

## ECOLOGICAL ASSESSMENT OF THE KAYALIKA RIVER WITHIN THE MARITSA RIVER WATERSHED USING MACROZOOBENTHOS AS A BIOINDICATOR

Radoslava ZAHARIEVA<sup>1,2</sup>, Petya ZAHARIEVA<sup>1,2</sup>, Diana KIRIN<sup>1,2</sup>

<sup>1</sup>National Institute of Geophysics, Geodesy and Geography (NIGGG),  
Hydrology and Water Management Research Center, Bulgarian Academy  
of Sciences, 3 Acad. G. Bonchev Street, 1113, Sofia, Bulgaria

<sup>2</sup>Agricultural University - Plovdiv, 12 Mendelev Blvd, 4000, Plovdiv, Bulgaria

Corresponding author email: radoslava.zaharieva7@gmail.com

### Abstract

The study aims to conduct hydrobiological monitoring of the Kayaliika River (Maritsa River watershed) based on the biological quality element macrozoobenthos. The monitoring was performed following methodologies approved by the European Union and Bulgaria. Macrozoobenthos samples were collected in the autumn of 2024 from four locations (biotopes) along the Kayaliika River near the village of Ezerovo, Bodrovo, Varbitsa and Skobeleva. The number and taxonomic composition of the macrozoobenthos were determined, with the highest number of taxa found in the Kayaliika River biotope near the village of Skobeleva – 28 taxa, and the lowest – in the Kayaliika River biotope near the village of Varbitsa – 12 taxa. Basic indices for species diversity and abundance were calculated. The number of macrozoobenthos taxa by saprobity groups and sensitivity groups from the four biotopes of the studied river ecosystem was presented and compared. The results provide valuable insights into the ecological status of the Kayaliika River and contribute to regional water quality assessments.

**Key words:** biological quality elements (BQE), ecological assessment, Kayaliika River, macrozoobenthos, water quality.

### INTRODUCTION

The territory of Bulgaria covers an area of 110,994 km<sup>2</sup> (Metodieva et al., 2024; Kilifarska et al., 2025), characterized by a complex hydrogeographic network (Belkinova et al., 2013). The country is divided into four river basin management regions: Danube, Black Sea, East Aegean and West Aegean (Cheshmedjieva et al., 2010). The East Aegean region covers 31% of Bulgaria's territory and encompasses the catchments of four rivers (Maritsa, Tundzha, Arda and Byala). The Maritsa River is the largest river on the Balkan Peninsula and has over 100 tributaries (Belkinova et al., 2013), one of which is the Kayaliika River.

The Kayaliika River originates in the Rhodope Mountains near the village of Zhalt Kamak. It flows for 39 km, passing through the villages of Iskra, Bregovo, Dragoyanova, Ezerovo, Bodrovo, Varbitsa and the village of Skobeleva, before discharging into the Maritsa River (Kiradzhiev, 2013). According to the East Aegean River Basin Directorate (2018),

the Kayaliika River is classified as type R5 ("Semi-mountainous river").

Water is a valuable natural resource. River waters are used for irrigation and domestic and industrial needs. However, various anthropogenic activities contribute to water pollution (Gartsianova et al., 2022; Gartsianova et al., 2024). Benthic macroinvertebrates a key component of aquatic ecosystems, are widely used as bioindicators due to their low mobility, long life cycles, and sensitivity to pollution. Their abundance and diversity depend on the physicochemical properties of the water and substrate. Any environmental changes directly influence macrozoobenthos communities, making them essential for biological monitoring (Rashid & Pandit, 2014). According to the Water Framework Directive (Directive 2000/60/EU), the leading role in assessing the ecological state of surface water is played by the biological quality elements (BQEs) (algae, macrophytes, benthic macroinvertebrates, fish) listed in Annex V of the Directive. Physicochemical and

hydromorphological quality elements have a complementary role and provide information about the causes that led to the deterioration of the ecological state. The final ecological state assessment is determined according to the “one out – all out” rule (Belkinova et al., 2013). Despite extensive hydrobiological studies in Bulgaria, no prior research has assessed the ecological state of the Kayaliika River using biological quality elements.

This study aims conduct hydrobiological monitoring of the Kayaliika River using macrozoobenthos as a biological quality element, contributing to regional water quality assessments and ecological status evaluation.

## MATERIALS AND METHODS

Hydrobiological monitoring of the Kayaliika River was carried out in the autumn of 2024. Four biotopes located along the Kayaliika River were selected and surveyed: the Ezerovo biotope ( $42^{\circ}00'49.0"N$   $25^{\circ}17'24.2"E$ ); the Bodrovo biotope ( $42^{\circ}01'41.6"N$   $25^{\circ}18'54.6"E$ ); the Varbitsa biotope ( $42^{\circ}03'07.8"N$   $25^{\circ}20'40.2"E$ ) and the Skobelovo biotope ( $42^{\circ}05'35.4"N$   $25^{\circ}22'33.8"E$ ) (Figures 1-2; Table 1).

Macrozoobenthos samples were collected using standardized methods (Cheshmedjieva et al. 2011; EN ISO 10870:2012; EN 16150:2012; Regulation No. H-4 of 14.09.2012; Belkinova et al., 2013). Sampling was conducted using a kick net sampler. The collected material was preserved in 70% ethyl alcohol until subsequent laboratory processing. In laboratory conditions, the taxonomic affiliation of the macrozoobenthos was determined using a Micros Austria MZ 1240 stereomicroscope. The following metrics were calculated:

- The total number of taxa;
- The number of taxa from the orders Ephemeroptera, Plecoptera and Trichoptera (EPT);
- Margalef species richness index (Dmg);
- Shannon-Weaver species diversity index ( $H'$ );
- Pielou's evenness index (E);
- Simpson's dominance index (C);
- Abundance metrics (% (Oligochaeta & Diptera), % Filtering feeders, % EPT taxa);
- Saprobic index (SPUB), trophic index (RETI) and
- Adapted Biotic Index (BI) according to Regulation No. H-4 of 14.09.2012 and Belkinova et al. (2013).

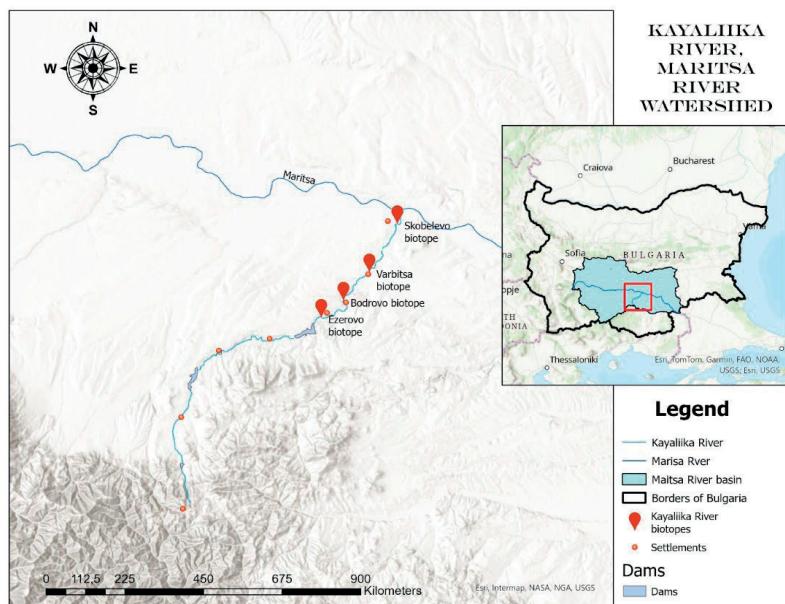


Figure 1. Researched biotopes along the Kayaliika River, Maritsa River watershed



Figure 2. Views of the studied biotopes from the Kayaliika River (from left to right: 1) near the village of Ezerovo; 2) near the village of Bodrovo; 3) near the village of Varbitsa and 4) near the village of Skobelevо)

Table 1. Characteristics of the studied biotopes from the Kayaliika River

Biotopes	Altitude	Location	Bottom substrate and current speed
Ezerovo	197 m	Along the Kayaliika River, southwest of the village of Ezerovo, after the Ezerovo Dam	Sandy/muddy bottom and slow current
Bodrovo	180 m	Along the Kayaliika River, northwest of the village of Bodrovo	Sandy/muddy bottom and slow current
Varbitsa	173 m	Along the Kayaliika River, within the borders of the village of Varbitsa	Sandy/rocky bottom and slow current
Skobelevо	148 m	Along the Kayaliika River, east of the village of Skobelevо, before flowing into the Maritsa River	Sandy/rocky bottom and slow current

## RESULTS AND DISCUSSIONS

During the hydrobiological monitoring of the Kayaliika River, a total of 970 specimens of 42 taxa were identified. These taxa included: (*Asellus (Asellus) aquaticus* (Linnaeus, 1758); *Anodonta cygnea* (Linnaeus, 1758); *Atherix ibis* (Fabricius, 1798), larva; *Baetis* sp., nymph; *Bithynia tentaculata* (Linnaeus, 1758); *Caenis horaria* (Linnaeus, 1758), nymph; Ceratopogonidae; Chironomidae, larva and pupa; *Chironomus plumosus* (Linnaeus, 1758), larva; *Cloeon* sp., nymph; *Corixa* sp., nymph; *Culex* sp., larva; *Dytiscus* sp., larva; *Enallagma cyathigerum* (Charpentier, 1840), larva; *Gammarus pulex* (Linnaeus, 1758); *Gerris* sp., nymph; *Glossiphonia* sp.; *Gomphus* sp., larva; *Gyraulus (Gyraulus) albus* (O.F.Müller, 1774); *Gyrinus substriatus* Stephens, 1829; *Haemopis sanguisuga* (Linnaeus, 1758); *Hydropsyche ornata* McLachlan, 1878, larva; *Hydroporus* sp.; *Hydropsyche* sp., larva; *Hyphydrus ovatus* (Linnaeus, 1761); *Ilyocoris cimicoides* (Linnaeus, 1758), nymph; *Lethocerus patruelis* (Stål, 1854); *Limnodrilus hoffmeisteri* Claparède, 1862; *Lumbricus rubellus* Hoffmeister, 1843; *Notonecta* sp., nymph; *Peregrina peregra* (O.F.Müller, 1774) (syn.

*Radix peregra*); *Physella acuta* (Draparnaud, 1805); *Platambus maculatus* (Linnaeus, 1758); *Plea minutissima* Leach, 1818; *Radix (Radix) auricularia* (Linnaeus, 1758); *Rhantus frontalis* (Marsham, 1802); *Schmidtea polychroa* (Schmidt, 1861); *Sialis lutaria* (Linnaeus, 1758); *Simulium* sp., larva; *Sphaerium (Sphaerium) corneum* (Linnaeus, 1758); *Tipula* sp., larva; *Valvata (Cincinnia) piscinalis* (O.F.Müller, 1774)). These taxa belonged to 18 orders and were found in the four studied biotopes, including: Amphipoda, Arhynchobdellida, Coleoptera, Crassiclitellata, Diptera, Ephemeroptera, Gastropoda, Hemiptera, Isopoda, Littorinimorpha, Megaloptera, Odonata, Rhynchobdellida, Sphaeriida, Trichoptera, Tricladida, Tubificida, Unionida. Insects in the order Hemiptera do not undergo a larval and pupal stage; instead, they develop through several nymph stages or "instars". The highest number of macrozoobenthos taxa was found in the Skobelevо biotope, and the lowest – in the Varbitsa biotope (Figure 3). The dominant macrozoobenthos taxon in the Ezerovo and Varbitsa biotopes is *En. cyathigerum*, larva (order Odonata), with 103 and 40 specimens, respectively. Dominant in the Bodrovo biotope

is *G. pulex* (order Amphipoda) with 40 specimens, and in the Skobelev biotope – *Cloeon* sp., nymph (order Ephemeroptera) with 141 specimens. According to the total number of taxa metric, the ecological state of the Kayaliika River was classified as very good in three of the studied biotopes and good in the Varbitsa biotope.

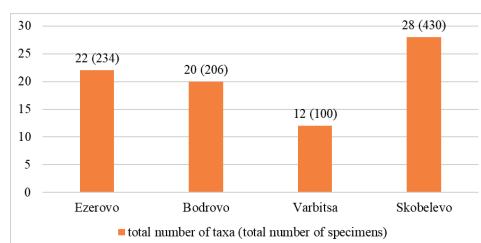


Figure 3. Total number of macrozoobenthos taxa by biotope

In general, across all four studied biotopes, there were few representatives of the Ephemeroptera and Trichoptera. Plecoptera taxa were absent, which contribute to the classification of a moderate ecological state for Ezerovo, Bodrovo and Skobelev biotopes and a bad ecological state for the Varbitsa biotope (Figure 4).

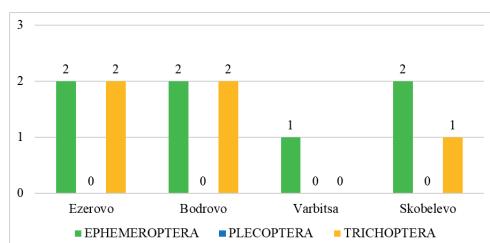


Figure 4. Number of taxa from the orders Ephemeroptera, Plecoptera and Trichoptera by biotope

The percentage of Oligochaeta and Diptera, as well as the percentage of Filtering feeders, is lowest in the Ezerovo biotope, while the percentage of EPT taxa is relatively high, indicating a weakly influenced river ecosystem. In contrast, the Varbitsa biotope shows the highest percentage of Oligochaeta and Diptera and the lowest percentage of EPT taxa, suggesting anthropogenic pressure on the aquatic environment in this section of the river (Figure 5).

The value of the German trophic index RETI increases downstream along the Kayaliika River, with the highest value observed in the Skobelev biotope ( $RETI_{Skobelev} = 0.79$ ), indicating a stable and undisturbed river ecosystem in this biotope. According to the RETI index, the ecological state of the Kayaliika River is classified as good across all four biotopes ( $RETI_{Ezerovo} = 0.51$ ;  $RETI_{Bodrovo} = 0.62$ ;  $RETI_{Varbitsa} = 0.67$ ).

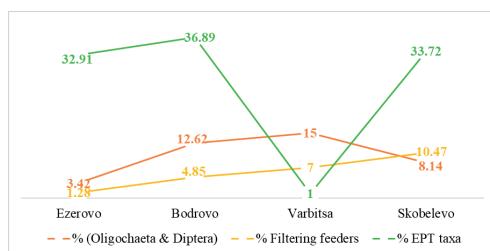


Figure 5. Macrozoobenthos abundance metrics by biotopes

The macrozoobenthos identified in this study belong to 4 sensitivity groups – Group B (less sensitive forms), Group C (relatively tolerant forms), Group D (tolerant forms) and Group E (the most tolerant forms). No taxa were identified from Group A (sensitive forms). In three of the studied biotopes (Ezerovo, Bodrovo and Skobelev), the majority of macrozoobenthos taxa belonged to Group C. In the Varbitsa biotope, an equal number of taxa for Groups C and D were identified (Figure 6).

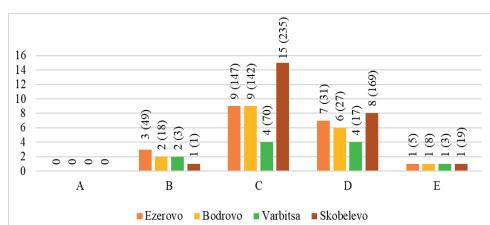


Figure 6. Distribution of macrozoobenthos taxa by sensitivity groups

In the Ezerovo biotope, indicator macrozoobenthos for  $\beta$  and  $\beta$ - $\alpha$ -mesosaprobic conditions prevails (6 taxa each). In the Bodrovo biotope, taxa indicating  $\beta$ -mesosaprobic conditions were dominant (6 taxa), while in the Varbitsa biotope taxa

indicating for 0- $\beta$  and  $\beta$ - $\alpha$ -mesosaprobic conditions (3 taxa each). In the Skobeleva biotope taxa characteristic of 0- $\beta$  and  $\beta$ -mesosaprobic conditions dominated (6 taxa each) (Figure 7).

According to the obtained values of the saprobic index (SPUB), the ecological state of

the Ezerovo, Bodrovo and Varbitsa biotopes was classified as very good ( $SPUB_{Ezerovo} = 1.89$ ;  $SPUB_{Bodrovo} = 1.69$ ;  $SPUB_{Varbitsa} = 1.80$ ), while in the Skobeleva biotope the ecological state was assessed as good ( $SPUB_{Skobeleva} = 2.07$ ).

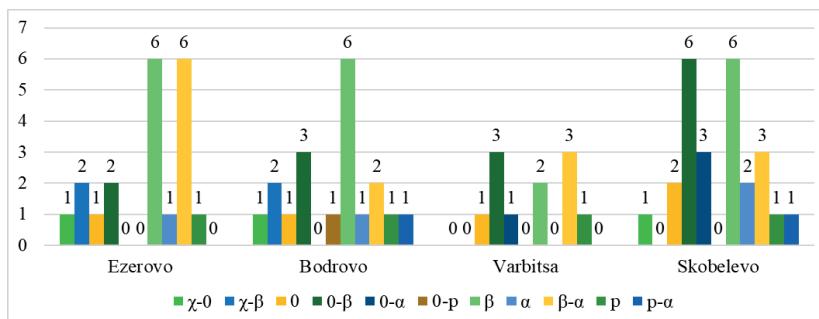


Figure 7. Distribution of macrozoobenthos taxa by saprobity groups

The Margalef species richness index ( $Dmg$ ) in all studied biotopes was below 8, indicating that the environmental conditions in the studied river ecosystem are not optimal. The Shannon-Weaver species diversity index ( $H'$ ) indicated  $\beta$ -mesosaprobic conditions in three of the studied biotopes (Ezerovo, Bodrovo and Skobeleva) and  $\alpha$ -mesosaprobic conditions in the Varbitsa biotope. The calculated Pielou's evenness index ( $E$ ) and Simpson's dominance index ( $C$ ) showed the most favorable conditions in the Bodrovo biotope ( $E = 0.845$ ;  $C = 0.106$ ) (Figure 8).

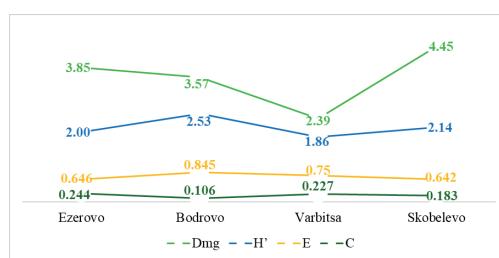


Figure 8. Species diversity indices (Margalef species richness index ( $Dmg$ ), Shannon-Weaver species diversity index ( $H'$ ), Pielou's evenness index ( $E$ ), Simpson's dominance index ( $C$ )) by biotope

The biotic index (BI) indicates a bad ecological state in the Varbitsa biotope – BI (nEQR) = 2 (0.4) and a moderate state in the other three biotopes – BI (nEQR) = 2.5 (0.5).

## CONCLUSIONS

During the hydrobiological monitoring of the Kayaliika River, a total of 42 taxa of macroinvertebrate organisms were identified, represented by 970 specimens, from the four studied biotopes. The highest number of macrozoobenthos taxa was recorded in the Skobeleva biotope (28 taxa), while the lowest number was observed in the Varbitsa biotope (12 taxa). Based on the assessment of biological quality elements (BQE), particularly macrozoobenthos, the ecological state of the river was classified as moderate in the Ezerovo, Bodrovo, and Skobeleva biotopes, and bad in the Varbitsa biotope, reflecting potential anthropogenic pressures.

These findings underscore the importance of regular biological monitoring to detect early signs of ecological degradation and guide targeted conservation measures. Further research integrating physicochemical parameters and land-use impacts is recommended to support comprehensive water management strategies for the Kayaliika River.

## ACKNOWLEDGEMENTS

This research is supported by the Bulgarian Ministry of Education and Science under the national Program “Young Scientists and

Postdoctoral Students-2". The study results have been published with financial support (project No. 17-12) of the Centre of Research, Technology Transfer and Protection of Intellectual Property Rights at the Agricultural University-Plovdiv.

## REFERENCES

Belkinova, D., Gecheva, G., Cheshmedzhiev, S., Dimitrova-Dyulgerova, I., Mladenov, R., Marinov, M., Teneva, I., Stoyanov, P., Ivanov, P., Mihov, S., Pehlivanov, L., Varadinova, E., Karagyozova, Ts., Vassilev, M., Apostolu, A., Velkov, B., & Pavlova, M. (2013). *Biological analysis and ecological assessment of surface water types in Bulgaria*. Plovdiv, BG: Univ "P. Hilendarskii" Publishing House (in Bulgarian).

Cheshmedzhiev, S.D., Karagiozova, T.L., Michailov, M.A., & Valev, V.P. (2010). Revision of river & lake typology in Bulgaria within Ecoregion 12 (Pontic Province) and Ecoregion 7 (Eastern Balkans) According to the Water Framework Directive. *Ecologia Balkanica*, 2, 75–96.

Cheshmedzhiev, S., Soufi, R., Vidinova, Y., Tyufekchieva, V., Yaneva, I., Uzunov, Y., & Varadinova, E. (2011). Multi-habitat sampling method for benthic macroinvertebrate communities in different river types in Bulgaria. *Water Research and Management*, 1(3), 55–58.

Directive 2000/60/EU of the European Parliament and of the Council (2000). *Establishing a framework for Community action in the field of water policy*. Luxembourg, 23.

East Aegean River Basin Directorate (2018). <https://earbd.bg/> (in Bulgarian).

EN ISO 10870:2012 *Water quality - Guidelines for the selection of sampling methods and devices for benthic macroinvertebrates in fresh waters (ISO 10870:2012)*

EN 16150:2012 *Water quality - Guidance on pro-rata Multi-Habitat sampling of benthic macroinvertebrates from wadeable rivers*

Gartsianova, K., Kitev, A., Varbanov, M., Georgieva, S., & Genchev, S. (2022). Water quality assessment and conservation of the river water in regions with various anthropogenic activities in Bulgaria: A case study of the catchments of Topolnitsa and Luda Yana rivers. *International Journal of Conservation Science*, 13(2), 733–742.

Gartsianova, K., Genchev, S., & Kitev, A. (2024). Assessment of water quality as a key component in the water-energy-food nexus. *Hydrology*, 11(3), 36.

Kilifarska, N.A., Metodieva, G.I., & Mokreva, A.C. (2025). Detection and Attribution of a Spatial Heterogeneity in the Temporal Evolution of Bulgarian River Discharge. *Geosciences*, 15, 12.

Kiradzhiev, S. (2013). *Encyclopedic Geographical Dictionary of Bulgaria*. Sofia, BG: Iztok-zapad Publishing house (in Bulgarian).

Metodieva, G., Mokreva, A., & Kilifarska, N. (2024). Parametric and non-parametric estimations of Bulgarian rivers' outflow trends. *Sixth International Conference, CAWRI-BAS "Global Warming's Imprints on the Elements of the Climatic System", Hisarya, 24-28 September 2024*, 109–116.

Rashid, R., & Pandit, A.K. (2014). Macroinvertebrates (oligochaetes) as indicators of pollution: A review. *Journal of Ecology and the Natural Environment*, 6(4), 140–144.

Regulation No. H-4 of 14.09.2012 on the characterization of surface waters. *Promulgated in the Official Gazette, No. 22 of 5.03.2013, amended and supplemented, No. 67 of 04.08.2023*.