Microbiological water quality of the Nišava River

D. I. Čučak, N. V. Marković and D. V. Radnović

ABSTRACT

Microorganisms are the ideal indicators of the organic pollution of the surface waters because of their ability to promptly respond to environmental changes. Due to poor economic situation and lack of law implementation, most wastewaters in Serbia are released directly into a surface water recipient without any, or a limited, treatment. This practice has created numerous highly polluted surface waters in the region. Therefore, we conducted seasonal monitoring of the bacteriological quality of the Nišava River located in southeastern Serbia in order to assess the extent of its organic and faecal pollution. A total number of heterotrophs, *Escherichia coli* and intestinal enterococci counts were determined by standard cultivation methods in samples from five locations along the river. In most instances, bacteriological quality of Nišava water belonged to the classes of low or moderate organic and faecal pollution. The samples taken downstream of the city of Niš were the most polluted. Intestinal enterococci and *E. coli* counts indicated a moderate faecal contamination. Since bacterial counts can reveal a presence of faecal pollution and therefore presence of potentially pathogenic bacteria, a proper microbiological monitoring of surface waters used as a source of drinking water, like the Nišava River itself, is critically important.

Key words | *Escherichia coli*, intestinal enterococci, microbiological indicators, Nišava, surface water quality

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INTRODUCTION

Pollution of the surface waters with organic substances of anthropogenic origin is a global problem. Due to the poor economic situation in Serbia, most of the rivers and lakes act as the recipients of raw sewage from the settlements, animal farms or fields and pastures leading to a drastic decline in their water quality. In order to monitor and achieve a better quality of surface waters it is necessary to conduct a microbiological survey defining their microbiological quality. Due to their metabolic diversity and ability to respond quickly to environmental changes, bacteria are the ideal indicators of the pollution of surface waters (Kavka *et al.* 2006). The aim of this work was to determine the level and seasonal dynamics of organic and faecal pollution of the Nišava River located in southeastern Serbia using microbiological techniques.

There are several existing classification systems used for assessing microbiological quality of surface waters. One of the most recent classification systems proposed by Kavka *et al.* (2006) modifies and combines previous classifications developed by Kohl (1975), Kavka & Poetsch (2002), as well as the guidelines from EU Bathing water quality directive 2006/7/EEC (Council Directive 2006/7/EEC) and 76/160 EEC (Council Directive 76/160/EEC). This particular system combines indicators of faecal and organic pollution and classifies water into one of five levels of pollution. Classes I and II correspond to good bathing water quality according to mentioned EU directives. Target values for achieving such good quality are the following counts: *Escherichia coli* < 1,000, intestinal enterococci <400, total coliforms <10,000 (per 100 ml) and heterotrophic plate count (HPC) at 22 °C <10,000 per ml. The system is also used

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by Joint Danube Survey – survey of overall quality of the Danube River along its entire flow throughout Europe.

The Nišava is the largest river in the southeastern part of Serbia, with a length of 248 km. Its annual average flow rate is 28.43 m^3/s with variation from 19.19 to 37.67 m^3/s. Many small tributaries contribute to the basin area of 3,870 km^2 (Ducić & Luković 2009). Water of the Nišava River is indirectly used as a source of drinking water for around 240,000 people in the city of Niš and surrounding areas, which emphasizes its importance as a safe and healthy water supply for a large population (JKP Naissus 2015). Water is drawn from three different supply systems: Mediana, Studena and Ljuberađa-Niš. The Mediana is a groundwater supply system which is artificially infiltrated with the water from the Nišava River in order to increase its capacity. The other two supply systems are of the wellhead type and are not directly affected by the Nišava River water quality. However, the use of the Nišava water to artificially increase amount of groundwater available to the water factory highlights a need for maintaining its high physical, chemical and microbiological quality. In addition, the fact that the Nišava is a tributary of the Južna Morava River which is also the water supply source for a large central part of Serbia, further emphasizes the importance of maintaining its good water quality.

The municipal wastewaters of the city of Niš and surrounding settlements and industrial wastewaters are released into the Nišava without any treatment. The Ministry of agriculture and environmental protection of Serbia with aid of the EU funding plans to build wastewater treatment facilities in all Serbian cities with more than 100,000 equivalent inhabitants until the year 2020 including the city of Niš. However, as it is now, wastewaters are discharged directly into the Nišava without any treatment.

Moreover, the poor protection of water quality currently in place is further jeopardized by an insufficient and inadequate monitoring. Historically, municipal monitoring of the Nišava River is largely based on physical and chemical water parameters. Total coliform bacteria are the only measured microbiological parameter, which is certainly not sufficient for an accurate determination of microbiological quality, especially in the light of the newer European legislation and classification used in this study. Therefore, the aim of this study was to determine microbiological quality of the Nišava River throughout the entire season by using a wider range of bacteriological indicators when compared with the standard municipal monitoring program.

MATERIAL AND METHODS

A total of 20 samples from five locations (Dimitrovgrad, Pirot, Bela Palanka, Krupac, Niš) along the River's length (Figure 1) were taken seasonally from mid-September 2013 to April 2014. Samples were taken by submerging sterile glass bottles

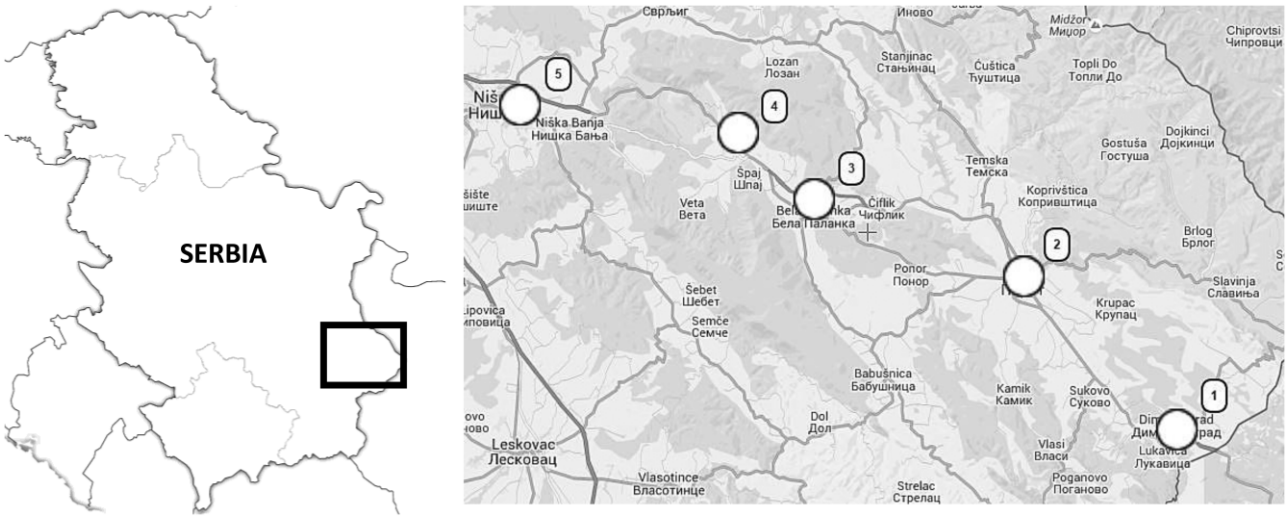

Figure 1 | Sampling sites along the Nišava River: 1–Dimitrovgrad, 2–Pirot, 3–Bela Palanka, 4–Krupac, 5–Niš.

10–20 cm under the surface of the water against the flow of the river. Depth of water column was 50–100 cm depending on the location. Distance from the shore was approximately 2–3 m. Samples were kept in a hand refrigerator until arrival at the laboratory. Microbiological analyses were performed no later than 24 h after sampling. The number of aerobic heterotrophic bacteria (HPC) was determined by spread plate technique on nutrient agar (Torlak, Serbia) according to the standard method APHA 9215 (APHA 2000). The counts of *E. coli* were determined by membrane filtration method using ChromoCult® Coliform Agar (Merck KGaA, Darmstadt, Germany). Intestinal enterococci counts were determined by the standard method (ISO 7899:2 2000). A minimum of three replications were conducted for each of the groups indicated above.

In order to interpret the microbiological water quality data obtained, we used the novel classification system which defines the five microbiological water quality categories (Kavka *et al.* 2006) (Table 1).

RESULTS

The obtained aerobic heterotrophic bacteria counts present in the water of the Nišava River were between 430 and 19,000 CFU per ml. A majority of the samples (90%) were below the value of 10,000 colonies per 1 ml (Table 2). Therefore, according to the used classification system, the water of the Nišava River belonged to the class II or the class of moderately polluted waters exposed to a weak pollution by an easily degradable organic matter (Kohl 1975 classification modified by Kavka *et al.* 2006). According to the results published by Petrović *et al.* (1996), the Nišava had significantly higher HPCs belonging to the class III or IV according to Kohl's classification system. In that period the lowest water quality was observed downstream from the fertilizer manufacturing plant, which was no longer in operation during our sampling period. A comparison of the results by Petrović *et al.* (1996) with our results shows an improvement of the Nišava River water quality in the past 20 years, probably due to a decreased level of industrial and agricultural pollution.

In order to determine faecal contamination, the counts of *E. coli* and intestinal enterococci were used. The counts of *E. coli* at most sampling sites (90%) were below target value of 1,000 CFU per 100 ml (Table 3).

Based on the *E. coli* counts, the water of the Nišava River was classified as class I or II meaning that the examined waters are under the influence of low or moderate levels of faecal pollution. Critical levels of faecal contamination were observed at the sites of Krupac and Niš only.

The number of intestinal enterococci also points to a little or moderate level of faecal pollution of downstream parts of the river (Table 4). The lowest water quality from a sanitary point of view was recorded at the sampling site in the vicinity of the city of Niš indicating its negative influence on the microbiological water quality of the Nišava. The target value for good bathing water quality of <400 intestinal enterococci per 100 ml was achieved in all but two samples from Niš.

Table 1 | Classification system of water quality according to Kavka *et al.* (2006), which uses faecal and organic pollution indicators. Classes I and II correspond to good bathing water quality and target values are underlined

Class	I	II	III	IV	V
Level of pollution	Little	Moderate	Critical	Strong	Excessive
Color	Blue	Green	Yellow	Orange	Red
Fecal pollution indicators (CFU or MPN per 100 ml)					
Escherichia coli	≤100	>100–1,000	>1,000–10,000	>10,000–100,000	>100,000
Intestinal enterococci	≤40	>40–400	>400–4,000	>4,000–40,000	>40,000
Total coliforms	≤500	>500–10,000	>10,000–100,000	>100,000–1,000,000	>1,000,000
Organic pollution indicator (CFU per 1 ml)					
HPC 22 °C	≤500	>500–10,000	>10,000–100,000	>100,000–750,000	>750,000

Please refer to the online version of this paper to see this table in color: http://dx.doi.org/10.2166/ws.2016.089.

Table 2 | Number of heterotrophic bacteria along the Nišava River (CFU/1 ml; modified Kohl classification according to Kavka *et al.* (2006))

	Sampling site				
Season (month)	**Dimitrovgrad**	**Pirot**	**Bela Palanka**	**Krupac**	**Niš**
Summer (September)	1,300	1,000	1,400	430	540
Autumn (October)	3,400	1,000	1,600	990	740
Winter (February)	1,900	3,700	3,500	4,100	19,000
Spring (April)	1,400	1,400	970	1,400	1,400

Legend: Classification of organic pollution: blue color: class 1 – low; green: class II – moderate; yellow: class III – critical; orange: class IV – high; red: class V – excessive organic pollution. Please refer to the online version of this paper to see this table in color: http://dx.doi.org/10.2166/ws.2016.089.

Table 3 | Counts of *Escherichia coli* along the Nišava River (CFU/100 ml; classification system according to Kavka *et al.* (2006))

	Sampling site				
Season (month)	**Dimitrovgrad**	**Pirot**	**Bela Palanka**	**Krupac**	**Niš**
Summer (September)	0	300	100	1,100	300
Autumn (October)	0	0	0	0	0
Winter (February)	0	400	100	300	300
Spring (April)	0	600	200	900	2,300

Legend: Classification of faecal pollution: blue color: class 1 – low; green: class II – moderate; yellow: class III – critical; orange: class IV – high; red: class V – excessive faecal pollution. Please refer to the online version of this paper to see this table in color: http://dx.doi.org/10.2166/ws.2016.089.

Table 4 | Counts of intestinal enterococci along the Nišava River (CFU/100 ml; classification system according to Kavka *et al.* (2006))

	Sampling site				
Season (month)	**Dimitrovgrad**	**Pirot**	**Bela Palanka**	**Krupac**	**Niš**
Summer (September)	227	40	26	0	633
Autumn (October)	29	0	5	153	413
Winter (February)	21	33	3	67	73
Spring (April)	23	39	15	18	96

Legend: Classification of fecal pollution: class 1 – low; green: class II – moderate; yellow: class III – critical; orange: class IV – high; red: class V – excessive faecal pollution. Please refer to the online version of this paper to see this table in color: http://dx.doi.org/10.2166/ws.2016.089.

DISCUSSION

Regular monitoring and protection measures are necessary for an optimal watershed management in order to provide safe and sanitary drinking water supply. Although cultivable population of microorganisms encompasses less than 1% of total population (Staley & Konopka 1985; Amann *et al.* 1995; Hugenholtz *et al.* 1998), indicator organisms are valuable tools in assessing water quality. Indicator groups are chosen specifically because their abundance correlates to influences such as faecal and organic pollution. In our study, we focused on well-established internationally used microbial parameters and systems of classification: HPCs, *E. coli* and intestinal enterococci, and the classification system according to Kavka *et al.* (2006).

HPCs recorded in our study indicate that the Nišava is under a moderate influence of easily degradable organic matter. The majority of samples had HPCs below the target value of 10,000 CFU/ml set as limit for a good bathing water quality. The city of Niš itself does not contribute noticeably to an increase of HPCs, since the results obtained were similar at all five sampling points across the river's length. Only one sample from Niš had HPCs in class III – critically polluted water.

Based on the observed abundance of the faecal pollution indicators (*E. coli* and intestinal enterococci), the city of Niš showed a clearly negative effect to the microbiological quality of the sampled waters due to the emission of the municipal wastewaters. The class III (critical pollution) was only recorded at sampling site Niš, while all other sampling sites remained within classes I or II. Thus, upstream portions of river have good bathing water quality according to EU Bathing water quality directive 2006/7/EEC and the 76/160 EEC directive.

Earlier studies conducted by the Municipality of Niš used only total coliform counts for microbiological quality assessment. This is now replaced in European legislation and has become somewhat obsolete. The Serbian Environmental protection agency additionally records the counts of intestinal enterococci, but unfortunately, frequency of their samplings is fairly irregular. Therefore, a comparison of the historical data with the results we have obtained is rather limited. The past assessments of the water quality of

the Nišava River were based on water quality index (WQI) as: very low, low, good, very good or excellent quality. This method is based on the 10 physico-chemical and microbiological parameters which are subsequently combined into a single quality index. According to WQI, the Nišava had 'very good' status in March and June 2014, while according to annual reports from 2011, 2012 and 2013 the WQI status was 'very good' or 'good'. In the annual report of the Serbian Environmental Protection Agency (SEPA) for 2013 microbiological data were not provided while in 2012 intestinal enterococci varied from 9 to 2,400 CFU/100 ml, and total coliforms ranged from 880 to 4,600 per 100 ml to even more than 240,000 at Niš sampling site. HPC was not determined (SEPA 2012, 2013). Other historical data for microbiological quality of the Nišava River are fairly limited or are publicly unavailable. Still, our results show an improved microbiological quality of the Nišava River when compared to the historical data.

The methods we used (HPCs, *E. coli* and intestinal enterococci) together with the classification system based on the five basic water quality classes indicate that the Nišava is under a moderate influence of easily degradable organic matter with a low or moderate level of faecal contamination. As expected, the indicators of faecal pollution have confirmed negative impact of the untreated municipal wastewater of the city of Niš leading to the deterioration of the microbiological water quality. In the portion of river upstream from the city of Niš good bathing water quality is maintained. Therefore, this work emphasizes the importance of using up–to-date classification systems in accordance with current international legislation and monitoring practices for reliable and comprehensive surface water quality assessment.

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