

## Effects of land use change on macroinvertebrate community composition in upper reaches of the Chehel-Chai catchment, Iran

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### ABSTRACT

Urbanization of catchment regions is a major cause of freshwater ecosystem degradation worldwide. As catchments become more developed and river ecosystems become increasingly engulfed in various land use activities, there is a growing need to understand these impacts on freshwater ecosystems. Macroinvertebrate are extensively used as indicators of ecosystem health and have been an instrument tool in ecosystem monitoring and management. Five stations with different types of land use (reference station, forest, recreational, agricultural, urban area) of Chehel Chai River, Iran were sampled in 2018. A total of 2040 macroinvertebrate belonging to 6 orders from 12 families were identified. The highest abundance was related to the diversity of the Ephemeroptera (Batidae). According to the results, the abundance of macroinvertebrate was significantly different in the seasons and sampling stations. The maximum value of diversity indices was measured to the Shannon index ( $S1= 1.06$ , autumn), Simpson's Index ( $S1= 0.63$ , autumn), Margalef richness ( $S1= 0.9$ , spring) and Pielou's index ( $S3= 0.92$ , summer). Stations in up-stream (forest area) recorded highest species richness, abundance and proportion of sensitive macroinvertebrates compared to the agricultural and urban development area. Cluster and nMDS analysis revealed that among the macroinvertebrates, Ephemeroptera were distinct in the forest sites and Diptera and Oligochaeta in urban area of Chehel Chai River. This finding suggests that present environment problem (such as: agriculture, tourism and urban area) caused serious impacts on the biodiversity of Chehel Chai River.

**Keywords:** Benthic macroinvertebrates, Land use, Diversity, Chehel Chai River.

### INTRODUCTION

Land-use changes and vegetation cover are one of the most important factors in hydrological regime change, erosion process, and biodiversity status. Since land-use type can positively and negatively affect water quality and biological communities, therefore it is necessary to determine the extent of impact and contribution of different types of land use in surface water supply areas. Environmental impacts of land-use change on current water ecosystems increase environmental impacts at large spatial scales. Agricultural activities can lead to soil erosion and runoff, which causes large amounts of soft sediment deposition in streams and rivers (Palmer *et al.* 2002). Investigation of quantity and quality of pollutant sources, determination of qualitative status and presentation of a suitable model for investigating spatial and temporal variations of pollutants are the most important components of water quality (Imanpour Namin *et al.* 2013; Gholizadeh 2021). The pollution of rivers with pollutants caused by anthropological factors can be considered as an indicator of environmental pollution. Because rivers are the only water resources that go a long way through cities, villages, agricultural and industrial areas and are used more than any other sources for various applications, they can have adverse effects on the environment (Rosenberg & Resh 1993). Therefore, identifying the status of river water quality is an important issue. Currently, most of the available fresh water in Iran is flowing into the lotic ecosystems. Therefore, proper management is essential. The basis of water resources management is its evaluation (Xiao-jun *et al.* 2012). The first step is to identify living

organisms and the environmental factors that inhabit that ecosystem. The basis of this recognition is its ecological examination. Physical and chemical changes in water indicate contamination at the time of sampling. However, ecological data show long-term changes in the ecosystem due to water pollution (Barakat *et al.* 2016). According to the US Environmental Protection Agency, the best way to evaluate aquatic ecosystems is to use the ecological relationships of living organisms with the same ecosystems as ecological indicators called ecological evaluation (Flotemersch *et al.* 2017). A good water quality assessment should include important aquatic life, such as macroinvertebrates (Aazami *et al.* 2015). Many studies have been conducted to evaluate the ecological aquatic ecosystems due to the effects of the physical, chemical and biological factors on benthic macro-invertebrates (Nessimian *et al.* 2008; Bucker *et al.* 2010; Jun *et al.* 2011; Foomani *et al.* 2019). Because of their inability to escape contamination, relatively long life, easiness of sampling and identification, low cost, and variable susceptibility to pollution are good indicators for understanding water health (Van Ael *et al.* 2015). They illustrate the cumulative effects of multiple stressors such as habitat degradation that are not observable by traditional water quality (physico-chemical) assessment methods. Macroinvertebrates have a sensitive place in the food chain of the water courses and are therefore easily sampled and identified. Macro-invertebrates have a sensitive place in the food chain of the water currents and are therefore easily sampled and identified (Aazami *et al.* 2015). Chehel Chai River located in Minoodasht City, Golestan Province, Iran and plays an important role in the social and economic life of the inhabitants in this region and is also important for agricultural, aquaculture and water supply purposes. Since no research has been conducted on the health status of this ecosystem, the present study has considered this issue. The main purpose of this study was to evaluate the quality status of Chehel Chai River using macroinvertebrate communities and biological indices.

## MATERIALS AND METHODS

### Study area and sampling procedure

The Chehel Chai catchment area is approximately 25,000 hectares in the northern slopes of eastern Alborz. The study basin is located at the east longitude of  $55^{\circ} 22' 30''$  to  $55^{\circ} 37' 30''$  and the north latitude of  $36^{\circ} 57' 30''$  to  $37^{\circ} 15' 00''$ . The annual rainfall is 766.5 mm. The hydrological flow of the Chehel Chai basin has a significant impact on the economy of the basin and out of the basin as well as the fertile and desirable lowlands of the Gorganroud River (Lakzaei *et al.* 2018). The dominant vegetation of the basin in the northern part is the forest and in the south, it changes to dry and grassland type. The height difference between the highest and lowest points is 1380 meters (Department of Agriculture, Gorgan and Gonbad 2005). The discharge of the Chehel Chai River is minimal during the summer months due to the exploitation of rivers to irrigate the riverside farms (Golestan Watershed Management 2014). In addition, about 90% of water in Chehel Chai basin is used for agricultural activities and another portion is used for industrial use (Ravanab Consulting Engineering Company 2005).

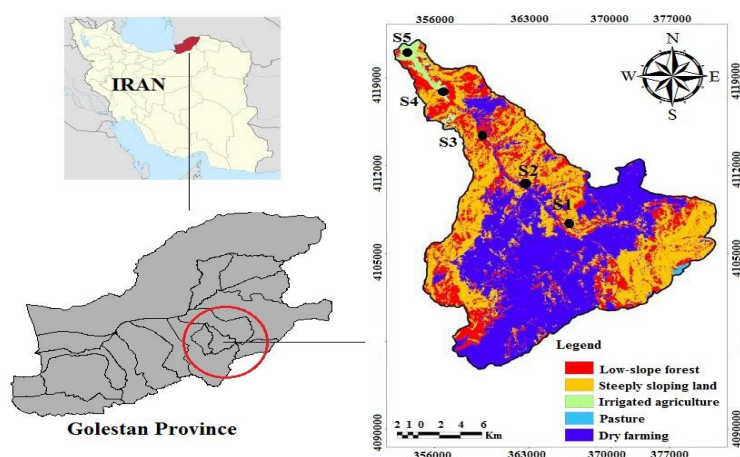


Fig. 1. Location of the sampling stations in the Chehel Chai River, 2018.

Its watershed can be divided into five geomorphological areas according to the type of land use: 1- native forest area in upstream region; 2- a wide range of highland agriculture from the upper to middle region; 3- as well as tourist and recreational area in the lower region; and 5- residential area (Minodasht City). To prepare land use map of Chehel Chai watershed from satellite image of Landsat 7, ETM<sup>+</sup> band in 2015 with a spatial resolution of

30 m was used. Regarding the field visit and considering the low land use diversity in the study area, the normalized difference vegetation index was selected for image classification. This indicator reveals the difference between coverage and non-coverage parts. So, image bands were separated and was imported into IDRISI software. Then, by controlling the threshold for change of land use, the detection of image changes was performed and the image was classified according to the supervised method. To evaluate the accuracy of the maps provided with terrestrial reality, point terrestrial control points were randomly (using global positioning system and point coordinates) transferred to ArcGIS 10.6.1 environment for definition and interpretation. We implemented Pearson correlation analysis (SPSS v24) to assess the correlation between the major land use categories and the macroinvertebrate community. Macroinvertebrates were sampled seasonally in 4 times (May, August, November and February) during 2018 from five stations using Surber sampler (0.09 m<sup>3</sup>) with 3 replications in the complete water mixing area. The benthic specimens collected after separating the unwanted material using standard sieves with a 500-micron mesh were fixed in 4% formalin and transferred to the Laboratory in Gonbad Kavous University. The locations of the sampling stations are shown in Fig. 1 and the geographical coordinates of the stations are displayed in Table 1. After transferring the specimens to the laboratory and washing for separation, the specimens were counted and identified using stereo microscope according to the Family Identification Keys (Thorp & Covich 2009; Needham 1976).

**Table 1.** Characteristics of the sampling station in the Chehel Chai River.

| Stations | Land use           | Geographic coordinates       | Width (m) | Altitude (m) |
|----------|--------------------|------------------------------|-----------|--------------|
| S1       | Reference area     | 36° 59' 77" E, 41° 09' 91" N | 2.5       | 135          |
| S2       | Native forest      | 36° 37' 09" E, 41° 13' 74" N | 8.4       | 210          |
| S3       | Recreational area  | 36° 13' 40" E, 41° 16' 69" N | 6.7       | 324          |
| S4       | Agriculture impact | 35° 84' 42" E, 41° 20' 52" N | 3.6       | 511          |
| S5       | Urban area         | 35° 53' 17" E, 41° 20' 48" N | 2.3       | 721          |

### Statistical processing and data analysis

Two-Way ANOVA and Tukey's HSD test were carried out using the software SPSS 24. The biological indices of Shannon-Wiener ( $H'$ ), Simpson ( $1-D$ ), Margalef richness index and Pielou were calculated in terms of abundance using the PRIMER v.6. The difference of macroinvertebrates was investigated using cluster analysis and non-metric multidimensional scaling (n.MDS) and also using Bray-Curtis relative similarity index in PRIMER v.6. ANOSIM and SIMPER analyses were conducted using the PRIMER v.6 (Clarke & Gorley 2006). The statistical significance of the species indicator values was estimated using a random reallocation procedure (1000 randomizations; Monte Carlo test) of sites among site groups. A species is considered a group indicator if the results are significant to a level of 0.05 (Zintzen *et al.* 2008). The IndVal coefficient was calculated using the PC-ORD v.4.0 program for Windows (McCune & Mefford 1999).

### RESULTS AND DISCUSSION

Six orders with 12 families consisting of 2040 individuals in Chehel-Chai River were recorded during the sampling period (Table 2). Baetidae (695 ind, 34.07%) and Chironomidae (460 ind, 22.55%) were the dominant families in this study. Aquatic insect larvae were the most abundant among the large population of macroinvertebrates. The highest diversity belonged to Diptera order with 5 families, as well as Ephemeroptera and Gastropoda orders with 2 families, respectively. The role of aquatic invertebrates in the transmission of energy flow in aquatic ecosystems has particular importance and the study of benthic communities is an appropriate criterion for assessing the ecological status of aquatic ecosystems (Gholizadeh & Heydarzadeh 2019). Based on the comparison of the upstream (St. 1) to downstream (St. 5, in urban area) of Chehel Chai River, the susceptible organisms were more abundant and diverse in the upper stations (in pristine and forest area with good water quality) than the lower stations (in urban area). The opposite was observed for resistant organisms. Other studies have shown that human activities such as rural, industrial and agricultural effluents import large amounts of pollutants into the aquatic environment and affect the diversity and abundance of macroinvertebrates (Martzeni & Esteve 2007). In this study, resistant organisms (Simuliidae and Chironomidae) were more abundant in stations 4 and 5 than in upstream stations (St. 1-2). The most important factors in the abundance increase of these groups at the stations 4 and 5 were agricultural and urban wastewaters. Chironomidae is a common organism in most aquatic habitats and is one of the resistant communities in the disturbances of the aquatic environments. This

benthos is used in the detection of human disturbances in medium-sized rivers (Çetinkaya & Bekleyen 2017). Anthropogenic activities in the middle part of the river can lead to an increase in filtering groups (*Simulium* sp.), resistant groups (Oligochaeta and Diptera orders), and a decrease in susceptible groups (such as Perlidae). The number of Ephemeroptera species was highest at four upstream stations and decreased at St. 5 (in urban area) which indicates an increased water pollution from upstream to downstream. Gastropoda and Tubificidae species were observed at stations 2 (in forest) and 4 (in agricultural area) that are able to withstand adverse conditions such as low soluble oxygen and high concentrations of pollutants (Deborde *et al.* 2016). The Perlidae was the only identified family belong to this order at the studied stations. Psychodidae and Dixidae were not observed in autumn. Oligochaeta was only observed in downstream stations (St. 4-5) that were most affected by Anthropogenic activities, including high agricultural activity and urban area (Table 2).

**Table 2.** The abundance of macro-invertebrates (ind) identified in Chehel Chai River, 2018.

| Order         | Family            | S1  | S2  | S3  | S4 | S5  |
|---------------|-------------------|-----|-----|-----|----|-----|
| Plecoptera    | Perlidae          | 3   | -   | -   | -  | -   |
| Ephemeroptera | Baetidae          | 172 | 233 | 108 | 68 | 122 |
|               | Caenidae          | 31  | 134 | 67  | 42 | 8   |
| Tricoptera    | Hydropsychidae    | 66  | 126 | 138 | 25 | 6   |
| Diptera       | Pupa-Chironomidae | -   | -   | 5   | 7  | 3   |
|               | Chironomidae      | -   | 28  | 86  | 80 | 266 |
|               | Simuliidae        | -   | 15  | 32  | 21 | 40  |
|               | Ceratopogonidae   | -   | -   | 4   | 8  | -   |
|               | Psychodidae       | -   | -   | 10  | 16 | -   |
| Gastropoda    | Dixidae           | -   | -   | 5   | 8  | -   |
|               | Physidae          | -   | 7   | -   | 1  | -   |
|               | Lymnaeidae        | -   | 3   | -   | 1  | -   |
| Oligochaeta   | Tubificidae       | -   | -   | -   | 14 | 39  |

The results showed that Ephemeroptera (977 ind, 47.89%) and Diptera (634 ind, 31.08%) had the highest abundance, while Plecoptera (3 ind, 0.15) and Gastropoda (12 ind, 0.59) exhibited the lowest one (Fig. 2). Plecoptera order (3 individual) had 0.15% of the macrobenthic invertebrate population in this river, and Gastropoda order (4 individual) by 0.59% was observed only in spring and autumn. The abundance of macroinvertebrates in the seasons and sampling stations are shown in Fig. 3. The highest abundances of macroinvertebrates were observed in St. 2 (229 ind, 25.78%) and St. 5 (140 ind, 13.68%) in winter. Also, the lowest percentages were observed at St. 1 (0.54%) and St. 4 (91%) in spring followed by St. 4 (1.72%) in summer. Fig. 4 depicts the highest abundance in winter (844 ind, 41.3748%) and autumn (555 ind, 27.21%), respectively. In addition, macroinvertebrates displayed a significant difference between sampling stations and seasons ( $P > 0.05$ ) (Table 3). The highest abundance and diversity of macroinvertebrate communities were observed in winter and autumn due to the provision of suitable environmental conditions such as reduced agricultural activities, increased discharge, as well as river self-refining and food availability. The lowest abundance was observed in spring (12%) and summer (15%) (Fig. 3b). The entrance of agricultural water drains (rice, rapeseed, and wheat) containing chemical fertilizers, elevates river water phosphate, and also unfavorable river conditions including low discharge cause environmental problems (decreased diversity and increased resistant species) in summer (Department of Agriculture, Gorgan and Gonbad 2005).

**Table 3.** The Two-Way ANOVA results on the influence of stations, seasons and interaction between stations and seasons on the abundance of macroinvertebrates within Chehel Chai River.

| Variable         | df | Mean square | Sum of squares | F    | P-value |
|------------------|----|-------------|----------------|------|---------|
| Season           | 3  | 24977       | 74933          | 7239 | 0.001   |
| Station          | 4  | 6215        | 24863          | 1801 | 0.001   |
| Station × Season | 12 | 1852        | 22229          | 536  | 0.001   |
| Residual         | 20 | 3           | 69             | -    | -       |
| Total            | 40 | 24977       | 528118         | -    | -       |

Df = degrees of freedom, F = MS factor/MS residual and p = probability of significance;  $\alpha = 0.05$ .

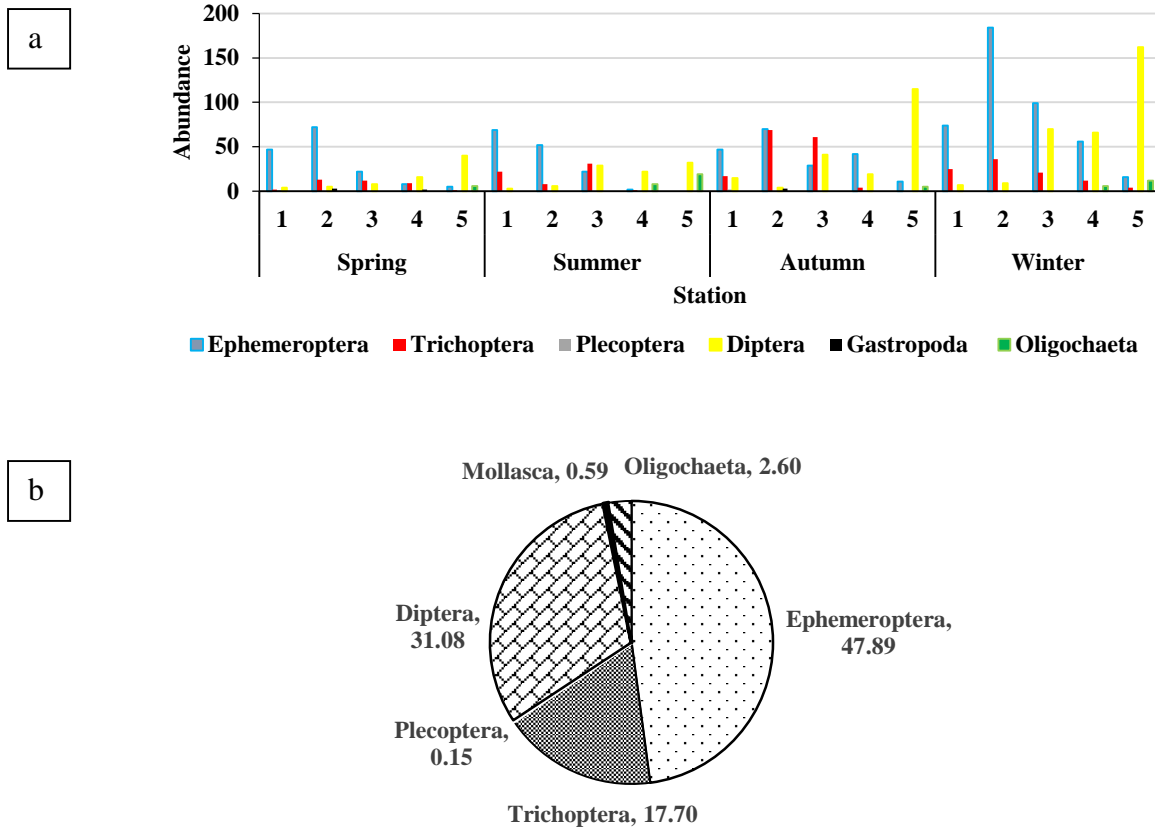


Fig. 2. Abundance (a) and percentage (b) of macroinvertebrates during the sampling period.

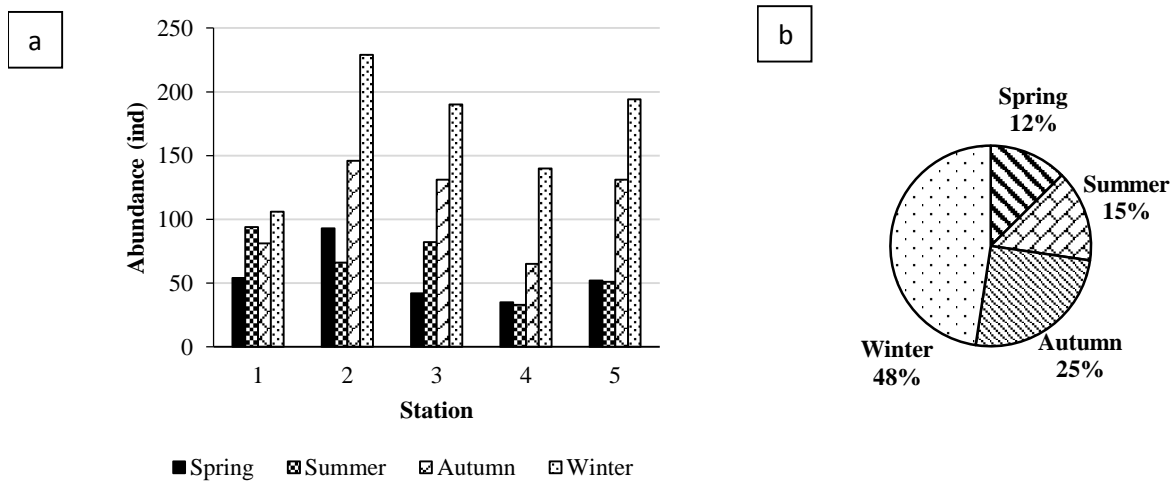


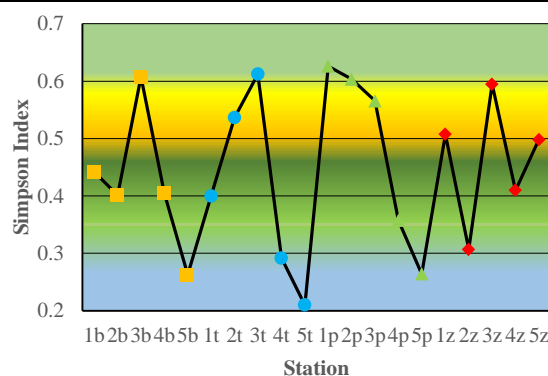
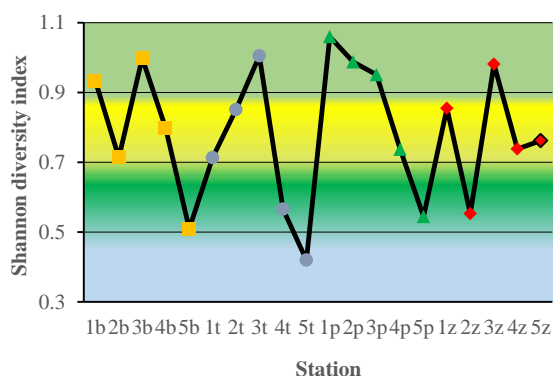
Fig. 3. Abundance (a) and percentage (b) of macroinvertebrates at different sampling stations and seasons.

The diversity indices based on Shannon-Weiner and Simpson Indexes showed the same results comparing aquatic insects between the five sampling stations (Fig. 4). The value was highest in St. 1 (in reference area) during autumn (1-D = 0.63;  $H' = 1.06$ ) and in St. 3 (in recreational area) during summer (1-D = 0.62,  $H' = 1.01$ ) compared to St. 5 (in urban area) during summer (1-D = 0.21,  $H' = 0.42$ ) and station 5, spring (1-D = 0.26,  $H' = 0.51$ ). The results were similar with taxa richness for both highest and lowest values. The Shannon-Wiener diversity values found in this study ranged from 0.42 to 1.06. Studies with a more even station distribution amongst season may obtain more useful Shannon diversity results. Stations in spring, summer, autumn and winter revealed an average Shannon diversity scores of 0.79, 0.71, 0.86 and 0.78 respectively (Fig. 4).

Minor relationships can be observed between the sampling stations in different seasons and the Margalef index results. For instance, some of the highest Margalef index values were recorded in St. 1 during spring ( $d = 0.9$ ). Stations 1 and 2 (in reference and forest areas) during winter ( $d = 0.33, 0.35$ ) exhibited a lower average Margalef index than other stations. However, the station-to-station variation is undeniable, with no obvious patterns (Fig. 4). The Pielou index values found in this study ranged from 0.38 to 0.92. The stations in spring, summer, autumn and winter displayed an average Pielou index values of 0.64, 0.62, 0.69 and 0.68, respectively (Fig. 4).

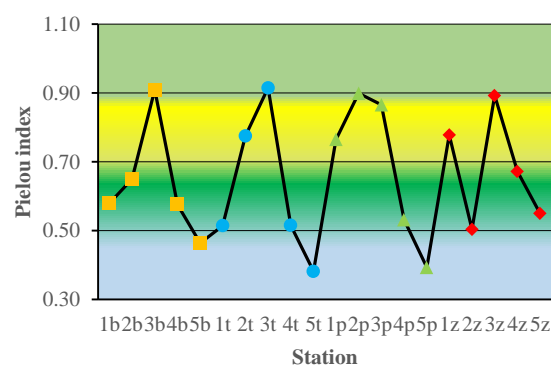
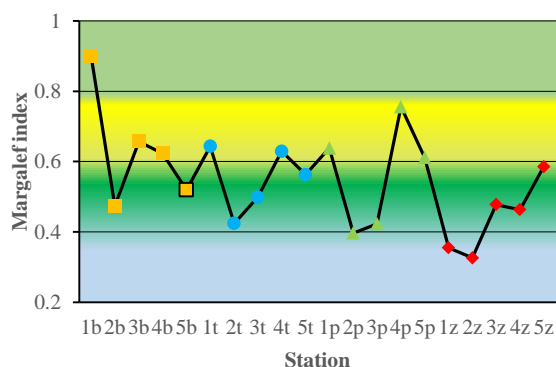
| Shannon-Weiner Index | Studied stations |                |
|----------------------|------------------|----------------|
|                      | Rate (%)         | No. of station |
| <0.5                 | 5                | 1              |
| 0.5-0.7              | 20               | 4              |
| 0.7-0.9              | 40               | 8              |
| >0.9                 | 35               | 7              |

| Simpson Index | Studied stations |                |
|---------------|------------------|----------------|
|               | Rate (%)         | No. of station |
| <0.3          | 20               | 4              |
| 0.3-0.4       | 10               | 2              |
| 0.4-0.5       | 35               | 7              |
| 0.5-0.6       | 15               | 3              |
| >0.6          | 20               | 4              |



| Margalef index | Studied stations |                |
|----------------|------------------|----------------|
|                | Rate (%)         | No. of station |
| <0.4           | 15               | 3              |
| 0.4-0.6        | 45               | 9              |
| 0.6-0.8        | 35               | 7              |
| >0.8           | 5                | 1              |

| Pielou index | Studied stations |                |
|--------------|------------------|----------------|
|              | Rate (%)         | No. of station |
| <0.5         | 15               | 3              |
| 0.5-0.7      | 45               | 9              |
| 0.7-0.9      | 30               | 6              |
| >0.9         | 10               | 2              |



**Fig. 4.** Diversity index values for each sampling site, displayed upstream to downstream, along the Chehel-Chai River. Numbers = stations, b = spring, t = summer, p = autumn, z = winter. Reference station: number 1, Native forest: number 2, Recreational Land: number 3, Agriculture impact: number 4, Urban station: number 5.

The community structure index is a composite measure of biological structure that combines the number of species (richness) and the distribution of individuals among species (evenness). Diversity indices depend on the quality and availability of habitats (Barbour *et al.* 1999). They reflect the impact of all stressors independent of ecological boundaries. According to the results, the highest values of Shannon-Wiener (H) and Simpson (1-D) diversity were observed at St. 1-2, which may be due to the rich and intact habitat structure. Also, the lowest values were observed

in the downstream (at St. 5). Low values of these indices indicate pollution or turbulence in the river environment. The Shannon-Wiener diversity values found in this study ranged from 0.42 to 1.06. As with the other macro-invertebrate indices, most of the sampling stations in different seasons do not show an obvious relationship to the Shannon diversity index. For instance, the stations during spring, autumn and winter all fall within similar Shannon diversity ranges, whereas summer contains a high variety of results. Studies with a more even station distribution amongst season may obtain more useful Shannon diversity results. Other biological indicators follow a similar trend and show an overall increase in nutrient contamination, especially along the downstream river. The results of indicator species analysis showed that different land use areas were different in terms of species, such that an indicator species for each land use can be introduced. In this study, forest and urban areas had 4 indicator species, while the recreational and agricultural areas exhibited 2 and the reference station only 1 (Table 4). Considering the definition of stations into groups using land use, the indicator value (IndVal) coefficient revealed thirteen significant indicator taxa. However, based on IndVal scale proposed, only four species revealed high indicator values (> 50%). The Perlidae belonged to group 1 (in reference station), Caenidae belonged to group 2 (in forest), Chironomidae and Tubificidae belonged to group 5 (in urban area). The results showed that there was a significant difference in the abundance of macro-invertebrates between the stations and the different seasons. However, there was no significant difference in the biodiversity between the different seasons, although there was a significant difference between stations. In addition, the diversity of benthic species decreased from the upstream to downstream stations, similar to results reported by Gholizadeh & heydarzadeh (2019) and Van Ael *et al.* (2015).

**Table 4.** Indicator value (IndVal) coefficient for significant species related to the categorical groups.

| Indicator species | Group* | Indicator value (IndVal) | P-value |
|-------------------|--------|--------------------------|---------|
| Perlidae          | 1      | 51                       | 0.054   |
| Baetidae          | 2      | 33.5                     | 0.26    |
| Caenidae          | 2      | 55                       | 0.015   |
| Hydropsychidae    | 3      | 38.2                     | 0.223   |
| pupa Chironomidae | 3      | 25                       | 0.579   |
| Chironomidae      | 5      | 58.5                     | 0.025   |
| Simuliidae        | 5      | 32.9                     | 0.435   |
| Ceratopogonidae   | 4      | 33.3                     | 0.414   |
| Psychodidae       | 5      | 29.2                     | 0.377   |
| Dixidae           | 4      | 34.1                     | 0.131   |
| Planorbidae       | 2      | 37.5                     | 0.152   |
| Physidae          | 2      | 37.5                     | 0.275   |
| Tubificidae       | 5      | 73.6                     | 0.009   |

\*. Groups: 1- Reference station, 2- Native forest, 3- Recreational land, 4- Agriculture and 5- Urban.

Table 5 shows the area of forest, agricultural, urban and tourism land uses based on the Landsat satellite map.

**Table 5.** Area of land use classes in year 2015.

| Land use     | Area (hectare) | Area (%) |
|--------------|----------------|----------|
| Forest       | 14450.56       | 54.87    |
| Agricultural | 11229.97       | 42.64    |
| Urban        | 653            | 2.48     |
| Tourism      | 2              | 0.01     |

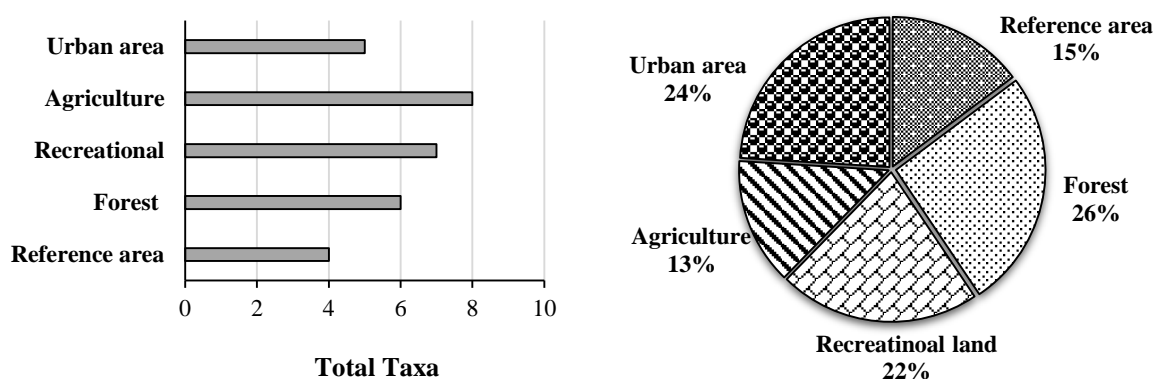
The results showed that the different land use types have a significant effect on macroinvertebrate communities. These effects include the negative effect of urban area and the positive effects of reference station and forest area on species diversity and biomarkers. These results are consistent with other studies on the impact of urban areas on macroinvertebrate communities (Sharifinia *et al.* 2012; Gholizadeh & Alinejad 2018; Villeneuve *et al.* 2018). Reduction in the diversity of aquatic insect communities at St. 5 confirmed that deforestation and deterioration of natural habitat quality by urban development trends is affecting aquatic insect diversity (Che Salmah *et al.* 2013; Gholizadeh & Boveiri 2019). Among the four land use variables considered, forest land use was positively correlated with Perlidae, while negatively correlated with Simuliidae. The agriculture land use was positively

correlated with Caenidae, Hydropsychidae, Planorbidae and Physidae. The urban land use was negatively correlated with Baetidae, while positively correlated with Ceratopogonidae and Tubificidae. Tourism was negatively correlated Caenidae and Dixidae, while positively correlated with Chironomidae and Psychodidae (Table 5).

**Table 6.** The Pearson correlation coefficients between macroinvertebrate community (all variables were ln-transformed) and watershed land use classes (area).

| Indicator species | Forest | Indicator value | Agriculture | Urban  | Tourism |
|-------------------|--------|-----------------|-------------|--------|---------|
| Perlidae          | 0.99*  | -0.33           | -0.33       | -0.33  | -0.33   |
| Baetidae          | 0.89   | -0.11           | -0.73*      | -0.05  | -0.05   |
| Caenidae          | -0.16  | 0.86*           | 0.13        | -0.79* | -0.79*  |
| Hydropsychidae    | 0.08   | 0.9*            | -0.38       | -0.6   | -0.6    |
| pupa Chironomidae | -0.63  | 0.27            | 0.63        | -0.17  | -0.17   |
| Chironomidae      | -0.61  | -0.15           | -0.19       | 0.95*  | 0.95*   |
| Simuliidae        | -0.78* | 0.52            | -0.32       | 0.59   | 0.59    |
| Ceratopogonidae   | -0.42  | 0.21            | 0.84*       | -0.63  | -0.63   |
| Psychodidae       | -0.6   | -0.41           | 0.14        | 0.88*  | 0.88*   |
| Dixidae           | -0.66  | -0.45           | -0.35       | -0.76* | -0.76*  |
| Planorbidae       | -0.33  | 0.99*           | -0.33       | -0.33  | -0.33   |
| Physidae          | -0.33  | 0.99*           | -0.33       | -0.33  | -0.33   |
| Tubificidae       | -0.48  | -0.48           | 0.93*       | 0.03   | 0.03    |

The highest number of taxa was found in agriculture (8 taxa), while the lowest in reference (4 taxa) areas. The highest percentage of aquatic insects was observed in the forest (26%), while the lowest in the urban development (St. 5) areas (Fig. 5).

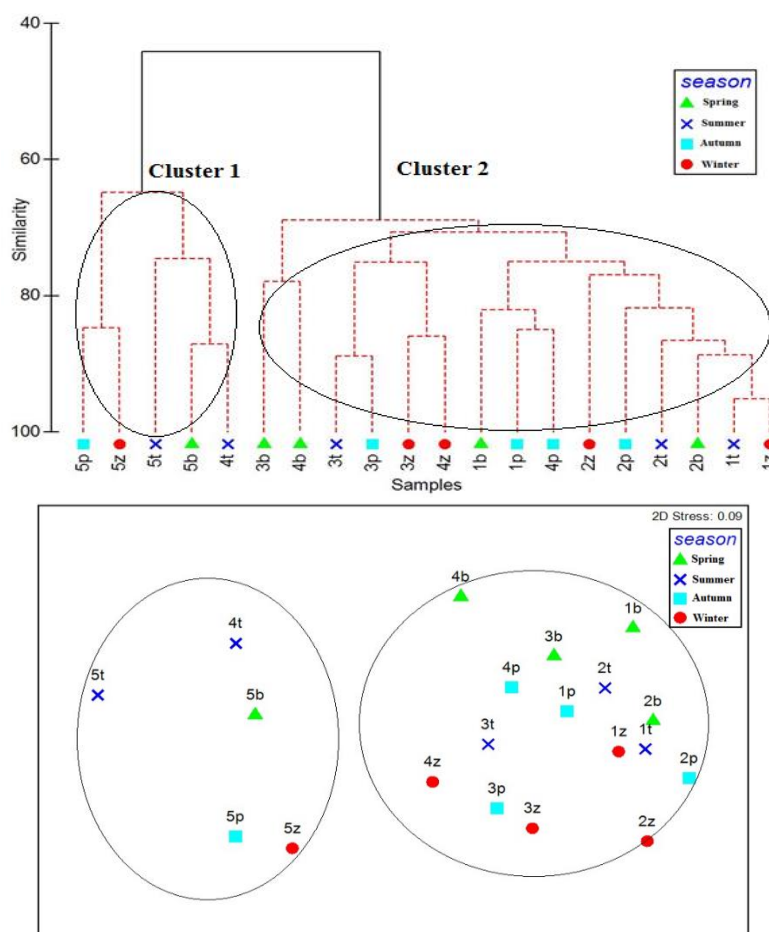


**Fig. 5.** Total taxa and percentage of macroinvertebrates in different land uses.

The ANOSIM results indicated significant differences ( $p < 0.05$ ) between each pair of the land use groups in terms of the macroinvertebrate composition (Table 7). No significant differences were found between reference station and native forest, and also between recreational land and agriculture as well as between agriculture and urban ( $p > 0.05$ ). SIMPER results revealed the highest average dissimilarity (65.65%) to be observed between St. 1 and St. 5 with the top seven contributor families including Chironomidae (24.79%), Baetidae (18.94%), Tubificidae (13.62%), Hydropsychidae (12.29%), Caenidae (8.08%) and Simuliidae (7.7%) and Psychodidae (5.12%). Sampling stations in agriculture and urban area revealed the lowest average dissimilarity (30.7%) with top three contributor families including Hydropsychidae (29.1%), Tubificidae (14%) and Caenidae (12.7%). Within each season, the highest dissimilarity of benthic macroinvertebrate community was observed between autumn and summer (SIMPER average dissimilarity = 50.28%) with top four contributor families including Hydropsychidae (18.99%), Baetidae (17.91%), Chironomidae (16.73%) and Caenidae (14%), Simuliidae (11.69%), Tubificidae (9.04%). Based on clustering diagrams and non-metric multidimensional scaling (n.MDS) of identified samples in different land uses (reference station, forest, recreational land, agricultural and urban area), macroinvertebrate groups were divided into two groups at 64.79 similarity distance (Fig. 6). Sampling



stations of benthic invertebrate groups in a cluster indicate that the diversity and abundance of organisms in these locations are similar: Cluster 1 consists of downstream stations in the urban area (St. 5, mainly affected by the city effluent). Cluster 2 is also diverse and includes other sampling stations. In addition, cluster 2 has sub-clusters with short lengths which indicate intra-group similarity in these clusters.



**Fig. 6.** Cluster and nMDS analysis of grouping (UPGMA, Bray-Curtis) based on square root transformed macrobenthos abundance data in sampling stations with different land use influences in Chehel-Chai River. Numbers = stations, b = spring, t = summer, p = autumn, z = winter. Reference station: number 1, Native forest: number 2, Recreational land: number 3, Agricultural impact: number 4, Urban area: number 5.

**Table 7.** ANOSIM results comparing macroinvertebrate taxa collected from different land use.

| Groups            | Rho   | P-value           |
|-------------------|-------|-------------------|
| Reference area    |       |                   |
| Forest            | -0.12 | 0.77 <sup>a</sup> |
| Recreational land | 0.46  | 0.03              |
| Agriculture       | 0.38  | 0.03              |
| Urban             | 0.97  | 0.01              |
| Forest            |       |                   |
| Recreational land | 0.56  | 0.03              |
| Agriculture       | 0.44  | 0.03              |
| Urban             | 0.96  | 0.01              |
| Recreational land |       |                   |
| Agriculture       | 0.16  | 0.14 <sup>a</sup> |
| Urban             | 0.89  | 0.03              |
| Agriculture       |       |                   |
| Urban             | 0.26  | 0.14 <sup>a</sup> |

## CONCLUSION

The presence of some special species of macroinvertebrates, especially in the contaminated and non-polluted parts of a river, can be used as a potential indicator for river evaluation. Since the study of macroinvertebrates is not comprehensive in Iran, identification of these organisms in the Chehel Chai River is useful for further studies. More taxonomic work is usually needed to identify organisms at the species level. The study of Chehel Chai River showed that this river is not rich in diversity of benthic. Abundance was also high in some stations, while low in others. The application of bio-indicator can well explain the impact of anthropogenic activities on water quality changes at different stations. This method was used in the biological evaluation of the Chehel Chai River to assess the water quality at each station under major pollution factors (urban wastewater, agricultural and recreational area). In this study, the results of population composition and bio-demographic indices in different stations showed more severe effects of anthropogenic activities in downstream than in upstream stations. The destructive effects of human sewage and its impact on the aquatic ecosystem are not hidden to anyone, so it seems that proper planning and management can reduce the severity of the damage.

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**Bibliographic information of this paper for citing:**

Gholizadeh, M, Rezvani, S.A, Zibaei, M 2021, Effects of land use change on macroinvertebrate community composition in upper reaches of the Chehel-Chai chatchment, Iran. *Caspian Journal of Environmental Sciences*, 19: 523-533

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