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BIOLOGICAL AND MICROBIOLOGICAL MONITORING OF THE QALITY OF THE RIVER NIŠAVA

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Abstract. *This paper presents the degree of disturbance of the localities on the Nišava River, which are located near Bela Palanka and Niš (after the output of untreated water from city collectors). Quality assessment parameters were macroinvertebrates and microbial indicators of faecal pollution of water. The results show that there is a continuous pollution of the river, especially at the site near Niš, which imposes the need to build a wastewater treatment plant.*

Key words*: macroinvertebrates, microbial indicators, surface water*

1. INTRODUCTION

The Nišava, whose source is in adjacent Bulgaria, in its watercourse from the border to Niš with the length of 144 km, passes through Dimitrovgrad, Pirot, Bela Palanka, Niška Spa and Niš. The Nišava basin is in a mountainous area with a continental and mountain climate [1]. According to the regulation pertaining to watercourse categorization (Official Gazette SRS 5/68) the Nišava is categorized as a class II river in its whole course from the Bulgarian border to the mouth into the Južna Morava.

Using aquatic organisms with the purpose of determining water quality is a century old approach [2, 3]. Many methods have been developed for measuring biological conditions in aquatic ecosystems [4, 5]. Macroinvertebrates were used to evaluate the effects of anthropogenic stressors at all levels of biological organization, from molecular to ecosystemic [6]. One of our goals is to determine the level of the Nišava localities' disruption near Bela Palanka (Krupac) and Niš. It's also been our goal to compare this data to the results of microbiological examination of water quality at these localities considering that surface water is a reservoir of a spectre of pathogenic microorganisms [7] including indicators of fecal water contamination: coliform bacteria of fecal origin, enterococci, etc. Fecal pollution

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of anthropogenic origin is considered to be the main contaminant in the entire river basin [8], and therefore detailed data about the amount and origin of microbiological pollution is of vital importance for managing the Nišava water. Ascertaining fecal indicator concentrations along the Danube and its tributaries facilitated the first creation of a clear picture of contamination types along the entire length profile of this important international river which affected the proper implementation of new wastewater treatment facilities [9].

Even in the areas with the most advanced wastewater treatment, there may be high concentrations of microbiological contamination of fecal origin [10], unless the wastewater treatment facilities as a final step include disinfection such as UV radiation [11]. For that reason, in this paper, a special emphasis is put on the locality near Niš, before flowing into the Južna Morava, knowing that all wastewater of Niš, including fecal, is released into the river directly or through the sewage system, via the two main collectors, without treatment.

2. Research Objective

The aim of this research is to examine the biological and microbiological quality of the river Nisava before and after the discharge of waste water from city collectors.

3. Materials and Methods

A macroinvertebrates sample is taken through the use of kick-net – the net is placed perpendicularly in relation to the water current, a layer is disturbed directly above the frame so that the macroinvertebrates are carried by the water current into the net. Every locality was tested once a month over a period of one year. The first locality – near Bela Palanka (at Krupac) is marked with number 3 and the other locality, near Niš (at Medoševac), is marked with number 10 (Fig. 1).

Fig. 1 The Nišava- locality 3 and locality 10

Macroinvertebrates are identified through the use of identification keys for every group discretely. For observing and measuring very small body components, the instruments used were a binocular magnifying glass Leica MZ -16A Stereomicroscope with Leica DFC320 Digital Camera and Leica System Microscope DM2500 with Leica DFC490 Digital Camera.

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Diversity assessment is based on the Shannon diversity index (*H*'), Margalef's index (*d*) and Simpson's dominance index (*D*).

Shannon's diversity index was calculated through the following formula:

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

where $p_i = n_i / N$, n_i – number of units of a species, N – the total number of units, S – the total number of species.

Margalef's index was calculated according to the next formula:

$$d = \frac{S-1}{\ln N}$$

where N – the total number of units, S – the total number of species.

Simpson's dominance index was calculated according to the following formula:

$$D = -\sum_{i=1}^{S} (n_i/N)^2$$

where n_i is – the number of units of i kind, N – the total number of units, S – the total number of species.

Water samples for microbiological examination were taken by experts in accordance with the standards SRPS EN ISO 5667-1:2008, SRPS EN ISO 5667-3:2017, SRPS ISO 5667-6:1997 i SRPS EN ISO 19458:2009. One sample a month from both localities (3 and 10) was taken during 2017 and at the beginning of 2018. A microbiological analysis of the samples was carried out in the accredited lab PUC Naissus Niš (SRPS ISO/IEC 17025:2006). The examination encompassed the following parameters: indicators of fecal contamination: the total number of coliform bacteria (TC), coliform bacteria of fecal origin (FC), Escherichia Coli (*E. coli*) and enterococci (streptococci of fecal origin – SF). The first three parameters were examined according to the method SRPS EN ISO 9308-2 2015 (Colilert-18 /Quanti-Tray/), and SF according the the method ASTM D6 5030-14 (Enterolert-DW-18 /Quanti-Tray/). Escherichia coli and intestinal enterococci are marked by the World Health Organization as general indicators of fecal contamination [12].

An evaluation of the microbiological quality of water was based on the regulation concerning contaminant limits for surface and underground water as well as sediment and time windows for reaching those limits (Sl.gl.RS 50/12)- for a good ecological status, i.e., for class 2 – type 2 river. It was also based on the watercourse categorization regulation (Sl. gl SRS 5/68), inter-republic watercourse, interstate water and Yugoslavian coastal water categorization regulation (Sl. l. SFRJ 6/78). Finally, it was based on the regulation pertaining to the parameters for the ecological and chemical status of surface waters and the parameters for the chemical and quantitative status of surface waters (Sl.gl. RS 64/11) – for class II and III - type 2 rivers.

4. RESULTS AND DISCUSSION

Within the macroinvertebrates community at locality three, 32 species were detected whereas at locality 10 just 8 species during spring. In summer the species number ratio at these localities was 23:18, in autumn 26:12 and in winter 33:7. It is evident that locality 3, which is, based on numerous parameters, a locality with a better water quality and a greater species diversity. In percentage, the most spread is the Ephemeroptera group during spring, summer and autumn, while in winter the prevalent groups are Trichoptera and Dipera.

As it can be seen in table 1, the Simpson's dominance index is greater in all seasons at locality 10 than locality 3. This confirms the fact that macroinvertebrate communities are more uniform with a greater dominance of fewer species at localities which are more polluted. On the other hand, Margalef's and Shannon's indexes yield greater values in all seasons at the unpolluted locality, that is locality 3. This indicates that all three indexes are suitable for the goal of determining the level of disturbance of a certain locality in a river's ecosystem.

Table 1 Seasonal changes of indexes in macroinvertebrate communities:
The Simpson's dominance index; Margalef's index; Shannon's diversity index

	S3	S10	M3	M10	Sh 3	Sh 10
Spring	0.13	0.37	5.83	1.36	2.53	1.27
Summer	0.24	0.38	4.76	3.46	2.13	1.68
Autumn	0.16	0.5	4.99	2.25	2.43	1.16
Winter	0.12	0.66	5.61	1.28	2.6	0.8

Taking into account the statistically significant difference between average annual concentrations of all air pollutants, the observed places of measurements were found to be appropriate for tackling of the chronical effects of air contaminants on the health of the population exposed to them.

Biological monitoring results concur with the microbiological results where the amount of bacteria at the locality downstream from Niš (locality 10) is greater after the release of waste from the city collectors. The greatest levels of E.Coli and other bacteria have been observed in spring and summer while the microbiological pollution of the river is at the lowest in winter at both localities (table 2). The levels of E.coli at locality 3 varied from 5350/100ml (winter) to 217600/100ml (summer), while at locality 10 the values went from 162400/100ml to more than 1209800/10ml in summer. The total amount of coliform bacteria and other coliform bacteria of fecal origin at locality 10 in spring can be even bigger than 2419600/100ml while the amount of enterococci is somewhat smaller at this locality and varies from 35500-480300/100ml (table 2). The research carried out in Austria indicates that the utilization of microbiological contamination markers with a view to monitor the Danube's fecal bacteria contamination represents a powerful tool for goaloriented water quality management in rivers [13]. Research from around the world pertaining to other rivers, indicates that these bacteria are the most significant microbiological contaminant and that they are an indicator of water quality [14, 15].

Table 2 Microbiological examination values of the Nišava at localities 3 near Bela Palanka (Krupac) and 10 near Niš

		Parameter (cfu/100ml)			
	Locality	TC	E. Coli	FC	SF
Spring	3	153800	78250	67700	2700
	10	>2419600	1413000	>2419600	360900
Summer	3	523100	217600	217600	6060
	10	>1209800	>1209800	>1209800	480300
Autumn	3	42850	12250	6700	4304
	10	727000	344800	547500	88400
Winter	3	15250	5350	2400	392
	10	298700	162400	235900	35500

The Nišava's degradation is evident based on the microbiological examination which suggests that in the flow past Niš, throughout the year, all parameter values have seen more than a tenfold increase relative to the class II values indicated in the aforementioned regulations (TC: 10000/100ml, FC-1000/100ml, SF-400/100ml). This statement is in accordance with the biological examination results pointing to a low self cleansing capacity of the river.

5. Conclusions

Biological and microbiological water quality monitoring of the Nišava at the examined localities shows that there is a progressive river pollution, especially after the inflow of untreated water from the city collectors. Water quality deteriorates during summer when temperatures are higher and when hydrological conditions are unfavorable i.e. when the water level substantially declines. Such state of the Nišava watercourse is unsustainable and it suggest the necessity for a system which would treat the city's wastewater before its inflow into the recipient, with the goal to preserve the environment and human health.

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BIOLOŠKI I MIKROBIOLOŠKI MONITORING KVALITETA VODE REKE NIŠAVE

U ovom radu prikazan je stepen narušenosti lokaliteta na reci Nišavi, koji se nalaze ispod Bele Palanke i ispod Niša (nakon uliva neprečišćene vode iz gradskih kolektora). Parametri procene kvaliteta bili su makroinvertebrate i mikrobiloški indikatori fekalnog zagađenja vode. Rezultati pokazuju da postoji kontinuirano zagađenje reke, posebno na lokalitetu ispod Niša, što nameće potrebu izgradnje postrojenja za prečišćavanje otpadnih voda.

Ključne reči: *makroinvertebrate, mikrobiološki indikatori, površinska voda*