BIODIVERSITY ASSESSMENT OF AQUATIC MACROINVERTEBRATES

FOR THE NEW ALIGNMENT OF THE MATESEVO-ANDRIJEVICA SECTION

SUB-SECTION: TRESNJEVIK TUNNEL – ANDRIJEVICA

October-November 2024

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I INTRODUCTION

The EBRD Bank has engaged PASECO S.P. Ltd Greece (hereinafter referred to as the "Leading Consultant") to provide consultancy services and implement the project "Montenegro: Construction of the Bar-Boljare Highway – Environmental and Social Assessment" (hereinafter referred to as the "Project"), in accordance with EBRD Performance Requirements. As part of the Project, a biodiversity assessment was conducted in 2019–2021 for the Matesevo-Andrijevica section. Biodiversity assessment for the purposes of the Project covered the following aspects:

- Habitats and flora
- Bats and other mammals
- Ichthyofauna and benthic fauna
- Amphibians and reptiles
- Ornithofauna

Due to recent changes in the preliminary design for an approximately 12 km stretch of the route, from the entrance of the Tresnjevik tunnel to Andrijevica, it became necessary to conduct a complementary biodiversity assessment for this new alignment, Tresnjevik-Andrijevica (hereinafter referred to as the "Sub-Project"). To carry out the Sub-Project, the Leading consultant engaged E3 Consulting Ltd. Montenegro (hereinafter referred to as the "Local consultant") to recruit a local team of biologists and provide key findings. The Sub-Project was conducted by a multidisciplinary team of national experts during October–November 2024, including both fieldwork and reporting in accordance with the methodology provided by the Leading consultant. The Sub-Project covered following biodiversity aspects:

- Flora and Habitats
- Bats
- Mammals
- Ornithofauna
- Icthyiofauna and benthic fauna
- Terrestrial invertebrates
- Aquatic macroinvertebrates
- Amphibians and reptiles

The Sub-Project involved the following tasks:

1. Rapid review of the surveys conducted in the framework of conceptual/preliminary design (for the old alignment) for the Sub-Section (done in 2019-2021). These surveys covered:
   a. Habitats and flora
   b. Bats and other mammals
   c. Ichtyiofauna and bentic fauna
   d. Amphibians and reptiles
   e. Ornithofauna

Local consultant will assess the zones that were researched during the surveys 2019-2021 (e.g. flora and vegetation (habitats) surveys included 500m to the left and right of the planned Project) with the new alignment. The aim is to express the expert opinion on the degree on which the previous surveys cover the new alignment and determine the extent of the new campaign.

2. For the biodiversity features (among (a) to (e) above), for which it is estimated that tne new alignment is not covered by the previous surveys, Local consultant will conduct an additional survey, as follows:
   i. The survey will be conducted until the end of October 2024
   ii. Five on-site days are foreseen
   iii. The survey will follow the same methodology as the 2019-2021 research

3. Drafting of report with the findings. The report will have the main structure as per previous surveys and will be detailed enough to comply with EBRD ESP requirements. The report will include:
   i. Bibliographical data on biodiversity in the zone of the new alignment
   ii. Period of surveys and Methodology used
   iii. Findings of surveys
   iv. Assessment of protection status of habitats/species according to EU legislation, international agreements and national legislation
   v. Significant impact of the highway construction on habitats and species and proposal of relevant mitigation measures
   vi. Maps and photographical documentation

The Sub-Project resulted in the submission of eight separate reports, accompanied by supporting maps and photographic documentation, detailing key biodiversity aspects related to the Sub-Project, with a focus on autumnal research findings.

II BIBLIOGRAPHICAL DATA ON BIODIVERSITY IN THE ZONE OF THE NEW ALIGNMENT

Macrozoobenthos is frequently used as a qualitative biological indicator for assessing freshwater ecosystem conditions and evaluating anthropogenic impacts on water. In addition to habitat-specific factors, broader environmental influences, such as riparian vegetation, land use, and shading, play a significant role in shaping these communities. The life cycles of macrozoobenthos organisms are adapted to the habitats they occupy, and substrate composition and structure significantly influence species diversity, abundance, and distribution in various microhabitats. Studying macrozoobenthos communities is crucial for understanding the impact of hydromorphological changes on aquatic ecosystems.

Macrozoobenthos refers to all bottom-dwelling aquatic invertebrates that cannot pass through a mesh with openings approximately 100 to 500 µm in diameter. This group includes a diverse range of organisms found across various aquatic habitats. Changes in the quality of these habitats affect macrozoobenthos differently, as they exhibit varying levels of tolerance to shifts in hydromorphological elements, physical and chemical parameters, substrate composition and structure, and the presence of algae and aquatic macrophytes. The presence or absence of particular macrozoobenthos species can indicate the health of a water body. By analyzing the composition and structure of macrozoobenthos communities, it is possible to assess environmental pressures affecting these organisms.

The macroinvertebrate fauna of the studied area remains poorly understood (Malicky 1981, Oláh 2010, 2017), and baseline species inventory work is needed. Two species of the caddisfly genus Drusus (D. siveci and D. krusniki), which inhabit spring habitats and the first order of mountain streams, were originally described from the Gnjili Potok stream (Oláh 2017).

For the purposes of the Sub-Project a biodiversity survey was conducted in October 2024 to assess potential impacts on the macrozoobenthos communities in the Kraštica River and Gnjili Potok stream areas.

This report evaluates whether future highway construction poses a threat to the benthic community, including the status, survival, or conservation of specific protected or endangered species. Samples were collected once from five sampling sites within the investigated water bodies.

III SURVEY PERIOD AND METHODOLOGY

3.1. Benthic Invertebrate Sampling, Sorting, and Identification

Quantitative sampling of macroinvertebrate fauna was conducted at four selected localities within the planned highway's zone of influence (Figure 1) using Surber's net, following methods described in Pešić and Tomović (2009). For additional qualitative analysis, a hand net was employed. Collected samples were preserved in 95% alcohol and subsequently transported to the laboratory, where benthic macroinvertebrates were identified and counted from quantitative samples under a Stemi 2000-C stereomicroscope. The specimens are stored in the collection of the Department of Biology, University of Montenegro.

To assess water quality, the EPT Index (%EPT) was applied. This index, commonly used to evaluate organic pollution levels (Resh & Jackson, 1993), is based on the relative abundance of Ephemeroptera, Plecoptera, and Trichoptera (EPT) species, which are generally sensitive to organic pollutants and, thus, reliable water quality indicators. The EPT Richness Index provides an estimation of water quality: values above 50% indicate good quality, values between 50% and 25% indicate moderate quality, and values below 25% indicate poor quality. Additionally, the percentage of Chironomidae (% Chironomidae) was calculated, as this group is less sensitive to environmental changes (Plafkin et al., 1989). Samples with over 50% Chironomidae are typically indicative of eutrophic conditions.

3.2. Research area

Montenegro's landscape is predominantly hilly and mountainous, featuring complex geomorphologic characteristics and altitudes ranging from sea level to 2,500 meters above sea level. In terms of water resources per square meter, Montenegro ranks among the highest in Europe and globally. The Piva and Tara rivers alone contribute nearly 40% of the Drina River's total water volume, despite accounting for only 20% of its basin area. Including contributions from the Lim and Ćehotina rivers, which also originate in Montenegro, these water bodies collectively make up approximately 63% of the Drina's flow at its confluence with the Sava River.

Montenegro serves primarily as an upstream region; over 95% of its surface and groundwater flow originates within its borders, while only 5% consists of transitory waters. The Montenegrin hydrological system is divided between the Black Sea basin (7,260 km^2 or 52.5%) and the Adriatic Sea basin (6,267 km^2 or 47.5%). Key Montenegrin rivers flowing into the Black Sea basin include the Piva, Tara, Lim, Ćehotina, and Ibar, while those flowing into the Adriatic Sea basin are the Morača, Zeta, and Bojana. Significant portions of these waters discharge into the Adriatic or Black Sea through neighboring countries, as is the case with the Piva, Tara, Lim, Ćehotina, Ibar, and Bileća Lake, which is partially located in Montenegro (Radunović, 2008).

The Lim River, the largest tributary of the Drina River, has its upper basin in Montenegro, with its middle and lower basin extending into Serbia and Bosnia and Herzegovina. Covering a catchment area of 5,785 km^2 (of which 115 km^2 lies in Albania), the Lim's main tributaries include the Zlorečica, Šekularska, Ljubovića, Lješnica, Bjelopoljska Bistrica, Mileševka, and Beranska Bistrica.

As part of this study, field research focused on the tributaries Kraštica and Gnjili Stream to assess potential environmental impacts.

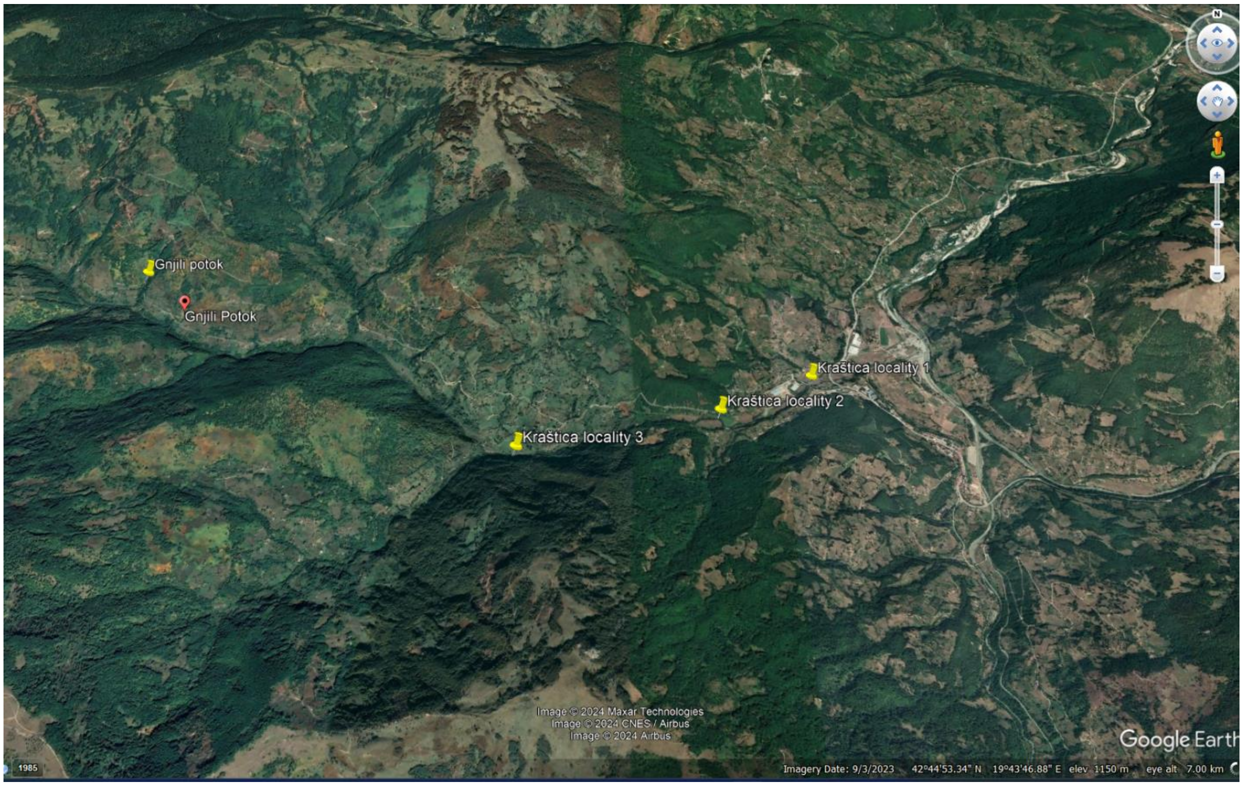

Figure 1. Investigated sampling sites in Kraštica (L1, L2, L3) and Gnjili Potok (L4)

Table 1. Characteristics of investigated sampling sites (rst—rocks and stones; st—stones, gp—gravel, pebble; s—sand; m—mud; d—detritus).

Sampling site	Coordinates	Substrate type (sand, stone, etc)	Water flow speed (medium, high, low)	Socio – economic activity
L1- Kraštica	42°44'20.75"N 19°46'50.89"E	st – 40%, gp – 30%, s – 20%, m – 10%	lotic ecosystem, medium	Urban area
L2 - Kraštica	42°44'8.81"N 19°46'19.03"E	rst–15%, st – 35%, gp – 15%, s – 20%, m – 10%, d– 5%	lotic ecosystem, medium	Rural area
L3 - Kraštica	42°43'56.26"N 19°45'12.75"E	rst – 10%, st – 20%, gp – 30%, s–30%, m–10%	lotic ecosystem, high	Rural area
L4 – Gnjili Potok		rst–5%, st – 25%, gp – 20%, s – 50%	lotic ecosystem, medium to high	Rural area

3.3 Data analysis methods

1) EPT Index (Plafkin et al., 1989) - The Ephemeroptera, Plecoptera, and Trichoptera index (EPT) displays the taxa richness within the insect groups which are considered to be sensitive to pollution, and therefore should increase with increasing water quality. The EPT index is calculated as the % of the individuals in the sample which belong to the aquatic insect orders Ephemeroptera, Plecoptera and Trichoptera.

% EPT Abundance = Total No. of EPT individuals/Total No. of individuals in the entire sample

Table 2. Reference value for EPT Index

EPT index	
Index value	**Water Quality**
> 50%	good
25 – 50%	moderate
< 25%	poor

2) Ratio of Ephemeroptera, Plecoptera, and Trichoptera and Chironomidae (EPT/C) The abundance of EPT and Chironomidae is indicator of balance of the benthic community, since EPT are considered to be more sensitive and Chironomidae less sensitive to environmental stress (Plafkin *et al*., 1989). An even distribution among these four groups indicate community which is in good biotic condition, while high numbers of Chironomidae in community may indicate environmental stress (Plafkin *et al*., 1989). The EPT/C index is calculated by dividing the sum of the total number of Ephemeroptera, Plecoptera, and Trichoptera individuals by the total number of Chironomidae individuals.

3) Family Biotic Index (FBI) (Hilsenhoff, 1982)

$$FBI = \Sigma (x_i \times t_i) / n$$

x_i – number of individuals within a taxon

t_i – tolerance value of a taxon

n – total number of organisms in the sample

FBI is usually used for estimation of organic pollutants in water but may be applicable for toxic pollutants as well.

Table 3. Reference value for Family Biotic Index

Family Biotic Index		
Index value	**Water Quality**	**Degree of Organic Pollution**
0.00–3.50	Excellent	No apparent organic pollution
3.51–4.50	Very good	Possible slight organic pollution
4.51–5.50	Good	Some organic pollution likely
5.51–6.50	Fair	Fairly substantial pollution likely
6.51–7.50	Fairly poor	Substantial pollution likely
7.51–8.50	Poor	Very substantial pollution likely
8.51–10.0	Very poor	Severe organic pollution likely

4) Biological Monitoring Working Party (BMWP) (Friedrich et al., 1996)

The Biological Monitoring Working Party score (BMWP) provides single values, at the family level, representative of the organisms' tolerance to pollution; the greater their tolerance towards pollution, the lower the BMWP scores. BMWP was calculated by adding the individual scores of all families, and subclass Oligochaeta represented within the community. The BMWP index is calculated by summation of the tolerant values of each taxa presented in the sample. The tolerance values were for each taxa were taken from SNIFFER WFD72A: Revision and testing of BMWP scores.

Table 4. Reference value for BMWP index

BMWP index	
Index value	**Water quality**
> 151	very clean
100–150	clean
51–99	moderate
16–50	polluted
0–15	very polluted

5) Average Score per Taxon (ASPT) (Armitage et al., 1983), (Friedrich et al., 1996)

The Average Score per Taxon (ASPT) represents the average tolerance score of all taxa within the community and was calculated by dividing the BMWP by the number of families represented in the sample.

ASPT=BMWP score/number of families

Table 5. Reference value for ASPT index

ASPT index	
Index value	**Biological water quality**
> 5.41	excellent
4.81–5.40	very good
4.21–4.80	good
3.61–4.20	medium
3.01–3.60	poor
< 3	very poor

IV KEY RESEARCH FINDINGS

Qualitative and quantitative analysis of benthic community

Sampling Site L1 – Kraštica stream

The macroinvertebrate community abundance in the Kraštica stream at locality 1 in October 2024 was 803 individuals per square meter (Table 6). The generally lower abundance compared to other watercourses in Northern Montenegro (see Pešić et al. 2020 for an overview) is likely due to the sampling period (late October 2024) and the higher water level of the stream, which directly impacts macroinvertebrate abundance.

At the investigated site, Trichoptera were the dominant group, with an abundance of 231 individuals per square meter, followed by Plecoptera with 220 individuals per square meter. Additionally, Ephemeroptera (99 individuals per square meter) and Hydrachnidia (69 individuals per square meter) were also present in significant numbers.

Table 6. Qualitative-quantitative composition of macroinvertebrate community at the locality Kraštica stream – L1 (October 2024)

Taxon	ind/m²
Lebertia sp.	22
Torrenticola	33
Woolastookia	11
Atractides	33
Oligochaeta	11
Ephemeroptera (Heptagenidae)	55
Ephemeroptera (Lepthobdeliidae)	44
Plecoptera (Perloididae)	121
Plecoptera (Leuctridae)	99
Hydraenidae	55
Elmidae	11
Coleoptera larvae	22
Trichoptera (Limnephilidae)	209
Trichoptera (Micrasema sp.)	22
Chironomidae	55
TOTAL:	803
EPT %	**68.5%**
%Chironomidae	**6.8%**

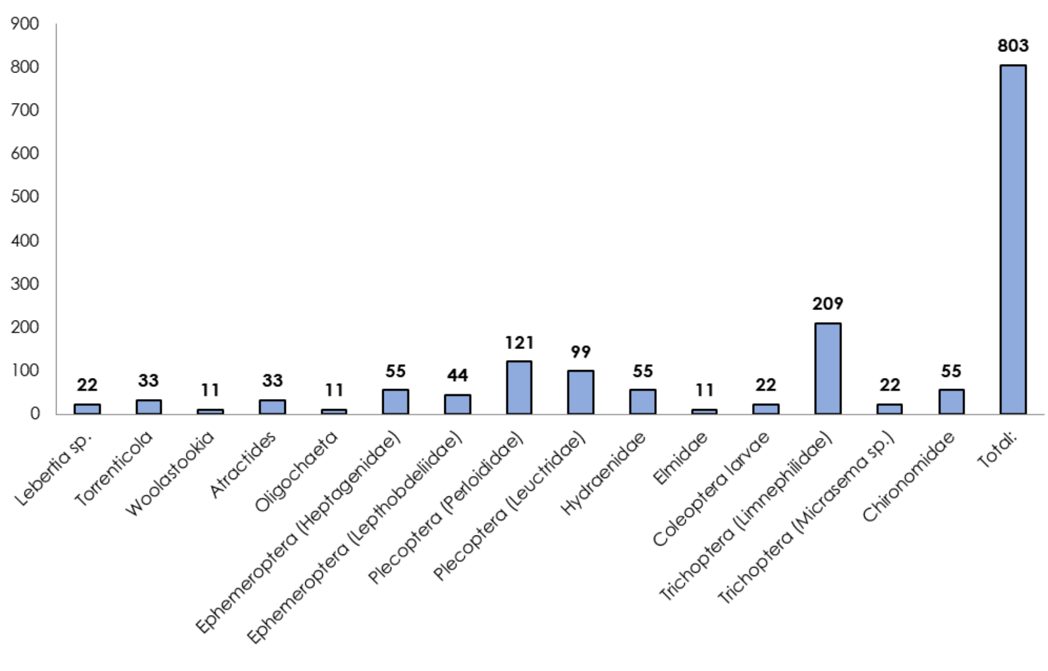

Figure 2. Quantitative composition of macroinvertebrate community at the locality Kraštica stream (ind/m²)– L1 (October 2024)

The high value of EPT index score (68.5%) are indicative for good water quality of Kraštica river at the sampling site 1.

Sampling Site L2 – Kraštica stream

The abundance of the macroinvertebrate community in the Kraštica River at locality 2 in October 2024 was 704 individuals per square meter (Table 7). Similar to the previous sampling site, the generally lower abundance compared to other watercourses in Northern Montenegro (see Pešić et al. 2020 for an overview) is attributed to the sampling period (late October 2024) and the higher water level of the stream, both of which directly impact macroinvertebrate abundance.

Trichoptera were the dominant group, with an abundance of 330 individuals per square meter, followed by Plecoptera, which were also numerous at 121 individuals per square meter, and Hydrachnidia with 99 individuals per square meter.

Table 7. Qualitative-quantitative composition of macroinvertebrates at the locality Kraštica stream – L2 (October 2024)

Taxon	ind/m²
Lebertia sp.	22
Torrenticola	33
Woolastookia	11
Atractides	33
Ephemeroptera (Heptagenidae)	22
Ephemeroptera (Leptophlebiidae)	22
Plecoptera (Perloididae)	55
Plecoptera (Leuctridae)	66
Hydraenidae	22
Elmidae	55
Coleoptera larvae	11
Trichoptera (Hydropsichidae)	22
Trichoptera (Limnephilidae)	308
Chironomidae	22
TOTAL:	704
EPT %	**70.3%**
%Chironomidae	**3.1%**

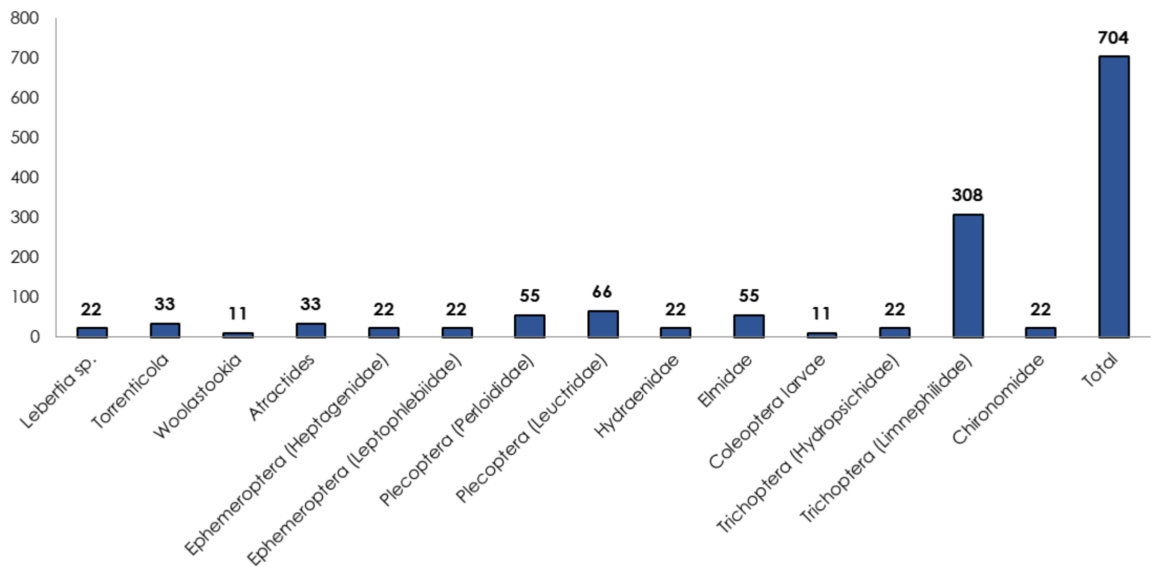

Figure 3. Quantitative composition of macroinvertebrates at the locality Kraštica stream (ind/m²)– L2 (October 2024)

The high value of EPT index score (70.3%) are indicator of good water quality of Kraštica river at the sampling site 2.

Sampling Site L3 – Kraštica stream

The abundance of macroinvertebrate assemblage of the Kraštica stream at the locality 3 in October 2024 was 1232 ind/m2 (Table 8).

Representatives of Trichoptera dominated quantitatively with an abundance of 671 ind/m^2. Representatives of Plecoptera were also numerous, with an abundance of 187 ind/m^2, as well as representatives of Hydrachnidia (110 ind/m^2).

Table 8. Qualitative-quantitative composition of macroinvertebrate at the locality at the locality Kraštica stream – L3 (October 2024)

Taxon	ind/m²
Lebertia sp.	44
Torrenticola	22
Atractides	44
Radix sp.	11
Ephemeroptera (Heptagenidae)	44
Ephemeroptera (Baetidae)	44
Ephemeroptera (Leptophlebiidae)	22
Plecoptera (Perloididae)	44
Plecoptera (Leuctridae)	143
Hydraenidae	11
Trichoptera (Hydropsichidae)	143
Trichoptera (Limnephilidae)	418
Trichoptera (Micrasema sp.)	66
Trichoptera (Rhyacophilidae)	44
Limonidae	11
Atherix sp.	77
Stratiomidae	11
Chironomidae	33
U k u p n o:	1232
EPT %	**78.6%**
%Chironomidae	**2.7%**

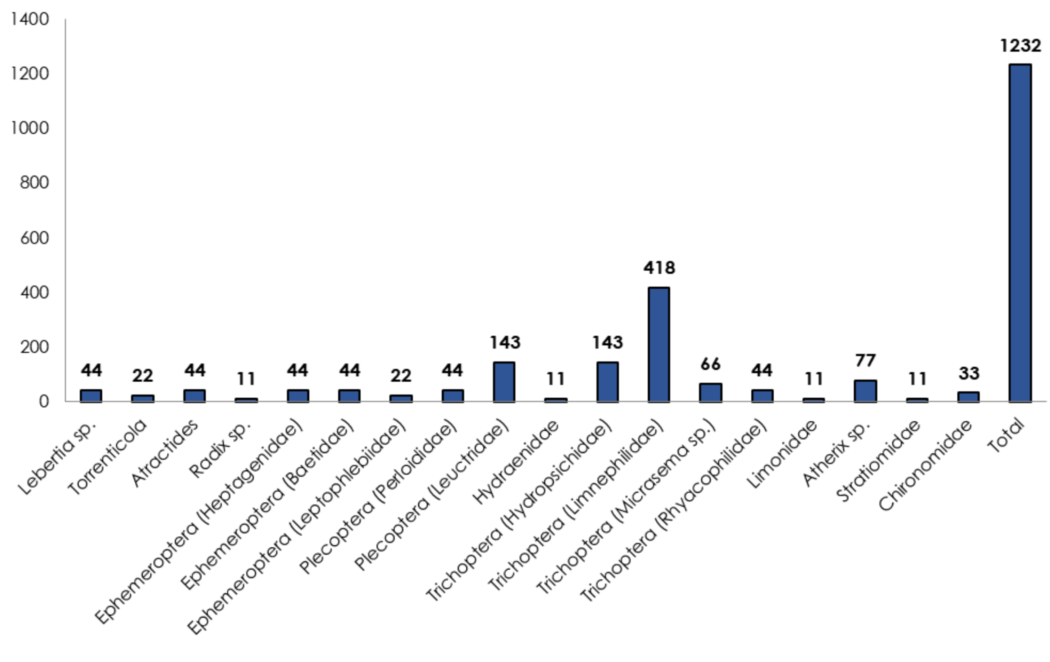

Figure 4. Quantitative composition of macroinvertebrates at the locality Kraštica stream (ind/m²) – L3 (October 2024)

The high value of EPT index score (78.6%) is indicator for good water quality of Kraštica river at the sampling site 3.

Sampling Site L4 – Gnjili Potok

Due to the characteristics of the sampling site, a quantitative analysis using a Surber net was not feasible, so sampling was conducted with a hand net.

At the investigated locality, representatives of prong-gilled mayflies (Leptophlebiidae) dominated. Drusinae larvae, likely of the species *Drusus siveci*, were also present in the sample. *Drusus siveci* Malicky, 1981, a rare Balkan endemic, was originally described from a single specimen collected from Gnjili Potok in 1981 (Malicky, 1981. This species is critically endangered and inhabits spring areas and headwaters of mountain streams, with Gnjili Potok serving as its locus typicus.

Additionally, larvae of *Siphonoperla torrentium* were found. This species of stonefly, belonging to the family Chloroperlidae, is commonly found in small streams. Another rare species which can be found in Gnjili Potok is *Drusus krusniki* Malicky, 1981, endemic to the Western Balkans, inhabiting the southeastern parts of the Dinaric Alps in Montenegro and Kosovo and the Prokletje Mts. in Albania (Oláh 2010).

Table 9. Qualitative composition of macroinvertebrate at the locality Gnjili Potok -L4 (October 2024)

Taxon	ind
Oligocheata (Lumbricidae)	1
Oligocheata aq.	1
Ephemeroptera (Leptophlebiidae)	13
Plecoptera, *Siphonoperla* sp.	2
Plecoptera (Leuctridae)	1
Trichoptera (Limnephilidae), Drusus sp. probably *D. siveci*	4
Trichoptera (Rhyacophilidae, Rhyacophila sp. probably *Rhyacophila akutila*)	3
EPT %	**88%**
%Chironomidae	**0%**

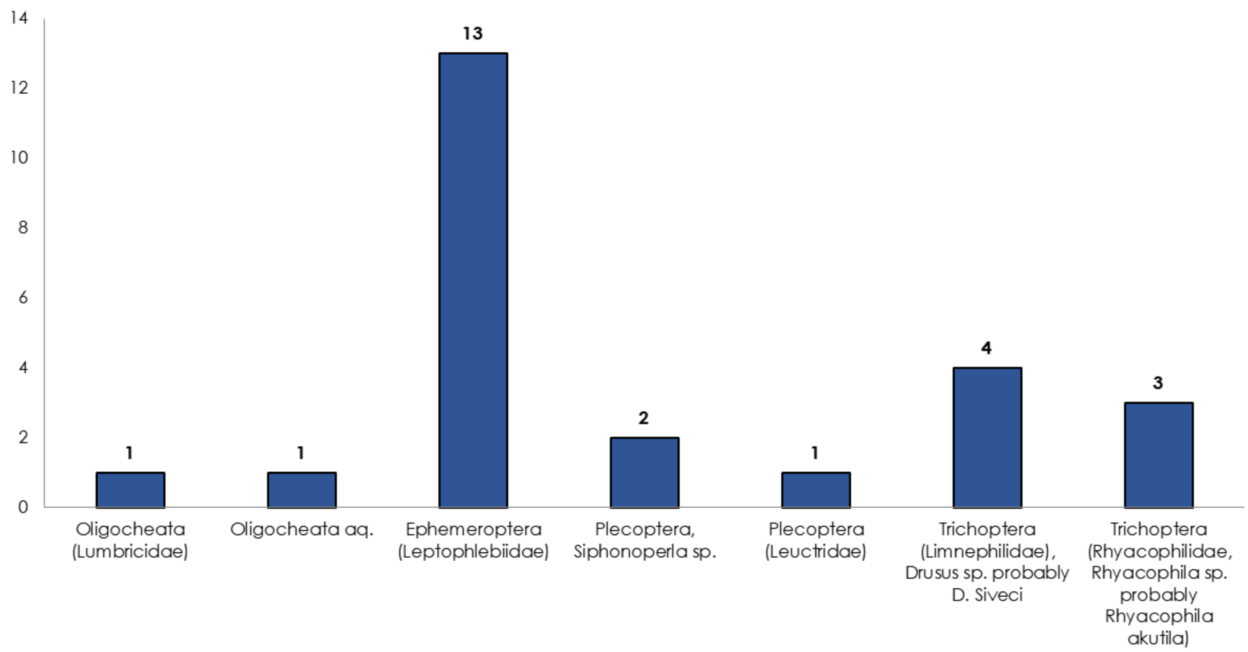

Figure 5. Quantitative composition of macroinvertebrates at the locality Gnjili Potok -L4 (ind) (October 2024)

Due to the characteristics of the sampling site, it was not possible to do a quantitative analysis by Surber net, so the sampling was conducted using a hand net.

Table 10. Proportions of Ephemeroptera, Plecoptera and Trichoptera (%EPT), Chironomidae (%C), and ratio of Ephemeroptera, Plecoptera and Trichoptera and Chironomidae (EPT/C) at the sampling sites

	Kraštica L1	**Kraštica L2**	**Kraštica L3**	**Gnjili Potok L4**
% EPT	68.5	70.3	78.6	88
% Chironomidae	6.8	3.1	2.7	0
Ratio EPT/Chironomidae	10.07	22.6	49,74	-

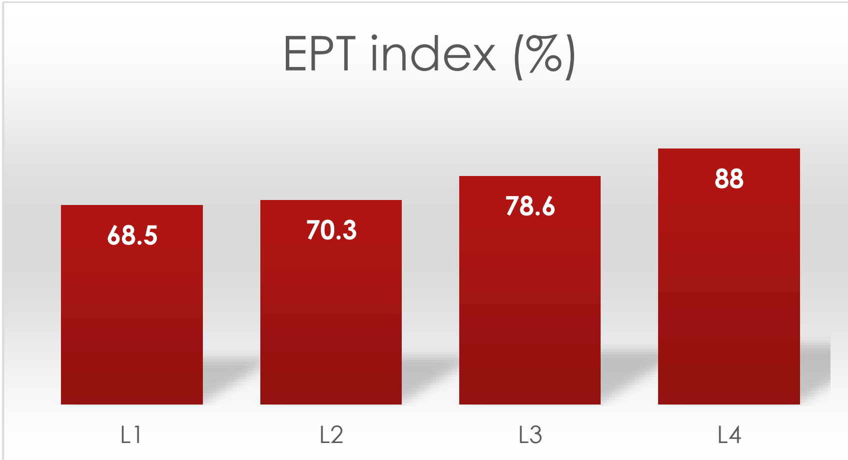

Figure 6. Values of EPT index (%) at investigated sampling sites

The EPT index with value of 68,5-88,00 indicated very good condition of benthic community at this sampling sites. The low number of Chironomidae in sample (0-6,8%), as well as EPT/C ratio (10,07-49,74) indicates benthic community which is in good biotic condition.

Table 11. Values of FBI, ASPT and BMWP Indices at investigated sampling sites

	L1	L2	L3	L4
FBI index	3,2	3,8	4,1	5,1
BMWP	105,00	126,00	130,00	152,00
ASPT	5,21	5,27	5,33	5,5

Values of FBI index (from 3,2 -5,1) indicate very good water quality with possible slight organic pollution, at L1 sampling site. The values of ASPT index (5,21-5,5) indicated excellent water quality, what is confirmed by BMWP (105-152) whose value indicates very clean water (Table 11).

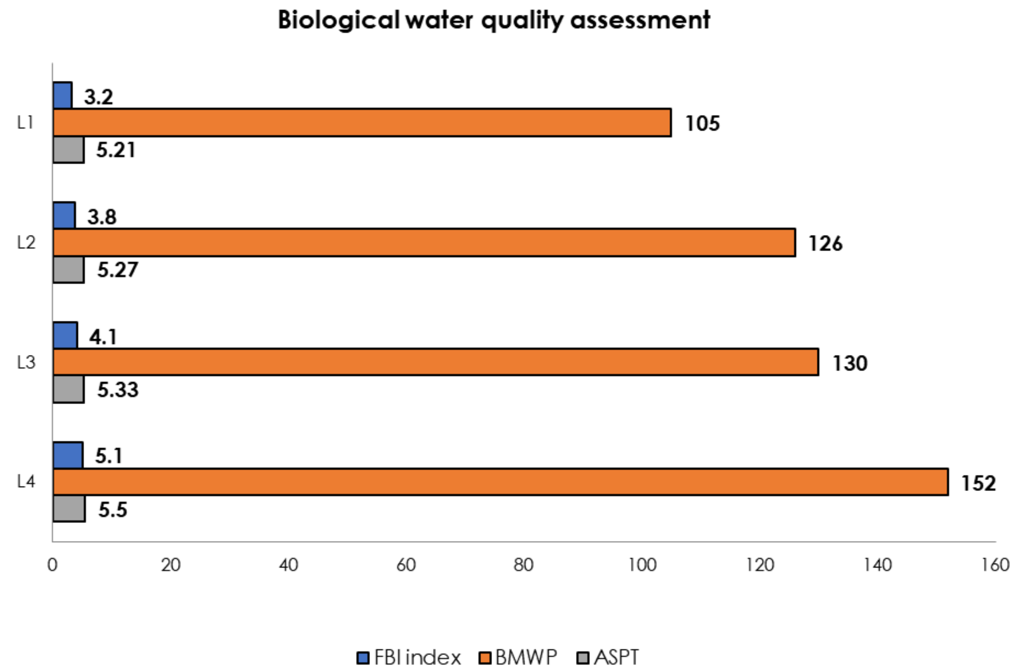

Figure 7. Values of FBI, BMWP and ASPT indices at sampling sites

V ASSESSMENT OF PROTECTION STATUS OF SPECIES

Larvae of *Drusus siveci*, were found in the sample from Gnjili Potok. Drusus siveci Malicky, 1981 is a rare Balkan endemic species described from a single specimen collected from Gnjili Stream in 1981. Another rare species orginally described from the same lovality is Drusus krusniki. The both speciea are inhabitants of spring areas and headwaters of mountain streams. These two endemic species are critically endangered and Gnjili Stream represents its locus typicus. The protection of the Gnjili Potok stream, which represents the locus typicus for two species of the genus Drusus, requires special care, and in this sense, continuous monitoring during the entire construction process is highly recommended. Siphonoperla torrentium larvae were also found in the material. This species of stoneflies belongs to the family Chloroperlidae, and can be found in small streams.

VI IMPACTS OF THE HIGHWAY CONSTRUCTION ON HABITATS AND SPECIES AND MITIGATION MEASURES

There might be significant impact on the fauna when the highway route is constructed in close proximity to the riverbed, particularly when direct construction activities are carried out on the riverbanks, such as stabilizing them to prevent collapse or reinforcing them for highway stability. These construction activities and excavations alter the natural habitat and appearance of the riverbanks, and may even change the morphology of the riverbed. Consequently, these actions might directly lead to habitat degradation and the potential loss of habitats for river organisms. The impact on both aquatic habitats and the ecotone (the transitional zone between land and water) affects the entire river ecosystem, and for invertebrates, it results in a reduction in biodiversity. In addition to these direct habitat alterations, the construction works can also introduce other transient water quality changes, such as increased turbidity, pollution, and vibrations during the execution of the project.

6.1 Impacts and mitigation measures during construction phase

Expected Impact of Excavations

Construction works can significantly alter the water regime, which in turn affects the ecosystem in several indirect ways, such as habitat loss, difficulties in finding shelter, and changes in physical and chemical characteristics, including temperature fluctuations, changes in gas regimes, pH, and more.

Excavation activities near water bodies can also lead to accidental spills of harmful substances, such as oil derivatives, into the environment. In larger quantities, these substances can cause the death of all organisms in the affected area.

Another potential negative impact at construction sites is the introduction of large quantities of concrete particles into the river system. The presence of these small suspended particles can cause their deposition in deeper layers of sand and gravel, leading to the formation of solid conglomerates that inhibit the development of the hyporheic zone (stigoritron), where organisms live in the sand and gravel substrate.

It is well-known that for the hyporheic interstitial zone, the composition of sand particles is far more important than the chemical composition of the water. Therefore, disturbances in the substrate structure can significantly alter the structure of the biocenosis. These particles can lead to the complete sealing of the lower substrate layers (concreting) and/or prevent oxygen circulation, which in turn hinders the development of living organisms.

In addition to the impacts on the aquatic environment, the surrounding soil and invertebrates can also be affected by changes in the immediate area due to construction activities. The clearing of larger forest areas reduces the terrain's ability to absorb water, leading to surface runoff. During rainy periods, this can cause an increase in the speed of water flow, destabilizing the banks and promoting erosion. These changes can alter the watercourse's hydrology, river channel morphology, suspended sediment levels, and the chemical and biological properties of the water. As a result, this can directly and indirectly affect various species, including fish, by eliminating critical habitats and impacting other organisms. Increased runoff from the surrounding terrain and significant runoff of sediment from the roadway can lead to torrents, which, like the suspended matter caused by construction work, have an erosive effect on the substrate and living organisms.

Mitigation Measures

To prevent and minimize harmful impacts during construction, it is essential to implement all suggested measures aimed at preventing erosion in the excavation areas, as well as the leakage of oil and grease from construction machinery.

Changes in the physical and chemical characteristics of water—provided that construction site organization and work procedures adhere to environmental protection guidelines outlined in the project documentation—should only cause accidental pollution from spills of hazardous substances. Therefore, controlled access for machinery to watercourses and other surface waters is necessary.

It is strictly prohibited to dispose of excess excavation material in the beds of streams, rivers, riverbanks, or on agricultural land.

6.2 Impacts and mitigation measures during operational phase

Expected Impact of Pollutants/Chemicals

During construction activities along the highway route, various actions can potentially harm the flow regime and the quality of surface water. The greatest potential threats include:

- **Construction works** (such as blasting, deep excavations, removal of surface layers, etc.), which can disrupt natural water recharge channels and create new catchment areas by removing surface layers.
- **Construction machinery**, which poses a potential risk for the spillage or accidental release of oil and oil derivatives, as well as the improper management of waste oil, batteries, and similar materials.
- **Uncontrolled disposal of excavated material** and the placement of machinery maintenance sites near surface watercourses.
- **Use of inappropriate construction materials**.
- **Deposition of exhaust gases**, tire wear, bodywork damage, cargo leakage, and spillage.
- **Disposal of organic and inorganic waste**, including atmospheric deposition brought by wind or generated by passing vehicles.

Several harmful substances are often found in the water runoff from road surfaces in concentrations that exceed the maximum allowed levels for discharge into water bodies.

These pollutants primarily include fuel components such as hydrocarbons, organic and inorganic carbon, nitrogen compounds (nitrates, nitrites, and ammonia), and a range of heavy metals, such as:

- Lead (from fuel additives)
- Cadmium
- Copper
- Zinc
- Mercury
- Nickel

In addition, solids with varying structures and characteristics—such as sedimentable, suspended, and soluble substances—are also present. Corrosion protection materials may also introduce pollutants, along with **polyaromatic hydrocarbons** (PAHs), such as **benzo-a-pyrene** and **fluoranthene**, which are highly carcinogenic substances resulting from incomplete combustion of fuel and motor oil.

To identify the presence of these pollutants, a range of macro indicators can be monitored, such as pH, electrical conductivity, suspended and sedimentary substances, COD, BOD, fats and oils, etc.

Mitigation Measures

Failure to apply protective measures in a timely and appropriate manner can result in contamination of water and land habitats from dust, pollutants from construction machinery, and waste from labor camps. These contaminants can have both direct and indirect harmful effects on invertebrates, leading to long-term consequences for their habitats. Substances such as heavy metals and nitrogen compounds can cause direct exposure mortality, developmental anomalies, and increased metabolic rates, all of which can lead to demographic changes with negative impacts on populations.

Therefore, it is essential to conduct regular surface water monitoring to assess the impact of highway construction and operation and to ensure the preservation of surface water quality within the project corridor.

The protection of the Gnjili Potok stream, which represents the locus typicus for two endemic species of the genus Drusus, requires special care, and in this sense, continuous monitoring during the entire construction process is highly recommended.

VII SUMMARY OF FINDINGS

The abundance of the macroinvertebrate community in the Kraštica stream at locality 1 in October 2024 was 803 ind/m². In this locality, representatives of Trichoptera dominated with an abundance of 231 ind/m², followed by representatives of Plecoptera with an abundance of 220 ind/m². Additionally, there were notable numbers of Ephemeroptera (99 ind/m²) and Hydrachnidia (69 ind/m²).

At locality 2, the macroinvertebrate community abundance of Kraštica River was 704 ind/m². Trichoptera again dominated, with 330 ind/m², followed by Plecoptera (121 ind/m²) and Hydrachnidia (99 ind/m²).

At locality 3, the abundance of the macroinvertebrate community was significantly higher at 1,232 ind/m². Trichoptera again dominated quantitatively with an abundance of 671 ind/m². Plecoptera were also abundant (187 ind/m²), as were Hydrachnidia (110 ind/m²).

At locality 4, representatives of prong-gilled mayflies (Leptophlebiidae) dominated. The sample also contained Drusinae larvae, likely of the species Drusus siveci, a rare Balkan endemic species described from a single specimen collected from Gnjili Stream in 1981. This species is critically endangered, and Gnjili Stream represents its locus typicus. Siphonoperla torrentium larvae were also present in the sample.

The score of the %Chironomidae metric was low at all investigated sites, indicating that there is no significant organic pollution at these localities. On the other hand, the %EPT metric score was relatively high, suggesting good water quality at all studied sites and generally healthy benthic communities.

Metrics used for rapid assessment of water quality, such as the FBI, BMWP, and ASPT, which are based on the composition and abundance of the benthic community, indicated high water quality at all investigated sites.

Construction works near water bodies can lead to accidental events, such as the spillage of harmful chemical substances into the environment, e.g., oil derivatives. These substances, in large quantities, can cause the death of organisms in the affected area.

One potential negative impact at construction sites is the introduction of large quantities of concrete particles into the river system. Therefore, it is necessary to conduct surface water monitoring to assess the impact of highway construction and operation, as well as to preserve the quality of surface water in the corridor.

VIII KEY CONCLUSIONS

Based on the results presented in this study, several conclusions can be drawn regarding the diversity value of individual macroinvertebrate groups, as well as the overall communities they form within hydrological features along the longitudinal transect:

- The class Insecta is the most diverse group across the investigated sites, represented by the greatest number of families. As expected, given the nature of the study sites, EPT taxa (Ephemeroptera, Plecoptera, and Trichoptera) dominated each location. Notably, EPT taxa dominance is characteristic of mountain streams, such as the watercourses investigated in this study.
- Trichoptera, Ephemeroptera, Plecoptera, Diptera, and Hydrachnidia were observed across all sites along the transect. These groups contribute both quantitatively and qualitatively to the macroinvertebrate communities in the study sites.
- The results of the %EPT metric were consistently high across all sites, indicating good water quality and generally healthy benthic communities.
- The %Chironomidae metric score was low at each site, suggesting an absence of organic pollution in the investigated areas.

- Sites 1 and 2 demonstrated the greatest richness in terms of both quantity and diversity of the aquatic macroinvertebrate community.
- A relatively high proportion of Plecoptera, Trichoptera, and Ephemeroptera taxa at the study sites indicates an important food resource for fish communities within these hydrological features.
- The relatively lower abundance values of the macroinvertebrate community across the sites may be attributed to the sampling period (late October) and elevated water levels in the studied watercourses, both of which directly impacted the macroinvertebrate community abundance.
- The one-time survey results suggest that all sampled sites in the studied watercourses exhibit good ecological quality.
- Aquatic invertebrates play a significant role in maintaining the ecological balance of the ecosystems in which they reside. They serve as an essential food source for other organisms while also regulating the populations they prey upon. Additionally, they are valuable indicators of ecosystem health. The data from this study provide a baseline for future monitoring to detect potential changes in the community due to planned highway construction activities.
- Quarterly monitoringof aquatic macroinvertebrates should be conducted annually to capture seasonal variations. This approach will provide a comprehensive view of the diversity within macroinvertebrate communities in areas that will fall under the direct influence of the planned highway construction. The protection of the Gnjili Potok stream, which represents the locus typicus for two endemic species of the genus Drusus, requires special care, and in this sense, continuous monitoring during the entire construction process is highly recommended.

IX MAPS AND PHOTOGRAPHICAL DOCUMENTATION

Photo 1-3. Photographs of selected representatives of macroinvertebrates from studied sites. Plecoptera larvae. Original photos by V. Pešić

Photo 4. Photograph of selected representatives of macroinvertebrates from studied sites. From left to right: elmid beetles, caddisfly larvae and prong-gilled mayflies larvae. Original photos by V. Pešić

Photo 5. Photo of sampling site (Locality 1)

Photo 6. Photo of sampling site (Locality 2)

Photo 7. Photo of sampling site (Locality 3)

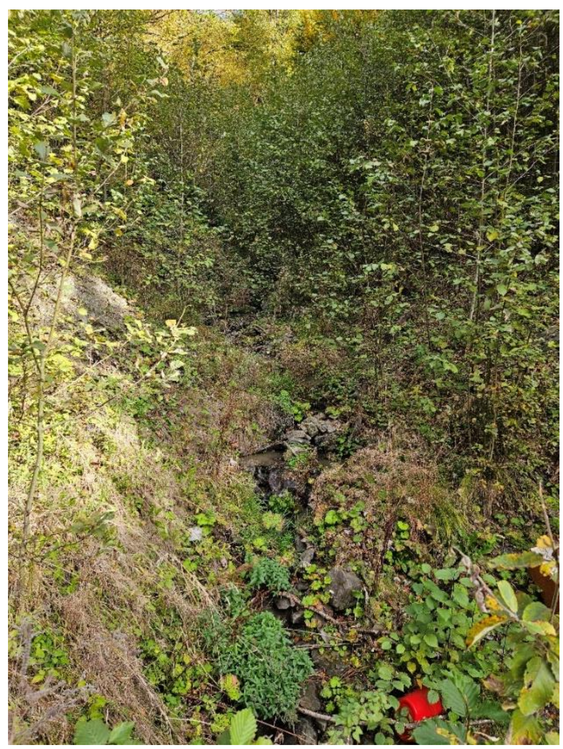

Photo 8. Photo of sampling site (Locality 4)

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