Arch Biol Sci. 2021;73(2):291-301 https://doi.org/10.2298/ABS210314023C

Assessment of the impact of copper mining and related industrial activities on aquatic macroinvertebrate communities of the Pek River (Serbia)

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Received: March 14, 2021; **Revised**: May 10, 2021; **Accepted**: May 11, 2021; **Published online**: May 31, 2021

Abstract: The upper stretch of the Pek River is located in proximity to one of the largest mining sites in Serbia. To estimate the influence of copper mining on river biota, the quantitative and qualitative macroinvertebrate community structure was analyzed, and the ecological status of the river was assessed using standard biological indices. Data was gathered at 6 sites along the river. A total of 75 macroinvertebrate taxa were recorded. The reference site had the highest number of recorded taxa (48) and a high biodiversity (diversity index H=2.3). In contrast, the lowest number of taxa (8), a low diversity index value (H=1.15), and a very poor overall ecological status were recorded at site 2, which is heavily influenced by mining discharge. The Ephemeroptera, Plecoptera and Trichoptera group (EPT) constituted 46.30% of the community at site 1 but was completely absent at site 2 where Diptera (70%) and Oligochaeta (20%) were dominant, reflecting a strong impact of the mining industry on the upper river stretch. A gradual improvement of water quality downstream of the pollution sources indicates that this medium-sized river has the capacity to overcome intensive pollution and to revitalize itself along its course.

Keywords: bioindicators; diversity depletion; macroinvertebrates; mining pollution

INTRODUCTION

Freshwater ecosystems are a valuable natural resource, and yet they are under diverse negative anthropogenic pressures. Modern society relies on these resources for community water supply, but at the same time uses them as recipients of solid and liquid waste. The Water Framework Directive (WFD) [1] provides a legal framework for sustainable utilization of waterbodies while supporting both users and ecosystem welfare. According to its guidelines, it is essential to establish regular monitoring of watercourses and to assess their current status based on physical, chemical, hydromorphological and biological parameters so that adequate measures for quality improvement and preservation of the waterbodies can be developed.

While physical and chemical parameters provide information about the current status and water properties at the moment of sampling, analysis of biological elements provides a long-term status of the ecosystem, as well as an overview of the cumulative effects of different pollutants. Benthic macroinvertebrates, which were considered in this study of the Pek River, form a reliable biological element in the assessment of the ecological status of a waterbody. Macroinvertebrates have a body size of over 500 µm and spend their life cycle, either entirely or partially at the bottom of aquatic habitats [2]. They are often used in environmental monitoring because they possess a number of suitable traits such as low mobility, a long life span, high diversity, different sensitivity to stress, and they are easily collected and have a well-studied taxonomy [3].

The main polluters in the Pek River basin are the Majdanpek Copper Mine (MCM) which discharges its waste waters in the Veliki Pek and Mali Pek rivers, the

How to cite this article: Čanak Atlagić J, Andjus S, Đuknić J, Milivojević A, Kulić K, Pokrajac J, Tubić B, Paunović M. Assessment of the impact of copper mining and related industrial activities on aquatic macroinvertebrate communities of the Pek River (Serbia). Arch Biol Sci. 2021;73(2):291-301.

Copper Pipe factory (CPF) and the stone pit “Kaona” in the Pek River itself. MCM is an active polluter and a source of potential hazardous incidents, with the last major incident occurring in 1974 when 10 million m^3 of tailings were spilled into the Pek River [4]. This accident polluted the whole river and the tailings spread all the way downstream to the confluence with the Danube [5]. In previous research of the Pek River, increased turbidity caused by elevated concentrations of dissolved solid matter was reported repeatedly, and high levels of copper, sulfates, nitrogen, ammonia, orthophosphates, iron and manganese have been recorded both in the water and sediment [6,7]. Recent studies assessing the influence of mining industries on river biota [8-11] have addressed elevated acidity or alkalinity and increased metal ion concentrations in the water and substrate, with the aim of discovering harmful factors and the mechanism of their influence.

The aim of the present study was to record the macroinvertebrate diversity of the Pek River and to correlate mining pollution and consequent habitat alterations with changes in the macroinvertebrate community, as well as to assess whether this river can be used as a model for future research into the influence of mining on river biota. To investigate this influence, the structure of the macroinvertebrate community was analyzed at six localities and the ecological status of the Pek River was assessed based on quantitative and qualitative analysis of the aquatic macroinvertebrate community.

MATERIALS AND METHODS

Study area

The Pek River is the Danube’s right tributary located in eastern Serbia, and comprises the Veliki Pek River, which originates near the village Lipovac in the Homolje Mountains, and the Mali Pek River, which sources from Rajko’s cave. The Mali Pek flows through the town of Majdanpek and over the open pit of the MCM. It joins the Veliki Pek in the village Debeli Lug to form the Pek River. Upstream from the confluence site, the MCM filtration plant discharges its wastewater into the Veliki Pek River. The Mali Pek River receives wastewaters from the mine’s open pit. Another pollutant at the site is the CPF, which is located on the right bank of the Pek River, while the stone pit “Kaona” is located on the right river bank in the middle section of the Pek’s course (Supplementary Fig. S1).

Physicochemical parameters

At each site, the parameters measured were the water temperature and pH (measured with a Hanna HI9126 device (USA)), the oxygen concentration, measured with the Hanna HI 9146N, conductivity and total dissolved solid particles (TDS) that were measured with a MARTINI EC59 probe (Milwaukee, USA). The GPS Garmin Geko 201 device (USA) was used to determine the coordinates and altitudes at all sites.

Macroinvertebrate sampling, processing and identification

Benthic macroinvertebrate samples were collected in August 2015 from 6 localities along the course of the Pek River (Supplementary Fig. S1). The first site of the investigation was selected as the reference site (“near natural site”) and is located on the Veliki Pek upstream of the MCM filtration discharge. Samples were taken using a benthos hand net (25x25 cm, mesh size diameter 500 μm) following the Assessment System for the Ecological Quality of Streams and Rivers throughout Europe using Benthic Macroinvertebrates (AQEM) protocol, from all available habitats, according to the percentage fraction of different habitats at the site (multihabitat sampling procedure) [12]. Collected samples were conserved in 70% ethanol. Identification of the macroinvertebrates was performed based on morphological characteristics using ZEISS Stemi 2000C (x50) (Germany) and Nikon SMZ 800N (×75) (Japan) binocular stereoscopic magnifiers, the ZEISS Axio Lab. A1 (×630) microscope, and appropriate identification keys [13-15].

Ecological status assessment

According to national legislation [16] and the given class boundaries for metrics, the following parameters for assessing the ecological status were used: the total number of taxa (N), the Shannon-Wiener diversity index, the Zelinka-Marvan saprobic index, the Biological Monitoring Working Party (BMWP) procedure and the American Society of Plant Taxonomists

(ASPT) net. Saprobity was assessed using bioindicator lists of organisms by Moog [15]. Calculation of biological indices was performed using the ASTERICS 4.04 software package [12].

RESULTS

Physicochemical parameters

The recorded values of physical and chemical parameters of water are shown in Fig. 1. The oxygen concentration was lowest at the most polluted site (8.11 ppm); the highest concentration was registered at site 5, Mišljenovac (10.25 ppm) (Fig. 1A). The measured pH at all localities varied between 7.52-7.81 (Fig. 1A), which is within the given boundaries of the first water quality class according to the national legislative [16]. A gradual increase in water temperature in the downstream direction was observed (Fig. 1A). Considerable changes of TDS and conductivity were recorded along the river course. At the first site, which is not under significant anthropogenic influence, high conductivity was measured (625 µS/cm). At site 2, where the river receives industrial wastewater, the conductivity was two-fold higher (1229 µS/cm). At site 4, where the stone pit "Kaona" is located, a conductivity of 1151 µS/cm was measured (Fig. 1B). Conductivity changes were proportional to the changes in TDS, with the highest TDS value recorded at site 2 and the lowest at site 1. An increase in TDS value was also recorded at site 4, the stone pit "Kaona" (Fig. 1B).

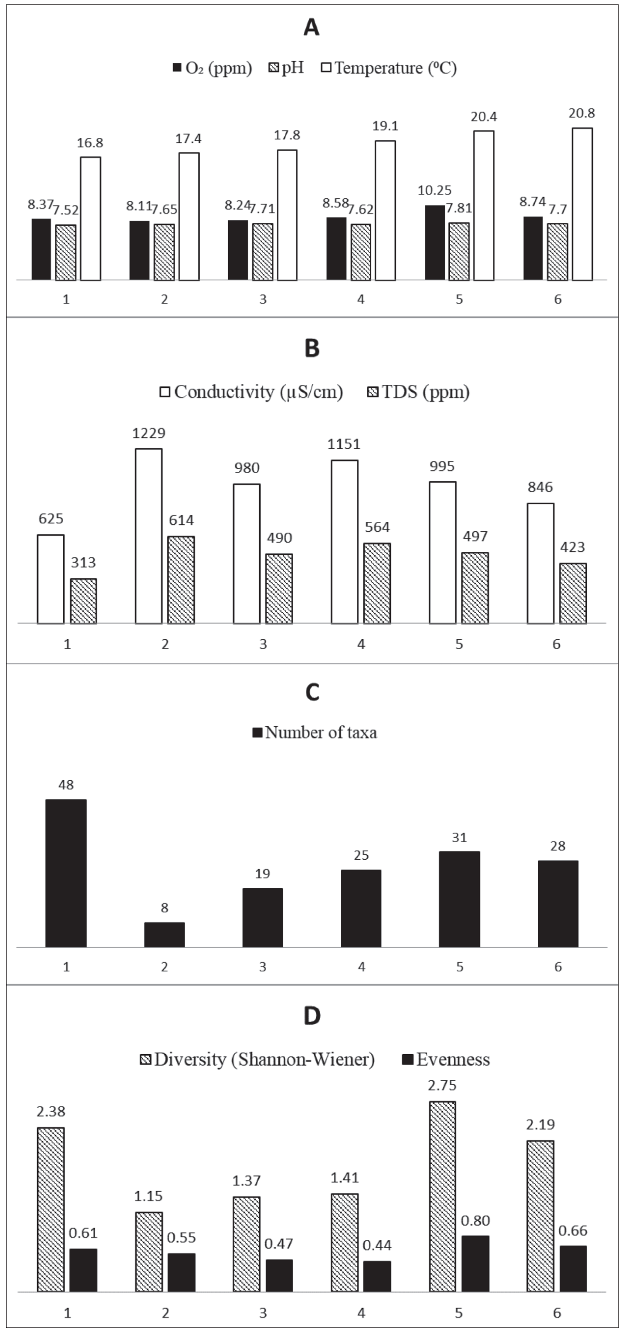


Fig. 1. Selected indicators of mining pollution. Recorded values of oxygen, pH and temperature (**A**), conductivity and total dissolved solid (TDS) particles (**B**), number of taxa (**C**), diversity and evenness at the investigated localities (**D**).

Macroinvertebrate community analyses

A total of 75 taxa were identified in the macroinvertebrate community of the Pek River (Table 1). Aquatic insects were the most dominant group in the community with respect to taxa richness and the number of individuals (89.73%, 72 taxa), while the rest of the community consisted of Crustacea (family Gammaridae) (7.22%), Oligochaeta (2.99%) and Mollusca (0.06%) (Fig. 2). The most distributed groups, present at all sites, were Oligochaeta, Diptera, Odonata and Coleoptera. Insect orders with the highest number of recorded taxa and the highest percentages in the total community were Ephemeroptera (16.5%, 19 taxa), Trichoptera (12.23%, 17 taxa),

Table 1. List of recorded taxa at the researched localities; (+) stands for taxa recorded at a certain locality.

Higher taxonomic rank	Taxon name	Site					
		1	**2**	**3**	**4**	**5**	**6**
Annelida	Oligochaeta	+	+	+	+	+	+
Mollusca	*Ancylus* sp.						+
	Pisidium sp.	+					
Crustacea	*Gammarus balcanicus* Schäferna, 1922	+		+	+	+	+
Ephemeroptera	*Baetis lutheri* Müller-Liebenau, 1967	+			+	+	
	Baetis scambus Eaton, 1870	+				+	
	Baetis sp.	+		+		+	+
	Baetis vernus Curtis, 1834				+	+	
	Caenis horaria (Linnaeus, 1758)				+		
	Caenis luctuosa (Burmeister, 1839)	+				+	+
	Caenis macrura Stephens, 1835					+	+
	Caenis pseudorivulorum Keffermüller, 1960	+			+	+	+
	Caenis rivulorum Eaton, 1884				+	+	
	Caenis robusta Eaton, 1884					+	+
	Caenis sp.	+		+	+	+	+
	Centroptilum sp.	+				+	
	Cloeon dipterum (Linnaeus, 1761)						+
	Cloeon sp.	+					
	Ecdyonurus sp.	+					
	Ephemera danica Müller, 1764					+	
	Ephemera sp.	+					
	Heptagenia sp.	+					
Plecoptera	*Perla* sp.	+					
	Leuctra fusca (Linnaeus, 1758)	+					
Trichoptera	*Beraea pullata* (Curtis, 1834)	+					
	Beraea sp.	+					
	Cheumatopsyche lepida (Pictet, 1834)	+		+	+	+	
	Diplectrona sp.	+					
	Ecnomus sp.	+		+		+	
	Hydropsyche angustipennis (Curtis, 1834)				+	+	
	Hydropsyche guttata Pictet, 1834	+					
	Hydropsyche incognita (Pitsch, 1993)	+		+	+	+	
	Hydroptila sp.	+			+	+	+
	Lepidostoma sp.	+					
	Leptoceridae Gen. sp.					+	
	Leptocerus sp.	+			+		
	Molannodes sp.	+					
	Mystacides sp.			+	+		+
	Oecetis sp.	+					
	Rhyacophila sp.	+					
	Tinodes sp.				+		
Odonata	*Calopteryx splendens* (Harris, 1780)				+		+
	Calopteryx virgo (Linnaeus, 1758)	+	+	+		+	+
	Coenagrionidae Gen. sp.			+			
	Gomphidae Gen. sp.	+		+			
	Gomphus sp.		+				
	Ischnura elegans (Vander Linden, 1820)						+
	Onychogomphus forcipatus ssp. (Linnaeus, 1758)			+	+	+	+
	Ophiogomphus cecilia Fourcroy, 1785				+	+	+
	Platycnemis pennipes (Pallas, 1771)	+			+	+	+

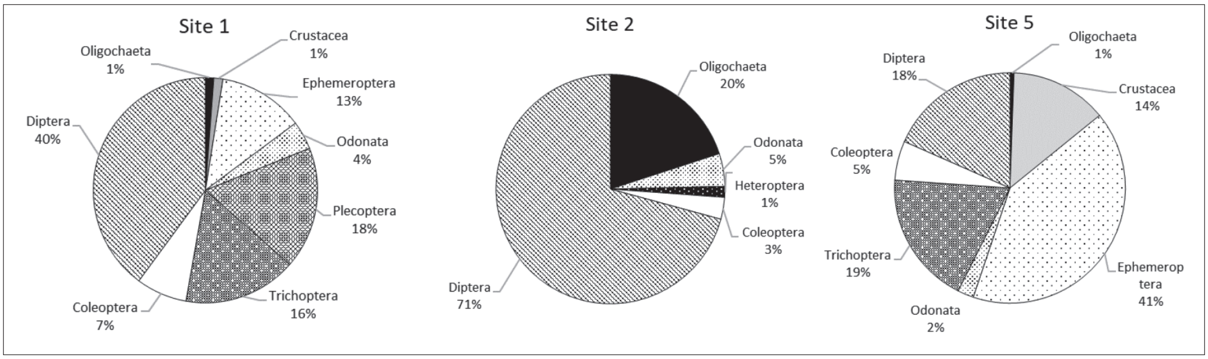
Table 1. continued

Diptera	*Antocha* sp.					+	
	Atherix ibis (Fabricius, 1798)	+				+	+
	Atrichops crassipes (Meigen, 1820)	+		+	+		+
	Chelifera sp.	+	+				
	Chironomidae spp.	+	+	+	+	+	+
	Chrysops sp.	+					
	Hemerodromia sp.	+	+	+	+		
	Ibisia marginata (Fabricius, 1781)					+	+
	Limoniidae Gen. sp.					+	
	Psyschodidae Gen. sp.	+					
	Tabanus sp.			+		+	
	Tipula sp.	+		+			
Coleoptera	*Dryops* sp.	+					
	Elmis aenea (Muller, 1806)	+					
	Esolus angustatus (Muller, 1821)	+				+	+
	Hydraena gracilis Germar, 1824	+					
	Laccobius sp.	+		+			
	Limnius sp.	+					
	Limnius volckmari (Panzer, 1793)	+	+	+	+	+	+
	Limnoxenus sp.						+
	Potamophilus acuminatus (Fabricius, 1792)						+
Hemiptera	Corixidae Gen. sp.				+		+
	Gerris lateralis Schummel, 1832				+		
	Veliidae Gen. sp.		+				+
Megaloptera	*Sialis sp.*	+					

Diptera (42.18%, 12 taxa), Odonata 6.83%, 9 taxa) and Coleoptera (5.47%, 9 taxa) (Fig. 2). Collectively, Diptera were the most numerous in the entire watercourse, with Chironomidae as the dominant family making up 96% of all individuals from this group. The highest number of taxa (48) was recorded at site 1, while the lowest number of taxa (8 taxa) was recorded at site 2 (Fig. 1C). Downstream from site 2, the number of taxa gradually increased, reaching 31 taxa (site 5). The highest diversity (2.75) and evenness (0.8) were recorded at site 5, while their values were lowest at site 2 (1.15, 0.55, respectively) (Fig. 1D).

Regarding the presence of the main taxonomic groups at the investigated localities, the dominant group was Diptera, which were the most abundant constituent of the recorded community at sites 2 (70.77%), 3 (69.12%) and 4 (66.60%) (Fig. 2). At sites 5 and 6, the share of Diptera in the community decreased significantly (18.39% and 23.19%, respectively). Plecoptera were present only at the first site, contributing to 17.50% of the community. Ephemeroptera, Trichoptera and Plecoptera (EPT taxa) at site 1 constituted an important component of the community, and together represented almost half of the community (46.30%). At site 2, EPT taxa were completely absent but once more attained a high proportion of the community at site 5 (41.02%).

In relation to the number of taxa, the most diverse groups were Ephemeroptera, Trichoptera, Odonata and Coleoptera (Fig. 2). At the 5th locality, 12 Ephemeroptera taxa were present. At site 1, which was the site with the highest number of recorded taxa, 11 Ephemeroptera taxa were present. The Trichoptera group had the highest number of present taxa at the 1st site (13) and was present at all localities except at site 2. Members of the order Plecoptera (3 taxa) were present only at the 1st site. The greatest number of taxa within the orders Coleoptera, Odonata and Diptera were also recorded at the 1st site (3, 6 and 8 taxa, respectively). The species *Gammarus balcanicus* Schäferna, 1922, was the only representative of the Crustacea group and was common throughout the river (except at site 2) (Fig. 2).



	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6	
	[%]	N	[%]	N	[%]	N	[%]	N	[%]	N	[%]	N
Diptera	39.82	8	70.77	3	69.12	5	66.60	3	18.39	5	23.19	4
Plecoptera	17.50	3	0	0	0	0	0	0	0	0	0	0
Trichoptera	16.30	13	0	0	9.07	4	3.11	7	18.67	6	3.26	2
Ephemeroptera	12.32	11	0	0	2.45	2	5.81	6	41.02	12	13.41	7
Coleoptera	7.32	6	3.08	1	2.45	2	0.21	1	5.52	2	12.32	4
Odonata	4.07	3	4.62	2	14.46	4	13.9	4	2.26	4	6.16	6
Crustacea	1.30	1	0	0	2.21	1	0.21	1	13.58	1	35.51	1
Oligochaeta	1.20	1	20	1	0.25	1	9.75	1	0.57	1	4.35	1
Bivalvia	0.09	1	0	0	0	0	0	0	0	0	0	0
Megaloptera	0.09	1	0	0	0	0	0	0	0	0	0	0
Heteroptera	0	0	1.54	1	0	0	0.42	2	0	0	1.45	2
Gastropoda	0	0	0	0	0	0	0	0	0	0	0.36	1

Fig. 2. Community structure at the investigated localities. Presence of invertebrate groups expressed as the percentage of the total sampled community [%] and the number of taxa [N]. Three most explanatory communities (at sites 1, 2, and 5) are shown as pie-charts.

The composition of the community from 3 sites stands out (Fig. 2) as follows: the reference site (site 1), the site under the most intense anthropogenic pressure (site 2), and the site with the highest recorded diversity and evenness (site 5). Differences in the distribution of the recorded invertebrate groups were evident. Site 1 was characterized by a diverse community composition with a uniform representation of different groups of insects. At this site, Ephemeroptera, Trichoptera and Plecoptera together constituted a significant part of the community (46.11%). At site 2 there was a notable decrease in diversity and a complete absence of the EPT group. At this site, Diptera was the dominant group in the community with over 70%, followed by Oligochaeta (20%). According to our results, the community completely recovered at site 5, which was characterized by increased diversity, the reappearance of Ephemeroptera and Trichoptera (41.02% and 18.67%, respectively), and a decrease in Diptera number (Fig. 2).

When the entire river community was assessed according to zonation preferences, the dominant components were epipotamal, hyporhithral and metharithral organisms, which contributed to 34.5% of the total community (Fig. 3A). A similar distribution pattern was determined at sites 1 and 5 (Table 2).

The structure of the community with respect to its adaptation to different habitat types is shown in Fig. 3B. The most prevalent were pelal taxa (30% of the total), especially at the 2nd, 3rd and 4th localities, followed by lithal taxa (15.32%), with the largest portion at site 5 (32%) and site 1 (27%) (Table 2). The presence of organisms preferring a phytal habitat was relatively high at sites 1-4 (15-19%) (Table 2).

With regard to the main feeding types, the dominant groups were collectors, scrapers, predators and filtrators (Fig. 3C). Collectors were the most numerous at sites 2 and 5, representing over 40% of the

Table 2. Taxa preferences to zonation, microhabitat and feeding types for each researched locality.

Zonation [%]	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site 6**
Crenal	1.037	0	0	0	0	0
Hypocrenal	2.602	0	0.025	0.021	1.726	1.268
Epirhithral	8.806	6.769	6.789	7.075	8.501	8.696
Metarhithral	16.417	7.692	9.461	9.834	15.149	6.123
Hyporhithral	14.435	8.923	10.441	10.83	18.416	6.377
Epipotamal	15.12	7.077	10.098	16.079	18.642	8.007
Metapotamal	8.556	6.462	7.01	9.398	7.935	4.783
Hypopotamal	3.833	6.462	6.642	6.722	2.885	3.152
Littoral	9.88	12.923	13.946	14.627	5.318	6.522
Profundal	7.648	12.923	13.284	13.174	3.465	4.348
no data	11.667	30.769	22.304	12.241	17.963	50.725
Microhabitat preference						
Pelal	24.898	45.385	44.289	43.983	11.74	15
Argyllal	0.167	0.308	2.5	0.041	1.754	0.399
Psammal	1.185	7.692	6.029	8.257	4.427	4.674
Akal	3.759	4.615	0.76	5.166	7.454	5.797
Lithal	27.37	6.154	3.652	8.029	32.249	14.493
Phytal	15.685	15.538	16.078	18.942	9.222	9.855
Pom	4.148	1.231	1.127	0.622	5.842	1.884
Other	9.361	12.923	14.044	13.506	7.511	4.783
no data	13.426	6.154	11.52	1.452	19.802	43.116
Feeding types						
Grazers and scrapers	26.62	15.385	17.475	16.39	18.472	15.652
Miners	3.86	6.462	6.642	6.722	1.881	2.754
Xylophagous	0.03	0	0	0	0	0.362
Shredders	6.25	0.308	1.912	0.332	8.175	21.92
Gatherers/Collectors	29.62	40.462	24.142	33.734	43.663	34.638
Active filter feeders	7.815	12.923	13.284	13.154	3.522	4.275
Passive filter feeders	6.63	0	4.093	1.058	8.571	0
Predators	14.44	18	25.368	21.992	10.863	10.797
Parasites	3.82	6.462	6.642	6.577	1.711	2.138
Other Feeding types	0.26	0	0.441	0.041	2.716	7.101
no data	0.648	0	0	0	0.424	0.362

community, while scrapers were dominant at site 1 (26.62%). Predators were the most numerous at sites 3 and 4. Xylophagous taxa were absent at all sites except sites 1 and 6. Active filtrators were prevalent in the middle section, while passive filtrators were the most frequent at site 5 and were not detected at sites 2 and 6 (Table 2).

Ecological status assessment

The water quality at the investigated sites was assessed based on the calculated indices (Table 3) and is in agreement with the current legislation [16]. According to all indices, sites 1 and 5 were assessed as those with the best water quality and ecological status, while site number 2 stood out as the locality with the poorest ecological status.

DISCUSSION

The Institute for Public Health “Timok” is occasionally engaged by the MCM to monitor the quality of waste effluent from the MCM, as well as the water quality of the Veliki Pek, upstream and downstream from the mine’s drainpipes. However, at the time this research was conducted, the effluent had not been treated in an adequate way [17,18]. Polluters such as CPF and the “Kaona” stone pit also have a strong influence on the Pek River, and these three sources’ combined discharge released notable amounts of suspended solids into the river.

Overall diversity with 75 recorded taxa is rather high, which is in agreement with a previous study of this river [19] where 84 taxa of macroinvertebrates were recorded. The ecological characteristics of the recorded community at the reference site correspond to communities of this type of river [16]. At three localities (1, 2 and 5), the structure of the zoobenthic communities and their notable downstream degradation clearly signal anthropogenic impact on the Pek River. The macroinvertebrate community of the Pek has a characteristic prevalence of organisms that, according to their feeding types, are classified as gatherers, scrapers and predators. Organisms inhabiting rithral and epipotamal habitats are the most important part of the community. As far as microhabitats are concerned, it is interesting that a significant part

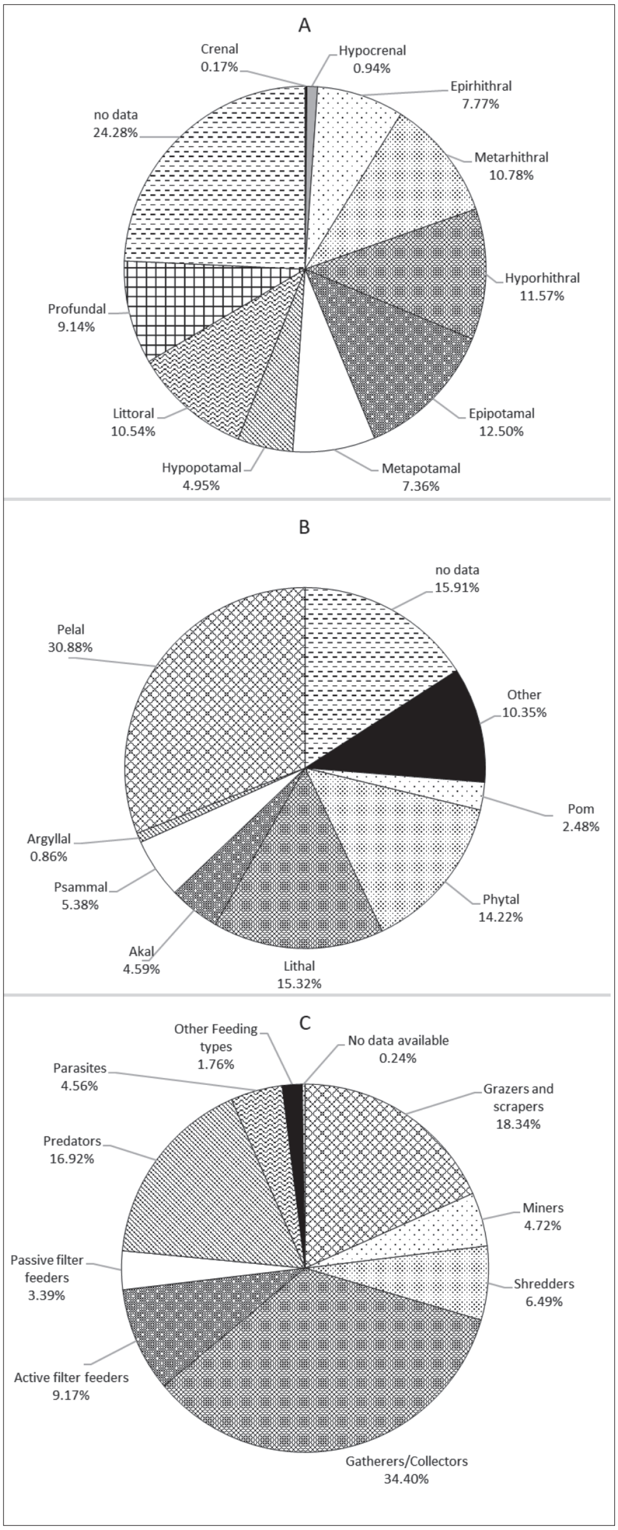


Fig. 3. The Pek River community structure based on zonation (**A**), microhabitats (**B**), and feeding types (**C**).

Table 3. Values of biological indices and assessed water quality class for each locality according to the National Legislative of the Republic of Serbia.

	1	2	3	4	5	6
Number of taxa	48	8	19	25	31	28
	I	IV	I	I	I	I
Diversity (Shannon-Wiener)	2.376	1.153	1.369	1.413	2.751	2.186
	I	IV	III	III	I	II
Saprobic Index (Zelinka & Marvan)	1.888	1.667	2.075	2.033	1.928	1.671
	I	I	II	II	I	I
BMWP	175	24	75	86	91	82
	I	IV	I	I	I	I
ASPT	6.731	4.8	5.769	5.733	6.067	5.467
	I	III	II	II	I	II
Total status	**I**	**IV**	**III**	**III**	**I**	**II**

of the community (30%) were organisms that inhabit pelal habitats. This ecological component is mainly present in the most polluted parts of the river (sites 2, 3 and 4). This finding can be interpreted as a community response to the presence of notable amounts of effluent with high concentrations of particles from the mine's flotation, which have changed the natural morphology of this habitat. At sites 1 and 5, organisms that inhabit rocky substrate were dominant. These two localities show the highest diversity, with the first locality being upstream from all the main polluters and the 5th downstream, where the impact of polluters was reduced.

The difference in the recorded taxa between the reference site and site 2 is a strong confirmation of the impact of the polluters (48 taxa at site 1 compared to 8 taxa at site 2). A large portion of the invertebrate community was not taken into consideration for the saprobic index calculation because of data deficiency (as much as 95% at the second locality). These results should not therefore be considered for an assessment of organic pollution (the values are within the given boundaries for classes I and II of water quality). The macroinvertebrate community of the Pek River has reacted to the current anthropogenic impact with a decrease in diversity, which, however, improved further downstream from the main polluters. Better treatment of the wastewaters from the filtration complex of the MCM that carry most of sediment, could help to improve the ecological status of the water and biodiversity. Several technical solutions for upgrading the wastewater system were proposed in Šerbula et al. [18].

Some of the findings among the recorded species are worth mentioning. Namely, *Potamophilus acuminatus* (Fabricius, 1972) (Coleoptera), found at the 6th locality, is considered rare and endangered in many European countries. The species' rarity was already noted in Serbia [19] and our record represents the first finding of the species in the Iron Gate area. Although this riffle beetle has been considered as sensitive to pollution and degradation of habitat [20], recent findings in the region suggest that *P. acuminatus* could have a wider ecological tolerance [21]. The presence of *Ophiogomphus cecilia* Fourcroy, 1785 (Odonata), a species rare and protected in Serbia [22] but recorded in a previous research [19], was reconfirmed. Considering its low saprobic valence according to Moog [15], this species is sensitive to pollution. Its presence in the lower stretch of the river, along with a higher macroinvertebrate diversity in comparison to sites closer to the pollution source, demonstrates the river's autopurification potential. In contrast, the structure of the community at site 2 consists mostly of Diptera and Oligochaeta, which are indicators of increased water pollution.

The measured high conductivity indicated an unusually high concentration of ions (both natural and from pollution). At the first site, where the anthropogenic impact is low, the higher value (625 μS/cm) could be explained as due to the geological substrate that is rich in metals such as copper, iron and zinc [18], as well as by rain runoff and drainage from the mine's pit. The highest conductivity, which coincides with the values recorded by Petrović and Marković [7], was measured at the 2nd locality (1229 μS/cm). Petrović and Marković conducted their research from November 2011 to May 2012 at a locality 30 m downstream from the confluence of the Veliki and Mali Pek rivers, at a site that corresponds to site 2 in this study.

The highest values of TDS (total dissolved solids) were measured at the 2nd locality, beside the MCM and CPF, and locality 4, beside the "Kaona" stone pit. Previous research [4,5,7,17] confirmed that the river is constantly overloaded with suspended particles and ions, which was also confirmed in the present study. The absence of the whole EPT group in the community at the 2nd locality was most likely the result of habitat alteration caused by increased concentrations of suspended solids and ions. Nevertheless, at the 4th locality where high conductivity and TDS were measured as well (caused by the "Kaona" stone pit), the community was more resilient and EPT were present, although in lower numbers. Presumably, wastewaters from the MCM filtration plant and the adjacent CPF carry more metal ions or other compounds that additionally negatively influence the community.

The MCM's wastewaters constantly contribute to the increase in concentrations of metal ions in the river, mostly copper, cadmium and iron [6,17], which is also detected as higher water conductivity. Although they are important micronutrients, metal ions in high concentrations represent an ecological risk because of their non-degradable and cumulative nature. Sensitive organisms react very rapidly to elevated concentrations of these elements in water, and soon disappear from the community, while metals accumulate in resistant organisms and then enter the food web [2]. Sediment analysis of the Pek River revealed the presence of high concentrations of copper and cadmium (870 mg/kg and 6.26 mg/kg, respectively) [7]. According to these results, the Pek was declared the most copper-polluted river in Serbia, which is a direct consequence of the influence of the Majdanpek mining complex. In our research, the highest values of conductivity and TDS, as well as of EPT taxa absence and the lowest diversity of macroinvertebrates, were recorded at site 2.

Although the results of this study show that the pH values at all localities were within the given boundaries of class I water quality [16], the results of earlier studies have recorded high pH values for wastewaters released into the Veliki Pek. In Šerbula et al. [18], research that was conducted between 2008 and 2012, the average pH value of wastewater was measured, as well as the average pH values of the Veliki Pek water both upstream and downstream from the filtration plant's drainpipes. The average pH value of the effluent was very alkaline (pH 10.24), while the average pH value upstream from the plant was 7.95 (reference value), and just below the plant it was 8.49. Downstream from the effluent release point, the pH value was within the allowed boundaries, with the exception of 2010 when the recorded values were above the maximum allowed concentration [18]. The fact that the pH value of the Pek's water downstream was within the boundaries of class I water shows that the Pek has the potential to overcome this aspect of pollution.

The largest number of taxa was recorded at site 1, the reference locality, in contrast to site 2, which is under considerable anthropogenic influence and where the community exhibited a notable decrease in diversity with the absence of taxa sensitive to pollution, such as the EPT group and Crustacea. The EPT group is sensitive to metal contamination and other elements of mining pollution [8,9], as are Crustacea [23-25]. A gradual increase in diversity downstream of site 2, with the reappearance of groups such as Ephemeroptera, Trichoptera and Gammaridae, as well as increased diversity of Coleoptera, Diptera and Odonata, point to a successful recovery of the Pek River macroinvertebrate community. The highest values of diversity and evenness indices recorded at site 5 indicate successful community recovery. Such recovery of the community at the localities downstream of the pollution sources could be the result of the dilution and/or sedimentation of the pollutants, which lead to the decrease in their concentration.

CONCLUSIONS

The results of this study confirm a notable negative anthropogenic influence caused by mining and related pollution of the Pek River in Serbia. Changes in the macroinvertebrate community structure along the watercourse indicate that the Pek River can be used as a suitable model system for a more comprehensive investigation into the impact of mining pollution on river biota. The collected information about the current state can be used as a baseline for setting up a framework for future studies, which would analyze mining impacts on the Pek River in detail and address additional aspects for a better understanding of the processes and subsequent implementation of measures to reduce the negative impacts of pollution. Extended monitoring of the entire biota and abiota, with focus on metal concentrations in the water and sediment and on suspended particles should be conducted and analyzed in more detail.

Funding: The study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant Nos. OI176018, TR370009 and 451-03-9/2021-14/ 200007.

Acknowledgements: We are grateful to Prof. Ivana Živić from the Faculty of Biology, University of Belgrade, for the generous support.

Author contributions: JČA: development of the study concept, field work, data analyses, writing of the manuscript; SA: sample processing, data analyses, writing of the manuscript; JĐ: field work, identification of organisms; AM: sample processing and identification of organisms; KK: sample processing and identification of organisms. JP: sample processing and identification of organisms; BT: Supervision of the identification of organisms; MP**:** team leader and supervisor.

Conflict of interest disclosure: the authors declare no conflict of interest.

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Supplementary Material

The Supplementary Material is available at: http://www.serbiosoc.org.rs/NewUploads/Uploads/Canak%20Atlagic%20et%20al_6380_Supplementary%20Material.pdf