

# DIVERSITY OF AQUATIC MACROINVERTEBRATE COMMUNITIES IN THE MISOČA RIVER: THE ARGUMENT FOR CONSERVATION

BELMA NAHIĆ\*, AMINA OMERAGIĆ, ADI VESNIĆ, MAHIR GAJEVIĆ  
& ALDIJANA MUŠOVIĆ

Faculty of Natural Sciences and Mathematics, University of Sarajevo, Zmaja od Bosne 33-35,  
71000 Sarajevo, Bosnia and Herzegovina

Nahić, B., Omeragić, A., Vesnić, A., Gajević M. & Mušović, A.: Diversity of aquatic macroinvertebrate communities in the Misoča River: the argument for conservation. *Nat. Croat.*, Vol. 33, No. 1, 13–27, Zagreb, 2024.

In the present study the macroinvertebrate composition in the Misoča River, its diversity and abundance were analyzed. The study was conducted in autumn and spring, in order to determine the influence of seasonal dynamics on the structure of macroinvertebrate communities and water quality. A biotic element analysis revealed a high abundance and diversity of macroinvertebrate taxa. Significant differences in the structure of communities with respect to seasons and sampling sites were observed, as well as in species dominance, which was confirmed by the Bray-Curtis similarity analysis. The results of the EPT% index, EPT/Chironomidae index, Shannon-Weaver and Simpson's biodiversity indices showed the good ecological status of water at studied sites. The Pantle-Buck saprobic index indicated an oligo to  $\beta$ -mesosaprobic category, or slightly contaminated water. The presence of the Natura 2000 species *Austropotamobius torrentium*, and of six Trichoptera species new to the fauna of Bosnia and Herzegovina and, among others, the species *Hydropsyche botosaneanui* was recorded in samples. This study represents the first literature data on macroinvertebrate diversity for the protected area of the Misoča River.

**Key words:** biodiversity, macrozoobenthos, freshwater ecosystems, protection, Bosnia and Herzegovina

Nahić, B., Omeragić, A., Vesnić, A., Gajević M. & Mušović, A.: Raznolikost zajednica makrozoobentosa u rijeci Misoči: argument za zaštitu. *Nat. Croat.*, Vol. 33, No. 1, 13–27, Zagreb, 2024.

Analiziran je sastav makrozoobentosa u rijeci Misoči, njihova raznolikost i brojnost. Istraživanje je provedeno u jesen i proljeće, kako bi se utvrdio utjecaj sezonske dinamike na strukturu zajednica makrozoobentosa i kakvoću vode. Na osnovu rezultata analize biotičkih elemenata utvrđena je velika brojnost i raznolikost makrozoobentosa. Uočene su primjetne razlike u strukturi zajednica s obzirom na godišnja doba i mjesta uzorkovanja, kao i u dominaciji vrsta, što je potvrđeno Bray-Curtisovom analizom sličnosti. Rezultati EPT% indeksa, EPT/Chironomidae indeksa, Shannon-Weaver i Simpsonovog indeksa bioraznolikosti pokazali su dobar ekološki status vode na istraživanim lokalitetima. Pantle-Buck saprobni indeks je ukazao na oligo- i  $\beta$ -mesosaprobnu kategoriju ili blago zagađenu vodu. U uzorcima je zabilježeno prisustvo Natura 2000 vrste *Austropotamobius torrentium*, te šest vrsta reda Trichoptera koje su nove za faunu Bosne i Hercegovine, između ostalih i vrsta *Hydropsyche botosaneanui*. Ova studija predstavlja prve literaturne podatke o raznolikosti makrozoobentosa za rijeku Misoču – zaštićeno područje.

**Ključne riječi:** biodiverzitet, makrozoobentos, slatkovodni ekosistemi, zaštita, Bosna i Hercegovina

---

\*Corresponding author

## INTRODUCTION

An important indicator of an ecosystem's condition is biological diversity (REDŽIĆ, 2009). Biodiversity loss can be manifested in the extinction of entire populations and in some cases in the extinction of species, with the disappearance of the "roles" and functions they performed, leading to a decline in ecosystem functionality and the sustainability of planet Earth (BARUDANOVIĆ, 2012). In order to identify and prevent the impact of anthropogenic factors on biodiversity, various methods are applied, one of them based on the use of bioindicators. Unlike physicochemical parameters, bioindicators can integrate and reflect environmental changes over a long-term period. The importance of bioindicators is also reflected in their being the end-recipients of the degradation and pollution of freshwater ecosystems and therefore important indicators of their "health" (BARBOSA, 2001).

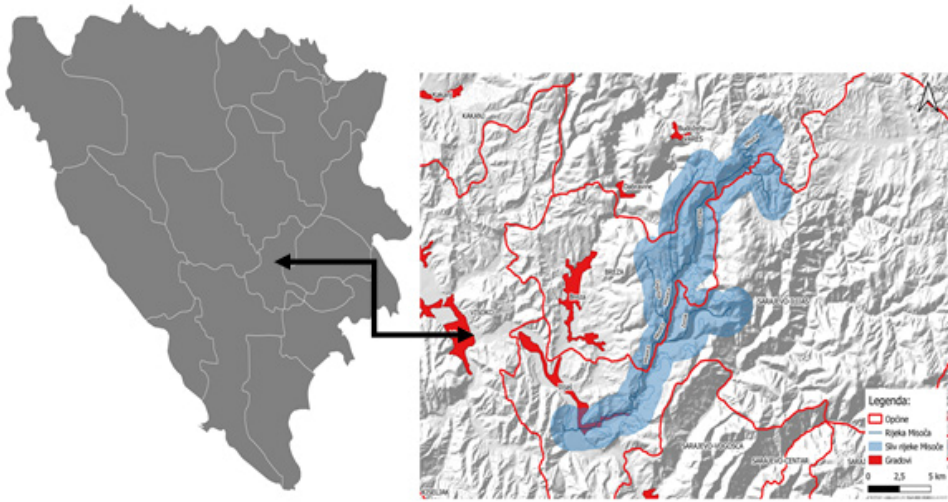
Communities of aquatic insects, macrozoobenthos, are of great importance, representing a heterogeneous group of different taxa that show changes in aquatic ecosystems in several aspects (BONADA *et al.*, 2006; RESH, 2007; KALYONCU, 2010). The composition and structure of communities are the results of specific spatio-temporal interactions of abiotic and biotic factors, i.e. they depend on the ecological conditions of a given biotope (GILLER & MALMQVIST, 1998). Environmental changes affect the distribution patterns of macroinvertebrate communities and this can be used in biomonitoring and restoration of degraded ecosystems (HEPP *et al.*, 2013).

With a large number of representatives, and already developed and advanced indices of water quality and biodiversity, it is not surprising that most European countries have chosen macroinvertebrates as one of the most reliable bioindicators of freshwater ecosystem quality. This approach represents a substitute for much more expensive methods with the same efficiency and can monitor the impact on specific habitats (DEWALT & HEINOLD, 2005). Significant constituents of macrozoobenthos are aquatic insects, which are specific due to biodiversity, population size, and emergence processes. Emerging insects represent a link between two ecosystems, aquatic and terrestrial (DAVIES, 1984).

Freshwater habitats and aquatic fauna are among the most endangered in the world as they receive and amplify the contaminants in water (HOLZENTHAL *et al.*, 2007). Freshwater ecosystems are significantly endangered due to anthropogenic impacts (TROŽIĆ-BOROVAC, 2005). The Misoča River is located in the central part of Bosnia and Herzegovina, in the Sarajevo Canton, Ilijaš Municipality (Fig. 1). The river is formed by the confluence of the Blaža River and Kunosički Brook below the mountain Okruglica. The Government of the Federation of Bosnia and Herzegovina, at the proposal of the Federal Ministry of Agriculture, Water Management and Forestry based on Article 68. Paragraph 4 of the "Law on Waters" (2010) made a decision for the protection of the Misoča River, which is the main source of water supply for residents of the municipality of Ilijaš.

From the aspect of biodiversity and the composition of macroinvertebrates, the Misoča River is insufficiently investigated, as evidenced by the lack of literature sources on this important ecosystem.

The aim of this study was to assess macroinvertebrate diversity based on their qualitative-quantitative composition and selected biodiversity indices and to compare the diversity among the investigated sites. Using several water quality indices that include



**Fig. 1.** Physical and geographical position of the researched area of River Misoča in Bosnia and Herzegovina (Left picture – Bosnia and Herzegovina (©Vermaps); right picture – Misoča River).

macroinvertebrates, the task was also to assess the water quality of the Misoča River. The motive was to assess the significance of this protected area and its insufficiently referenced research.

## MATERIAL AND METHODS

This study was conducted on the Misoča River, located in central Bosnia. The total length of the Misoča River watercourse is 43 km, consisting of numerous tributary streams of which its largest tributary is the River Bara (NEFIĆ, 1997). Geographical parameters of sampling sites were determined: latitude, longitude and altitude of the five sampling site locations (S1 – “Three waterfalls”, S2 – “Cliff”, S3 – “Slopes”, S4 – “Rocker”, S5 – “Bridge”) were recorded with a Garmin Oregon 600 (Tab. 1).

### Biotic data sampling

Biotic data sampling was in accordance with the Water Framework Directive. Macrozoobenthos samples were collected at five selected sites marked: S1-S5. Collection of macrozoobenthos samples and measurement of various parameters were carried out once in each of two seasons, autumn (October 26, 2019) and spring (May 10, 2020).

**Tab. 1.** Distribution of sampling points on the Misoča River

Sites	River parts	Lat.	Lon.	Altitude meters
S1	Upper	44.01624	18.33599	664
S2	Upper	44.01464	18.33695	666
S3	Middle	44.01266	18.33676	667
S4	Middle	44.01027	18.33563	662
S5	Lower	43.95859	18.30674	525

The sampling sites represent segments of the upper, middle and lower parts of the Misoča River. A "kick sampling" method was used to sample macrozoobenthos in the studied parts of the Misoča watercourse. The collected samples were preserved in the field with 4% formaldehyde. The analysis of samples was performed in the Laboratory for Systematics, Hydrobiology and Evolutionary Entomology at the Department of Biology, Faculty of Science, University of Sarajevo. After separation of the preimaginal stages of the macroinvertebrates using a stereozoom microscope, further taxonomic analysis to the species level was addressed using relevant identification keys: ELLIOT *et al.*, 1988; NAGEL, 1989; STUDEMANN *et al.*, 1992; BAUERNFEIND, 1994; SOLDAN & LANDA, 1999; HAMADA & COUCEIRO, 2003; OLIFIERS *et al.*, 2004; BIRMINGHAM *et al.*, 2005; Stresemann *et al.*, 2011; WARINGER & GRAF, 2013; KRISKA, 2014. Early-juvenile stages or damaged individuals were not identified.

## Data analysis

The number of identified specimens was recorded. Number of taxa and individuals was analyzed in Microsoft Office Excel and PRIMER (version 6.1.16). by ratio of number of species to total abundance. of number of species to total number of individuals. To determine the diversity of macroinvertebrates in the studied sites, the following indices were used: the Shannon-Weaver Index (SHANNON & WEAVER, 1949; FEDOR *et al.*, 2013) and the Simpson Index (SIMPSON, 1949).

Saprobiological analysis was performed using the Pantle-Buck saprobic index. The given index is based on the total tolerance of the species that make up the community to a certain saprobic level (PANTLE & BUCK, 1955; STOYANOVA, 2010).

The EPT% index is based on the presence of three orders of aquatic insects that are easily identified and most commonly used in water quality assessment (LENAT, 1988). It relates the total number of Ephemeroptera, Plecoptera and Trichoptera (EPT) orders to the total number of all macroinvertebrates taxa found. The value obtained summarizes the richness of taxa within the given ranks that are considered sensitive to any type of pollution. The EPT% & Chironomidae index represents the ratio between the abundance of the EPT% group and the abundance of Chironomidae. It compares the number of pollution-sensitive individuals (EPTs) to those that are tolerant to pollution (Chironomidae) (KLEMM *et al.*, 1990).

The Bray-Curtis analysis is performed with the aim of establishing faunal similarity between sampling sites (CLARKE & WARWICK, 2001). The values obtained by Bray-Curtis similarity analysis were used for cluster analysis using the single linkage method, and the values were standardized by  $\log(x + 1)$ . The analysis was performed in the EXAMPLE 5 program.

One-way ANOSIM analysis and pairwise test was used to test the differences among the research sites on the base of the number of EPT taxa collected during the autumn and spring seasons. Bray-Curtis similarity index was used for statistical analyses.

## RESULTS AND DISCUSSION

The results of sampled macroinvertebrates at five sampling sites of the Misoča River during spring and autumn are represented by 60 invertebrate taxa. In the samples of the Misoča River, 5266 individuals were recorded; 3061 individuals belonging to 44 taxa in autumn, 2205 individuals belonging to 48 taxa in spring (Tab. 2; App. 1).

**Tab. 2.** Absolute abundance of aquatic macroinvertebrates in Misoča River during autumn and spring

Taxa	Site S1 autumn	Site S1 spring	Site S2 Autumn	Site S2 spring	Site S3 autumn	Site S3 spring	Site S4 autumn	Site S4 spring	Site S5 Autumn	Site S5 spring	Total autumn	Total spring
Tricladida	-	-	-	-	1	-	-	-	-	-	1	0
Architaenioglossa	4	15	8	11	8	12	5	10	2	1	27	49
Lumbriculida	28	25	27	34	31	24	24	11	7	5	117	99
Trombidiformes	7		1		3				28		39	0
Decapoda	4	5	2	2	5	4		3			11	14
Ephemeroptera	199	198	278	374	436	394	159	108	294	103	1366	1177
Odonata	-	-	-	-	-	-	-	-	-	5	-	5
Coleoptera	148	53	171	71	351	81	58	23	152	71	880	299
Plecoptera	28	6	38	7	54	11	13	5	128	195	261	224
Trichoptera	40	25	2	22	27	38	6	3	9	20	84	108
Diptera	53	25	57	56	105	81	19	22	41	46	275	230
<b>TOTAL</b>	<b>511</b>	<b>352</b>	<b>584</b>	<b>577</b>	<b>1021</b>	<b>645</b>	<b>284</b>	<b>185</b>	<b>661</b>	<b>446</b>	<b>3061</b>	<b>2205</b>

The order Ephemeroptera was the most dominant taxa represented by 2543 individuals (48.2%), followed by the order Coleoptera (22.5%), which in both seasons makes a higher contribution than other aquatic insect orders: Plecoptera (9.2%), Trichoptera (3.6%), Diptera (9.6%) and Odonata (0.1%). Of the other aquatic groups in the macroinvertebrate community, it is important to point out the presence of species belonging to the following taxa: Rhabditophora, Gastropoda, Oligochaeta (4.1%), Arachnida and Malacostraca (0.5%), whose relative presence is significantly lower (6.8%) than that of aquatic insects.

The fauna of the Misoča River is characterized by high biodiversity and abundance of individual species, with the dominance of aquatic insects (93.2%). In spring the aquatic insects were represented by 93.6% and in autumn by 92.7% of the total 5266 individuals that were recorded. A review of the values of the analyzed indices at the researched sites of the Misoča River is shown in Tab. 3, Fig. 2.

The macroinvertebrate assemblage and distribution along the river indicates the level of disturbance and water quality variation, which can be best seen from the indices given below. The Shannon-Weaver (2.33-2.01) and Simpson Diversity indices (0.89-0.79) clearly show higher values at the survey sites in the upper Misoča River (Tab. 3). The Shannon-Weaver index belongs to type I diversity indices that are most sensitive to changes in rare species in a sample from an investigated community, while type II indices, like the Simpson Diversity index, are the most sensitive to changes in more frequent species (Peet, 1974). Reference values of the Shannon-Weaver Biodiversity Index are usually in the range of 1.5 to 3.5, very rarely exceeding values above 5.

Simpson's index reference values range from 0 to 1, and increasing values indicate greater diversity of macrozoobenthos communities.

The values of the saprobic indices (S value) of the Misoča River vary during the seasons as can be seen from Fig. 2. Lower saprobic values are recorded in the autumn, while in the spring they are slightly increased, which changes the categorization in certain sites (Tab. 4). Based on this, we can conclude that water quality decreases in warmer seasons (ZAHRAĐKOVA & SOLDAN, 2008). Based on the categorization of water

quality (LIEBMANN, 1962), the degree of saprobity for S5 - "Bridge" in the autumn season indicates pure water (oligosaprobic). The values at other sites of the same season are slightly higher, and according to the degree of saprobity, they belong to the oligo- $\beta$ -mesosaprobic category or slightly contaminated water (Tab. 3).

The saprobic index is one of the most important indices used in the assessment of a freshwater ecosystem water quality. Research conducted over a single year on the Krivaja River showed that the river water quality (Tab. 4). belongs in the oligo-to- $\beta$ -mesosaprobic category, i.e. slightly to moderately polluted water. Also, the values of the lower course of the river indicated  $\beta$ -mesosaprobic category water quality, which confirms the fact that the Krivaja is becoming more and more burdened longitudinally (CΙΚΟΤΙĆ, 2005). In correlation with this research, according to the values of the diversity indices and the water quality index, the Misoča River is characterized by specific faunal communities and good water quality.

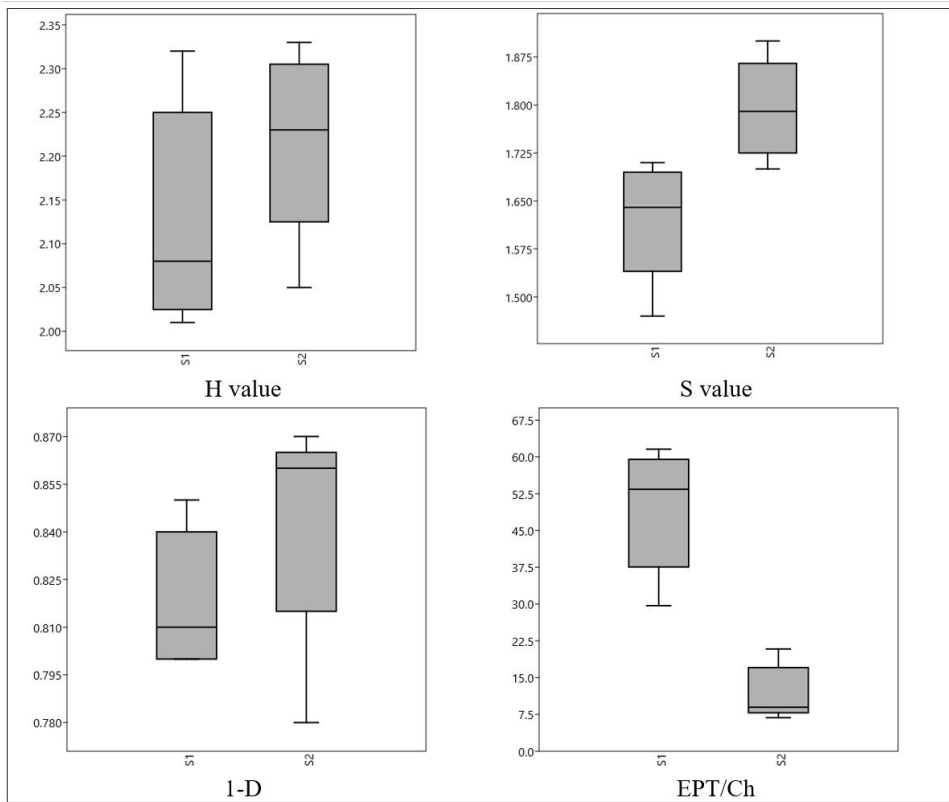
Distribution, seasonal dynamics and ecological tolerance of EPT group species are in correlation with the site categorization (upper, middle, lower parts of river). Ephemeroptera, Plecoptera, and Trichoptera are known to be highly sensitive to pollution; a high number of EPT species implies high water quality (LENAT, 1993). Mayfly species

**Tab. 3.** Values of the analyzed indices at the investigated sites of the Misoča River: H – Shannon-Weaver index; 1-D – Simpson's index; S – Pantle-Buck saprobic index; EPT% – index; EPT/Ch – EPT/Chironomidae index

Sites	Index	26.10.2019.	10.05.2020.
S1 "Three waterfalls"	H	2.32	2.33
	1-D	0.85	0.86
	S	1.68	1.83
	EPT	52.14%	65.24%
	EPT/Ch	267/5	229/11
S2 „Cliff“	H	2.08	2.2
	1-D	0.81	0.85
	S	1.64	1.79
	EPT%	54.45%	69.12%
	EPT/Ch	318/7	403/45
S3 „Slopes“	H	2.18	2.28
	1-D	0.83	0.86
	S	1.61	1.75
	EPT%	50.14%	68.36%
	EPT/Ch	517/9	443/50
S4 „Rocker“	H	2.04	2.23
	1-D	0.80	0.87
	S	1.71	1.90
	EPT%	62.67%	62.70%
	EPT/Ch	178/6	116/17
S5 „Bridge“	H	2.01	2.05
	1-D	0.80	0.78
	S	1.47	1.70
	EPT%	65.20%	71.30%
	EPT/Ch	431/7	318/24

**Tab. 4.** Classification of water quality according to the saprobic index (LIEBMANN, 1962).

Saprobic level	Value SI	Category	Saprobity
Oligosaprobic	1,0 - 1,5	I	Clear water
Oligo do $\beta$ -mesosaprobic	>1,5 - 1,8	I - II	Slightly polluted
$\beta$ -mesosaprobic	>1,8 - 2,3	II	Moderately polluted
$\beta$ do $\alpha$ -mesosaprobic	>2,3 - 2,7	II - III	Medium pollution
$\alpha$ -mesosaprobic	>2,7 - 3,2	III	Medium pollution - polluted
$\alpha$ do polysaprobic	>3,2 - 3,5	III - IV	Polluted
Polysaprobic	>3,5 - 4,0	IV	Highly polluted



**Fig. 2.** Box-plot seasonal variation of analysed indices at the sites of the Misoča River: H – Shannon-Weaver index; 1-D – Simpson's index; S – Pantle-Buck saprobic index; EPT/Ch – EPT/Chironomidae index; (S1 - autumn, S2 - spring; medina + quartile (min-max))

*Paraleptophlebia submarginata* was dominant in the collected samples from the autumn at all sites. The value of the saprobic index for *Paraleptophlebia submarginata* is 1.6, which characterizes oligosaprobic waters (Moog *et al.*, 1997). This claim was confirmed in later studies by GRANDJEN *et al.* (2011) which confirmed the connection between the presence of *Paraleptophlebia submarginata* in clean watercourses in which the intolerant and endangered species *Austropotamobius pallipes* occur. According to the case study in

Poitou-Charentes region in France that looked closely at the relationship between the occurrence of *Austropotamobius pallipes* and those of different Ephemeroptera species it was detected that a high presence of *Paraleptophlebia submarginata* is correlated with a high presence of white crayfish as they share similar preferences as to environmental conditions (cold water temperature, high levels of dissolved oxygen, etc.). In contrast, the study sites that did not have the presence of white crayfish had a high presence of Baetidae species such as *Centroptilium luteolum* (TROUILHÉ *et al.*, 2012). The prevalence of *Paraleptophlebia submarginata* decreases significantly in the spring. In the spring we recorded *Centroptilium luteolum*, family Baetidae, which is less sensitive to organic and inorganic pollution. Species *Centroptilium luteolum* is typical for  $\beta$ -mesosaprobic waters, which explains its occurrence in the spring season, since all localities approached this categorization with their saprobic value (GRANDJEN *et al.*, 2011). It is also worth noticing that *Paraleptophlebia submarginata* usually has a monovoltine life cycle with the highest flight emergence around May, while *Centroptilium luteolum* in Europe shows two types of cycles, but usually a bivoltine life cycle, with the highest flight emergence in May and July (MARTYNOV, 2016).

The results of the EPT% index showed that all the surveyed localities in both seasons are in excellent ecological conditions, i.e. the EPT% index at each locality exceeds 50%. The highest value was recorded in the spring at site 5 (71.30%), while the lowest was recorded at S3 in the autumn (50.14%).

We used a Chi-square test to test the difference of number of EPT individuals between seasons. Chi-square for the number of EPT individuals by season shows statistically significant differences  $\chi^2$  (4, N=3220), 36.2297,  $p < 0.001$ .

This means that the number of EPT individuals is significantly different in the different seasons, a higher number of EPT individuals being observable in spring. Seasonal changes in the number of EPT individuals can be the result of various factors, such as water temperature, food availability, amount of light, amount of oxygen in the water and other factors that can affect the ecosystem (ŠPORKA *et al.*, 2006).

Based on the EPT% index, we can conclude that the Misoča River is of oligosaprobic water quality.

Dominance of Chironomidae taxa in samples over the EPT group indicates a degraded state of the ecosystem and the presence of organic pollution in the study area. When we observe the EPT/Chironomidae index, the dominance of the EPT taxa in relation to Chironomidae is evident, which confirms the results of the analysis of the EPT% index on the excellent state of the River Misoča. The number of Chironomidae increases in the spring and at sampling site S3 the highest recorded number was 50 individuals. In relation to the number of EPT taxa at site 3 and all other sites, the number of Chironomidae does not indicate increase in organic or other pollution agents.

Periphyton can also impact the change in saprobic categorization on the investigated sites. Development of a large periphyton biomass in rivers and streams mostly depends on two factors: water velocity and nutrient concentration. Large influxes of nitrogen and phosphate based nutrients can cause eutrophication of periphyton which in correlation can degrade the ecological conditions of freshwater ecosystems (SARAVIA *et al.*, 1998). There is an also interesting link between abundance of Chironomidae taxa and high concentration of periphyton biomass. A study on the River Cvrčka, BiH, noted that a higher occurrence of Chironomidae was connected with the increase of perip-



hyton concentration (DMITROVIĆ, 2017). As was discussed earlier, dominance of Chironomidae taxa is usually found in degraded freshwater ecosystems and their occurrence heavily depends on the periphyton biomass because it is important food source (DMITROVIĆ, 2017). It should be also noted that the seasonal dynamics of periphytic communities in the temperate region shows that in the spring bloom there is a prevalence of diatoms while green algae and cyanobacteria are dominant in the late summer periphyton communities (VERMAAT, 2005).

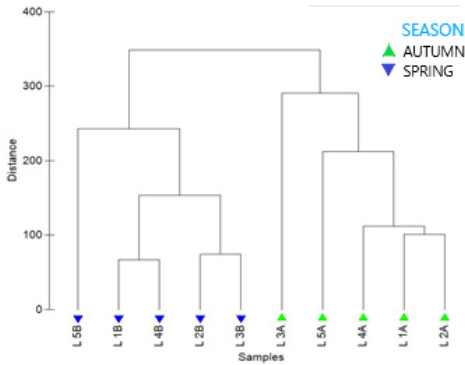
Since the EPT group is usually dominant in the macroinvertebrate community of freshwater ecosystems, the study of these three orders has proved quite successful in determining the general state of given ecosystems (HAMID & RAWI, 2017). When the EPT% index is used, in general, a higher percentage represents a better state of the studied ecosystem. The value of the indicators of this group is reflected in the fact that the number of EPT taxa is used as a sufficient and effective measure to determine the ecological status of the river. Considering that there is a justified suspicion of serious anthropogenic impacts on the Misoča River (quarry, sediment separation, landfill, picnic areas), it was necessary to perform a biological analysis that would indicate the degradation of the Misoča habitat. The accepted method for investigating changes in the composition of macroinvertebrate communities is the EPT/Chironomidae index (MANDAVILLE, 2002; KLEINE & TRIVINHO-STRIXINO, 2005).

It is also worth noticing that the relatively high presence of the members of the order Trombidiformes could indicate good water quality in the River Misoča (VASQUEZ *et al.*, 2022). Water mites are not as common an indicator as EPT fauna but studies that took a closer look to assemblage of water mites in riffles and streams concluded that their high abundance and biodiversity in most cases (not including the species that have developed a high tolerance to pollution) shows healthy freshwater ecosystems (GROWNS, 2001; GOLDSCHMIT, 2016). If we take a closer look to a study carried out in the River Wieprz, Poland, it was established that, based on the occurrence of certain communities of water mites, rivers can be zoned according to species that are sensitive to species that are tolerant to water pollution (KOWALIK & BIESIADKA, 1981). Looking at research in the River Misoča, species of *Hydrachna* genus were recorded only in the autumn, when the value of the saprobic index indicated the oligo to  $\beta$ -mesosaprobic category of water quality, which can mean that the representatives from the order Trombidiformes found in the Misoča River are more sensitive to pollution, because their occurrence was not recorded when water quality decreased.

Comparative analysis based on the Bray-Curtis similarities indicated differences of faunal community structures for the two seasons (Fig. 3). Based on the cluster analysis of the composition of the macroinvertebrate community, the upper and middle course sites are grouped in one clade, except for the fifth locality in different sampling seasons.

Based on the number of individuals from the EPT group, one-way ANOSIM analysis (Permutation N = 9999; Mean rank within = 9.6; Mean rank between = 24.68; R = 0.67;  $p < 0.01$ ) was performed and differences among sampling sites were tested. The Bray-Curtis similarity index was used. Results showed significant differences among sites.

The results of the analysis show that the R value (which ranges from -1 to 1) is 0.67, which indicates a relatively large difference among sites. Also, the p value (less than 0.01) indicates that these differences are statistically significant. This means that there



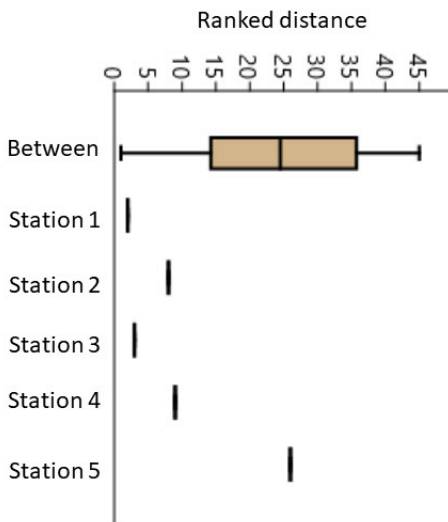
**Fig. 3.** Hierarchical clustering dendrogram based on the Bray-Curtis analysis for macrozoobenthos communities from September 2019 to May 2020 in the five sampling sites in the Misoča River (L1-L5 – represents location number; A- autumn, B – spring).

is a difference in the composition of EPT communities among the different localities, which may indicate differences in the quality of their aquatic ecosystems.

Likewise, pairwise test R value showed significant differences.

Pairwise test R-value closer to values  $R = -1$  or  $1$  indicates significant differences in similarity between the compared stations macroinvertebrate community compositions. This means that the two stations are significantly different in the composition of their communities of organisms. The closer the R-value is to zero ( $R = 0$ ), the less significant differences in similarity between the two compared groups. If the R-value is zero ( $R = 0$ ), it means that there are no significant differences in similarity between the two stations compared (Fig. 4).

In the context of macroinvertebrate community diversity in the Misoča River, this indicates that there is a large diversity of organisms in different sites, which can be positive because it means that there are a large number of different species that can survive in the investigated ecosystem. However, these differences in communities may be due to various factors such as water quality, food and habitat availability, pressures from



**Fig. 4.** Variation of values of Pairwise test showing the differences between stations based on the number of individuals from the EPT group.

anthropogenic activities, and so on. Therefore, these differences can also indicate problems in the ecosystem, such as habitat degradation and pollution (AMARAL *et al.*, 2015).

In order to understand the causes of differences in community similarity, additional research is needed that will take into account the various factors that may affect the ecosystem. This will help in understanding the processes that lead to different communities and in assessing the health and sustainability of ecosystems.

The highest diversity of benthic invertebrates was recorded at S1 "Three waterfalls" and S3 "Slopes", while lower values were recorded at S5 - "Bridge", which belongs to the lower reaches of this river. Upstream stations with high species diversity suggest unpolluted environments, whereas downstream stations with low species diversity imply environmental stress.

The greatest species diversity was shown by the order Trichoptera (18 species), whose morphological identification results are the most interesting. The endemic species *Hydropsyche botosaneanui* Marinkovic-Gospodnetic, 1966 was morphologically identified in the River Misoča, which was previously found only in the Rama River in Bosnia and Herzegovina (MARINKOVIĆ-GOSPODNETIĆ M., 1966; 1978). Six additional species of Trichoptera order that have not been reported in any previous study in Bosnia and Herzegovina have been found in our study: *Beraeamyia hrabei/squamosa*, *Halesus radiatus* Curtis, 1834, *Hydropsyche bulbifera* McLachlan, 1878, *Hydropsyche siltalai* Dohler, 1963, *Rhyacophila dorsalis* Curtis, 1834 and *Micrasema morosum* (McLachlan, 1868).

## CONCLUSION

Based on the results of our research, we can conclude that the Misoča River is characterized by favourable ecological conditions and a large diversity of macroinvertebrate communities. In the, there is an increase in the anthropogenic activities that disturb the balance of ecosystems and consequently affect the water quality of the Misoča River, which is characterized by a change in saprobic categorization of all sites and their approach to  $\beta$ -mesosaprobic category.

This study records the first literature data on macroinvertebrate diversity for this area, and the research itself showed the high diversity and abundance of the macrozoobenthos community. Analysed macrozoobenthos communities in the Misoča River show differences in structure and composition with greater diversity and abundance of macrozoobenthos at the sites of the upper and middle course compared to the lower course.

This study also determined the presence of protected and endemic species of macrozoobenthos, and the presence of species from the order Trichoptera, to which there is no reference in the literature with respect to the territory of BiH.

It is, therefore, necessary to prevent all the possible negative impacts that might disrupt the natural balance of the ecosystem of the Misoča River watercourse, and thus jeopardize the survival of certain species and the preservation of biodiversity. In order to preserve the Misoča River, it is important to develop a watercourse management plan, which would establish new goals in solving problems in water management and solving environmental problems. All these are necessary preconditions for the protection of this extremely important area.

## REFERENCES

- AMARAL, P., SILVEIRA, L., ROSA, B., OLIVEIRA, V. & ALVES, R., 2015: Influence of Habitat and Land Use on the Assemblages of Ephemeroptera, Plecoptera, and Trichoptera in Neotropical Streams. *Journal of Insects* **15**(1), 1-7.
- BARBOSA, F. A., CALLISTO, M. & GALDEAN, N., 2001: The diversity of benthic macroinvertebrates as an indicator of water quality and ecosystem health: A case study for Brazil. *Aquatic Ecosystem Health & Management* **4**(1), 51-59.
- BARUDANOVIĆ, S., 2012: Dostignuća Internacionalne godine biodiverziteta. *Biodiverzitet – Teorijski i Praktični Aspekti* **22**, 333-347.
- BAUERNFEIND, E., 1994: Bestimmungsschlüssel für die Österreichischen Eintagsfliegen (Insecta, Ephemeroptera), 2. Teil. Bundesanstalt für Wassergüte des Bundesministeriums für Land und Forstwirtschaft **92**, Wien.
- BIRMINGHAM, M. *et al.*, 2005: Benthic Macroinvertebrate Key. IOWATER Volunteer Water Quality Monitoring.
- BONADA, N., RIERADEVALL, M., PRAT, N. & RESH, V. H., 2006: Benthic macroinvertebrate assemblages and macrohabitat connectivity in mediterranean-climate streams of northern California. *Journal of the North American Benthological Society* **25**, 32-43.
- CIKOTIĆ, M., 2005: Saprobna i biološka analiza vode rijeke Krivaje. *Voda i mi* **44**, 28-36.
- CLARKE, K. R. & WARWICK, R.M., 2001: Change in Marine Communities: An Approach to Statistical Analysis and Interpretation. Plymouth Marine Laboratory, UK, 2, p. 2-5.
- DAVIES, I.J., 1984: Sampling aquatic insect emergence. In: DOWNING, J.A. & RIGEL, F.H. (eds.), *A manual on methods for the assessment of secondary productivity in fresh waters*. Oxford: Blackwell scientific publications. p. 161-227.
- DEWALT, E. & HEINOLD, B., 2005: Summer emerging Ephemeroptera, Plecoptera, and Trichoptera of Abrams Creek, Great Smoky Mountains National Park. *Proceedings of the Entomological Society of Washington* **107**, 34-48.
- DMITROVIĆ, D., 2017: Makrozoobentos odabranih krenona sliva rijeke Cvrčke, Doktorska disertacija. Prirodno-matematički fakultet, Univerzitet u Banjoj Luci.
- ELLIOT, J. M., HUMPRESCHE, U. H. & MACAN, T. T., 1988: Larvae of the British Ephemeroptera: a key with ecological notes. Ambleside: Freshwater Biological Association.
- FEDOR, J.P. & SPELLERBERG, I.F., 2013: Shannon-Wiener Index. Reference Module in Earth Systems and Environmental Sciences.
- GILLER, P.S. & MALMQVIST, B., 1998: *The Biology of Streams and Rivers*. Oxford: Oxford University Press.
- GOLDSCHMIDT, T. & MELZER, R.R., 2011: An interesting water mite fauna in springs near the city of Munich (Bavaria, Germany) – a pilot study for the monitoring of prealpine and alpine springs (Acari, Hydrachnidia). *Spixiana* **34**, 153-194.
- GRANDJEAN, F., JANDRY, J., BARDON, E., COIGNET, A., TROUILHÉ, M., PARINET, B., BRULIN, M., 2011: Use of Ephemeroptera as bioindicators of the occurrence of white-clawed crayfish (*Austropotamobius pallipes*). *Hydrobiologia* **671**(1), 253-258.
- GROWNS, J.E., 2001: Aquatic mites as bioindicators, with an Australian example. In: HALLIDAY, R.B., WALTER, D.E., PROCTOR, H. *et al.* (eds.), *Acarology: proceedings of the 10th International Congress*. Melbourne: CSIRO Publishing. p. 136-142.
- HAMADA, N. & COUCEIRO, S. R., 2003: An illustrated key to nymphs of Perlidae (Insecta, Plecoptera) genera in Central Amazonia, Brazil. *Revista Brasileira De Entomologia* **47**(3), 477-480.
- HAMID, S. A. & RAWI, C. S., 2017: Application of Aquatic Insects (Ephemeroptera, Plecoptera and Trichoptera) in Water Quality Assessment of Malaysian Headwater. *Tropical Life Sciences Research* **28**(2), 143-162.
- HEPP, L. U., RESTELLO, R. M., MILESI, S. V., BIASI, C. & MOLOZZI, J., 2013: Distribution of aquatic insects in urban headwater streams. *Acta Limnologica Brasiliensia* **25**(1), 1-9.
- HOLZENTHAL, R. W., BLAHNIK R. J., PRATHER, A. L. & KJER, K. M., 2007: Order Trichoptera Kirby, 1813 (Insecta), Caddisflies. In: ZHANG, Z.-Q. & SHEAR, W.A. (eds.), *Linnaeus Tercentenary: Progress in Invertebrate Taxonomy*. Zootaxa 1668. p. 1-766.
- KALYONCU, H. & ZEYBEK, M., 2011: An application of different biotic and diversity indices for assessing water quality: A case study in the Rivers Çukurca and Isparta (Turkey). *African journal of agricultural research* **6**(1), 19-27.

- KLEINE, P. & TRIVINHO-STRIXINO, S., 2005: Chironomidae and other aquatic macroinvertebrates of a first order stream: Community response after habitat fragmentation. *Acta Limnologica Brasiliensia* **17**(1), 81-90.
- KLEMM, D. J., LEWIS, P. A., FULK, F. & LAZORCHAK, J. M., 1990: Macroinvertebrate field and laboratory methods for evaluating the biological integrity of surface waters. Environmental Protection Agency EPA 600, 4-90.
- KOWALIK, W. & BIESIADKA, E. 1981: Occurrence of water mites (Hydracarina) in the river Wieprz polluted with domestic industry sewage. *Acta Hydrobiologica* **23**, 331-348.
- KRISKA, G., 2014: *Freshwater Invertebrates in Central Europe: A Field Guide*. SpringerVerlag, Wien.
- LENAT, D., 1993: *Freshwater Biomonitoring and Benthic Macroinvertebrates*. Journal of the North American Benthological Society **12**, 220-222.
- LENAT, D.R., 1988: Water quality assessment using a qualitative collection method for benthic macroinvertebrates. *Journal of the North American Benthological Society* **7**, 222-233.
- LIEBMANN, H., 1962: *Handbuch der Fischwasser und Abwasser-biologie*. Oldenbourg, Fischer.
- MANDAVILLE, S.M., 2002: *Benthic Macroinvertebrates in Freshwaters-Taxa Tolerance Values, Metrics, and Protocols*. Soil & Water Conservation Society of Metro. Halifax, Canada.
- MARINKOVIĆ-GOSPODNETIĆ M., 1966: Nove vrste Trichoptera iz Jugoslavije (New species of Trichoptera from Yugoslavia.) in *Bulletin Scientifique, Conseil Academy. RSF Yougoslavie* **11**(4-6), 110-112.
- MARINKOVIĆ-GOSPODNETIĆ, M., 1978: Some characteristics of the Yugoslav fauna of Trichoptera. In: CRICHTON, M.I. (eds.), *Proceedings of the 2nd International Symposium on Trichoptera*. Springer, Dordrecht. p. 83-88.
- MARTYNOV, A., 2016: *The Life Cycles of Mayflies (Insecta: Ephemeroptera) of the Eastern Ukraine*. Second Report. Proceedings of the National Museum of Natural History **14**, 86-94.
- MOOG, O., Bauernfeind, E. & Weischelbaumer, P., 1997: The use of Ephemeroptera as saprobic indicators in Austria. In: LANDOLT, P. & M. SARTORI (eds.), *Ephemeroptera and Plecoptera: Biology, Ecology, Systematics*. MTL, Fribourg. p. 254-260.
- NAGEL, P., 1989: *Bildbestimmungsschlüssel der Saprobien: Makrozoobenthon*.
- NEFIĆ, E., 1997: *Rijeka Misoča: opće fizičko-geografske karakteristike*. Diplomski rad.
- OLIFIERS, M. H., DORVILLE, L. F., NESSIMIAN, J. J. & HAMADA, N., 2004: A key to Brazilian genera of Plecoptera (Insecta) based on nymphs. *Zootaxa* **651**(1), 1.
- PANTLE, R. & BUCK, H., 1955: Die Biologische Überwachung der Gewässer Und die Darstellung der Ergebnisse. *Gas und Wasserfach* **96**, 604-624.
- PEET, R. K., 1974: The measurement of species diversity. *Annual Review of Ecology and Systematics* **5**(1), 285-307.
- REDŽIĆ, S., BAŠIĆ, H. & BARUDANOVIĆ, S., 2009: *Ekološki sistemi i modeli / Ecological systems and models*. University book. Sarajevo: Grafika Šaran.
- RESH, V. H. & JACKSON, J. K., 1993: Rapid assessment approaches to biomonitoring using benthic macroinvertebrates. In: ROSENBERG, D. M. & RESH, V. H. (eds.), *Freshwater Biomonitoring, Benthic Macroinvertebrates*. New York: Chapman and Hall. p. 159-194.
- SARAVIA, L., MOMO, F., & LISSIN, L., 1998: Modeling periphyton dynamics in running water. *Ecological Modelling*, **114**, 35-47.
- SHANNON, C.E. & WEAVER, W., 1949: *The Mathematical Theory of Communication*. Urbana: University of Illinois Press.
- SIMPSON, E. H., 1949: Measurement of diversity. *Nature* **163**, 688.
- SOLDAN, T. & LANDA, V., 1999: A key to the Central European species of the genus *Rhithrogena* (Ephemeroptera : Heptageniidae). *Klapalekiana* **35**, 25-37.
- ŠPORKA, F., VLEK, H., BULÁNKOVÁ, E. & KRNO, I., 2006: Influence of seasonal variation on bioassessment of streams using macroinvertebrates, *Hydrobiologia* p. 543-555
- STOYANOVA, T., TRAYKOV, I., YANEVA, I. & BOGOEV, V., 2010: Ecological Quality Assessment of Luda River, Bulgaria. *Natura Montenegrina, Podgorica* **9**(3), 341-348.
- STRESEMANN, E., HANNEMANN, H., KLAUSNITZER, B. & SENGLAUB, K., 2011: *Wirbellose: Insekten*. Heidelberg: Spektrum Akademischer Verlag.
- STUEDEMANN, D., LANDOLT, P., SARTORI, M., HEFTI, D. & TOMKA, I., 1992: *Ephemeroptera, Insecta Helvetica*. In: *Societe entomologique Suisse* (eds.), *Fauna* **9**, Freiburg. p. 174.
- TROUILHÉ, C. M., FREYSSINEL, G., JANDRY, J., BRULIN, M., PARINET, B., SOUTY-GROSSET, C. & GRANDJEAN, F., 2012: The relationship between Ephemeroptera and presence of the white-clawed crayfish (*Austro-*

- potamobius pallipes*). Case study in the Poitou-Charentes region (France). *Fundamental and Applied Limnology / Archiv für Hydrobiologie* **179**, 293-303.
- TROŽIĆ-BOROVAC, S., 2005: Biodiverzitet vodenih cvjetova (Insecta: Ephemeroptera) u BiH i njihov značaj u ocjeni kvaliteta vode. *Voda i mi, JP za „Vodno područje slivova rijeke Save“*, Sarajevo **82 (41)**, 60-67.
- TROŽIĆ-BOROVAC, S., GAJEVIĆ, M. & BOROVAC, B., 2019: Makrozoobentos krenona (izvorišta) tekućica. *Voda i mi* 55-68.
- VASQUEZ, A.A., KABALAN, B.C. & MILLER, C.J., 2022: First data on water mite (Acari, Hydrachnidia) assemblages of Point Rosa Marsh, Harrison Township, Michigan, USA, and their use as environmental bioindicators of aquatic health. *Acarologia* **62 (3)**, pp.653-665.
- VERMAAT, J. E., 2005: Periphyton dynamics and influencing factors. *Periphyton ecology, exploitation and management*. Cambridge: CABI Publishing, p. 35-49.
- WARINGER, J. & GRAF, W., 2013: Key and bibliography of the genera of European Trichoptera larvae. *Zootaxa* **3640 (2)**, 101-151.
- ZAHRADKOVA, S. & SOLDAN, T., 2008: Saprobic System. In: *Encyclopedia of Ecology* (eds.), p. 3141-3143.

### Appendix 1.

Spreadsheet of found species in River Misoča in both seasons (autumn, spring).

TAXON	SEASON 1 (AUTUMN) 6.10.2019.					SEASON 2 (SPRING) 10.05.2020.				
	LS1	S 2	S 3	S 4	S 5	S 1	S 2	S 3	S 4	S 5
<b>TRICLADIDA</b>										
<i>Dugesia gonocephala</i> (Duges, 1830)			1							
<b>ARCHITAENIOGLOSSA</b>										
<i>Viviparus contectus</i> (Proso, 1813)	4	8	8	5	2	15	11	12	10	1
<b>LUMBRICULIDA</b>										
Lumbriculidae	28	27	31	24	7	25	34	24	11	5
<b>TROMBIDIFORMES</b>										
<i>Hydrachna</i> sp.	7	1	3		28					
<b>DECAPODA</b>										
<i>Austropotamobius torrentium</i> (Schrank, 1803)	4	2	5			5	2	4	3	
<b>EPHEMEROPTERA</b>										
<i>Centroptilium luteolum</i> (Müller, 1776)					6	9	11		8	
<i>Ecdyonurus venosus</i> (Fabricius, 1775)	60	40	50	18	33	98	117	161	41	51
<i>Epeorus sylvicola</i>		1			1					
<i>Epeorus</i> sp.	2	15	2		13	2			1	1
<i>Rhithrogena semicolorata</i> (Curtis, 1834)	7	5		10	5	7	19		2	
<i>Ephemera danica</i> (Müller, 1764)	17	10	77	14		26	121	80	27	4
<i>Ephemera vulgata</i> (Linnaeus, 1758)	11	13	34	7		9	24	17	6	
<i>Ephemerella notata</i> (Eaton, 1887)	22	28	132	14	4	22	50	72	23	26
<i>Paraleptophlebia submarginata</i> (Stephens, 1835)	80	166	141	106	233	26	31	22	8	9
<i>Habrophlebia fusca</i> (Curtis, 1834)					4	15	12	2	2	
<b>ODONATA</b>										
<i>Onychogomphus forcipatus</i> (Linnaeus, 1758)										5
<b>COLEOPTERA</b>										
<i>Nebrioporus elegans</i> (Panzer, 1794)			2						1	
<i>Austrolimnius</i> sp.		4								
<i>Elmis</i> sp. larva	125	117	120	38	37	36	50	48	20	11
<i>Elmis maugeti</i> (Latreille, 1802)		2		2	1	3	2		44	
<i>Esolus parallelepipedus</i>		5	13		34			2		2

TAXON	SEASON 1 (AUTUMN) 6.10.2019.					SEASON 2 (SPRING) 10.05.2020.				
	LS1	S 2	S 3	S 4	S 5	S 1	S 2	S 3	S 4	S 5
<i>Esolus</i> sp. larva	18	33	190	15	10	10	12	25	3	8
<i>Limnius</i> sp. larva	5	8	22	2	43	5	6	4		2
<i>Hydraena riparia</i> (Kugelann, 1794)		2	4	3	26	1				3
<b>PLECOPTERA</b>										
<i>Leuctra braueri</i> (Kempny, 1989)	16	10	33	8	29				3	
<i>Nemurella picteti</i> (Klapalek, 1900)	3	5	10		3					
<i>Protonemura intricata</i> (Ris, 1902)						2	3		1	3
<i>Amphinemura standfussi</i> (Ris, 1902)										182
<i>Perla burmeisteriana</i> (Pictet, 1833)			3		2	1		2		2
<i>Perla marginata</i> (Panzer, 1799)	3	19	2	5	5	3	4	8	4	5
<i>Isoperla grammatica</i>								1		
<i>Isoperla rivulorum</i> (Pictet, 1841)	6	4	6		89					
<b>TRICHOPTERA</b>										
<i>Beraemyia hrabei/squamosa</i> grupa								2		
<i>Micrasema morosum</i> (McLachlan, 1868)								1		1
<i>Hydropsyche angustipennis</i> (Curtis, 1934)	6		8	1	4					
<i>Hydropsyche botosaneanui</i>	7				1		18	1	13	
<i>Hydropsyche bulbifera</i> (McLachlan, 1878)	2									
<i>Hydropsyche fulvipes</i> (Curtis, 1934)	2									
<i>Hydropsyche instabilis</i> (Curtis, 1934)	2									
<i>Hydropsyche siltalai</i> (Dohler, 1963)					1					
<i>Halesus radiatus</i> (Curtis, 1834)					11	14				
<i>Odontocerum</i> sp.							3			
<i>Rhyacophila aurata</i> (Brauer, 1857)	27				2		2	3	2	
<i>Rhyacophila dorsalis</i> grupa (Curtis, 1834)			1							
<i>Rhyacophila laevis</i> (Pictet, 1834)					1					
<i>Rhyacophila oblitterata</i> (McLachlan, 1863)			1					1		1
<i>Rhyacophila pubescens</i> (Pictet, 1835)			7	5	2	6	1	4		3
<i>Rhyacophila stigmatica</i> (Kolenati, 1859)			5							
<i>R. tristis, bosnica</i> grupa (Pictet, 1834)			3			6		8		
<b>Porodica Sericostomatidae</b>										
<i>Sericostoma personatum, flavicorne</i> grupa			2				1			
<b>DIPTERA</b>										
Athericidae	17	4	42		9	7				
<i>Atherix ibis</i> (Fabricius, 1798)	16	11	11	10	23	1	4	13	3	19
Ceratopogonidae						1		2		1
Chironomidae	5	7	9	6	7	12	45	50	17	24
Clinocera sp.							2			
<i>Antocha vitripennis</i> (Meigen, 1830)	12	33	43	2	2	1	3	11	2	1
<i>Limnophila</i> sp.								5		
<i>Dicranota</i> sp.	2					1				
<i>Pericoma</i> sp.	1	1		1			1			
<i>Oxycera</i> sp.		1				2	1			1
<b>Number of taxa</b>	27	30	33	19	28	32	29	31	19	33
<b>Number of Individuals</b>	511	584	1021	284	661	352	577	645	185	446

