

SEASONAL SUCCESSIONS OF BENTHIC MACROINVERTEBRATE COMMUNITIES IN THE BOSNA RIVER IN THE AREA OF THE CITY OF ZENICA

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Abstract

This research describes seasonal successions of the qualitative-quantitative composition of benthic macroinvertebrate communities in the Bosna River in the area of the city of Zenica. Collection of benthic macroinvertebrates was conducted from October 2014 to May 2015 at 5 measurement locations along the course of the Bosna River in the city of Zenica. Biological diversity indices (Shannon–Weaver index, Simpson's index), a species evenness indicator, and Bray–Curtis cluster analysis was used for data analysis. The study of benthic macroinvertebrates identified 34 taxa and 4224 individuals, with a dominance of preimaginal stages of aquatic insects with 21 taxa. The highest observed Shannon–Weaver diversity index was 2.26 in the spring aspect at the Balnozi site, while the lowest was 0.27 in the summer aspect at the Raspotočje site. Using the Simpson's index, values ranged from 0.07 in the summer period at the Raspotočje site to 0.82 in the winter period at the Balnozi site. Analysis of species evenness between communities at the investigated sites revealed similarity in benthic fauna between the first (0.64) and second (0.67) sites, as well as between the fourth (0.76) and fifth (0.70) sites. According to the results of the cluster analysis, the investigated sites have similar macroinvertebrate communities classified by seasons of benthic macroinvertebrate examination.

Key words: benthic macroinvertebrates, Bosna River, biological indices

INTRODUCTION

The area of the city of Zenica is situated between 17.30 and 18.00 degrees east longitude and 44.00 and 44.30 degrees north latitude. It is located in the Zenica-Doboj Canton and the Central Bosnia region, in the hilly-mountainous belt between low mountains belonging to the foothills of two mountain massifs. The average relative air humidity, based on multi-year measurements, is 83% in winter and 72% in summer. The northern part of the basin has completely changed its natural appearance - it is the area of the ironworks, coal mine, and the

narrower urban area of Zenica. All industries are located in the northern part of the Zenica basin and are connected to the urban zone of the city, forming a unique industrial-urban spatial entity.

The Bosna River is a right tributary of the Sava River and belongs to the Black Sea basin. It originates from about thirty karst springs in the village of Vrutci near Sarajevo, at the foot of the Igman mountain, at an altitude of about 500 meters above sea level. The watershed area is approximately 10,500 km². In the upper course, the Bosna River flows through the Sarajevo, Visoko, Kakanj, and Zenica fields. Water pollution is becoming increasingly intense, especially near larger settlements and industrial centers, as exemplified by the Bosna River. In addition to pollution from industrial wastewater, the Bosna River is a site for the disposal of various types of waste. In the Zenica area, ecological incidents occur, manifesting in the mass mortality of fish populations in the Bosna River. Considering these facts, it is important to monitor the biological monitoring of benthic macroinvertebrates in the Bosna River and its tributaries to determine water quality.

Benthic macroinvertebrates constitute the invertebrate fauna of the bottom. Benthic macroinvertebrates include groups of aquatic insects that are highly sensitive to pollution (Plecoptera, Ephemeroptera, Trichoptera, Diptera), as well as other macroinvertebrates that spend their entire lives in the water (Coleoptera, Heteroptera, Megaloptera, Hydrozoa, Gastropoda, Bivalvia, Turbellaria, Oligochaeta, Crustacea, Hirudinea). Macroinvertebrates serve as biological indicators of water quality, integrating environmental conditions over an extended period and requiring less frequent sampling compared to chemical analyses, which typically reflect only the current state of water quality and demand frequent sampling. Monitoring of water quality and the results of research on benthic macroinvertebrates of the Bosna River with its tributaries began in the 1950s and continued until today (Šenk, 1956; Kačanski *et al.*, 1980; Trožić-Borovac, 2001). Insect orders were investigated in Bosnia and Herzegovina: Plecoptera (Kačanski, 1972; Kačanski, 1978), Ephemeroptera (Tanasijević, 1981), Simuliidae (Kačanski, 1970).

The research report on macroinvertebrates, physico-chemical, and bacteriological indicators of the Bosna River in the Zenica area and its tributaries (Babina River, Kočeva, and Gračanica) was conducted during 1977-1988 and 1989-1990 by the Metallurgical Institute "Hasan Brkić" Zenica (Habibović, *et al.*, 1988; Bursać *et al.* 1990). Continuous measurements of air and water pollution of the Bosna River in Zenica were carried out during the period 2000-2001 by the Metallurgical Institute "Kemal Kapetanović" in Zenica, but without the inclusion of biological indicators (Duran and Salkić, 2001).

MATERIALS AND METHODS

A portion of the selected sites was adopted from previous biological-ecological research conducted at the Metallurgical Institute "Hasan Brkić" Zenica during the periods 1977-1988 and 1989-1990. Sampling was carried out at 5 sites (L): Drivuša (L1), Raspotočje (L2), Hotel Metalurg (L3), below the Coastal Channel of Zenica Ironworks (L4), and Banlozi-Jelina (L5). Sampling was carried out during 2014 and 2015 and covered all seasons: autumn (October 7th, 2014) winter (January 15th, 2015), spring (May 6th, 2015) and summer (June 23rd, 2015). For the sampling of benthic macroinvertebrates in the research area, the kick sampling method was

employed, utilizing a benthic net. Three cross-sections were sampled at each site, taking care to include both quieter sections of the flow and areas with rapids. The net is positioned perpendicular to the rock, which is lifted upstream, and the water flow is waited for to flush the organisms into the net. It is necessary to wait for the water to clear. The entire procedure is repeated at all three cross-sections of each site. In the laboratory, the separated sample is isolated from other impurities using tweezers and needles, and the individuals are transferred to small glass bottles filled with 70% alcohol and identified using taxonomic keys: (Aubert, 1959; Bole, 1969; Berthélemy and Laur, 1975; Consiglio, 1980; Belifore, 1983; Kerovec, 1986). The biological characteristics of the investigated area are presented through species of the corresponding taxon, the number of individuals per taxon, and the relative abundance calculated according to the formula:

$$A = \frac{ni}{N} \times 100;$$

As it is:

A – The value of the relative contribution, where n_i is the number of individuals of the i -th taxon, taxon

N – the total number of individuals in the sample.

The number of species in a specific habitat and the total abundance of individuals in that habitat are the primary parameters for creating diversity indices. If there are more species in a sample, it will be richer. A sample with a more similar abundance of individuals for each species will exhibit greater evenness.

The diversity of macroinvertebrate communities will be represented by the Shannon–Weaver diversity index (H) (Shannon and Weaver, 1949), which utilizes the relative abundance of individual taxa:

$$H' = -\sum P_i \log_2 P_i \quad (\text{where } P_i = \frac{n_i}{N};)$$

As it is:

H' – the value of the diversity index,

P_i – the relative representation of that taxon in the sample, and $\log_2(x) = \log(x)/\log 2$.

The Shannon-Weaver diversity index belongs to type I diversity indices, which are most sensitive to changes in rare species in a sample from a community, while type II indices are most sensitive to changes in more common species (Peet, 1974). This index starts from zero if only one species is present in the sample, and as the number of species increases, the index value also increases, indicating increased diversity. The values of the Shannon-Weaver index have been applied to assess water quality, and the interpretation of index values is as follows: $H > 3$ - clean water (Category I water), $H = 2-3$ - slightly polluted (Category II water), $H = 1-2$ - moderately or moderately polluted (Category III water), $H < 1$ - heavily polluted (Category IV water).

Simpson's diversity index, calculated by the formula (Krebs, 1999):

$$1 - D = \sum_{i=1}^s p_i^2$$

As it is:

(1 - D) - Simpson's diversity index,

p_i - is the proportion of individuals of the i -th species in the community, and

s - the total number of species in the sample.

The Simpson diversity index is a representative of type II indices. The Simpson diversity index (1-D) expresses the probability that two randomly selected individuals from the community belong to different categories (species), derived from the basic Simpson index (D). The basic Simpson index (D) (Simpson, 1949) expresses the probability that two randomly selected individuals from the community belong to the same category.

Evenness for all indices is defined as the ratio of observed diversity to the maximum attainable diversity. In the study, a very simple evenness measure was used, calculated by the formula (Pielou, 1969):

$$E = \frac{H'}{\ln S}$$

Where:

E - evenness (or equitability) of species in the sample,

H' - Shannon-Weaver species diversity index in the sample, and

S - the total number of species in the sample

Hierarchical clustering of macroinvertebrate samples based on similarity/dissimilarity was tested using Bray-Curtis cluster analysis (Bray and Curtis, 1957), within which the group average linkage method was employed to form clusters or groups of the most similar samples. To determine the degree of similarity in the composition of macroinvertebrate samples from the same locality but different seasons and samples from different localities, cluster analysis based on the Bray-Curtis diversity index was utilized. All the mentioned indices were calculated using the Biodiversity prover 2 software package.

RESULTS AND DISCUSSION

Through the analysis of the qualitative-quantitative composition of macroinvertebrates in the Bosna River, 34 taxa were identified, with a total of 4224 individuals. The highest number of taxa (14) and individuals (1138) was observed in the sample at the Banlozi-Jelina site during the summer period, while the lowest number of individuals (3) was recorded in the autumn sample from the same locality. The lowest number of taxa (2) was observed at the Raspotočje, Hotel Metalurg, and Coastal Channel of Zenica Ironworks sites in January, as well as at the Banlozi site in October. The number of identified taxa in the Bosnia River is significantly lower than the number of taxa recorded in the Neretva research (Trožić-Borovac *et al.*, 2011), where 51 taxa were recorded, among which the larval stages of the orders Ephemeroptera, Plecoptera and Trichoptera dominate. The number of registered taxa is significantly lower than the number of macroinvertebrate taxa in the Una River, where Bakrač *et al.* (2021) identified 130 taxa. In the results of the Konjuha stream research, Skenderović *et al.* (2020) list 46 registered taxa. The number of taxa in the Bosna River is lower than the

number of macroinvertebrate taxa found in Kreševka, where Hafner and Trožić-Borovac (2012) registered 56 taxa.

Site 1 – Drivuša

At the Drivuša site, 8 taxa were observed with a total of 715 individuals. The highest number of individuals (603) and taxa (8) were found in the month of June, while the lowest number of individuals (7) and taxa (3) were found in January. Processing the material collected throughout the year (Table 1) reveals a dominance of Diptera larvae from the Chironominae subfamily, with a total of 603 individuals, reaching peak abundance in the month of June.

Table 1. Qualitative-quantitative composition of benthic macroinvertebrates at Site 1 - Drivuša

| Taxon | Autumn | | Winter | | Spring | | Summer | |
|-----------------------------------|----------|------------|----------|------------|-----------|------------|------------|------------|
| | No/ind. | % | No/ind. | % | No/ind. | % | No/ind. | % |
| OLIGOCHAETA | | | | | | | | |
| Lumbriculidae | | | | | 8 | 8.33 | 5 | 0.83 |
| Tubificidae | | | | | 2 | 2.08 | | |
| HIRUDINEA | | | | | | | | |
| <i>Glossiphonia complanata</i> | | | | | | | 7 | 1.17 |
| CRUSTACEA | | | | | | | | |
| Amphipoda | | | | | | | | |
| <i>Gammarus</i> sp. | | | | | | | 5 | 0.83 |
| INSECTA | | | | | | | | |
| Ephemeroptera | | | | | | | | |
| <i>Heptagenia lateralis</i> | | | 1 | 14.29 | | | | |
| <i>Baetis muticus</i> | | | | | | | 13 | 2.16 |
| <i>Baetis rhodani</i> | | | 5 | 71.43 | 17 | 17.71 | 7 | 1.16 |
| <i>Ephemerella ignita</i> | | | | | 3 | 3.13 | | |
| <i>Paraleptophl. submarginata</i> | | | | | | | 14 | 2.32 |
| Plecoptera | | | | | | | | |
| <i>Isoperla grammatica</i> | | | | | 12 | 12.50 | | |
| Trichoptera | | | | | | | | |
| <i>Hydropsyche instabilis</i> | 4 | 44.44 | | | | | 2 | 0.33 |
| Diptera | | | | | | | | |
| Chironomidae | | | | | | | | |
| Chironominae | 3 | 33.33 | | | 49 | 51.04 | 550 | 91.21 |
| Tanytarsinae | 1 | 11.11 | 1 | 14.29 | 5 | 5.21 | | |
| Simulidae | 1 | 11.11 | | | | | | |
| Number of individuals | 9 | 100 | 7 | 100 | 96 | 100 | 603 | 100 |
| Number of taxa | 4 | | 3 | | 7 | | 8 | |

Site 2 - Raspotočje

At the Raspotočje site, 7 taxa were identified with a total of 573 individuals. The highest number of individuals (497) occurred in the summer aspect, while the lowest number of individuals (3) was observed in the winter aspect. The representation of the percentage participation of benthic macroinvertebrates indicates the dominance of Diptera (96%) in the summer aspect (Table 2).

Table 2. Qualitative-quantitative composition of benthic macroinvertebrates at Site 2 - Raspotočje

| Taxon | Autumn | | Winter | | Spring | | Summer | |
|-------------------------------|-----------|------------|----------|------------|-----------|------------|------------|------------|
| | No/ind. | % | No/ind. | % | No/ind. | % | No/ind. | % |
| GASTROPODA | | | | | | | | |
| <i>Valvata cristata</i> | 1 | 4.55 | | | | | | |
| <i>Planorbis planorbis</i> | | | 2 | 66.67 | | | | |
| BIVALVIA | | | | | | | | |
| <i>Spherium corneum</i> | | | 1 | 33.33 | | | | |
| OLIGOCHAETA | | | | | | | | |
| Lumbriculidae | 8 | 36.36 | | | | | 4 | 0.80 |
| Lumbricidae | 4 | 18.18 | | | | | | |
| Tubificidae | 7 | 31.82 | | | | | | |
| HIRUDINEA | | | | | | | | |
| <i>Erpobdella octoculata</i> | 1 | 4.55 | | | | | | |
| INSECTA | | | | | | | | |
| Ephemeroptera | | | | | | | | |
| <i>Heptagenia lateralis</i> | | | | | 1 | 1.96 | | |
| <i>Heptagenia sulphurea</i> | | | | | 1 | 1.96 | | |
| <i>Baetis muticus</i> | | | | | | | 2 | 0.40 |
| <i>Baetis rhodani</i> | | | | | 25 | 49.02 | 6 | 1.21 |
| <i>Ephemerella ignita</i> | | | | | 4 | 7.84 | | |
| Plecoptera | | | | | | | | |
| <i>Isoperla grammatica</i> | | | | | 8 | 15.69 | | |
| Trichoptera | | | | | | | | |
| <i>Hydropsyche instabilis</i> | 1 | 4.55 | | | | | | |
| <i>Goera pilosa</i> | | | | | | | 5 | 1.01 |
| Diptera | | | | | | | | |
| Chironomidae | | | | | | | | |
| Chironominae | | | | | 7 | 13.73 | 480 | 96.58 |
| Tanytarsinae | | | | | 5 | 9.80 | | |
| Number of individuals | 22 | 100 | 3 | 100 | 51 | 100 | 497 | 100 |
| Number of taxa | 6 | | 2 | | 7 | | 5 | |

Site 3 - Hotel Metalurg

At the Hotel Metalurg site, 12 taxa were observed in the spring aspect, with a total of 1153 individuals. The highest number of individuals (820) was recorded in the summer aspect, while the lowest number of individuals (6 individuals and 2 taxa) was observed in the winter period (Table 3).

Table 3. Qualitative-quantitative composition of benthic macroinvertebrates at Site 3 – Hotel metalurg

| Taxon | Autumn | | Winter | | Spring | | Summer | |
|----------------------|---------|-------|---------|-------|---------|------|---------|-------|
| | No/ind. | % | No/ind. | % | No/ind. | % | No/ind. | % |
| GASTROPODA | | | | | | | | |
| <i>Viviparus</i> sp. | | | 2 | 33.33 | | | | |
| OLIGOCHAETA | | | | | | | | |
| Lumbriculidae | 17 | 56.67 | | | 8 | 2.69 | 87 | 10.61 |

| | | | | | | | | |
|--------------------------------------|-----------|------------|----------|------------|------------|------------|------------|------------|
| Tubificidae | 10 | 33.33 | | | | | | |
| HIRUDINEA | | | | | | | | |
| <i>Glossiphonia complanata</i> | 1 | 3.33 | | | 2 | 0.67 | 9 | 1.01 |
| INSECTA | | | | | | | | |
| Ephemeroptera | | | | | | | | |
| <i>Heptagenia lateralis</i> | | | | | 3 | 1.01 | | |
| <i>Heptagenia sulphurea</i> | | | | | 3 | 1.01 | | |
| <i>Baetis muticus</i> | | | | | | | 7 | 0.87 |
| <i>Baetis rhodani</i> | | | | | 41 | 13.80 | 3 | 0.37 |
| <i>Ephemerella ignita</i> | 1 | 3.33 | | | 4 | 1.35 | | |
| <i>Paraleptophlebia submarginata</i> | | | | | 1 | 0.34 | 13 | 1.59 |
| Plecoptera | | | | | | | | |
| <i>Isoperla grammatica</i> | | | | | 1 | 0.34 | | |
| Trichoptera | | | | | | | | |
| <i>Agapetus</i> sp. | | | | | | | 1 | 0.12 |
| Diptera | | | | | | | | |
| Chironomidae | | | | | | | | |
| Chironominae | 1 | 3.33 | 4 | 66.67 | 200 | 67.34 | 700 | 85.37 |
| Tanytarsinae | | | | | 28 | 9.43 | | |
| Psychodidae | | | | | 5 | 1.68 | | |
| Odonata | | | | | | | | |
| <i>Cordulegaster</i> sp. | | | | | 1 | 0.34 | | |
| Number of individuals | 30 | 100 | 6 | 100 | 297 | 100 | 820 | 100 |
| Number of taxa | 5 | | 2 | | 12 | | 7 | |

Site 4 - Coastal Channel of Zenica Ironworks

At the site below the Coastal Channel of Zenica Ironworks, 10 taxa were identified with a total of 539 individuals. The highest number of individuals (286) occurred in the summer aspect, while the lowest number of individuals (5) was observed in the winter aspect. In the summer sample, Diptera maintained dominance at 51%, but with a smaller number of individuals compared to the spring sample. The composition of benthic macroinvertebrates includes taxa that were not present in other aspects, such as Ephemeroptera - *Baetis muticus* and *Paraleptophlebia submarginata*, Trichoptera with *Glossosoma* sp., *Plectrocnemia conspersa*, and *Rhyacophila nubile*, as well as new groups of Hirudinea.

Table 4. Qualitative-quantitative composition of benthic macroinvertebrates at Site 4 - Coastal Channel of Zenica Ironworks

| Taxon | Autumn | | Winter | | Spring | | Summer | |
|--------------------------------|---------|-------|---------|-------|---------|------|---------|------|
| | No/ind. | % | No/ind. | % | No/ind. | % | No/ind. | % |
| GASTROPODA | | | | | | | | |
| <i>Bithynia</i> sp. | 1 | 7.69 | | | | | 3 | 1.05 |
| OLIGOCHAETA | | | | | | | | |
| Lumbriculidae | 5 | 38.46 | 3 | 60.00 | 7 | 2.98 | | |
| Lumbricidae | | | | | 2 | 0.85 | | |
| Tubificidae | 3 | 23.08 | | | | | | |
| HIRUDINEA | | | | | | | | |
| <i>Glossiphonia complanata</i> | | | | | | | 14 | 4.90 |

| | | | | | | | | |
|-----------------------------------|-----------|------------|----------|------------|------------|------------|------------|------------|
| INSECTA | | | | | | | | |
| Ephemeroptera | | | | | | | | |
| <i>Heptagenia lateralis</i> | | | | | 9 | 3.83 | | |
| <i>Baetis muticus</i> | | | | | | | 48 | 16.78 |
| <i>Baetis rhodani</i> | | | | | 27 | 11.49 | | |
| <i>Ephemerella ignita</i> | | | | | 6 | 2.55 | | |
| <i>Paraleptophl. submarginata</i> | | | | | | | 23 | 8.04 |
| Plecoptera | | | | | | | | |
| <i>Isoperla grammatica</i> | | | | | 9 | 3.83 | | |
| Trichoptera | | | | | | | | |
| <i>Hydropsyche instabilis</i> | | | | | 5 | 2.13 | 4 | 1.40 |
| <i>Glossosoma</i> sp. | | | | | | | 47 | 16.43 |
| <i>Plectrocnemia conspersa</i> | | | | | | | 1 | 0.35 |
| <i>Rhyacophila nubila</i> | | | | | | | 1 | 0.35 |
| Megaloptera | | | | | | | | |
| <i>Sialis lutaria</i> | 1 | 7.69 | | | | | | |
| Diptera | | | | | | | | |
| Chironomidae | | | | | | | | |
| Chironominae | 3 | 23.08 | | | 130 | 55.32 | 85 | 29.72 |
| Tanytarsinae | | | 2 | 40.00 | 40 | 17.02 | 60 | 20.98 |
| Number of individuals | 13 | 100 | 5 | 100 | 235 | 100 | 286 | 100 |
| Number of taxa | 5 | | 2 | | 9 | | 10 | |

Site 5 - Banlozi-Jelina

At the Banlozi-Jelina site, 14 taxa were identified with a total of 1244 individuals. The highest number of individuals (1138) occurred in the summer aspect while the lowest number of individuals (3) was observed in the autumn aspect. Throughout seasonal investigations there is an increase in the number of taxa from the winter aspect (5) to the spring (10) and summer (14) aspects (Table 5).

Table 5. Qualitative-quantitative composition of benthic macroinvertebrates at Site 5 - Banlozi-Jelina

| Taxon | Autumn | | Winter | | Spring | | Summer | |
|--------------------------------|---------|-------|---------|-------|---------|------|---------|------|
| | No/ind. | % | No/ind. | % | No/ind. | % | No/ind. | % |
| GASTROPODA | | | | | | | | |
| <i>Valvata cristata</i> | | | 1 | 12.50 | | | | |
| <i>Ancylus fluviatilis</i> | | | 1 | 12.50 | | | | |
| <i>Ferrisia</i> sp. | | | 1 | 12.50 | | | | |
| OLIGOCHAETA | | | | | | | | |
| Lumbriculidae | | | | | | | 10 | 0.88 |
| Lumbricidae | | | 2 | 25.00 | | | 1 | 0.09 |
| HIRUDINEA | | | | | | | | |
| <i>Glossiphonia complanata</i> | 2 | 66.67 | | | 2 | 2.11 | 2 | 0.18 |
| CRUSTACEA | | | | | | | | |
| Amphipoda | | | | | | | | |
| <i>Gammarus</i> sp. | | | | | 9 | 9.47 | 43 | 3.78 |
| INSECTA | | | | | | | | |
| Ephemeroptera | | | | | | | | |
| <i>Heptagenia lateralis</i> | | | 3 | 37.50 | 7 | 7.37 | | |

| | | | | | | | | |
|--------------------------------------|----------|------------|----------|------------|-----------|------------|-------------|------------|
| <i>Baetis muticus</i> | | | | | | | 3 | 0.26 |
| <i>Baetis rhodani</i> | | | | | 18 | 18.95 | | |
| <i>Ephemerella ignita</i> | | | | | 6 | 6.32 | | |
| <i>Paraleptophlebia submarginata</i> | | | | | | | 8 | 0.70 |
| Plecoptera | | | | | | | | |
| <i>Isoperla grammatica</i> | | | | | 4 | 4.21 | | |
| Trichoptera | | | | | | | | |
| <i>Hydropsyche instabilis</i> | 1 | 33.33 | | | 1 | 1.05 | 2 | 0.18 |
| <i>Plectrocnemia conspersa</i> | | | | | 1 | 1.05 | | |
| <i>Agapetus</i> sp. | | | | | | | 4 | 0.35 |
| <i>Rhyacophila nubila</i> | | | | | | | 1 | 0.09 |
| Diptera | | | | | | | | |
| Chironomidae | | | | | | | | |
| Chironominae | | | | | 38 | 40.00 | 800 | 70.30 |
| Tanytarsinae | | | | | 9 | 9.47 | 200 | 17.57 |
| Psychodidae | | | | | | | 3 | 0.26 |
| Tipulidae | | | | | | | 60 | 5.27 |
| Coleoptera | | | | | | | | |
| <i>Dytiscus</i> sp. | | | | | | | 1 | 0.09 |
| Number of individuals | 3 | 100 | 8 | 100 | 95 | 100 | 1138 | 100 |
| Number of taxa | 2 | | 5 | | 10 | | 14 | |

The seasonal dynamics of benthic macroinvertebrate abundance were determined based on the total presence of individuals at all sites during each field survey. The abundance of individuals per seasonal aspect and site is shown in Table 6. By comparing the number of individuals found during the four seasons at the five sampling sites of the Bosna River, it is evident that the Banlozi-Jelina location had the highest number of individuals (1244), while the lowest number of individuals (539) was found below the Coastal Channel of Zenica Ironworks.

Table 6. The abundance of macroinvertebrate individuals in the Bosna River in the Zenica area

| Sites | Number of individuals | | | | |
|-------------------------------------|-----------------------|--------|--------|--------|----------------|
| | Autumn | Winter | Spring | Summer | Total by sites |
| Drivuša | 9 | 7 | 96 | 603 | 715 |
| Raspotočje | 22 | 3 | 51 | 497 | 573 |
| Hotel Metalurg | 30 | 6 | 297 | 820 | 1153 |
| Coastal Channel of Zenica Ironworks | 13 | 5 | 235 | 286 | 539 |
| Banlozi-Jelina | 3 | 8 | 95 | 1138 | 1244 |

The percentage seasonal representation of taxa at the sampling sites throughout the entire research period is presented in Table 7.

Table 7. The abundance and percentage seasonal representation of taxa at the sampling sites

| Taxon | Autumn | | Winter | | Spring | | Summer | |
|-------------------------|-------------|------------|-----------|------------|------------|------------|-------------|------------|
| | No/ind. | % | No/ind. | % | No/ind. | % | No/ind. | % |
| GASTROPODA | 2 | 2.60 | 7 | 24.14 | 0 | 0.00 | 3 | 0.09 |
| BIVALVIA | 0 | 0.00 | 1 | 3.45 | 0 | 0.00 | 0 | 0.00 |
| OLIGOCHAETA | 54 | 70.13 | 5 | 17.24 | 27 | 3.49 | 107 | 3.20 |
| HIRUDINEA | 4 | 5.19 | 0 | 0.00 | 4 | 0.52 | 32 | 0.96 |
| CRUSTACEA | 0 | 0.00 | 0 | 0.00 | 9 | 1.16 | 48 | 1.44 |
| Ephemeroptera | 1 | 1.30 | 9 | 31.03 | 176 | 22.74 | 147 | 4.40 |
| Plecoptera | 0 | 0.00 | 0 | 0.00 | 34 | 4.39 | 0 | 0.00 |
| Trichoptera | 6 | 7.79 | 0 | 0.00 | 7 | 0.90 | 68 | 2.03 |
| Diptera | 9 | 11.69 | 7 | 24.14 | 516 | 66.67 | 2938 | 87.86 |
| Megaloptera | 1 | 1.30 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 |
| Odonata | 0 | 0.00 | 0 | 0.00 | 1 | 0.13 | 0 | 0.00 |
| Coleoptera | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 | 1 | 0.02 |
| Total per season | 77 | 100 | 29 | 100 | 774 | 100 | 3344 | 100 |
| Total | 4244 | | | | | | | |

During the study, fluctuations in the abundance and diversity of benthic macroinvertebrates were observed, which depended on the sampling period, as well as in examinations (Trožić-Borovac *et al.*, 2011; Hafner and Trožić-Borovac, 2012; Skenderović *et al.*, 2020). The highest number of individuals was found in the summer aspect, while the lowest was in the autumn and winter aspects. It should be noted that many insect species survive the unfavorable winter period in the egg or very small larval stage, causing their apparent absence from the benthic macroinvertebrate sample. A small number of Chironomidae individuals were found in the autumn and winter periods (7 individuals), while a large number was found in the spring and summer periods (2875 individuals) because, during the summer, most larvae are near the shore, while in winter, they retreat to the river's main channel.

Snails were recorded with the families Ancyliidae, Bithynidae, Planorbidae, Valvatidae, and Viviparidae. A total of 6 Gastropoda species were sampled, with a total of 12 individuals, where 7 individuals were found in the winter aspect. The highest number of individuals (4) was found at the location of the Coastal Canal of Zenica Ironworks. A total of 6 species of Gastropoda were sampled, with a total of 12 individuals, of which 7 individuals were found in the winter aspect. A similar finding is presented by Trožić-Borovac *et al.* (2011) for the lower reaches of the Neretva River. The class of bivalves is represented by the species *Spherium corneum*, found in the winter period at the Raspotočje location (Table 7). It occurs in cleaner waters and, in water quality assessments, has proven to be a relatively weak indicator (Wegl, 1983). Oligochaetes are present at all locations and during all sampling seasons with 193 individuals. In terms of abundance, Lumbriculidae are the most represented with 162 individuals, followed by Tubificidae (22) and Lumbricidae (9). Species from the family Tubificidae indicate heavy pollution and show greater tolerance to reduced oxygen saturation in water. They inhabit flowing waters with muddy sediment (Trožić-Borovac, 2001). Two leech species are present in benthic samples of the Bosna River at the investigated locations and in all sampling periods except January. Six leech species were found in the watercourse of the Bosna River (Vagner, 1997; Vagner and Meštrov, 1998).

The species *Erpobdella octoculata* was found at the Raspotočje location. These leeches are alpha-mesosaprobic indicators, and earlier research emphasized them as a constant species in the Bosna River (Vagner, 1997; Vagner and Meštrov, 1998). The dominant species is *Glossiphonia complanata* with 39 observed individuals. Leeches are known to inhabit locations with a considerable amount of organic waste as their food source (Moog, 1995). Amphipods appear in cleaner waters of higher quality (Moog, 1995). According to the mentioned research, these crustaceans inhabit sandy sediment and clean water with faster and moderate flow. The species *Gammarus* sp. was identified at two locations, Drivuša with 5 individuals and Banlozi with 52 individuals, during the spring and summer periods. During sampling at the Banlozi-Jelina location, near one transect, a small stream flows in without discharge of wastewater, so it is likely that 43 individuals of *Gammarus* sp. were found at a location that is not their original habitat due to accidental drift. Water flowers are represented by members of the families Baethidae, Ephemerellidae, Haptogeniidae, and Leptophlebiidae. There are 6 species with a total of 333 individuals at five locations. They are present in all aspects of the research, with the highest abundance in the spring period with 176 individuals. From the family Baethidae, the species *Baetis rhodani* is present, which has two generations: summer and autumn (Studeman *et al.*, 1992). *Baetis rhodani* has the highest representation with a total of 149 individuals at five locations, and *Baetis muticus* with 73 individuals.

Within this study, one representative of the family Ephemerellidae, *Ephemerella ignita*, was determined and occurs in the autumn and spring aspects. From the family Haptageniidae, two species, *Heptagenia lateralis* and *Heptagenia sulphurea*, are present with 4 individuals. The family Leptophlebiidae is represented by the species *Paraleptophlebia submarginata*. This species is a reliable indicator and prefers clean waters. It was recorded in the spring aspect (1 individual) and summer aspect (58 individuals). Comparing the composition of the fauna of water flowers from benthic samples of the Bosna River with (Trožić-Borovac, 2001), similarities are observed with the composition of recorded species in this study, with a higher proportion of water flower species in the period 1999-2000 due to more frequent sampling and a greater number of locations. Golden Stonefly were identified with the species *Isoperla grammatica* at all five locations in the spring aspect of the study, with a total of 34 individuals, most represented by 12 individuals at the Drivuša location. Of Alderflies, one species, *Sialis lutaria*, was found individually at the location of the Coastal Channel of Zenica Ironworks. This species is a predator, a weak indicator of water quality, and occurs in polluted waters. Among beetles, one species, *Dytiscus* sp., was found individually in the summer period at the Banlozi location. Water beetles are represented by 81 individuals with 6 species. From the family Hydropsychidae, the species *Hydropsyche instabilis* is present with 20 individuals at five locations. According to their feeding habits, they belong to the filter feeder group (Moog, 1995). From the family Glossosomatidae, the species *Glossosoma* sp. is present with 47 individuals in the summer period at the location of the Coastal Channel of Zenica Ironworks. From the family Goeridae (predators), the species *Goera pilosa* was found with 5 individuals in the summer period at the Raspotočje location. This species is a reliable indicator, but it has a broad valence for ecological factors, so it occurs even in polluted waters. From the family Rhyacophylidae, the species *Rhyacophila nubila* is present with 2 individuals. Although a good indicator of clean water, it occurs in polluted waters with a smaller number of individuals (Wegl, 1983).

Diversity indices

During the research, variations in the results of the Shannon-Weaver Diversity Index were observed, with lower values in the winter and summer aspects, while higher values were noted in the autumn and spring aspects (Table 8). In January, low index values were recorded due to the presence of snail representatives, while higher values were contributed by the presence of *Haptegenia lateralis*. In spring, the index reaches its maximum value (2.26) due to the presence of a large number of insect larval stages, and in the summer period, there is a subsequent decline from 0.2 to 2.08 due to the dominance of Chironominae and Oligochaeta.

Table 8. Shannon-Weaver Diversity Index (H') of macroinvertebrates in the Bosna River in the Zenica area

| Season | SITES | | | | |
|--------------|-------|-------|-------|-------|-------|
| | L1 | L2 | L3 | L4 | L5 |
| Autumn | 1.39 | 2.11 | 1.48 | 2.08 | 0.92 |
| Winter | 1.15 | 0.92 | 0.92 | 0.97 | 1.91 |
| Spring | 1.86 | 1.89 | 1.26 | 1.52 | 2.26 |
| Summer | 0.627 | 0.267 | 0.794 | 2.075 | 0.809 |
| Medium value | 1.26 | 1.30 | 1.11 | 1.66 | 1.47 |
| Min. value | 0.63 | 0.27 | 0.79 | 0.97 | 0.81 |
| Max. value | 1.86 | 2.11 | 1.48 | 2.08 | 2.26 |

Based on the average values of this index, the Bosna River in the area of the City of Zenica belongs to the III water category – moderately or moderately polluted. In the implementation of the scientific research project (Škrijelj *et al.*, 2007), the analysis of the benthic macroinvertebrate composition was conducted at 19 locations in the Bosna River basin. The upper locations of the Bosna River, such as Rimski Most downstream from the mouth of Zujevina, were investigated, where the II water category was determined (Shannon-Weaver index ranging from 2.11 to 2.06 - slightly polluted water). In the middle course of the Bosna River upstream from Zenica (in the area of the Raspotočje settlement, IV water category), and downstream near the Banlozi-Jelina settlement (III water category), the index was determined in the range of 0.86 to 1.32. At the location upstream from Maglaj, the IV water category was determined ($H=0.99$ - waste or heavily polluted water). The upper locations of the Bosna River show water with lower pollution and a higher number of registered taxa, while downstream, due to increased pollution and the creation of unfavorable conditions for the life of macroinvertebrates, the water quality is significantly worse with a lower number of taxa.

Considering the results of the Simpson Diversity Index, the highest value is present in the winter period (0.82) at the Banlozi site due to the least difference between communities. The lowest value indicates the greatest diversity between communities and is achieved in the summer period (0.07) at the Raspotočje site (Figure 1).

The highest mean evenness value (0.76) is recorded at the Coastal Channel of Zenica Ironworks because the abundance of individuals at this site is the most similar. Also, at this site, the highest index value (0.97) is observed in the winter aspect with the participation of two species with an equal number of individuals (Figure 1). By analyzing the evenness of species between communities at the investigated sites, a similarity in benthic fauna is noted

between the first (0.64) and second (0.67) sites, as well as between the fourth (0.76) and fifth (0.70) sites.

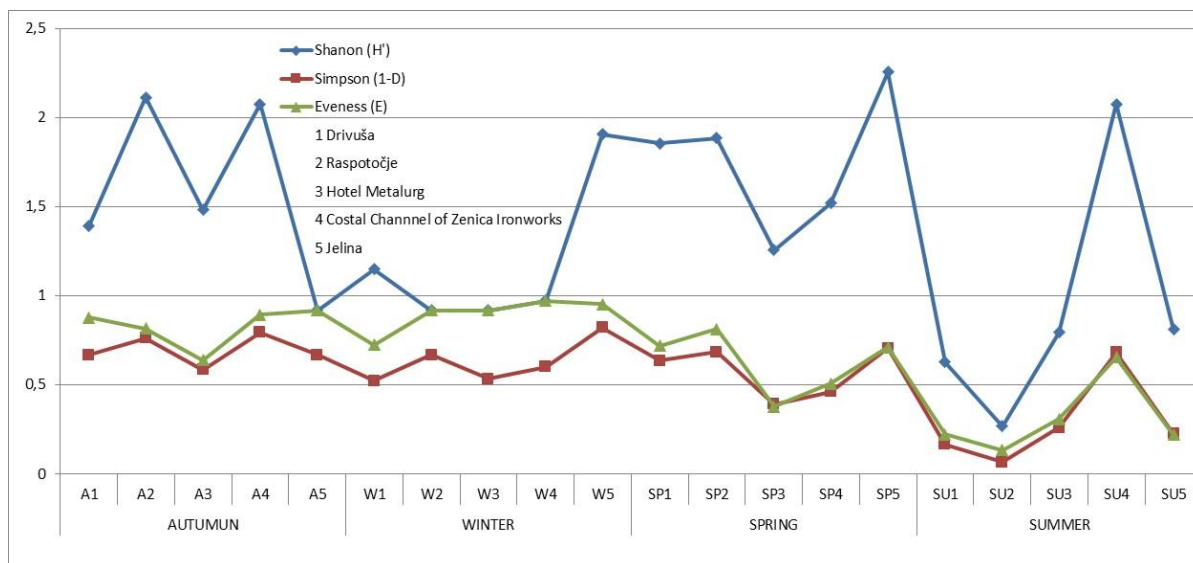


Figure 1. Diversity indices by locations and seasons

The Simpson Diversity Index and evenness of macroinvertebrates show similar values, with identical values achieved at the Banlozi site (0.71 and 0.22) in the spring and summer periods. The Shannon-Weaver Diversity Index shows congruence with evenness (0.92, 0.92, and 0.97) in the winter period at the Raspotočje, Hotel Metalurg, and Coastal Channel of Zenica Ironworks sites, while in other cases, it does not exhibit commonalities.

By applying cluster analysis to the macroinvertebrate communities of the Bosna River, three groups are observed based on the degree of similarity (Figure 2). The first group, showing similarity in the composition of benthic macroinvertebrates, consists of spring and summer samples from the investigated locations, while the second group is formed by autumn and winter samples from the locations. The communities of benthic macroinvertebrates in the winter aspect at the Raspotočje site and the autumn aspect at the Banlozi site exhibit the greatest distance (least similarity) from the other samples. The locations are grouped based on spatial proximity in terms of similar abiotic conditions and the composition of benthic macroinvertebrates.

Bray-Curtis Cluster Analysis (Single Link)

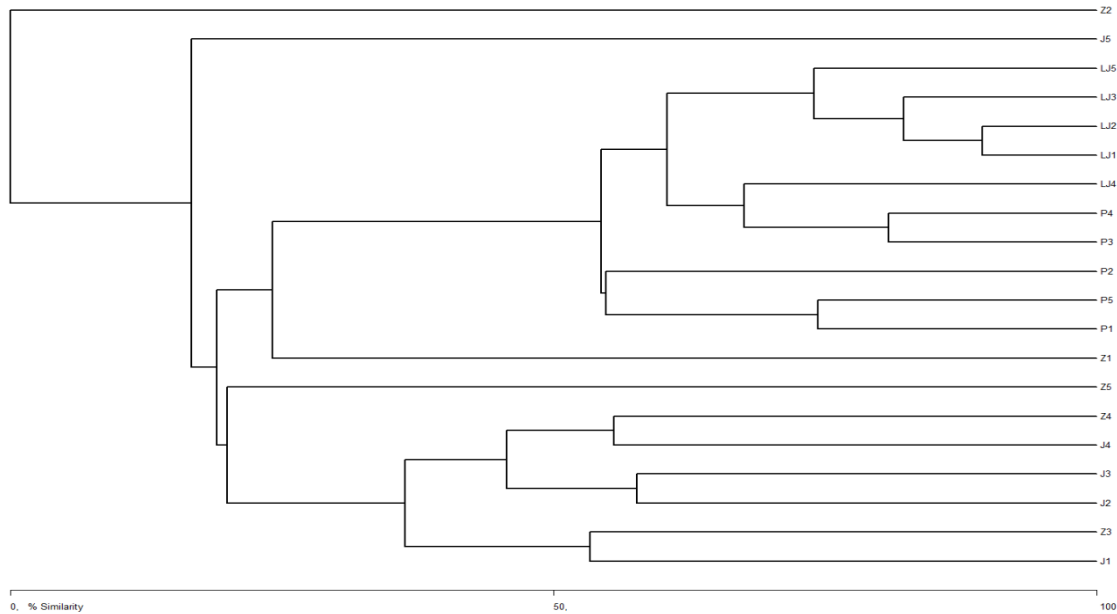


Figure 2. Display of cluster analysis of benthic macroinvertebrate samples of the Bosna River (Z – Winter, P – Spring, LJ – Summer, J – Autumn)

CONCLUSION

At five measurement points along the course of the Bosna River in the area of the City of Zenica during the period from October 2014 to May 2015, seasonal successions of the qualitative-quantitative composition of benthic macroinvertebrate communities were analyzed. The Banlozi-Jelina site had the highest number of taxa (14) and the highest number of individuals (1138). Based on the average values of the Shannon-Weaver index, the Bosna River in the Zenica area belongs to the III category of water – moderately or moderately polluted.

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SEZONSKE SUKESIJE ZAJEDNICA BENTOSKIH MAKROBESKIČMENJAKA U RIJECI BOSNI NA PODRUČJU GRADA ZENICE

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Sažetak

Ovim istraživanjem opisane su sezonske sukcesije kvalitativno-kvantitativnog sastava zajednica bentoskih makroinvertebrata u rijeci Bosni na području grada Zenice. Sakupljanje bentoskih makroinvertebrata obavljeno je od oktobra 2014. do maja 2015. godine na 5 mjernih

lokacija duž toka rijeke Bosne u gradu Zenici. Za analizu podataka korišteni su indeksi biološke raznovrsnosti (Shannon–Weaverov indeks, Simpsonov indeks), indikator ujednačenosti vrsta i Bray–Curtisova klaster analiza. Istraživanjem bentoskih makrobeskičmenjaka identificirana su 34 taksona i 4224 jedinke, s dominacijom preimaginalnih stadijuma vodenih insekata s 21 taksonom. Najveći uočeni Shannon–Weaverov indeks raznovrsnosti iznosio je 2,26 u proljetnom aspektu na lokalitetu Balnozi, a najniži 0,27 u ljetnom aspektu na lokalitetu Raspotočje. Korištenjem Vrijednosti Simpsonovog indeksa kretale su se od 0,07 u ljetnom periodu na lokaciji Raspotočje do 0,82 u zimskom periodu na lokaciji Balnozi. Analiza ujednačenosti vrsta među zajednicama na istraživanim lokacijama pokazala je sličnost bentoske faune između prvog (0,64) i drugog (0,67) lokaliteta, kao i između četvrtog (0,76) i petog (0,70) lokaliteta. Prema rezultatima klaster analize, istraživana mjesta imaju slične zajednice makrobeskičmenjaka klasifikovane po sezonama.

Ključne riječi: bentoski makrobeskičmenjaci, rijeka Bosna, biološki indeksi

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