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Original Article

# BMWP and ASPT biotic indices, useful tools to monitor stream water quality: Case study Ghale Rudkhan River, Guilan, Iran

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**Abstract:** The objectives of the present study were to evaluate the health status and extent of pollution in Ghale Rudkhan River using the biotic index BMWP/ASPT, Margalef species richness, and Simpson and Jaccard similarity indices. Five sampling sites were selected from upstream to downstream reach of the river, and the samples were collected in each site using a Surber sampler (900 $cm^2$ area). In total, 5134 individuals of macroinvertebrates were enumerated and identified, belonging to 35 species and 30 families, 10 orders, 4 classes, and 3 phyla. Orders of Diptera, Ephemeroptera, Trichoptera, and Plecoptera were the most dominant taxa, respectively. Site 1, located at the upstream reach of the river, exhibited better quality indexes. In contrast, site 5, located at the downstream reach, had the poorest water quality due to relatively high loads of pollutants from the river's upper reaches and domestic and agricultural wastes. The mean±SD of the evaluated indexes were: BMWP/ASPT as 5.78±1.09, Margalef index as 1.38±0.48, Simpson index as 0.56±0.16 and Jaccard and 0.48±0.08. According to the BWMP/ASPT index, the Ghale Rudkhan River is classified into three classes of pollution: clean water at sites 1 and 2 (with values of 6.02 and 6.65), slightly polluted water at sites 3 and 4 (with values of 5.63 and 5.68) and moderately polluted at sites 5 (with value of 4.96).

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## Introduction

Looking through the history of running water-quality assessments based on biological indicators, at least 100 indices have been developed, of which about 60% of biotic ones are based on macroinvertebrate analysis (De Pauw and Hawkes, 1993). Biotic indices are numerical expressions linking quantitative measures of species diversity with qualitative evidence of the ecological sensitivity of the taxa in question. Principally, they are based on macroinvertebrates, including Plecoptera, Ephemeroptera, Trichoptera, *Gammarus, Asellus*, and Chironomidae, and the fact that the number of taxonomic groups is reduced as pollution increases (Hellawell, 1986; Czerniawska-Kusza, 2005).

Aquatic habitats are adversely impacted by urbanization, deforestation, construction, irrigation, and pollution (Dudgeon, 2008; Clews and Ormerod, 2009). Freshwater organisms live almost continuously in the water and respond to all environmental stresses, including pollutants (Morse et al., 2007; Al-Shami et al., 2010a, b; Veroli et al., 2010). These ecosystems are among the most altered and threatened by humans due to the intense demand for water by constantly growing human populations and economies, resulting in widespread degradation (Abell et al., 2008; Limburg et al., 2011). Therefore, assessing the ecological state of aquatic ecosystems is critical for their effective management, protection, rehabilitation, and integrity of ecosystem services (Revenga et al., 2005; Balderas et al., 2016).

Assessing the quality of freshwater ecosystems, particularly streams, provides an insight into their health and the pressures imposed by the surrounding environment (Sandin, 2003). A significant advantage of biomonitoring is the ability of organisms to integrate their responses over space and time, providing insight into environmental conditions

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Table 1. Geographical coordinates of the sampling sites at the Ghale Rudkhan River.

| Sampling site | Longitude | Latitude |
|---|---|---|
| 1 | 49°14´965″ | 37°04´430″ |
| 2 | 49°14´964″ | 37°04´434″ |
| 3 | 49°15´555″ | 37°05´194″ |
| 4 | 49°15´553″ | 37°05´203″ |
| 5 | 49°16´333″ | 37°06´360″ |

before the sample is collected. Thus, biomonitoring or biological assessment is a relatively cost-effective method that is less dependent upon laboratory equipment and simultaneously provides acceptable results. It allows the detection of cumulative effects of pollutants and temporal effects of environmental stressors while causing minimal impact on the environment (Aazami, 2015). Many organisms, including macroinvertebrates, have been used for ecosystem monitoring (Blanchet et al., 2008). Worldwide experience has demonstrated that the most useful biological assessment methods for freshwater monitoring are based on benthic macroinvertebrates (Sivaramakrishnan, 2000; Dos Santos, 2011). Benthic macroinvertebrates possess several advantages, including characteristic sensitivity to pollution, limited mobility, and ease of collection and identification (Pan et al., 2015; Balderas et al., 2016; Mangadze et al., 2016). Therefore, any change in their population would impact the productivity of the entire ecosystem (Merz et al., 2005).

Of a large number of indices developed to evaluate water quality, BMWP (Biological Monitoring Working Party), ASPT (Average Score Per Taxon), and some others are used very commonly (Gerhardt et al., 2004). These indices use tolerance scores designated to each macroinvertebrate family or species. BMWP was developed in 1980 to assess the quality of running waters (Wright et al., 1989) and was then modified and used in different regions. BMWP employs a scoring system to evaluate the effects of pollutants and fish farm effluents on stream ecosystems (Blomqvist, 1991; Camargo and Gonzalo, 2007). The advantage of the score systems is that they are applicable at family levels and not limited to geographical regions (Armitage et al., 1983). Some researchers believe the average score per taxon (ASPT) is a more accurate and reliable water quality assessment system than BMWP (Armitage et al., 1983; Hawkes, 1998). The advantage of ASPT over BMWP is that it is not dependent on sample size, season, year, or sampling method (Armitage et al., 1983; Hawkes, 1998).

Environmental conditions are very fragile and deteriorated, at least in highly populated regions of Iran (e.g., Guilan Province). Considering the importance of stream ecosystems, this study compared diversity indices, richness indices, and similarity indices of the impacted/polluted and relatively untouched reaches of the Ghale Rudkhan River, Guilan Province, Iran. For this purpose, Simpson, Margalef, Jaccard, and BMWP/ASPT indices were used.

## Materials and Methods

**Study area:** The study area comprises the downstream and upstream of the Ghale Rudkhan River. The river is located at 665-715 m above mean sea level and drains an area of approximately 452.2 $km^2$ of high mountains and forests within the longitude 49°03´ to 49°25´E and 37°00´ to 37°24´N in Guilan Province, Iran. Tourism impacts almost all parts of the river except for small reaches upstream. Agriculture is a common activity along the river, and some rainbow trout farms have been established alongside it. Therefore, we selected five sampling stations along the river course, with two at the upstream reach, two in the middle reach, and one at the downstream reach of the river. These sampling sites provide a near-complete picture of the activities along the river and their corresponding impact on the river (Table 1).

Station 1 is located upstream of the river in a forested and canopy-covered area, far from human activities, and the ecosystem is relatively untouched. The site is not easily accessible to the general public and, therefore, has preserved its natural condition. Station 2 is located upstream but below the tourist complex of the Ghale Rudkhan and is heavily impacted by human activities. A large amount of waste is released into the river. Stations 3 and 4 are located in the middle reach of the river, slightly before and after a rainbow trout farm, and wastewater from the farm enters exactly before site 4. Station 5 is located about 5 km from site 4 at the downstream reach of the river and can provide the self-purification capacity of the river and changes in the macroinvertebrate communities concerned.

**Sampling:** The benthic macroinvertebrates were sampled monthly from May to December 2017 using a Surber sampler (900 $cm^2$ area). Triplicate samples of the benthic macroinvertebrates were collected at each site. Samples were stored in plastic bags, preserved in 96% Ethanol, and washed through a 0.5 mm mesh sieve in the Laboratory. Using valid references, macroinvertebrates were identified under a stereomicroscope (Usinger, 1963; Pennak, 1987; Merritt and Cummins, 1996; Mugnai et al., 2010; Hamada et al., 2014). The individuals of some insect orders, e.g., Ephemeroptera, Plecoptera, Trichoptera (EPT), and Diptera were identified to the genus level. Other taxa were identified at the level of family (other Insects) or subclass (Anellida). The biomass and abundance of each taxon were calculated in $m^2$.

**Physical and chemical habitat measures:** In each sampling reach, we recorded quantitative measures of the physical habitat following Peck et al. (2006). Those measures describe stream channel morphology (e.g., depth, wetted and bank full widths, etc.), habitat features (substrate size, amount of wood in the channel), riparian structure (canopy cover, vegetation type), and human alterations in riparian zones (e.g., presence of buildings, pasture, crops, roads, trash). Based on Kaufmann et al. (1999), we calculated metrics and indices combining field measurements into a single value. Water temperature, electrical conductivity, total dissolved solids, turbidity, and pH were measured at each stream reach using portable equipment (YSI Model 650). A water sample was collected and transported to the laboratory to determine the concentrations of dissolved oxygen, total nitrogen, and total phosphorus (APHA, 1998).

**BMWP/ASPT indices:** To apply BMWP, the collected macroinvertebrates are identified at family levels, and a corresponding tolerance score is assigned (Guntharee, 2003). Families with the highest sensitivity take the highest scores (e.g., Ephemeroptera and Plecoptera with 10), and tolerant families like worms receive the lowest scores (Guntharee, 2003). The scores range from 1 to 10, corresponding to tolerant and sensitive families (Artemiadou and Lazaridou, 2005).

A family-level BMAP/ASPT biotic index, commonly used for water quality classification, was used to detect the water quality of the Ghale Rudkhan River. A tolerance score is assigned to each taxon, reflecting its tolerance/sensitivity to pollution. The tolerance metrics were based on taxa sensitivity to organic pollution, where we assigned values ranging from 1 (most tolerant) to 10 (most sensitive) for each taxon following the scores proposed by Junqueira et al. (2000) and additional sources when the information was not available for a specific taxon. The following formula is used to calculate BMWP/ASPT values. The BMWP is calculated by summing the tolerance scores of all families recognized in the sample based on the formula of $BMWP/ASPT = \Sigma B(n)/N$, where N is the total number of macroinvertebrate families in each sampling site, n is the abundance of each family, and B is the tolerance score assigned for each family (Armitage et al., 1983; Walley and Hawke's1996; Sharifinia et al., 2012). The score values of different families ranged from 1 to 10 according to their tolerance to pollution (highly tolerant to highly sensitive; Hawkes, 1998). High BMWP scores indicate unimpacted sites, whereas low BMWP scores indicate sites with heavy organic pollution (Table 2).

**Margalef index:** Margalef's Index (Margalef, 1951) is focused on the number of species relative to the number of individuals. The higher the value of Margalef's index, the greater the diversity and, apparently, the cleaner the aquatic ecosystem. The

Table 2. Stream water quality categories based on BMWP/ASPT (Esmaeili Sari, 2000)

| Water quality | BMWP/ASPT |
|---|---|
| Clean Water | Over 6 |
| Slightly polluted water | 5-6 |
| Moderately polluted water | 4-5 |
| Heavily polluted water | below 4 |

Table 3. Abundance of benthic macroinvertebrates at the Ghale Rudkhan River sampling sites during 2018.

| Season/site | 1 | 2 | 3 | 4 | 5 | Total |
|---|---|---|---|---|---|---|
| Spring | 124 | 141 | 90 | 273 | 214 | **842** |
| Summer | 223 | 232 | 204 | 281 | 551 | **1491** |
| Autumn | 611 | 515 | 545 | 408 | 722 | **2801** |
| **Total** | **958** | **888** | **839** | **962** | **1487** | |

main inconvenience of Margalef's index is the absence of an evenness component, which may result in differences when compared with Shannon's and Simpson's indices. Using the formula of R = S-1/Ln(N), Margalef's index is calculated where R is Margalef's diversity index, S is the number of species observed, and N is the total number of species observed.

**Simpsons index:** Simpson's Index (D) measures the probability that two individuals randomly selected from a sample belong to the same species (or some category other than species). In this formula $(D = 1-(\sum n(n-1))/(N(N-1))$, D is the Simpson's diversity index, n is the number of each taxon in the sample, and N is the total number of species observed in the sample.

**Statistical analyses:** The data were analyzed using SPSS 22 software. The Shapiro-Wilks and Kolmogorov-Smirnov tests examined the normality of data, and normalization was applied where necessary. One-way and two-way analyses of variance (ANOVA) were performed to compare sampling sites and seasons. Post-hoc tests using Tukey and Scheffé revealed the sources of the differences. Microsoft Excel 2013 was used for some analyses and graph drawing.

## Results

The aquatic macroinvertebrates identified were assigned to 4 classes, 10 orders, and 30 families. A total of 5134 macroinvertebrate specimens were collected and analyzed. Insect orders were dominant in all sampling sites. The highest abundance was observed in autumn at site 5, whereas the lowest in spring at site 3 (Fig. 1; Table 3), and Insect orders of Diptera (60.03%), Ephemeroptera (19.18%), Trichoptera (17.72%) and Plecoptera (1.75%) were the most abundant and dominant taxa, respectively. Other groups accounted for only 1.01% of the combined abundance. Order Ephemeroptera (7 families and 11 genera), Diptera (8 families and nine genera), Trichoptera (5 families and five genera), and Plecoptera (2 families and two genera) were the most diverse benthic macroinvertebrate (Fig. 2, Table 4). *Simulium* sp. and *Chironomus* sp. were the first and second most abundant taxa within the order Diptera. Both taxa were observed in all sampling sites, while *Dicranota* sp. and *Atherix* sp. were present only at sites 1 and 4, along with *Hexatoma* sp. and *Tipula* sp. at sites 2 and 5. Mayflies or Ephemroptera were the second most abundant benthic taxa, while *Baetis* sp. and *Rhithrogena* sp. were the dominant groups. Genus *Isonychia* and *Tricorythodes* were found only at site 4, while *Ecdyonurus* sp. was found only at site 5. The family Hydropsychidae and the genus *Hydropsychae* were the most common representatives of the order Trichoptera. *Lepidostoma* was the second most frequent taxon observed in sampling sites 4 and 5. *Limnephilus* sp. was observed only at the upstream site 1. The order Plecoptera was observed at very clean parts of the river, with two representatives in *Isoperla* sp. observed at all sites and Chloroperla sp., which

Table 4. Taxa tolerance values of benthic macroinvertebrates used in BMWP (Wally and Hawkes, 1996, 1997).

| | Kingdom | Class | Order | Family | BMWP score |
|---|---|---|---|---|---|
| 1 | Annelidae | Clitellata | Haplotaxida | Lumbricidae | 1 |
| 2 | Mollusca | Gastropoda | Basommatophora | Lymnaeidae | 3 |
| 3 | Arthropoda | Crustacea | Amphipoda | Gammaridae | 6 |
| 4 | | | Decapoda | Potamidae | 10 |
| 5 | | Insecta | Ephemeroptera | Baetidae | 4 |
| 6 | | | | Heptageniidae | 10 |
| 7 | | | | Ephemerelidae | 10 |
| 8 | | | | Isonychiidae | 10 |
| 9 | | | | Oligoneuriidae | 10 |
| 10 | | | | Tricorythidae | 7 |
| 11 | | | | Caenidae | 7 |
| 12 | | | Trichoptera | Hydropsychidae | 5 |
| 13 | | | | Philopotamidae | 8 |
| 14 | | | | Rhyacophilidae | 7 |
| 15 | | | | Lepidostomatidae | 10 |
| 16 | | | | Limnephilidae | 7 |
| 17 | | | Pelecoptera | Perlodidae | 10 |
| 18 | | | | Chloroperlidae | 10 |
| 19 | | | Diptera | Tipulidae | 5 |
| 20 | | | | Pediciidae | 5 |
| 21 | | | | Blephariceridae | 10 |
| 22 | | | | Simulidae | 5 |
| 23 | | | | Chironomidae | 2 |
| 24 | | | | Tabanidae | 4 |
| 25 | | | | Dixidae | 4 |
| 26 | | | | Athericidae | 10 |
| 27 | | | Odonata | Gomphidae | 8 |
| 28 | | | | Cordulegastridae | 8 |
| 29 | | | | Coenagrionidae | 8 |
| 30 | | | Coleoptera | Diytiscidae | 5 |

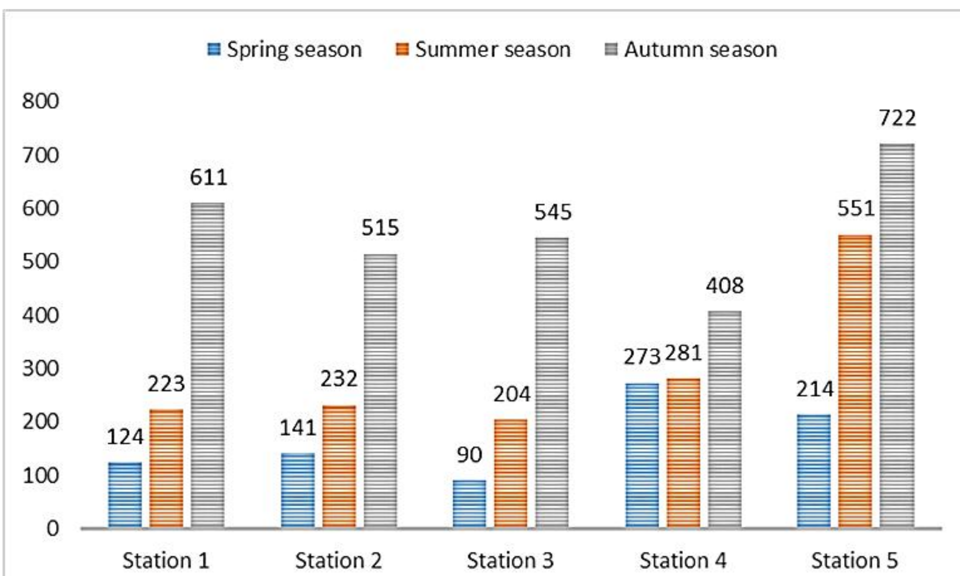


Figure 1. Distributions and abundance of benthic macroinvertebrates at sampling sites of the Ghale Rudkhan River 2018.

was present only at the upstream site 1.

Since the taxa mentioned above counted for almost 99% of the benthic population and their members were present at almost all sampling sites, analyses were mostly focused on these groups. There was a significant difference ($P \geq 0.05$) between sites 2 and 5

Table 5. BMWP/ASPT values at the Ghale Rudkhan River sampling sites during 2018.

| Season | Sampling sites | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | Mean |
| **Spring** | 8.39±068 | 7.30±1.29 | 5.54±0.34 | 6.22±1.37 | 5.25±0.26 | 6.54±1.40 |
| **Summer** | 6.50±0.71 | 5.90±0.92 | 6.38±1.42 | 5.49±0.61 | 4.8±0.29 | 5.81±0.98 |
| **Autumn** | 5.65±0.46 | 5.29±0.63 | 5.03±0.37 | 5.37±0.63 | 4.91±0.97 | 5.25±0.61 |
| **Mean** | 6.65±1.40 | 6.02±1.14 | 5.66±1.01 | 5.62±0.79 | 4.95±0.58 | |

Table 6. Values of Margalef species richness index in sampling sites and seasons- Ghale Rudkhan River during 2018.

| Season | Sampling sites | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | Mean |
| **Spring** | 1.83±0.30 | 2.10±0.24 | 1.44±0.19 | 1.86±0.26 | 1.05±0.86 | 1.66±0.51 |
| **Summer** | 2.22±0.28 | 1.02±0.50 | 1.23±0.46 | 1.00±0.60 | 1.55±0.64 | 1.40±0.59 |
| **Autumn** | 1.23±0.31 | 1.22±0.12 | 0.91±0.30 | 1.30±0.22 | 1.14±0.11 | 1.15±0.23 |
| **Mean** | 1.75±0.54 | 1.37±0.54 | 1.16±0.37 | 1.30±0.39 | 1.27±0.53 | |

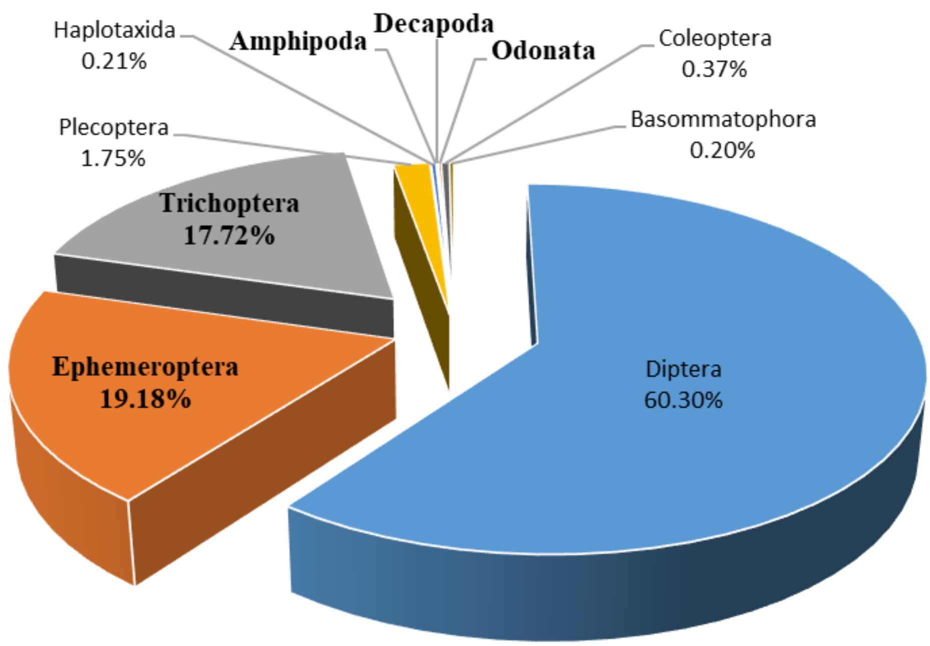


Figure 2. Distributions and abundance of benthic macroinvertebrates at sampling sites of the Ghale Rudkhan River 2018.

when Trichoptera was used for comparison. Otherwise, no differences were found between the sites. A comparison of the sites in different seasons based on benthic macroinvertebrate taxa showed significant differences between the sites based on their Diptera population, while there were no differences for other taxa. Based on BMWP/ASPT results, water quality in spring was "Good," while it was suspected of pollution in summer and autumn. According to these indices, no severe pollution or pollution threat was detected at any sampling site.

Table 4 shows the taxonomic details of benthic macroinvertebrates and their respective tolerance values used for BMWP/ASPT analyses. The results of BMWP/ASPT showed that water quality in the Ghale Rudkhan River falls in three categories, i.e., clean water at sites 1 and 2 (upstream), slightly polluted water at sites 3 and 4 (middle reach), and moderately polluted water at site 5 (downstream) (Fig. 3, Table 5). The highest and lowest values of BMWP/ASPT were observed in spring (8.93 and 5.25) and autumn (4.91 and 5.25) at sites 1 and 5, respectively. These values in the summer months were 4.8 and 6.50, indicating good-quality and moderately polluted water at these

Table 7. Values of Simpson index of species dominance and diversity in sampling sites and seasons- Ghale Rudkhan River during 2018.

| **Season** | **Sampling sites** | | | | | |
|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **Mean** |
| **Spring** | 0.73±0.09 | 0.66±0.05 | 0.53±0.25 | 0.74±0.21 | 0.56±0.27 | 0.64±0.17 |
| **Summer** | 0.75±0.14 | 0.51±0.04 | 0.49±0.24 | 0.38±0.16 | 0.6±0.09 | 0.55±0.18 |
| **Autumn** | 0.51±0.25 | 0.49±0.13 | 0.42±0.06 | 0.6±0.10 | 0.61±0.10 | 0.53±0.14 |
| **Mean** | 0.65±0.19 | 0.54±0.10 | 0.47±0.17 | 0.55±0.20 | 0.59±0.13 | |

Table 8. Values of Jaccard similarity index in sampling sites and seasons- Ghale Rudkhan River during 2018.

| **Season** | **Sampling sites** | | | | | |
|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **Mean** |
| **Spring** | 0.50±0.04 | 0.46±0.11 | 0.43±0.10 | 0.42±0.11 | 0.32±0.09 | 0.42 ± 0.06 |
| **Summer** | 0.40±0.05 | 0.43±0.07 | 0.51±0.07 | 0.44±0.07 | 0.46±0.10 | 0.44 ± 0.04 |
| **Autumn** | 0.66±0.12 | 0.56±0.17 | 0.55±0.07 | 0.54±0.09 | 0.61±0.07 | 0.58 ± 0.05 |
| **Mean** | 0.52±0.13 | 0.48±0.06 | 0.49±0.06 | 0.46±0.06 | 0.46±0.14 | |

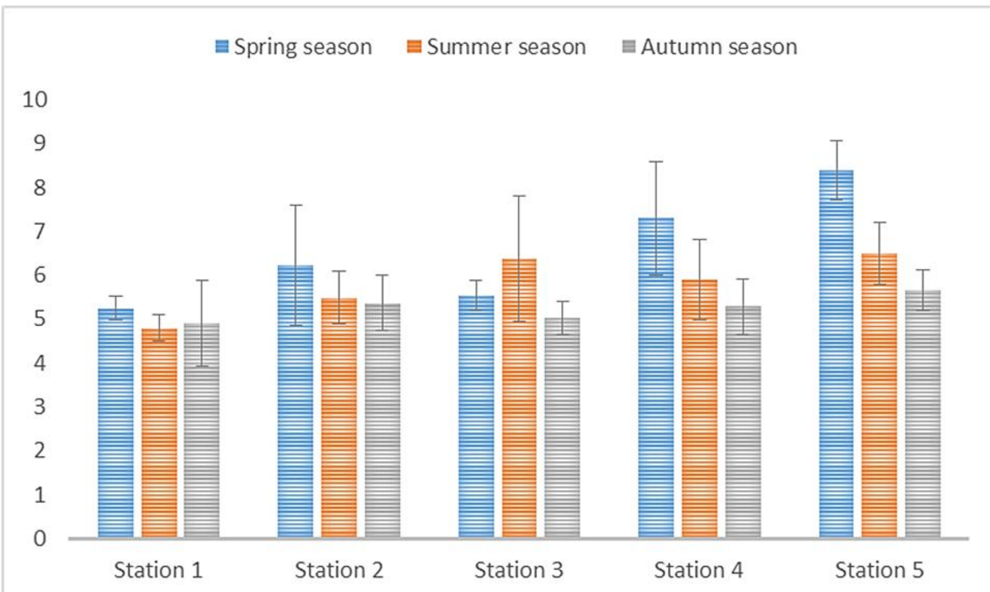


Figure 1. BMWP/ASPT values at the Ghale Rudkhan River sampling sites during 2018.

sites. Sites 3 and 4 showed a slightly polluted status throughout the study period.

The highest and lowest mean values for the Margalef index were observed at sites 1 (1.75±0.54) and 3 (1.16±0.37), respectively. Comparison of species richness in sampling seasons also showed the highest richness in spring (1.66±0.51), followed by summer (1.40±0.59) and the lowest in autumn (1.15±0.23). Comparison of sampling sites individually on single sampling occasions showed the highest Margalef index at site 1 (2.22) in summer, while the lowest (0.91) value was at site 3 in autumn (Table 6).

The Simpson index's highest and lowest mean values were observed at sampling sites 1 (0.65±0.19) and 3 (0.47±0.17), respectively, corresponding to the highest diversity at site 3 and the lowest at site 1. A seasonal comparison of the sites showed the highest value in spring (0.64±0.17) while the lowest in autumn (0.53±0.14) (Table 7). The Jaccard index was used to detect the similarity of sampling sites and seasons. The Jaccard index was used to detect the similarity between sampling sites and seasons. This index's highest and lowest mean values were observed at sites 1 (0.66±0.12) in autumn and 5 (0.32±0.09) in spring, respectively. The highest similarity values were observed in autumn, while the lowest was in spring (Table 8).

## Discussions

Benthic macroinvertebrates represent an integral part of lotic systems in understanding the effects of anthropogenic and natural stressors. Therefore, these organisms have been used in water quality assessment to understand the magnitude of stresses exerted by human activities (Pearson and Rosenberg, 1978; Perus et al., 2004). Based on the results, the negative impacts of urbanization on stream ecological health were apparent in the Ghale Rudkhan River. Significant variation in the macroinvertebrate community compositions was observed along urbanization and river gradient. A high proportion of susceptible species (e.g., EPT taxa) was observed in upstream reaches, where urbanization is low. In contrast, less-diverse communities dominated by tolerant species, e.g., *Simulium* sp. and *Chironomus* sp., were observed in relatively highly urbanized and tourist areas. Luo et al. (2017) noted that higher EPT (Ephemeroptera, Plecoptera, and Trichoptera taxa) is observed at low urbanization and dominant species at high urbanization levels.

BMWP is commonly used to evaluate the anthropogenic ecological impairment of freshwater ecosystems. In this study, the BMWP scores indicated the deterioration of water quality with increased urbanization. Several variables of habitat parameters, including nutrient concentrations, water flow, and substrate types, contributed to the taxonomic variation of macroinvertebrates along the river gradient. Increased nutrient concentrations resulting from human activities, urbanization, and trout farming favored species tolerant to organic pollution, allowing them to dominate the benthic community. In contrast, high water flow, substrate roughness, and heterogeneity (characteristic of upstream sites) promoted community taxon richness, diversity, and EPT richness. The region's rapid urbanization and pressure exerted by tourism activities are relevant to managing urban streams in the area or elsewhere. The impacts of urbanization and tourism on the Ghale Rudkhan River macroinvertebrate assemblages followed a general pattern; the sensitive species were gradually excluded, and tolerant species benefited from the disturbances to some extent, up to a certain threshold. In this study, macroinvertebrate species belonging to EPT taxa, known to be sensitive to pollution and environmental deterioration, were more abundant in upstream sites and gradually decreased in number and were replaced by more tolerant species of Diptera, e.g., *Simulium* and *Chironomus*.

Seasonal changes were not apparent in macroinvertebrate community compositions at sampling sites. There were also no significant differences in biological indices between the wet and dry seasons, even though macroinvertebrate communities typically exhibit changes (Huryn et al., 2008). The macroinvertebrate followed patterns observed in other urban streams subjected to increased pollution levels, characterized by the elimination of sensitive species (Kim et al., 2013) and a high abundance of tolerant taxa (Johnson et al., 2012). The tolerance score used at the family level in water quality assessment is the mean tolerance value given to species belonging to that family (Armitage et al., 1983). Order Ephemeroptera are sensitive to pollution and are indicators of good water quality. In this study, *Heptagenia* sp. and *Baetis* sp. were observed upstream.

BMW system assigns higher scores to sensitive EPT species and lower scores to tolerant organisms, e.g., Chironomids. BMWP gives higher weight to macroinvertebrate family numbers, which tend to change seasonally (Cooper and Knight, 1998); therefore, in the present study, the BMWP value in autumn was lower than in spring and summer. According to the BMWP index, the water quality of the Ghale Rudkhan River falls into three categories. In spring, it is categorized as clean water; in summer and autumn, it is categorized as slightly and moderately polluted water quality, respectively. In this study, nutrient loads, substrate types, roughness, and water flow regimes were correlated with catchment and riparian land uses. Therefore, changes in land use plans within the catchment could operate as filters limiting species by influencing in-stream habitat type and quality (Li et al., 2012; Nguyen, 2014; Wen et al., 2016).

The results showed a noticeable decrease in BMWP/ASPT values in sampling sites adjacent to the trout fish farm. It appears that micro-pollutants, dissolved nutrients, and suspended particles from the effluents of fish farms and domestic sources have been the main disturbance factor, thereby altering the macroinvertebrate composition from a sensitive EPT dominance to a tolerant Diptera (Chironomidae and Simulidae) dominance. Micro-pollutants typically occur at very low concentrations and enter surface waters through atmospheric deposition, polluted rainwater from roofs and sealed areas, untreated and treated wastewater, and other diffuse sources, such as field runoff carrying pesticides (Loos et al., 2013). Fish farm effluent and habitat modification resulting from human activities alter the community composition of macroinvertebrates. As a result, the population of tolerant species increases at the expense of eliminating sensitive species (Pipan, 2000). Other stressors that impact river organisms (e.g., macroinvertebrates) arise from multiple sources, including catchment land use, e.g., agriculture and urbanization (Rasmussen et al., 2012; Kuzmanović et al., 2016).

In the present study, BMWP/ASPT values decreased along the river gradient and urbanization levels. However, overall BMWP/ASPT values suggest that Ghale Rudkhan River retains relatively good-quality water, although the middle and downstream reaches are slightly and moderately polluted, respectively. A single biotic index cannot reflect the ecosystem health (Lydy et al., 2000). To assess the health status of the aquatic ecosystem, it is preferable to use diversity and similarity indices in conjunction with other metrics (e.g., multimetric approaches) to integrate the behavior of the elements and processes within biological systems (Karr, 1999; Balderas, 2016). Therefore, several biotic and population indices are used in the biological assessment of aquatic ecosystems. In the present study, we used Margalef's species richness, Simpson's species dominance and diversity, the Jaccard similarity index, and the BMWP/ASPT indices. They supplemented the assessment, and their results were almost in agreement. Margalef's index reflects the species richness and is used to compare biological communities (Washington, 1984). Higher values of the Margalef index indicate that the ecosystem is healthy. In this study, the higher Maraglef index was recorded at sites 1 and 2, located in the upstream reaches, which were healthier. Maraglef species richness followed a similar pattern to species diversity during the study period. The highest values of both Margalef and Simpson indices were observed in spring, while the lowest were in autumn.

Washington (2003) pointed out that calculating the diversity index of macroinvertebrates is an important structural property of a given ecosystem to understand its biological health. The use of diversity indices in water quality assessment assumes that macroinvertebrate community structure varies in correlation with environmental disturbances, where diversity is decreased as some species are severely influenced compared to others (Taylor, 1997). The higher the value of Margalef's index, the greater the diversity and, apparently, the cleaner the aquatic ecosystem. Simpson index is a common diversity index. According to Simpson's index, lower values correspond to higher diversity (Washington, 1984; Balderas, 2016). In the present study, the highest Simpson value (0.75) was observed at site 1 in summer, where water quality is good, whereas the lowest value (0.38) was at site 4, which is influenced by fish farm effluents. Two classical similarity indices are used for evaluating the similarity of samples: the Jaccard coefficient (Jaccard, 1901) and the Sorensen coefficient (Sorensen, 1948). Although there are some differences between these two coefficients, the results are roughly comparable.

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