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WATER QUALITY OF THE LEPENICA RIVER – OVERVIEW AND STATUS IN 2021

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ABSTRACT. The Lepenica River is the most important watercourse in the city of Kragujevac. Regardless of its importance, this river and its tributaries are extremely poorly investigated from a biological aspect. Only a few investigations were conducted from the end of the 20th century. After that, only physicochemical and microbiological parameters were monitored by scientists and Public Institutions. Our research was performed in 2021 at five localities at the Lepenica River and included an analysis of phytobenthos, macroinvertebrate, and fish communities, with the aim to assess ecological status/potential according to National Regulative. Our results indicate that the ecological status of this river was good (II class) only at one locality before the industrial and urbanized zone of the city of Kragujevac. From the entrance into the city of Kragujevac, the Lepenica River becomes highly polluted by multiple pollutants and belongs to the V class of ecological potential.

Keywords: water quality, water pollution, ecological status assessment, ecological potential assessment, algae, macroinvertebrates, fishes.

INTRODUCTION

In many countries, the rapid urbanization and industrialization in the Anthropocene era were not equally followed by equivalent environmental protection measures (ABRAHAM, 2011; ROY and SHAMIM, 2020). Rivers flowing through populated areas had become destroyed due to an enormous increase in human waste. This is extremely expressed in areas with dense human populations where watercourses are highly polluted by industrial wastewater, sewage, heavy metals, and garbage (POMPEU and ALVES, 2005; WEAR *et al*., 2021). Furthermore, the chemical contamination and pathogens of urban sewage origin may cause serious damage to aquatic ecosystems and public health, which represents the problem at the global level (STOJANOVIĆ-MILOSAVLJEVIĆ, 2002; MILANOVIĆ, 2007; MILANOVIĆ and KOVAČEVIĆ-MAJKIĆ, 2007; GARCÍA-ARMISEN *et al*., 2014). The endangerment of watercourses is primarily reflected in the presence of a large amount of waste material deposited along the riverbeds. The accumulation of organic pollutants in rivers stimulates microbial growth and leads to oxygen consumption, while the lack of dissolved oxygen in water results in the death of aquatic life (MILANOVIĆ, 2007).

Due to the intensive industrialization and urbanization of Kragujevac (the fourth largest city in Serbia), the surface waters of the city of Kragujevac are extremely exposed to anthropogenic pollution, which results in a series of negative effects on the environment, as well as an increased health population risk. The most important watercourse of the city of Kragujevac, the Lepenica River, was even in 1964 determined to be the fourth most polluted river in Serbia (STEPANOVIĆ, 1974). After that period, few studies were reporting chemical contamination of the river which mainly originated from the industrial sector (paints manufacturers, various chemical preparations, printing houses, dairies, slaughterhouses, laundries, stone quarries, and gravel pits) (STOJANOVIĆ-MILOSAVLJEVIĆ, 2002; MILANOVIĆ, 2007; MILANOVIĆ and KOVAČEVIĆ-MAJKIĆ, 2007), and the Institute of Public Health Kragujevac also reported several incidents of chemical contamination (GROUP OF AUTHORS, 2018a, 2019a, 2020a, b). In 2018, the Serbian Environmental Protection Agency (SEPA) found a large number of illegal waste dumping places along the entire course of the Lepenica River (GROUP OF AUTHORS, 2019b). A specific problem of the Lepenica River pollution represents the municipal waste dumpling of the city of Kragujevac, located only 50 m far away from the river. The SEPA noticed the existence of the public communal dumpling in Lapovo town, which also pollutes the Lepenica River (GROUP OF AUTHORS, 2019b).

Regardless of its importance, investigations on the biological aspects of the Lepenica River and its tributaries are poorly known. Data on aquatic communities of the Lepenica River are available from only a few scientific publications (RANKOVIĆ *et al.*, 1994; PETKOVIĆ, 1995; SIMIĆ V., 1996; SIMIĆ S., 2002; SIMIĆ *et al.*, 2008, 2015; ĐURETANOVIĆ, 2019), while data about hazardous substances in the Lepenica River are available in STOJANOVIĆ-MILOSAVLJEVIĆ (2002) and MILANOVIĆ (2007). Literature data on the research of the Lepenica River are summarized in Table 1.

This paper aimed to present a review of research on the water quality of the Lepenica River, as well as to present the water quality of this river in 2021 based on the analysis of biological parameters: phytobenthos, macroinvertebrates, and fishes. Additionally, we discussed anthropogenic pollution detected along the entire course of the Lepenica River.

MATERIALS AND METHODS

Study area

The Lepenica River is the biggest river in the city of Kragujevac. Since this city is poor in surface waters, this river is also its most important watercourse. It originates from the Studenac spring, located on the slopes of the Gledić Mountains, at 380 m above sea level, flows through the city of Kragujevac, and flows into the Velika Morava River after 55.4 km of the flow. Only a few kilometers in the upper stream part of the river have a natural flow, while from the beginning of the populated zone its flow has been regulated by concrete trough. Until the 1970s, this was a torrential river, often flooding, but today it is known as a low-water-level river, besides its 37 tributaries. Additionally, the intensive capture of springs in the upper stream part of its basin contributed to water impoverishment (STEPANOVIĆ, 1974; GAVRILOVIĆ and DUKIĆ, 2002; MILANOVIĆ, 2007).

The Lepenica River had become exposed to negative anthropogenic pollution from the beginning of urbanization and especially industrialization of the city from the 1950s (STEPANOVIĆ, 1974). To prevent further pollution from 1988 to 1991 seventeen plants for the pretreatment of industrial wastewater were built, but now only the treatment system "Cvetojevac" (located 7 km downstream from the city of Kragujevac) is in operation.

Nevertheless, purified water discharges into the Lepenica River although declared as IV class of water saprobity. But, Lepenica still contains a high concentration of chemicals not characteristic of municipal waters – heavy metals, sulfates, detergents, and zinc (GROUP OF AUTHORS, 2010).

Table 1. A review of investigations of the Lepenica River with a focus on water quality.

Year	Biological parameter	Water quality	Reference
1986	algae; macroinvertebrates	III-IV class	GROUP OF AUTHORS, 1986 GROUP OF AUTHORS, 1993
1993/94	macroinvertebrates	II-IV	SIMIĆ, 1996 SIMIĆ and SIMIĆ, 1999
1993/97	algae	III class	SIMIĆ, 2002
2001	algae; macroinvertebrates	IV class	*GROUP OF AUTHORS, 2002
2002	algae; macroinvertebrates	IV class	*GROUP OF AUTHORS, 2003
2003	algae; macroinvertebrates	IV class	*GROUP OF AUTHORS, 2004
2004	algae; macroinvertebrates	IV class	*GROUP OF AUTHORS, 2005
2005	algae; macroinvertebrates	IV class	*GROUP OF AUTHORS, 2006
2007	algae; macroinvertebrates	IV class	*GROUP OF AUTHORS, 2008
2018	diatoms; macroinvertebrates	V class	**GROUP OF AUTHORS, 2019b
2018	physicochemical; microbiological parameters	IV class	***GROUP OF AUTHORS, 2018a
2019	physicochemical; microbiological parameters	IV class	*** GROUP OF AUTHORS, 2019a
2020	physicochemical; microbiological parameters	V class	*** GROUP OF AUTHORS, 2020a
2020	physicochemical; microbiological parameters	V class	*** GROUP OF AUTHORS, 2020b

* monitoring conducted by the Serbian Republic Hydrometeorological Institute;
** monitoring conducted by the Serbian Environmental Protection Agency;
*** monitoring conducted by the Institute of Public Health Kragujevac.

Collecting samples and accompanying locality data

Field research of the Lepenica River was conducted in June 2021 at four localities: L1 – Goločelo village, L2 – Grošnica, L3 – old church and L5 – Lapovo, while L4 locality - outlet of purified water from the Wastewater Treatment System "Cvetojevac" was researched in September 2021 (Fig. 1, Tab. 2). Our research included sampling phytobenthos, macro-invertebrates, and fishes, as well as the measurement of physical and chemical parameters of water.

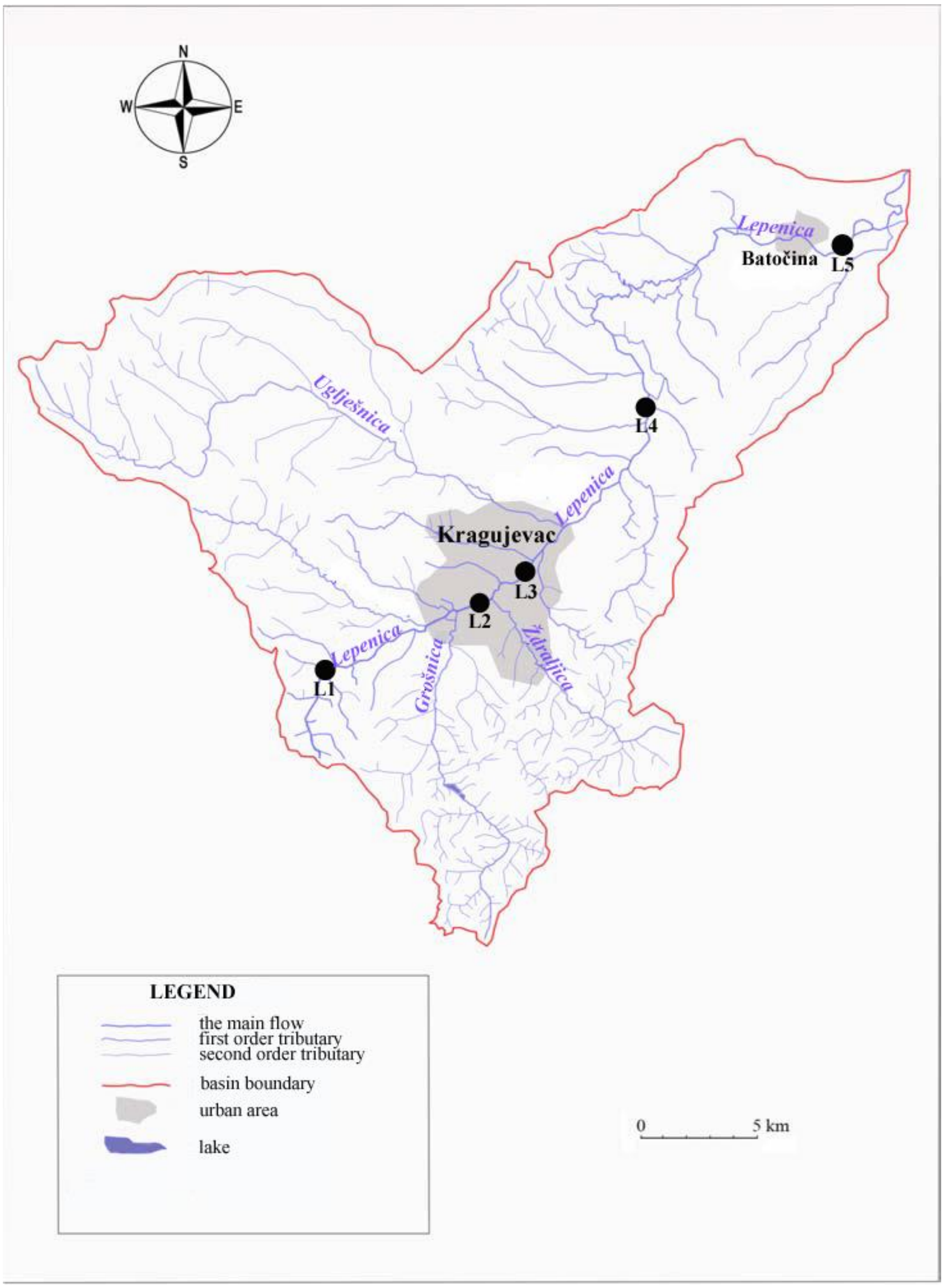

Figure 1. The Lepenica River Basin with position of investigated localities at the Lepenica River: L1 – Goločelo village; L2 – Grošnica; L3 – Old church; L4 - Outlet of purified water from the Wastewater Treatment System "Cvetojevac"; L5 – Lapovo.
(Modified from MILANOVIĆ, 2007)

Physical and chemical parameters of water

Physical and chemical parameters of water were measured by a set of field laboratory photometer system “AQUALITIC AL450”, according to the following standard EN 5667 1-19 (EN 5667 1-19: 2017). Temperature (°C), conductivity (µS/cm^3), water hardness (mg/l), pH, dissolved oxygen concentration (mg/l) and oxygen saturation (%) were measured directly on the field, while concentrations of phosphate (mg/l) and ammonia (mg/l) were determined in the Laboratory of the Center for Fishery and Biodiversity Conservation of Inland Waters – Aquarium, Faculty of Science, University of Kragujevac.

Table 2. Geographical position of investigated localities of the Lepenica River.

Locality	Locality abbreviation	Geographical coordinates	Altitude (m a.sl.)
Goločelo village	L1	43°57'42.54" N 20°48'41.33" E	260
Grošnica	L2	43°59'37.90" N 20°52'38.95" E	190
Old church	L3	44°00'31.00" N 20°54'47.20" E	172
Outlet of purified water from the Wastewater Treatment System "Cvetojevac"	L4	44°04'39.60" N 20°59'33.30" E	142
Lapovo	L5	44°08' 58.70" N 21°06'17.80" E	110

Phytobenthos

The samples of phytobenthos were collected depending on the type of morphological forms and substratum (scraping off from substrate, by tweezers, pipettes etc.), according to the following standard EN 15708 (EN 15708: 2009). Macroalgal aggregations were collected by tweezers, plates were collected by scraping off from rocks, while epipsamic community was collected by pipettes. The samples of epilithic diatoms for ecological status/potential assessment were collected at L1, L2, L3 and L5 localities according to the EN 19346 (EN 19346: 2014) standard. All collected samples were preserved in a 4% formaldehyde solution and stored at the Department of Biology and Ecology, Faculty of Science, University of Kragujevac.

Phycological samples were analyzed under the light microscope Motic BA310 with magnification up to 1000× with BRESSER (9 MP) digital camera and MicroCamLab software package. Morphological identification of recorded taxa was performed according to KRAMMER and LANGE-BERTALOT (1986, 1988, 1991), KOMÁREK and ANAGNOSTIDIS (1999, 2005), KRIZMANIĆ (2009), ELORANTA *et al.* (2011), JOHN *et al.* (2011), ANDREJIĆ (2012), and WEHR *et al.* (2015).

Quantification of epilithic diatoms was based on the relative abundance of identified taxa, represented by the valve percentage of each species relative to 400 counted valves on each permanent slide (EN 14407: 2014). Based on qualitative and quantitative analysis, diatom indices were calculated using OMNIDIA software (LECOINTE *et al*., 1993).

Aquatic macroinvertebrates

Samples of benthic macroinvertebrates were taken using an entomological benthos hand net (25x25 cm, mesh size diameter 500 μm), according to the EN 10870 (EN 10870: 2012) standard. Collected samples were conserved in 4% formaldehyde and subsequently taken to the Institute of Biology and Ecology collection, Faculty of Science, University of Kragujevac, Serbia. Thereafter, they were separated and identified to the lowest possible taxonomic level using NIKON SMZ 800 stereomicroscope with a MOTIC camera and Nikon Eclipse E100 microscope. The following identification keys were used to identify macroinvertebrates: CONCI and NILSEN, 1956; ROZKOŠNÝ, 1980; ELLIOTT et al., 1988; DOBSON, 2013.

Fishes community

The fishes were sampled with a standardized electrofishing method using equipment electrofisher "AquaTech" IG1300 on the 50 m of water flow.

Ecological status/potential assessment

The ecological status/potential assessment of the Lepenica River was performed according to the National Regulations in this field. Firstly, according to the ANONYMOUS (2010) it was determined that the Lepenica River at investigated localities except L1, belongs to the significantly changed water bodies of Type 3 – small and medium rivers, up to 500 m altitude and domination of large substrate granulation. After that, according to ANONYMOUS (2011) ecological status/potential of this river was assessed based on a threshold value of status classes for biological (epilithic diatoms and macroinvertebrates) water quality elements. Ecological status was assessed at locality L1, while at localities L2, L3, and L5 ecological potential were assessed.

Ecological status/potential assessment on the basis of phytobenthos as a biological quality element was assessed via IPS (CEMAGREF, 1982) and CEE (DESCY and COSTE, 1991) diatom indices, while for assessment based on macroinvertebrate community the following parameters were used: Zelinka and Marvan Saprobic Index (ZELINKA and MARVAN, 1961), Biological Monitoring Working Party (BMWP) Score (CHESTER, 1980), Shannon Weaver's Diversity Index (SHANNON, 1948), the total number of recorded taxa, Balkan Biotic Index (BNBI) (SIMIĆ and SIMIĆ, 1999), percentage participation of Oligochaeta/Tubificidae in the total macroinvertebrate community, and number of Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa. Calculation of biological indices was performed using the ASTERICS 4.04 software package. An indicative ecological status assessment was performed in accordance with the national legislation (ANONYMOUS, 2011).

According to the Serbian National Regulation (ANONYMOUS, 2011), ecological status assessment can be high (I class – blue color), good (II class – green color), moderate (III class – yellow color), poor (IV class – orange color), and bad (V class – red color), while ecological potential assessment can be good (II class – green color and dark gray stripes), moderate (III class – yellow color and dark gray stripes), poor (IV class – orange color and dark gray stripes), and bad (V class – red color and dark gray stripes) (DENIĆ *et al*., 2015).

RESULTS AND DISCUSSION

Physical and chemical parameters of water

The value of measured physical and chemical parameters of water of investigated localities of the Lepenica River are summarized in Table 3.

Values of measured physical and chemical parameters of water indicate that during the research period water of the Lepenica River was warm, hard, highly mineralized, slightly alkaline, and mostly poor with dissolved oxygen (Tab. 3). Concentrations of ammonium ions were extremely high at all localities, while concentrations of phosphates were high at all investigated localities except L1 locality (Tab. 3). During field research, oil and lubricant discharge from the exhaust service were noticed at locality L2. Solid large and small waste in riverbed and river coasts were noticeable at all investigated localities except locality L1.

Table 3. Physical and chemical water parameters of the Lepenica River.

Parameter / Locality	L1	L2	L3	L4	L5
Temperature (°C)	15.4	20.1	21.6	/	24.8
Conductivity (μS/cm3)	1040	820	680	/	790
Water hardness (CaCO3) (mg/l)	520	410	340	/	380
pH (0-14)	7.78	7.41	7.83	/	7.61
Oxygen concentration (mg/l)	7.9	3.1	8.8	/	5.9
Phosphates (mg/l)	P<0.06 PO4<0.06 P2O5<0.06	P=0.98 PO4=2.98 P2O5=2.24	P=0.70 PO4=2.14 P2O5=1.61	/	P=6.5 PO4=20 P2O5=15
Ammonium (mg/l)	N<0.02 NH3<0.02 NH4<0.03	N>50 NH3>60 NH4>64	N>50 NH3>60 NH4>64	/	N>50 NH3>60 NH4>64

Analysis of phytobenthos community

During research performed in June and September 2021 at five localities of the Lepenica River, 53 taxa belonging to five algal groups were identified: Cyanobacteria (11 taxa), Rhodophyta (1 taxon), Bacillariophyta (31 taxa), Euglenophyceae (6 taxa), and Chlorophyta (4 taxa) (Table 4).

Table 4. Qualitative analysis of algae from the Lepenica River (L1, L2, L3, L5 – June 2021; L4 – September 2021).

Taxa / Locality	L1	L2	L3	L4	L5
Cyanobacteria					
Arthrospira sp.				+	
Chroococcus sp.				+	
Leptolyngbya spp.				+	
Lyngbya sp.				+	
Merismopedia sp.				+	
Microcoleus sp.				+	
Microcoleus autumnalis (Gomont) Strunecky, Komárek & J. R. Johansen				+	
Microcoleus vaginatus Gomont				+	
Oscillatoria tenuis C.Agardh ex Gomont			+		
Oxynema sp.				+	
Phormidium spp.	+	+	+	+	+
Rhodophyta					
Audouinella pygmaea (Kützing) Weber Bosse	+				

Table 4. Continue

Taxa / Locality	L1	L2	L3	L4	L5
Bacillariophyta					
Achnanthidium saprophilum (H. Kobayashi & S. Mayama) Round & Bukhtiyarova				+	
Achnanthidium sp.				+	
Amphora copulata (Kützing) Schoeman & Archibald	+				
Amphora ovalis Kützing	+				
Amphora sp.					
Cocconeis placentula Ehrenberg	+				
Cyclotella meneghiniana Kützing		+	+	+	
Diatoma vulgaris Bory			+		+
Encyonema ventricosum (C. Agardh) Grunow			+		
Fallacia subhamulata (Grunow) D. G. Mann				+	
Gomphonema lagenula Kützing	+				+
Gomphonema parvulum (Kützing) Kützing	+		+		+
Gomphonema pumilum (Grunow) E. Reichardt & Lange-Bertalot	+				
Gomphonema sp.	+				
Gomphonella olivacea (Hornemann) Rabenhorst	+				
Meridion circulare (Greville) C.Agardh	+				
Navicula antonii Lange-Bertalot & Rumrich		+	+	+	+
Navicula associata Lange-Bertalot		+	+		+
Navicula cryptocephala Kützing				+	
Navicula gregaria Donkin	+	+	+	+	
Navicula lanceolata Ehrenberg		+	+		+
Navicula tripunctata (O.F. Müller) Bory	+				
Navicula radiosa Kützing	+				
Nitzschia palea (Kützing) W. Smith		+	+	+	+
Nitzschia spp.				+	
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot		+	+	+	+
Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot	+	+	+	+	
Sellaphora pupula (Kützing) Mereschkowsky				+	
Surirella brebissonii Krammer & Lange-Bertalot	+		+		
Surirella peisonis Pantocsek	+				
Ulnaria ulna (Nitzsch) Compère	+		+		+

Table 4. Continue

Taxa / Locality	L1	L2	L3	L4	L5
Euglenophyceae					
Lepocinclis spp.				+	
Euglena sp.				+	
Euglena texta (Dujardin) Hübner		+	+	+	+
Euglena viridis (O. F. Müller) Ehrenberg				+	
Phacus orbicularis Hübner				+	
Phacus spp.				+	
Chlorophyta					
Monoraphidium contortum (Thuret) Komárková-Legnerová				+	
Scenedesmus quadricauda (Turpin) Brébisson		+			
Stigeoclonium tenue (C. Agardh) Kützing					+
Ulothrix tenerrima (Kützing) Kützing				+	
Total number of taxa	**18**	**11**	**16**	**30**	**12**

As presented in Table 4, the highest number of taxa was recorded at locality L4, while the smallest number of taxa was recorded at L2 and L5 localities. The most diverse were members of Bacillariophyta (31), while from Rhodophyta phyla only one taxon was recorded. From the total number of diatoms, the largest (16 taxa) was recorded at L1, while the smallest number was recorded at the L2 locality (8 taxa). The highest number of taxa belonged to the genera *Navicula* (7) and *Gomphonema* (5). The percentage number of diatoms in the epilithic community at all investigated localities from June 2021 is presented in Table 5.

Based on the results of quantitative analysis of epilithic diatoms, the most dominant species were *Navicula antonii*, *N. associata*, *N. gregaria*, *N. tripunctata*, *Nitzschia palea*, *Planothidium frequentissimum*, and *Rhoicosphaenia abbreviata*. Most of these species are indicators of ß-mesosaprobic and α-mesosaprobic water (SLÁDEČEK, 1973; PÁL, 1998; KRIZMANIĆ, 2009). At all investigated localities of the Lepenica River, macroalgal aggregations were collected. *Phormidium* sp. was detected in the form of green plates on the stones at L1 locality. At localities L2 and L3, macroalgal aggregations in the form of gelatinous green plates of *Euglena texta* were detected. This alga is an indicator of ß-mesosaprobic water with high taxon indicator weight (SLÁDEČEK, 1973; PÁL, 1998). Macroalgal aggregations in a form of green coating, found at locality L4, were composed of cyanobacterial species *Microcoleus autumnalis* and *M. vaginatus*, euglenoids *Euglena texta*, *E. viridis*, *Lepocinclis* sp. and *Phacus orbicularis*, and diatoms *Fallacia subhamulata*, *Nitzschia palea*, and *Sellaphora pupula*. These algae are indicators of polisaprobic, α-mesosaprobic, and ß-mesosaprobic waters, high or very high taxon indicator weights (SLÁDEČEK, 1973; PÁL, 1998). Green alga *Stigeoclonium tenue* was found in a form of green tufts at locality L5. This alga is an indicator of α-mesosaprobic water with medium taxon indicator weight (SLÁDEČEK, 1973; PÁL, 1998). In the past, the algae of the Lepenica River was recorded only by RANKOVIĆ *et al.* (1994) and SIMIĆ (2002). During 1993, tri localities of the Lepenica River (upper stream, middle stream, and downstream) were investigated, and macroalgal aggregations of *Phormidium autumnale* Gomont (Cyanobacteria), *Vaucheria sessilis* (Vaucher) De Candolle (Xanthophyceae), *Cladophora glomerata* (Linnaeus) Kützing, *Oedogonium* sp., and *Stigeoclonium tenue* (Chlorophyta) were recorded.

Table 5. Quantitative analysis (percentages number of valves) of epilithic diatoms registered in the Lepenica River in June 2021.

Locality **Taxa**	**L1**	**L2**	**L3**	**L5**
Amphora copulata (Kütz.) Schoeman & Archibald	2.25			
Amphora ovalis Kützing	2.25			
Cocconeis placentula Ehrenberg	3			
Cyclotella meneghiniana Kützing		0.5	0.75	
Diatoma vulgare Bory			0.75	0.25
Encyonema ventricosum (C. Agardh) Grunow			2.5	
Gomphonema lagenula Kützing	1.25			3.5
Gomphonema parvulum (Kütz.) Kützing	3		5	6.75
Gomphonema pumilum (Grunow) E. Reichardt & Lange-Bertalot	5.25			
Gomphonema sp.	2			
Gomphonella olivacea (Hornemann) Rabenhorst	4.75			
Meridion circulare (Greville) C.Agardh	4.75			
Navicula antonii Lange-Bertalot & Rumrich		3.25	11.25	3.75
Navicula associata Lange-Bertalot		2.5	21.25	22.5
Navicula gregaria Donkin	0.75	26.75	1	
Navicula lanceolata Ehrenberg		0.75	3.75	0.25
Navicula tripunctata (O.F. Mueller) Bory	18.5			
Navicula radiosa Kützing	2.25			
Nitzschia palea (Kütz.) W. Smith		50.25	28.75	28.75
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot		12.5	11.25	33.75
Rhoicosphenia abbreviata (C.Agardh) Lange-Bertalot	47.75	3.25	9.5	
Surirella brebissonii Krammer & Lange-Bertalot	1.25		2.25	
Surirella peisonis Pantocsek	0.5			
Ulnaria ulna (Nitzsch) Compère	0.5		2	0.5

Analysis of aquatic macroinvertebrate community

A total of 28 taxa of aquatic benthic macroinvertebrates were collected in the investigation of the Lepenica River. The number of taxa per locality of the Lepenica River ranged from five (localities L2 and L5) to 18 (locality L1) taxa. The largest number of recorded taxa belonged to the order Diptera (seven species), followed by Ephemeroptera (six), Oligochaeta, Gastropoda, and Crustacea (three), Trichoptera (two), and Hirudinea, Odonata, Plecoptera and Coleoptera (each represented by one species) (Tab. 6).

In the locality L1 Insecta was the most dominant component of the community, except a significant participation of the amphipod crustacean *Gammarus balcanicus*. Compared to locality L1, a significant decrease in taxa number was observed at L2, L3, and L5 localities. Species diversity at localities L2, L3, and L5 were low (few species available with a large number of individuals). At localities L2, L3, and L5, the main taxa of macrozoobenthos were representatives of Oligochaeta from the family Tubificidae (*Limnodrilus hoffmeisteri*, *L. udekemianus*, *Tubifex tubifex*) and a representative of the Chironomidae family, *Chironomus thummi* (Table 6).

Table 6. Qualitative and quantitative analysis of aquatic macroinvertebrates from the Lepenica River.

Taxa / Locality	L1	L2	L3	L5
Oligochaeta				
Limnodrilus hoffmeisteri Claparède (1862)		10	84	47
Limnodrilus udekemianus Claparède (1862)		1	15	6
Tubifex tubifex Müller (1774)		7	26	10
Hirudinea				
Haemopis sanguisuga Linnaeus, 1758			1	
Gastropoda				
Bithynia sp.	1			
Lymnaea peregra Linnaeus (1774)			2	
Physa acuta Draparnaud (1805)			1	
Crustacea				
Asellus aquaticus Linnaeus (1758)	1			
Astacus astacus Linnaeus (1758)	1			
Gammarus balcanicus Schäferna (1923)	58			
Ephemeroptera				
Baetis rhodani Pictet (1843)	7			
Baetis sp.	1			
Ecdyonurus sp.	18			
Electrogena affinis Eaton (1883)	3			
Ephemerella ignita Poda (1761)	4		1	
Habrophlebia fusca Curtis (1834)	8			
Odonata				
Onychogomphus forcipatus Linnaeus (1758)			1	
Trichoptera				
Plectrocnemia conspresa Curtis (1834)	1			
Potamophylax sp.	10			
Plecoptera				
Isoperla difformis Klapálek (1909)	3			
Diptera				
Chironomus thummi Linnaeus (1758)		64	6	34
Orthocladius sp.			1	
Pedicia sp.	2			
Pericoma sp.	1			
Polypedilum sp.	1			
Prodiamesa olivacea Meigen (1818)	2			
Psychoda alternata Say (1824)		2	5	1
Coleoptera				
Hydraena gracilis Germar (1823)	1			
Total number of taxa	**18**	**5**	**11**	**5**

Further, the noble crayfishs, *Astacus astacus*, was collected in locality L1 on the Lepenica River. It is an autochthonous European species categorized as "vulnerable" on the Red List of Endangered Species of the International Union for Conservation of Nature and Natural Resources (IUCN Red List) with a trend of declining population and subpopulations

(ĐURETANOVIĆ *et al*., 2017). In Serbia, this species is classified as "endangered" and is a strictly protected species (SIMIĆ *et al*., 2008, 2015; ĐURETANOVIĆ *et al*., 2017).

Increased densities of Oligochaeta (representatives of the family Tubificidae) followed by a decrease in the diversity of other aquatic macroinvertebrates (primarily representatives of Ephemeroptera, Plecoptera, and Trichoptera) in running waters usually indicate organic enrichment (SLEPUKHINA, 1984). The present study revealed high densities of species *Limnodrilus hoffmeisteri*, *L. udekemianus* and *Tubifex tubifex* in the localities L2, L3, and L5, indicating pollution by organic matter in Lepenica River. Studies have shown that species of the family Tubificidae and many chironomids are generally tolerant to organic pollutants (MARTINS *et al*, 2008). These species are adapted to low concentrations of dissolved oxygen and can withstand complete anoxia. Their massive presence in polluted streams is not only due to their tolerance to the disturbed natural regime of gases in water but also to the relative decrease in the rates of competition and predation in such environments (BRINKHURST, 1966; MARTINS *et al*, 2008). At localities L2, L3 and L5, we identified the filter fly *Psychoda alternate*. The larvae of the genus Psychoda are often in the surface film of foul water, in sewage, as well as in wet, decaying, organic matter originating from various combinations of decomposing animal tissues (SATCHELL, 1947; AZMIERA *et al*., 2021).

According to research by SIMIĆ (1996), the habitats of the lower reaches of the Lepenica had a lower diversity of aquatic macroinvertebrates than the habitats of the upper reaches. Therefore, as in our research, a degraded community of macroinvertebrates was recorded with the dominance of paleophilic saprophagous and bacteriophage forms from the groups Oligochaeta (*Tubifex tubifex*, *Limnodrilus* sp.), Hirudinea (*Erpobdella* sp., *Helobdella* sp.) and Diptera (genera of *Chironomus*, *Trissocladius* and *Psychoda*) (SIMIĆ, 1996).

Analysis of fishes community

During field surveys performed in June and September 2021, the fish presence was not detected in the Lepenica River.

According to SIMIĆ *et al.* (2017) and SIMIĆ *et al.* (2020), during fish stock investigations of the Lepenica River, none of the fish was found in this river. The same authors assert that such a result is a consequence of strong organic and industrial pollution, so our results were expected. Truth be told, KOJADINOVIĆ (2020) noticed the presence of the Danube barbel *Barbus balcanicus* Kotlík, Tsigenopoulos, Ráb & Berrebi, 2002 in some tributaries of the Lepenica River (Grošnica and Petrovačka rivers), but stated that non of the fish species were found in the mouth of the Petrovačka river into the Lepenica River. The decades of pollution led the Lepenica River to become unsuitable even for the most resistant fish species.

Ecological status assessment

Based on parameters of phytobenthos analysis, the ecological status of the Lepenica River was assessed as good at L1 locality (II class), while at localities L2, L3, and L5 ecological potential of the Lepenica River was assessed as bad (V class) (Tab. 7).

Data about the water quality of the Lepenica River are available from an earlier investigation conducted by RANKOVIĆ *et al.* (1994). In this study, authors used standard saprobiological methods and determined that the water of the Lepenica River was ß-mesosaprobic to ɑ-mesosaprobic. According to SIMIĆ (2002), the Lepenica River belonged to II and II-III classes of water saprobity. Our data suggest that water at the L1 locality belongs to the II class of ecological status, while at L2, L3, and L5 localities it belongs to the V class of ecological potential, according to parameters of phytobenthos.

The ecological potential was not assessed at the L4 locality, but based on the taxa identified in samples collected from this locality, it can be concluded that water at this locality corresponds to α-mesosaprobic to polisaprobic.

Table 7. Ecological status/potential assessment of the Lepenica River based on epilithic diatom community.

Metric / Location	L1	L2	L3	L5
IPS index	15.3	4	6.6	5.6
CEE index	13.4	3.6	6.5	5.3
Ecologial status/potential assessment	good	bad	bad	bad
Class of ecological status/potential	II	V	V	V

good ecological status (II class)

bad ecological potential (V class)

Values obtained of the parameters for assessing the water quality based on aquatic macroinvertebrates of the Lepenica River are given in Table 8.

Table 8. Values of examined metrics and ecological status assessment of the Lepenica River based on aquatic macroinvertebrate community.

Metric / Location	L1	L2	L3	L5
Number of Taxa	18	5	11	5
Saprobic Index (Zelinka & Marvan)	1.88	3.532	3.484	3.503
BMWP Score	90	3	27	3
Diversity (Shannon-Wiener-Index)	1.92	0.656	1.343	1.17
Oligochaeta [%]	0	15.789	87.413	64.286
EPT-Taxa [%]	44.715	0	0.699	0
BNBI	3.5	1	1	1
Ecologial status/potential assessment	good	bad	bad	bad
Class of ecological status/potential	II	V	V	V

good ecological status (II class)

bad ecological potential (V class)

The composition of makrozoobenthos communities clearly reflects values of the saprobic index according to Zelinka and Marvan. The value of the saprobity index along locality L1 is 1.88, which corresponds with the II class (good ecological status). The values of the saprobity index at other Lepenica River localities are relatively uniform and show that the water quality is bad (V class) during the research (Tab. 8). In the majority of the sampling sites, the Tubificidae species were found at high densities, indicating pollution by organic matter in the Lepenica River. The total number of taxa in the Lepenica River per sample ranged from 18 (locality L1) to only five (localities L2 and L5). The presence of species from the Oligochaeta group was not recorded at the L1 locality, while their participation was dominant at the L2, L3, and L5 localitie, where the main macrozoobenthos taxa were representatives of the Tubificidae family, tolerant to high pollution and adapted to very low oxygen concentrations in water. The obtained results based on the EPT index indicate that the water at locality L1 corresponds to high status (I class). The disappearance of larvae of Ephemeroptera, Plecoptera, and Trichoptera was observed at other localities, due to which the water belongs to the V class (bad ecological potential) (Tab. 8). The BNBI index indicates that the water at locality L1 is rated as having good ecological status (clean water, in natural conditions), while at other localities it indicates very severe pollution (Tab. 8).

By analyzing the qualitative and quantitative composition of the macrozoobenthos, the water quality status in locality L1 corresponds with the II class (good ecological status). At sampling localities L2, L3, and L5, municipal wastewaters decrease water quality status and cause the presence of aquatic macroinvertebrates tolerant to pollution. Therefore, the ecological potential is rated as bad. In research by SIMIĆ (1996), the BNBI index based on aquatic macroinvertebrates showed the water in the Lepenica River varied from II to IV class, while the largest part of the river corresponded to the III class.

Ecological status/potential assessment of the Lepenica River based on parameters of biological quality elements (phytobenthos and macroinvertebrates) indicates that during our research ecological status at the L1 locality was good (class II), but at localities L2, L3, and L5 it was bad (class V) (Tab. 9).

Table 9. Ecological status/potential assessment at investigated localities of the Lepenica River based on biological quality elements (phytobentos and macroinvertebrates).

Location	**L1**	**L2**	**L3**	**L5**
Class of ecological status/potencial based on parameters of phytobenthos	II	**V**	**V**	**V**
Class of ecological status/potencial based on parameters of macroinvertebrate community	II	**V**	**V**	**V**
Ultimate ecological status/potential assessment	good	**bad**	**bad**	**bad**
Ultimate ecological status/potential class	II	**V**	**V**	**V**

good ecological status (II class)

bad ecological potential (V class)

The first data about the water quality of the Lepenica River are available from STEPANOVIĆ (1974) when it was the fourth most polluted river in Serbia. After the NATO bombing in 1999, a large concentration of polychlorinated biphenyls (PCBs) was found in the water and sediment of the Lepenica River and its tributaries, as well as in the Velika Morava River after the Lepenica River mouth. Chemical analyses have shown terrible water pollution with highly toxic PCB, which in animals and humans causes damage to reproductive organs and infertility. A year before the NATO bombing PCBs were not detected in the Lepenica River or its tributaries (STOJANOVIĆ-MILOSAVLJEVIĆ, 2002).

During 2004 and 2005 MILANOVIĆ and KOVAČEVIĆ-MAJKIĆ (2007) noticed a huge carelessness of the local population toward the watercourses of the city of Kragujevac. They observed a large amount of solid waste (car parts, plastic, and glass packing) in the Lepenica River, and in some places, the riverbed was turned into a damping. The same situation was noticed at some of its tributaries.

During the period 2001–2007, the Serbian Republic Hydrometeorological Institute conducted the Lepenica River monitoring at one locality and determined it belongs to the IV class of water saprobity (GROUP OF AUTHORS, 2002, 2003, 2004, 2005, 2006, 2008). The ecological status/potential assessment of the Lepenica River from the beginning of the Water Framework Directive implementation in Serbia was performed only in 2018 at one locality. According to biological and supporting physicochemical water quality elements its ecological potential was assessed as poor-bad (IV-V class of ecological potential) (GROUP OF AUTHORS, 2019b).

The Institute of Public Health Kragujevac conducts chemical and microbiological control of the raw water of the Lepenica River four times a year, usually at one locality near the industrial zone of the city of Kragujevac. According to the available data on the chemical and microbiological parameters, the water of the Lepenica River corresponded to IV and V-class water quality in the period from 2018 to 2020 (GROUP OF AUTHORS, 2018a, 2019a, 2020a, b).

According to a GROUP OF AUTHORS (2010), wastewater discharged into the Lepenica River contains high concentrations of ammonium, fats, and oils and low concentration of dissolved oxygen. Pretreatment of wastewater is not functioning, which causes the increasing concentration of different chemical substances not characteristic of municipal sewage. The lack of tertiary processing of sewage results in increased concentrations of ammonia, nitrates, nitrites, iron, and manganese in the effluent.

During our field research performed in June and September 2021, at all investigated localities of the Lepenica River, different types of pollution were noticed: communal waste-water discharge from unknown sources, car oil and lubricant discharge, solid large and small waste (tires, glass and plastic wrapping material, textiles, building materials etc). On the banks of the river, the existence of a large number of illegal waste dumping was recorded. Along the entire bank of the river, through populated areas, the SEPA also determined the existence of an abundance of illegal dumping in 2018. Except it the municipal waste damping of the city of Kragujevac, is located only 50 m far away from the Uglješnica River left tributary of the Lepenica River (GROUP OF AUTHORS, 2019b). The dumping leachates flow into the Uglješnica River, and through it into the Lepenica River. Contamination of the surface water of Uglješnica River with heavy metals was detected (MILIVOJEVIĆ *et al*., 2016). All these pollutants significantly make worse environmental conditions in the water habitats and, consequently, the composition of their hydrobiocenoses.

According to the SEPA (GROUP OF AUTHORS, 2013, 2014, 2015, 2018b, 2018c, 2019b, 2020c, 2021), the quality of Serbian surface waters is continually declining, and accordingly unsatisfactory. Approximately one-third of Serbian rivers belong to the II water class, while the largest number of watercourses is in the III class. According to the same source, none of the rivers flowing through cities had good ecological status/potential, while some of them also did not have good chemical status during the investigation period. For example, in the upper stream of the Raška and Ibar rivers, water belongs to the II class of water quality where the indicators of oligosaprobic waters were detected, such as *Bangia atropurpurea* (Mertens ex Roth) C. Agardh and *Paralemanea annulata* (Kützing) M.L. Vis & R.G. Sheath (SIMIĆ and ĐORĐEVIĆ, 2017; MITROVIĆ and SIMIĆ, 2021). Downstream of the first cities, these rivers become polluted and correspond to the V class of water quality (OCOKOLJIĆ *et al*., 2009; NIKOLIĆ *et al*., 2014; GROUP OF AUTHORS, 2018c). The Despotovica (Gornji Milanovac), Nišava (Niš), Moravica (Aleksinac), and Kolubara rivers are also examples of polluted rivers flowing through the cities, whose ecological status/potential was assessed as bad (V class) (GROUP OF AUTHORS, 2018c; SIMIĆ *et al*., 2018; SIMIĆ, 2019). Perhaps the most polluted river in Serbia is the Borska River, with a high content of potentially toxic heavy metals. This kind of pollution has an extremely negative impact on aquatic diversity and population health (MARINKOVIĆ *et al*., 2014).

The largest number of polluted watercourses in Serbia was recorded in the Basin of the Velika Morava River, to which belongs the Lepenica River, and the highest pollution was detected in the most densely populated and industrially developed parts (OCOKOLJIĆ *et al.*, 2009; ANDRIĆ, 2010). Our results on the analysis of phytobenthos, aquatic macroinvertebrates, and fish communities indicate that the Lepenica River in 2021 still represents one of the most polluted rivers in Serbia. Decades of pollution resulted in a significant reduction of biodiversity and the impossibility of using this largest watercourse in the city of Kragujevac for any purpose (irrigation, recreation, etc.).

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