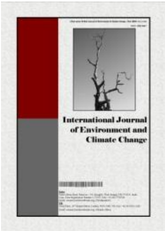

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Response of Freshwater Macroinvertebrate Communities to Various Anthropogenic Stressors in Lolab Streams- A Lotic System of the Indian Himalayan Region

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study evaluated the response of freshwater macroinvertebrate communities to several human-induced stresses in a characteristic temperate region with amassed population and population-related pollution. With the help of macroinvertebrate species associated with physicochemical parameters and contaminants in dissolved fractions, we aimed to establish an efficient bioassay approach for evaluating the water quality in the lotic ecosystems of Lolab streams. From the mouth of streams, a rapid scanning method was utilized and physicochemical analysis was done by utilizing APHA,2005. The standard method was used for macroinvertebrate collection and various indices were calculated using software like PAST, and Pearson correlation, CCA ,PCA were also calculated using PAST software. We discovered considerable differences in physicochemical parameters along a longitudinal gradient, with average mean values like air temperature (17.69℃), water temperature (13.2℃), pH (8.09) dissolved oxygen (7.38mg/L), free CO_2(131.96mg/L), total hardness (159.46 mg/L), Ammonical nitrogen(175.6 µg/L), Total phosphorus(62.85 µg/L) with the highest values recorded in populated and largely agriculturally developed areas in the catchment. A total number of 27 taxa were recorded belonging to 8 families. Trichoptera accounted for 36% of the total abundance studied, while Diptera accounted for 26%. Ephemeroptera accounts for 16% of all

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species. The macroinvertebrates displayed preferences for particular abiotic parameters, highlighting their potential utility in future research as dependable ecological indicators, molded by a synergistic mix of anthropogenic influences and land use intensity.

Keywords: Benthic; environmental stress; indicator; biomonitoring; contamination.

1. INTRODUCTION

The increase in human population and the increased need for resources and land have recently had a significant negative influence on aquatic ecosystems. Because increased pollution concentrations put more strain on species and the ecological health of watersheds, river ecosystems are actually at risk [1,2,3]. Quantifying the response of macroinvertebrate communities to stressors is a widely used method for assessing the effect of several pollutants on river habitats [4,5]. Monitoring programs typically include measurements of physico-chemistry of water and sediment together with the response of macroinvertebrate populations [6]. The bioassay investigations provide a supplementary thorough approach to chemical measurements of water alone [7]. The regularly measured stressors in biomonitoring programs are divided into management-relevant stressor groups, such as organic pollution, eutrophication, and hydro morphological changes [1,8,9].

Consequently, biological, hydro morphological, chemical, and physicochemical investigations are utilized to determine the ecological state of rivers [10]. Macroinvertebrates, fish, phytobenthic, and saprophytes are among the biological elements that are frequently examined in line with the Water Framework Directive [11]. In contrast to the limit value technique, this framework offers specific activities to accomplish the common goal of high water quality status [12]. Therefore, it is critical to assess how benthic macroinvertebrates respond to anthropogenic stresses in a lowland tributary river that flows through cities and agricultural areas and serves as the main receiver of wastewater from nearby wastewater treatment facilities. Major stressors are identified as organic matter load and chemicals, heavy metals, salts, and nitrogen compounds [13]. Studies have shown that groups of benthic macroinvertebrate organisms react quickly to changes in the water's chemical balance [14].

In this work, we looked at the effects of key contaminants on benthic macroinvertebrates in two temperate climate streams, as well as their reaction to water changes. According to the current research, it's possible to see how human-caused main pollutants and mixed land use (i.e. agricultural and urban areas) affect a river's longitudinal gradient from its headwaters to its mouth for the first time.

2. MATERIALS AND METHODS

2.1 Study Area

Jammu and Kashmir is home to the Himalayan sub-valley Lolab, where the Pir-Panjal mountains are found on all three sides. Some parts of the Lolab Valley have rocky and steep terrain. The Lahwal (Lalkul) river, which flows east to west and drains the Lolab Basin, is the source of water for the Lolab Basin. 34^{o} 43' 30"N to 34^{o}24'0" N latitudes and 74^{o}15'0"E to 74^{o}39'0" E longitudes are its latitude and longitude points. The research region's drainage area is 447 square miles. The Lolab's main axis extends westward for around 30 kilometers. One of the Lolab's lateral tributaries is the Kalaroos stream, which originates below the top of Nalgat (3645 m) and flows into the Lolab below Khumriyal. Morphologically, Lolab valley is flat to moderately sloping, at an elevation of around 1600 meters above mean sea level (AMSL). Temperatures typically range from –5 to 32 degrees Celsius at their coldest and warmest points. The study area was divided into 11 sub-sites for this investigation. Fig. 1 shows the geographical map and Table 1 shows the latitudes, longitudes, and altitudes of the study area.

2.2 Sampling Techniques

The eleven study locations were sampled every month from January 2018 to January 2019, from 9:00 am to 12:30 pm on each sample collection day.

2.2.1 Collection and analysis of macroinvertebrates

Using D-nets that were 30 cm broad and 30 cm long and had a mesh size of 0.5 mm, macroinvertebrates were captured [15,16]. The substrate's composition was considered

Table 1. Study sites with geographical Coordinates

Site Code	Altitude	Latitude	Longitude
I-(S1)	1594m	34°32'14.53"N	74°17'21.36"E
IL (S2)	1647m	34°3331.4N	74°19'16.21"E
III-(S3)	1555m	34°538701"N	74°33'58.36"E
Iv-(S4)	1666m	34°530754"N	74°37 83.57"E
v-(S5)	1675m	34°29°48 69"N	74°23'44.2"E
VI-(S6)	1692m	34°26°50 38"N	74°26°55,38E
VIL(S7)	1716m	34°32°05.15°N	74°24'47.83"E
VIII-(S8)	1781m	34°31°17.03"N	74°28'11.87°E
IX-(S9)	1604m	34°33°31.4"N	74°19'16.21"E
X-(S10)	1792m	34°33'31.3"N	74°19'16.21°E
XI-($11)	1799m	34°36°43.83 "N	74°24'26.32"E

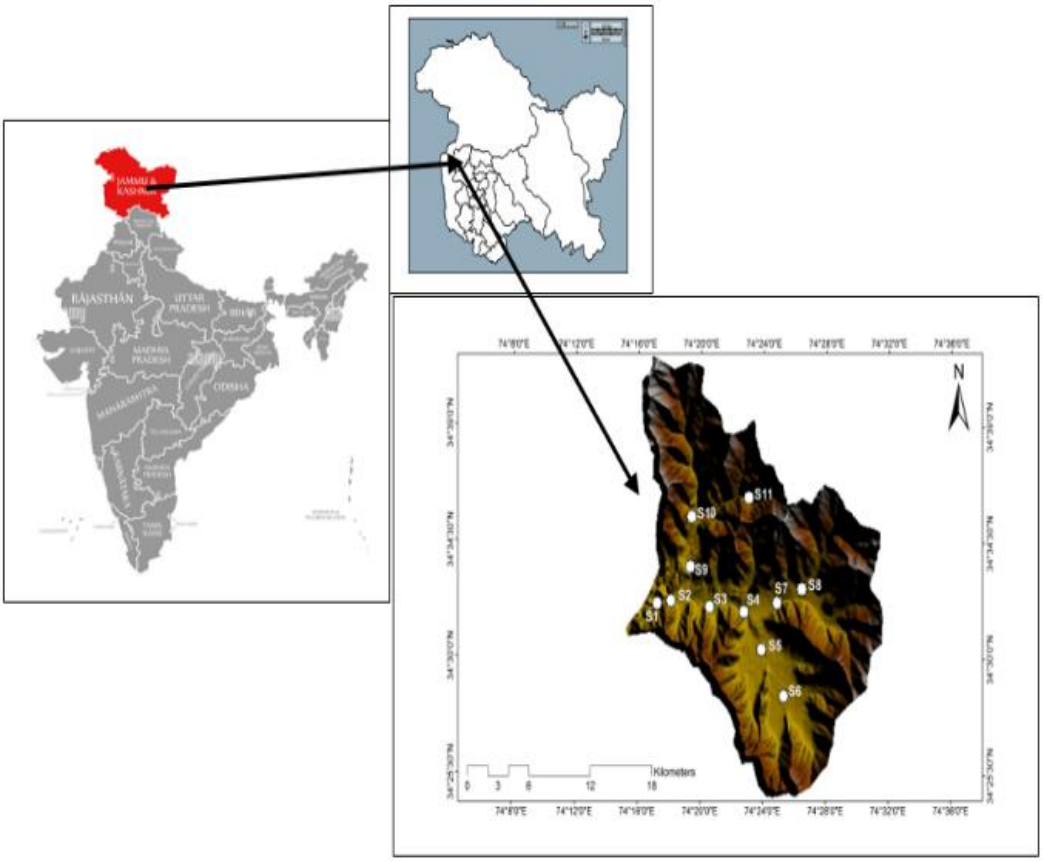

Fig. 1. Geographical map of Lolab watershed (Study Area)

when a semi-quantitative approach for hard-bottomed substrates was created. It is simple to collect samples because the natural flow of organisms in the streambed directs the sampling net upstream. The top layer of cobbles or pebbles was jolted off the bottom substrate for at least a minute to scrape the base bed [17,18]. Using each stage of the approach, one square metre was sampled at 15-meter intervals along the stream's path. Another approach for locating invertebrates in the wild is the rock pick method. Crustaceans were extracted from the slimy river bottoms using an 8-inch square metal box with spring-opened and spring-closed jaws [15,19,5,2]. To cover a 1 m2 area, it was applied three to four times at each site. Invertebrates, which had fragile bodies, were preserved in 70% alcohol, while molluscs, which had hard shells, were preserved in 4.5% formalin. Macroinvertebrates were observed and identified using a binocular microscope (x 6 magnification) [20,21,19,22,23]. Density [24], variety [25], taxonomic richness, dominance [26], evenness [27], and percentage densities of all macroinvertebrate orders are some of the estimated community properties of organisms.

2.2.2 Analysis of environmental variables

At each sampling site, we collected water samples, subsurface water temperatures, air temperatures, dissolved oxygen concentrations, and temperature. During each sampling, pH, depth, and flow velocity were all monitored on-site. When it came to taking an accurate reading, a mercury-in-glass thermometer came in handy. Precision Scientific Instruments Corporation India's PCS Tester 35 Eutech Multi-Parameter Tester was utilized to obtain readings for DO, and pH. Water samples were collected in acid-washed plastic bottles and sent to the laboratory on ice to preserve their integrity during transportation to the lab. Within 24 hours of collection, nitrates were examined in the laboratory, and phosphates were quantified spectrophotometrically after being reduced using appropriate solutions (APHA ,2005) as shown in Table 2.

Table 2. Physico Chemical parameters and methods followed

Parameter	Method
Air temperature	Mercury thermometer
Water temperature	Mercury thermometer
pH	PCS Tester 35
Dissolved oxygen	Iodometric method
Free CO^2	Titrimetric
Total hardness	EDTA titrimetric
Ammonical nitrogen	Spectrophotometry
Total phosphorus	Stannous chloride method

2.3 Data Analysis

The mean and standard error were computed for each parameter and research site. A one-way ANOVA was used to compare the stations' physical and chemical characteristics. The Shapiro-Wilk and Levene's tests were used to evaluate the assumptions of normality and homogeneity of variance before the use of ANOVA. When these presumptions were shown to be false, data were log (x + 1)-transformed, except for pH, and then analyzed using repeated measure ANOVA with sample month as a subfactor. After significant ANOVAs, post hoc Tukey Honest (HSD) testing was employed to find differences across station means ($P<0.05$).

3. RESULTS AND DISCUSSION

3.1 Environmental Conditions, Chemistry, and Pollutants

During the study, air and water temperature were recorded monthly for a year. The minimum temperature was recorded during January 2019 at site II at 1.8°C and, the maximum temperature was recorded in August 2019 at site II at 30.5°C. Typically, summer had the highest temperature, while winter had the lowest ($p = 0.0255$). Water temperature remained low throughout the year owing to the kryal nature of streams. Like air temperature, water temperature showed the same regime, the lowest temperature in January recorded was 1.4°C, and the highest temperature recorded in July was 30.1°C. Seasonally, summer saw the greatest temperatures and winter saw the lowest ($p = 0.042$).

pH was generally on the alkaline side, and values ranged from 7.6 at site VI in December to 8.67 at site I in March, reflecting limestone dissolution in the catchment. Seasonally, spring and autumn showed high pH, while summer recorded the lowest pH. Annual mean pH ranged from a minimum of 7.6 ± 0.4 to a maximum of 8.6 ± 0.3 from upstream to downstream. The importance of pH as a controlling factor in aquatic systems has long been acknowledged, and pH extremes have a detrimental effect on biological components [28,29]. The two metabolic processes that frequently have the greatest impact on pH in aquatic ecosystems are photosynthesis and respiration.

Dissolved oxygen is the most critical water quality variable in aquatic ecosystems [30]. During the study, the concentration of DO varied from 4.9 mg^{-1} to -12.6 mgL^{-1}. The highest concentration of 12.6 mgL^{-1} was recorded at site VII in February, while sites V and I recorded the lowest concentration of 4.9 mgL^{-1} in July and August, respectively. The annual mean concentration of DO was found to be highest at site VII with a value ± 10.1 $mgL^{-1,}$ while sites I and V recorded the lowest annual mean concentration with a value of 5.59 mgL^{-1}.DO showed a constant increase in values while approaching from human dominating areas (site I) towards wild areas (site VII). Seasonally, winter had the greatest record, followed by autumn and spring, while summer had the lowest. The lowest annual recorded field values of DO from all sites were found statistically significant ($p < 0.0025$).

The concentration of carbon dioxide in aquatic environments is an important feature that reflects both internal carbon dynamics and external biogeochemical processes in terrestrial ecosystems [31]. It affects the concentration of carbonates, bicarbonates, pH, and overall hardness in water as input parameters for the buffer system. In the present study, the monthly free CO_2 was recorded, and the values ranged from a minimum of 1.4 mgL^{-1} at site IV and at site III in August 2019 and a maximum of 8.4 mgL^{-1} at the site I and site II in June and July. The annual higher values of free CO_2 (5.4 ± 2.3 mgL^{-1}) were found at the site I, while the lowest level of CO_2 (3.2 ± 1.5 mgL^{-1})was found at site IV. When comparing the overall mean of free CO_2 values concerning seasons, spring and autumn are depicted as significantly lower than summer and winter. Subjected to statistical analysis, the Tukeys HSD test revealed that the monthly values of free CO_2 were not significantly different ($p < 0.005$), and the p-value for free CO_2 was found statistically insignificant($p = 0.23$).

Hardness is a significant consideration when using water for a variety of applications. Hardness may be caused by the natural accumulation of salts from soil and rocks. During the investigation, total minimum hardness was recorded as 40.6 mg L^{-1} at Site III in September, while maximum total hardness was found at Site IV in July as 316.9 mg L^{-1}. The annual average maximum concentration of total hardness was 201.64 mg L^{-1} at site VIII, while the minimum annual average concentration of total hardness was found at 53.34 mg L^{-1} at site I. Seasonally summer season recorded the highest concentration of total hardness while the spring season depicted the lowest. The concentrations of total hardness at all the sites during the study were found statistically significant ($p=0.007$).

During the study, Ammonical nitrogen concentration was found to vary from a minimum of 83.5 μgL^{-1} at site II in November to a maximum of 865.3 μgL^{-1} in July at site V. Further, the average annual concentration of Ammonical nitrogen showed the highest concentration of 273 μgL^{-1} at site V while as Site I recorded the lowest concentration of 139.9 μgL^{-1}. The highest value at site V is due to the direct washout of the water from paddy fields into the canal. All the values from study sites were found statistically significant ($p = 0.04$).

Owing to human-made inputs, nitrogen and phosphorus levels are frequently increased [32]. Examples of significant anthropogenic nitrogen and phosphorus additions to streams include agricultural fertilizers, air deposition, nitrogen-fixing plants, human and animal waste [33]. During the study, total phosphorus concentration was found to vary from a maximum of 885.3 μgL^{-1} at site V in June to a minimum of 78.6 μgL^{-1} in January at site VII. Further, the average annual concentration of total phosphorus showed the highest concentration of 286.33µgL-1 at site V while Site I recorded the lowest concentration of 112.17 μgL^{-1} at the site I. Seasonally, summer recorded the highest concentration of nitrate-nitrogen while winter recorded the lowest. All the values from study sites were found statistically significant ($p = 0.0010$). Linear (r) Pearson correlation between various physico-chemical parameters of the year 2018-2019 from Lolab Watershed is shown in Table 3.

3.2 Species Investigated and Diversity Indices

There are numerous ways in which macroinvertebrates can serve as accurate water quality indicators [34]. Natural environmental variation and anthropogenic stressors are likely to affect invertebrate communities in diverse river sectors in different ways [35]. Two general assumptions underlie the biotic indices: I) that more stable assemblages have higher diversity values, whereas stressors lead to lower diversity, and II) that diversity itself is a reasonable indication of environmental integrity because of the diversity it provides [36,37]. According to their stress tolerance and diversity values, taxa used as ecological indicators are graded when assessing the biological health of water bodies. Plecoptera, Ephemeroptera, Trichoptera, Decapoda, and Chironomidae are the taxonomic groupings utilized as indicators in the Extended Biotic Index, which uses pollution sensitivity as an indicator [38].

In comparison to their adults, mayflies, caddisflies, and dragonflies (including Odonata, Ephemeroptera, and Trichoptera) nymphs are more tolerant of pollution. The mayfly families Baetidae and Canidae, as well as other caddisfly families, have a wide range of pollution-resistant species. As pollution levels grow, tolerant organisms include non-biting midges, aquatic

Table 3. Linear (r) Pearson correlation between various Physico-chemical parameters during four seasons of the year 2018-2019 from Lolab Watershed

	Air T	Water T	pH	Dissolved Oxygen	Free CO_2	Total Hardness	Nitrate Nitrogen	Total Phosphorus
Air T*		0.009802	0.52199	0.14175	0.6841	0.66438	0.26863	0.39438
Water T	0.9902		0.47641	0.12484	0.76323	0.57609	0.36661	0.3672
pH	-0.47801	-0.52359		0.13979	0.42234	0.85453	0.95073	0.76621
Dissolved Oxygen	-0.85825	-0.87516	0.86021		0.45448	0.91781	0.58897	0.80196
Free CO_2	-0.3159	-0.23677	0.57766	0.54552		0.22351	0.57485	0.47197
Total Hardness	0.33562	0.42391	0.14547	-0.08219	0.77649		0.95829	0.12752
Nitrate Nitrogen	0.73137	0.63339	0.049266	-0.41103	-0.42515	-0.04172		0.55495
Total Phosphorus	0.60562	0.6328	0.23379	-0.19804	0.52803	0.87248	0.44505	

T = Temperature*

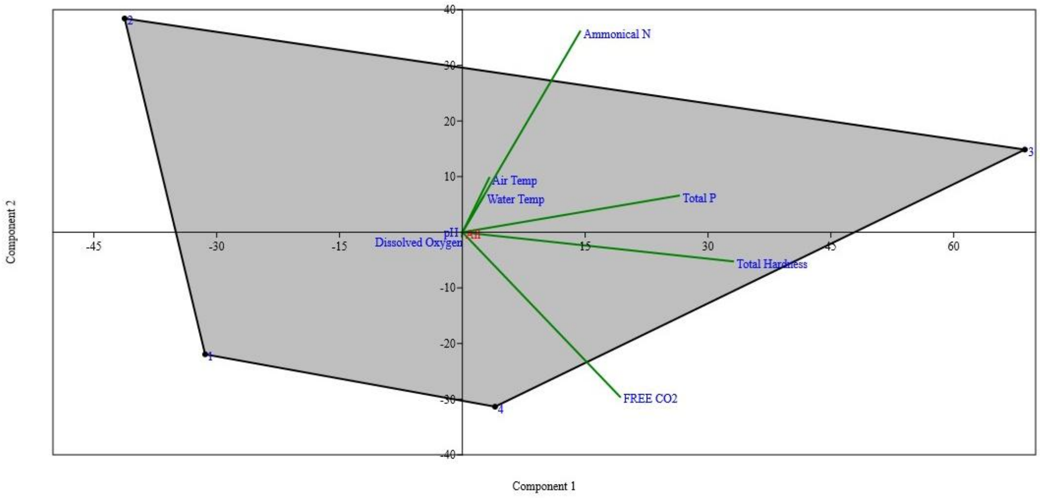

Fig. 2. Principal Component Analysis (PCA) ordination plot explaining the variation of physicochemical parameters among sampling sites

oligochaetes, and the isopod family, which includes the *Tubifex* sp., the biggest number of taxa (26) was observed at site VII due to the direct discharge of agricultural-rich effusive effluent, with a total of 27 taxa reported from all study sites. Trichoptera accounted for 36% of the total abundance studied, while Diptera accounted for 26%, Ephemeroptera accounts for 16% of all species. Percentage of Plecoptera, Coleoptera and Araneae comprise 4%, and the rest 1%. Figure 3 depicts the CCA ordinations that link taxa's frequency to several environmental factors in aquatic environments. As a consequence of the CCAs, it was determined that there were five major groups, each with distinct preferences for specific abiotic characteristics.

CCA was able to distinguish between the damaged and unaffected locations. The CCA ordination also revealed a substantial relationship between the macroinvertebrate fauna and environmental variables found in Lolab Streams. Site II had the highest nitrate concentration, pH, and hardness compared to the other sites, with site VI coming in second. A multiple-scale stressor could be recognized and described using a combination of factors. Numerous individual environmental variables have reasonably strong correlations with the axis for CCA, but these correlations were not statistically significant.

These predicted significances, however, might be the outcome of unmeasured environmental variables. At the other stations, the bulk of the tolerant dipteran groups—*Biliocephala sp., Chironomous sp., and Culex sp.,* as well as the *Trichoptera—Brachycentrus sp., Cheumatopsyche sp., and Hydroptilidae sp.,* were either rare or absent. Site II was an extreme outlier in our ordination analysis that is a sign of the river's declining biotic and ecological health.

Throughout the year of sampling, the species richness, diversity, and evenness indices at the various sampling sites seemed to match the water quality circumstances at each location. Low species diversity at site X indicated environmental stress as a result of gradually growing human influences on the water quality condition at these sites, but high species diversity at stations VIII and IX were associated with less contaminated circumstances.

The Simpson's Diversity Index is a measure for studying biodiversity that considers both the total number of species and their abundance. Species richness and evenness are two measures of diversity that improve together to produce a higher total dominance (D) it can take on a value between 0 and 1, inclusive. The D value during the study ranged from 0.68-0.73. Another popular indicator for doing so, the Shannon diversity index (H), measures the number of unique species within a certain area. Shannon's index, like Simpson's index, takes into account the overall diversity and abundance of species. The presence of many species is one way to boost diversity. Typically, the Shannon diversity index will take on a range of values between 1.5 and 3.5 when applied to ecological data from the actual world, and during the study, H- index showed a range between 1.69-2.168. Various diversity indices are shown in Table 4.

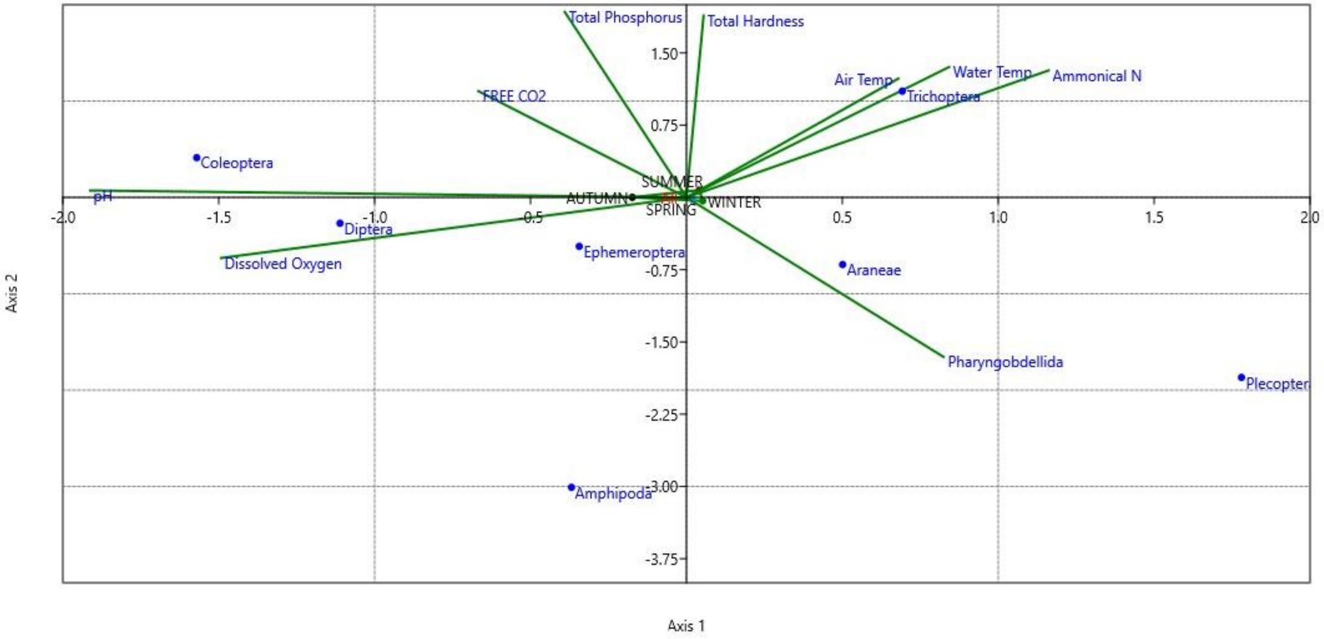

Fig. 3. CCA ordinations related the frequency of taxa to environmental parameters in water

Table 4. Various diversity indices are shown by macroinvertebrates in the study area (Lolab)

	SITE I	SITE II	SITE III	SITE IV	SITE V	SITE VI	SITE VII	SITE VIII	SITE IX	SITE X	SITE XI
Taxa_S	17	21	15	21	24	27	26	24	27	11	18
Individuals	256	228	220	136	238	520	670	458	244	92	168
Dominance_D	0.3119	0.3037	0.2743	0.2723	0.2737	0.2673	0.2694	0.2785	0.2821	0.2883	0.3044
Simpson_1-D	0.6881	0.6963	0.7257	0.7277	0.7263	0.7327	0.7306	0.7215	0.7179	0.7117	0.6956
Shannon_H	1.695	1.819	1.913	2.042	2.093	2.168	2.09	1.968	2.035	1.706	1.718
Evenness_e^H/S	0.3204	0.2936	0.4515	0.3671	0.3377	0.3237	0.311	0.2982	0.2833	0.5006	0.3098

4. CONCLUSION AND RECOMMENDATIONS

Rivers in the North Western Himalayas are highly appreciated for their rich biodiversity, however during the past two decades, urbanization in North India i.e., the Indian Himalayan Region has led to the disruption of freshwater ecosystems. The macroinvertebrates displayed preferences for particular abiotic parameters, highlighting their potential utility in future research as dependable ecological indicators, molded by a synergistic mix of anthropogenic influences and land use intensity. The Lolab streams are not exempted from the influence of a growing human population on the water and biotic quality of the river, primarily as a result of waste disposal. This study provides information on the current state of the Lolab Streams' water quality and acts as a baseline survey of the river's macroinvertebrate species. This study's findings can serve as the basis for a long-term evaluation of the stream and the use of bioindicators in the management of the river system.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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