Annals of the Institute of Biology - University of Sarajevo
AIBUS: 44: 71-84
Original Article UDC 574.587:592:578.68(497.6)

ISSN- ISSN 2831-0705 (Online)
http://aibus.pmf.unsa.ba/
DOI: 10.35666/2831-0705.2022.44.71
20 December 2022

Correspondence of hydrobiological and microbiological parameters in the water quality assessment of the middle course of the Misoča River

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Abstract

The water quality of the Misoča River was analyzed on two sites of the middle course, in a populated and in an unpopulated area. The research included sampling of macrozoobenthos and water for microbiological analysis, identification of macrozoobenthos taxa, application of hydrobiological indices for water quality assessment and microbiological analysis of water quality. The goal was to determine the compatibility and complementarities of different aspects of water quality analysis, and to demonstrate the expediency of a multi-aspect and multi-disciplinary approach for this type of research. Through this, an assessment of the water quality of a selected part of the Misoča River, which is very important for the water supply of the surrounding area, was carried out. According to the results of the macrozoobenthos analysis and the applied diversity and water quality indices, the Misoča River belongs to the β-mesosaprobic category, which indicates moderate pollution. The results of the analysis of microbiological parameters also indicate moderate pollution.

Key words: macrozoobenthos, water quality indices, bacteriological analysis, water categorization, Misoča River

1. Introduction

The specificity and complexity of the chemical composition of surface waters and quality indicators as a consequence of dissolved mineral and organic substances, gases, suspended substances and microorganisms indicate the importance of application of different methods for their evaluation and determination of common factor that encompasses quality as a whole. Polluted water deviates from natural/reference physical, chemical and biological properties, which limits the possibility of its use (Rosenberg, 1992). Changes in water characteristics caused by pollution lead to deterioration that very often cause diseases. For these reasons, it is necessary to limit the introduction of waste materials and energy and enable the maintenance of natural self-purification processes in watershed (Hartl and Clark, 1989).

Physical and chemical analyzes are very important in the assessment of water quality, but they only indicate the current state of inland waters. In order to determine the long-term status of a water body, various methods of hydrobiological characterization of water are applied, including diversity indices, biotic indices, saprobiotic methods, etc. Benthic macroinvertebrates and freshwater microorganisms are among the most important bioindicators.

One of the key biological elements for assessing the quality of water is macrozoobenthos. Macrozoobenthos is a community of macroscopic invertebrates that inhabit the bottom of aquatic ecosystems (Rosenberg and Reish, 1993). The largest group of the macrozoobenthos community consists of aquatic insect larvae. Various indices of quality and diversity have been developed, which, based on the structure of the macrozoobenthos community, provide a fairly reliable assessment of water quality. The aquatic ecosystem is never static, it is in a constant dynamic stage in which complex feedback mechanisms control the activity and abundance of microbes and other hydrobionts (Sladaček, 1973).

Bacteria with their enormous metabolic potential break down and transform non-living organic matter (Dalmacija et al., 2005). Research has shown that the number of bacteria changes under the influence of changed physical and chemical indicators in the watercourse. Various types of diseases occur by consuming untreated water (Yau, 2003). Aquatic pathogens can be divided into three main categories: viruses, bacteria and parasites (Bridle, 2014). As determinants of the presence of pathogenic organisms in water, the following microorganisms are used as bioindicators (WHO, 2011): the total number of heterotrophic bacteria or the total number of coliform bacteria, thermotolerant coliforms or Escherichia coli and index and model organisms - (e.g. E. coli which serves as an index for Salmonella species and F-RNA coliphage as a model of human enteric viruses).

The investigated area is located in the central part of Bosnia and Herzegovina, in the Sarajevo Canton, Ilijaš Municipality. The Misoča River is the most significant tributary of the Bosna River (Nefić, 1997) and it's formed by the confluence of Blaža River and Kunosički Brook under the mountain Okruglica. Misoča river, as a main water supply source for the municipality of Ilijaš is protected by the Federal legislation (Odluka u o zaštiti izvorišta vode za piće Mahmutovića rijeka i rijeka Misoča)

The aim of this research was to evaluate the complementarity, concordance and correlation of the analysis of several parameters for the purpose of assessing the water quality of the Misoča River. All the mentioned factors are part of the ecological factors, biotic and abiotic, of the investigated river, and at the same time reliable indicators of the quality of surface waters. Multidisciplinary approach in the context of use of several aspects/parameters of the analysis should enable to determine the degree of expediency of such analyses. The results of literature review indicate that the river Misoča was sporadically investigated.

One of the objectives was to assess the impacts of various human pressures.

2. Material and Methods

2.1. Field investigation

Water sampling was carried out in July 2020 at two sites: site 1 (S1) - near the dam and site 2 (S2) - near the bridge. Site 1 is a few kilometers upstream from the Misoča River dam, at geographic coordinates 43.96164°, 18.30762°. Site 2 is near the bridge and is located in a populated area. The geographical coordinates of this locality are 43.95725°, 18.29587° (Figure 1.).

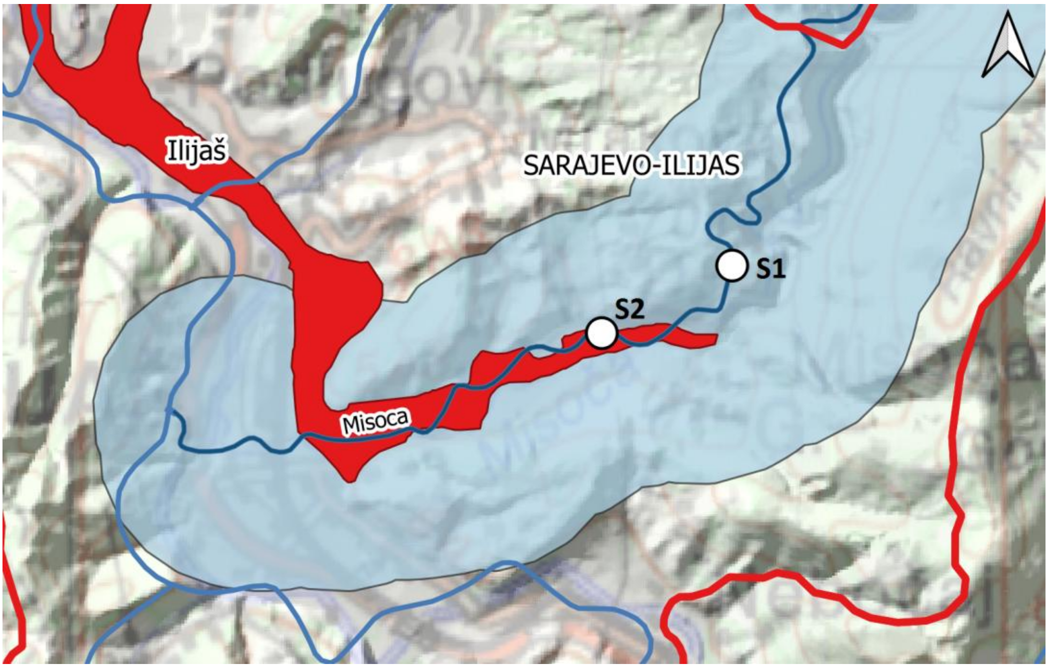


Figure 1. Research area – site 1 (S1) - near the dam and site 2 (S2) - near the bridge

The biotic data sampling was in accordance with the Water Framework Directive. A "kick sampling" method was used to sample macrozoobenthos in the studied parts of the Misoča river watercourse. Samples collected in the field were stored in plastic containers, labeled and fixed with 70% ethanol. Water samples for microbiological analysis were sampled in accordance with BAS EN ISO 19458 and transported in a hand-held refrigerator to the Water Microbiology Laboratory at the Faculty of Science, University of Sarajevo. Laboratory research included analysis of macrozoobenthos and microbiological analysis of collected samples from certain localities.

2.2. Laboratory analyses of macrozoobenthos

The analysis of macrozoobenthos samples was performed in the Laboratory for Systematics, Hydrobiology and Evolutionary Entomology at the Department of Biology, Faculty of Science, University of Sarajevo. After separation of the preimaginal stages of the macroinvertebrates using a stereozoom microscope, it was approached to further taxonomic analysis using relevant identification keys: Eliot et al., 1988; Nagel, 1989; Waringer & Graf, 2013; Kriska, 2014. Early-juvenile stages or damaged individuals have not been identified. The number of identified specimens was recorded. Number of taxa and individuals was analyzed in Microsoft Office Excel and PRIMER (version 6.1.16). The parameter that define the characteristics of a community is ratio of number of species to total number. The Shannon-Weaver index (Fedor et al., 2013) was used to determine the diversity of macroinvertebrates at the investigated sites. Saprobiological analysis was performed using the Pantle - Buck saprobic index. The Family Biotic Index was also applied.

The Shannon-Weaver diversity index is based on information theory (Shannon and Weaver, 1949). Water category values were estimated according to Liebmann (1962).

The Pantle-Buck index is defined on the basis of the representation and sample value of each species (Pantle and Buck, 1955). The given index is based on the total tolerance of the species that make up the community to a certain saprobic level (Stoyanova, 2010).

The family biotic index is also used to analyze water quality based on the composition of macrozoobenthos communities. The values of the index range from 0 to 10, with higher values indicating a higher degree of organic pollution (Hilsenhoff, 1988).

2.3. Micorbiological analyses

Microbiological analysis was performed within 24 hours, using standard procedures and methods. To determine the total number of aerobic/facultatively anaerobic heterotrophic bacteria (cfu/ml at 22ºC/68h) and mesophilic bacteria (cfu/ml at 35 ±1 0C/48h), the seeding method in an agar plate was used in accordance with BAS EN ISO 6222. Detection and determination of the number of total coliform bacteria and faecal (thermotolerant) coliform bacteria, i.e. *Escherichia coli*, the membrane filtration method and methods in accordance with ISO 9308-1:2014 were used. To determine the total number of coliform bacteria, a chromogenic coliform agar medium was used, and the plates were incubated at a temperature of 35ºC. The total number of thermotolerant coliform bacteria was determined by seeding the membrane filter on M-TEC agar, after which the petri dishes were incubated at a temperature of 44.0±0.5ºC. To confirm the presence of Escherichia coli on the suspected colonies, Kovač's reagent was applied, and the appearance of a red color was confirmation of the formation of indole and a positive reaction. To confirm *E. coli*, the reaction of the oxidase activity was determined by transferring a single suspect colony of the bacterium to a strip for determining the reaction with sterile gauze. After five seconds, the absence of dark blue color on the strip indicated a negative reaction that is characteristic of the E. coli bacteria. The total number and detection of fecal enterococci in 100 ml was performed by membrane filtration according to the ISO 7899-2:2000 method. Bile esculin azide agarand an incubation temperature of 44.0±0.5ºC were used to determine fecal enterococci.

3. Results and discussion

A total of 80 individuals from 12 families were recorded, and 10 species belonging to the genera: *Helobdella, Haptogenia, Rhitrogena, Leuctra, Beatis, Elmis, Ecdyonurus, Libellula* and *Elmi* were identified. The species *Leuctra sp*. dominates in both sites (S1 - 13, S2 - 15), followed by the species *Haptagenia sp.* (bridge -10, dam -6) and Baetis sp. (S1 - 5, S2 - 6). The species *Gammarus balcanicus, Hydropsyche sp., Cheumathopsyche sp., Hellobdela sp*. and *Elmis sp*. have the lowest abundance (both sites count only one individual of the given species) (Table 1).

According to Nahić (2020), 26 species of the *Ephemeroptera* order were found at the "Strmina" site of the Misoča River classified into 14 families, where the total number of individuals was 960. The most dominant species at the "Strmina" site were: *Paraleptophlebia submarginata* with a participation of 27%, then the *Ephemerella notata* with 26%. The species *Leuctra braueri* with a participation of 6% was more dominant in the autumn season, while within the order *Trichoptera* the species *Hydropsyche angustipennis* prevailed with 2%. Considering that the research was done during two seasons, spring and autumn, during the spring period the most dominant species is *Ecdyonurus venosus* (36%). Within the order of *Plecoptera*, the dominant species is *Perla marginata* (2%), while in the order of *Trichoptera*, dominant role plays *Hydropsyche botosaneuanui* (4%). During both seasons, the order *Ephemeroptera* prevails with 86%, while *Plecoptera* and *Trichoptera* have an equal share in the sample of 7%.

Table 1. Qualitative-quantitative analysis of the macrozoobenthos of the inhabited site (S2, bridge) and uninhabited site (S1, dam)

Taxa	**S1**	**Taxa**	**S2**
Rhynchobdellida		Amphipoda	
Glossiphoniidae		Gammaridae	
Helobdella stagnalis	1	*Gammarus balcanicus*	1
Ephemeroptera		Ephemeroptera	
Heptagenidae		Heptagenidae	
Heptagenia sp.	10	*Heptagenia sp.*	6
Rhitrogena sp.	4	*Rhitrogena sp.*	3
Ecdyonurus sp.	2	*Ecdyonurus sp.*	3
Baetidae		Baetidae	
Baetis sp.	6	*Baetis sp.*	5
Plecoptera		Plecoptera	
Leuctridae		Leuctridae	
Leuctra sp.	15	*Leuctra sp.*	13
		Trichoptera	
		Hydropsychidae	
		Hydropsyche sp.	1
		Cheumatopsyche lepida	1
Diptera		Diptera	
Chironomidae	1	Chironomidae	3
Coleoptera			
Elmis sp.	1		
		Odonata	
		Anisoptera	
		Libellulidae	
		Libellula sp.	4
Total of individuals:	**40**		**40**
Total of species:	**8**		**10**

The largest recorded number of individuals at site 1 belongs to the family *Leuctridae* with the species Leuctra sp. which counts 15 individuals, followed by the family *Heptagenidae*, with the species *Heptagenia sp*. with 10 individuals (Table 1.). Also, families such as *Baetidae* are present, with the species *Baetis sp*. which counts slightly fewer individuals (6), while the species *Rhitrogena sp.* counts 4, and *Ecdyonurus sp.* 2 individuals. The species *Helobdella stagnalis* and *Elmis sp.*, with only one individual each, and the family *Chironomidae*, where also only one individual was detected, have the lowest number of recorded individuals.

The family *Leuctridae*, with the species *Leuctra sp.* represents the leading link in the chain of zoobenthos of the uninhabited area (S1), where a total of 13 individuals were detected. The family *Heptagenidae* is represented with the species from genus with a representative of the species *Heptagenia sp.* (six individuals) (Table 1.). Also, species such as *Rhitrogena sp., Ecdyonurus sp.,* and the *Chironomidae* family are represented in a very small percentage. However, the smallest number of individuals is in the family *Gammaridae* with the species *Gammarus balcanicus*, and the family *Hystodroppsychidae*, with the species *Hydropsyche sp*., and finally the species *Cheumatosyche lepida*, which count only one present individual.

Based on the calculated indices, an analysis of water quality was performed at the researched sites.

The results have shown that the diversity index for the site „dam“ is 1.6, while for the site „bridge“ it is 1.7. According to freshwater categorization, these results indicate moderately polluted water. However, its primary purpose is to show the level of biodiversity.

The assessment of the water quality of the Misoča River was carried out using the Pantle-Buck saprobic index. In an uninhabited area, the concordance index was 2.14, while in a populated area it was 2.15. The saprobic index is elementary when it comes to water quality assessment, and the categorization according to Liebmann (1962) showed that both sites belong to the second quality class (β-mesosaprobic), i.e. moderately polluted water. Also, this index showed that there is no significant difference in water quality between the two researched sites. Generally speaking, and considering the anthropogenic impact, the water quality is still satisfactory, but it is also stress the need for measures necessary to prevent further deterioration.

The FBI index values calculated according to the number of individuals within macrozoobenthos families are as follows: 2.45 for the inhabited site - the bridge and 3.35 for the other site. According to the classification (Hilsenhoff, 1988), both sites belong to the first (I) class of waters - naturally clean waters., The higher value of this index in the uninhabited area, indicates that it does not play a key role when it comes to the load of the Misoča River. Also, it indicates that the sites are not far enough from each other. The fact is that the dam at/near the other location plays a significant role. Likewise, the values are approximately close for this and the other indices and indicate good water quality of the Misoča River, and in the case of the FBI index, water without significant organic load.

The families: *Haptagenidae, Baetidae, Leuctridae, Chironomidae* play a dominant role in the composition of zoobenthos. It is also important to point out the presence of a large number of individuals from the *Leuctride* family whose taxa tolerance to pollution is 0 (Table 2.).

Table 2. Number of individuals within families of macrozoobenthos at the investigated sites (S1, S2)

Family	Number of individuals (S1)	Family	Number of individuals (S2)
		Gammaridae	1
Glossiphoniidae	1		
Heptagenidae	16	Heptagenidae	12
Baetidae	6	Baetidae	5
Leuctridae	15	Leuctridae	13
Chironomidae	1	Chironomidae	3
Elmidae	1		
		Hydropsychidae	2
		Libellulidae	4
Total of individuals	**40**		**40**

Table 3 shows the comparative values of various indices obtained on the basis of macrozoobenthos composition for the two researched sites, the dam and the bridge. The obtain results show slight difference between the two sites. Area around S2 (the bridge) is more populated than the other, but both sites are under certain anthropogenic pressure. At the S1 (the dam), there are some other factors that influenced these values. However, all the analyzed indices show a good state of the water quality of the Misoča River (II or I class), as well as the diversity index.

Table 3. Correlative relations of the applied indices of diversity and quality

ANALYSIS	S1 – DAM	S2 – BRIDGE
Shannon-Weaver index	1.6	1.7
Pantle-Buck saprobic index	2.14	2.15
Familial Biotic Index	3.35	2.45

The results of the microbiological analysis of water at the researched sites of the Misoča River are shown in table 4, as well as the total number of heterotrophic bacteria at both localities. The obtained results are presented as the number of colonies in 1 ml of sample. The recorded number of colonies of heterotrophic bacteria was significantly higher in the populated area and amounted to 1210 cfu/ml, while 555 cfu/ml were counted in the unpopulated area.

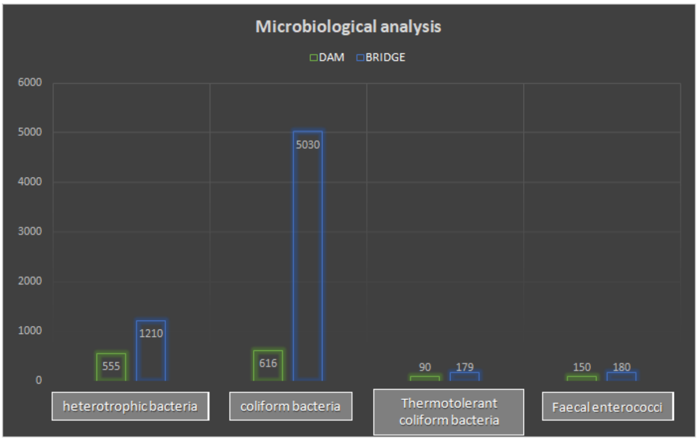
Based on the number of heterotrophic bacteria, both sites have the belong to the second class of surface water, and the number of colonies in the site 2 is significantly higher, which indicates a much higher load of organic matter compared to the site 1. Coliform bacteria, *Escherichia coli* and *Enterococcus sp*., which are significant indicators of fecal contamination of water, were isolated at both sites. The total number of coliform bacteria ranged from 616 cfu/100 ml at site 1, and 5030 cfu/100 ml at site 2. *Escherichia coli* is an indicator of faecal contamination originating from homeotherms, and the recorded values ranged from 260 cfu/100ml at site 1 and 3754 cfu/100ml in water samples from site 2. The total number of coliform bacteria and coliform bacteria of fecal origin *or E. coli* are indicators of fecal contamination.

According to Kohl (1975, modified), Kavka and Poetsch (2002, modified) and according to the EPCEU directives 2006/7 EEC, the recorded values of coliform bacteria indicate a moderate pollution of the tested liquid. Fecal enterococci are good indicators of old fecal load (Đug et al., 2020). The number of fecal enterococci ranged from 150 cfu/100ml in the first site, and 180 cfu/100ml in the water in the second site. Based on the recorded values of fecal enterococci, both localities belong to moderately polluted waters (Kohl, 1975; Kavka and Poetsch, 2002; EPCEU directives 2006/7 EEC). It is important to note that the growth tendency of coliform bacteria, *Escherichia coli* and faecal enterococci was recorded in the site located in the lower course of the examined stream and near the inhabited place. Since surface waters are not a natural habitat for fecal enterococci, their presence is considered to be precisely the result of fecal pollution (SCA, 2015). These results tell us that fecal enterococci can be the result of animal excrement, as well as the possibility of sewage spills. It is important to note that these bacteria are highly tolerant to different environmental conditions and less sensitive to certain disinfectants, so they are indicators of the potential presence of enteroviruses and parasitic protozoa (Gajević et al., 2021).

Table 4. Results of microbiological parameters at the researched sites

Site	Total heterotrophic bacteria (cfu/ml)	Total coliform bacteria (cfu/100 ml)	Total thermotolerant coliform bacteria (cfu/100 ml)	Total faecal enterococci (cfu/100 ml)
S1 (dam/uninhabited area)	555	616	90	150
S2 (bridge/inhabited area	1210	5030	179	180

Graph 1 shows the results of analyzed microbiological parameters at both sites. The results showed the highest number of coliform and heterotrophic bacteria for the site 1 (bridge), which belongs to the uninhabited part. The results of the total number of fecal enterococci also indicate greater fecal pollution in S2, i.e. in the inhabitated area.



Graph 1. Correlative relations of microbiological parameters

Hydrobiological methods for assessing water quality are based on determination of the degree of compatibility, the degree of diversity and the biotic index (Trožić-Borovac, 2011).

According to the overall results of the hydrobiological analysis, the water of the Misoča River belongs to the β-mesosaprobic category, which shows moderate pollution. This is also shown by the water classification based on microbiological parameters, according to which the water meets the quality of the second class of surface waters, that is, it belongs to the class of waters with moderate pollution. This matching of results also shows certain level of confirmation. When it comes to water quality assessment, it is most reliable to analyze several biological elements of water quality. In addition to confirming the results in this way, it also compensates shortcomings of the methodology of all elements, as well as the small number of sites and one-time sampling, which was the case in this study.

When it comes to load and pollution of freshwaters macroinvertebrates and microorganisms are the most reliable elements. The results of physical and chemical analyzes are undoubtedly important and must be included in every analysis of surface water, but it is known that they only show us the current state, which can be unreliable due to various circumstances. Although the presence of prohibited concentrations of certain bacteria also shows the current situation, it is clear that this has longer-term consequences and indicates anthropogenic impact.

4. Conclusions

On the basis of the obtained results of microbiological and hydrobiological analysis, it could be concluded that the water of the river Misoča belongs to the second category of surface water quality, that is, to the class of waters with moderate pollution. The results indicate that the water on the analyzed sites is suitable for swimming, recreation, various water sports, as well as for the cultivation of less noble fish species, i.e. cyprinids. Also, after certain treatments, this water can be used to supply the settlement with drinking water and in the food industry. It is important to emphasize that the site 2 (bridge) is heavily loaded with organic and fecal pollution. Given that this locality is located downstream and near the settlement, we believe that precisely these points are the sources of pollution. It is also important to point out that we expected greater differences in the results of the analyzes between sites. However, the fact is that the S1 (uninhabited area) is also under a certain anthropogenic pressure, firstly due to the proximity of the picnic area, and especially in the period when the research was carried out.

It was recorded that all the examined microbiological parameters had a tendency to increase on the longitudinal profile which indicates pollution sources of anthropogenic origin. The finding of Escherichia coli and intestinal enterococci indicates fecal pollution due to the discharge of raw wastewater or from diffuse pollution. Therefore, it could be concluded that substances of fecal origin reach the Misoča River, especially in the lower reaches, which indicates a correlation with the presence of pathogenic microorganisms that can cause diseases in humans.

The general condition of the water quality on the researched sites of the Misoča River, which indicates a slight load and pollution, was one of the goals of this study. Since it was confirmed through the analysis of two groups of significant water quality elements, and considering the importance of the researched area, it is clear why this river should be protected. Research and biomonitoring should continue. By matching the analysis of two significant parameters, the main goal of the work was realized, and thus the level of reliability of the results was moved to a higher level.

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