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### Fecal Bacteria as Bioindicators of the Quality of Freshwater Ecosystems

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#### Abstract

Monitoring of bacteria in surface and underground waters is used to detect the presence of pathogenic organisms. As the majority of microbial pathogens present in natural waters are of fecal origin, detection of fecal contamination of water is the main goal of water testing. The organisms most often used for microbiological monitoring are fecal indicators: bacteria that indicate the presence of fecal contamination of human or animal origin. Indicator bacteria can survive in the environment for a long time and thus provide a reliable indication of fecal contamination long after they have been

expelled from the digestive tract. This paper aims to assess the current state of water quality of the Buna River and to examine the influence of municipal wastewater on selected quality parameters. As microbiological indicators of water quality, within the scope of this research, the numbers of total coliform bacteria in 100 ml of water, *Escherichia coli* in 100 ml of water and fecal streptococci in 100 ml of water were analyzed. The results show that the concentration of total coliform bacteria is quite high, and in certain localities above the permitted limit.

**Keywords:** Thermotolerant Coliforms, Bioindicators, Water Quality

#### 1. Introduction

The quality of natural waters is one of the limiting factors for their use and maintenance of the natural balance of the environment. The most common human pathogens, coliform bacteria, are found in the gastrointestinal tract of warm-blooded animals. Historically, coliform organisms, especially *Escherichia coli*, have been used as indicators of fecal contamination of water and food (WHO, 2003; 2005) <sup>[5, 6]</sup>. *Escherichia coli* is the main thermotolerant coliform (97%) from the Enterobacteriaceae family. It was chosen as an indicator of fecal contamination of water resources, because it is the only thermotolerant coliform that is exclusively of fecal origin and does not reproduce outside its natural habitat - the intestinal tract of humans and animals, while other coliforms have the power of survival and growth outside the human or animal digestive tract (Prescott *et al.*, 2002) <sup>[1]</sup>. Fecal streptococci or enterococci are Gram-positive, elliptically elongated cocci, arranged in pairs and short chains. They have a group-specific polysaccharide and belong to Lancefield's group D. They do not create hemolysis zones on blood agar (Prescott *et al.*, 2002) <sup>[1]</sup>.

The subject of this research is the assessment of the water quality of the Buna River based on bacteriological parameters. Buna is a significant tributary of the river Neretva, the largest tributary of the Adriatic sea from the Balkans (Ridanovic & Ridanovic, 2016) <sup>[2]</sup>. Its source rises from under rocks several hundreds of meters high. It originates from the karst spring, located under the steep limestone. The spring's capacity is 43 m<sup>3</sup> of water per second, making it one of the strongest karst springs in Europe. It is a protected natural resource and a geomorphological phenomenon (Fig 1). Buna is an important trout hatchery. This area is also known for its diversity of flora and numerous endemic species. At lower altitudes there are many evergreen plant and deciduous species, while at higher altitudes in the hills there is a sparse forest. This paper will analyse bacteriological parameters with the aim of better understanding the water quality of the Buna River.



Fig 1: Source of Buna

**2. Materials and methods**

The length of the Buna River is 9 km. A total of four sites was selected on its watercourse, while two sites were chosen on the Neretva River (Fig 2), before and after the Buna's

estuary. Sampling was conducted during spring. Laboratory water analyses were carried out at the Institute for Public Health FBiH, by BAS EN ISO 9308-1/2015 method.

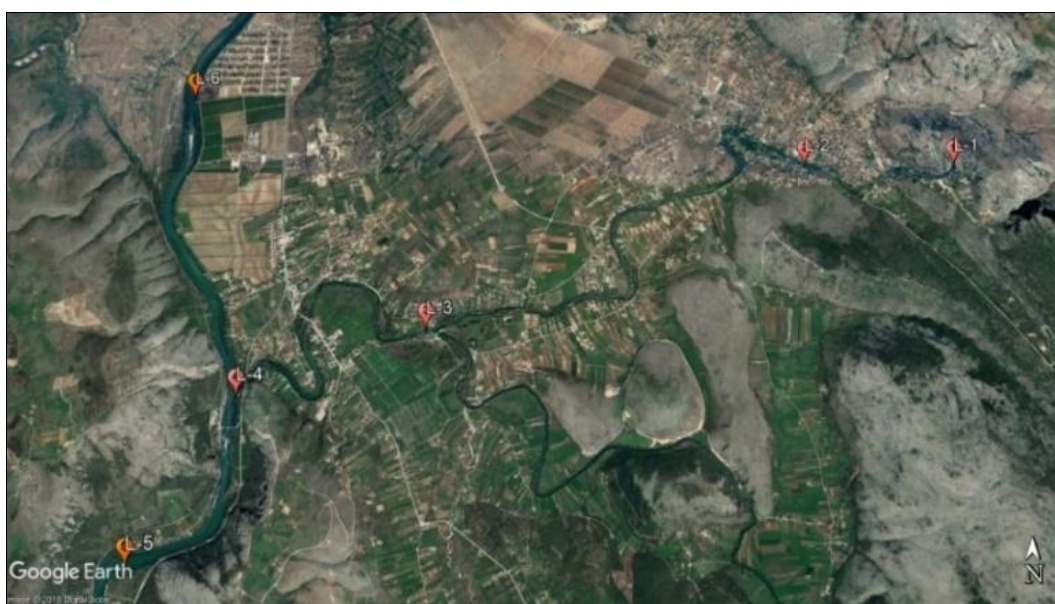


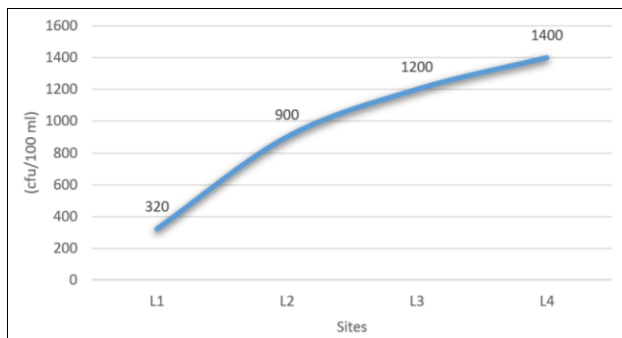
Fig 2: Research area: www.Google Earth Pro.com

**3. Results and discussion**

Bacteriological water pollution is a serious type of pollution. It indicates inadequate treatment of wastewaters, and can result in epidemiological diseases. The risk to human health arises from the presence of pathogenic microorganisms. Many of these microorganisms originate from water contaminated with human and animal feces, which may contain various enteric pathogens that cause disease in the community (WHO, 2004) [4]. Table 1 shows analytical values of tested parametres.

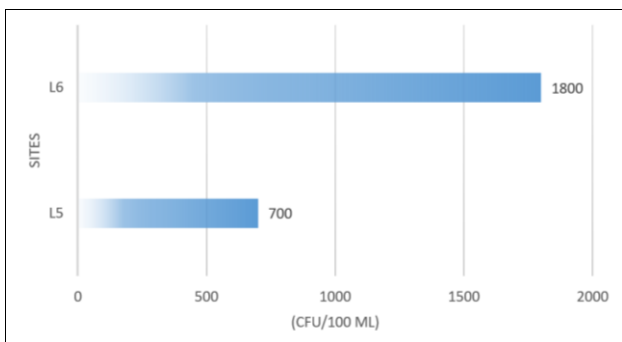
Table 1: Analytical Bacteriological parametres of water quality

	L1 (Buna)	L2 (Buna)	L3 (Buna)	L4 (Buna)	L5 (Neretva)	L6 (Neretva)
Total coliforms (cfu/100 ml)	3,2 x 10 <sup>2</sup>	9 x 10 <sup>2</sup>	1,2 x 10 <sup>3</sup>	1,4 x 10 <sup>3</sup>	7 x 10 <sup>2</sup>	1,8 x 10 <sup>3</sup>
<i>Escherichia coli</i> (cfu/100 ml)	2,1 x 10 <sup>2</sup>	60	80	50	1,9 x 10 <sup>2</sup>	1,5 x 10 <sup>2</sup>
Fecal streptococci (cfu/100 ml)	10	40	8	10	90	5,8 x 10 <sup>2</sup>



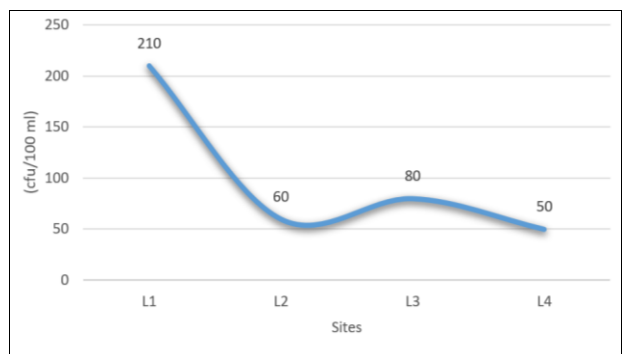
**Graph 1:** Concentration of total coliforms in the river Buna (cfu/100 ml)

The number of total coliform bacteria in the Buna River (Graph 1) ranges from 320 (cfu/100 ml) to 1400 (cfu/100 ml), with an average value of 955 (cfu/100 ml). The lowest value of coliform bacteria was recorded at L1 (source) 320 (cfu/100 ml), and the highest value at L4 (estuary) 1400 (cfu/100 ml).



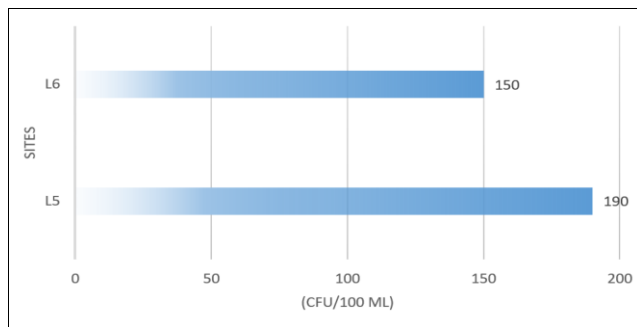
**Graph 2:** Concentration of total coliforms in the river Neretva

The number of total coliform bacteria at selected sites in the Neretva River is quite different. At L5 their concentration is 700 (cfu/100 ml), and at L6 it is 1800 (cfu/100 ml).



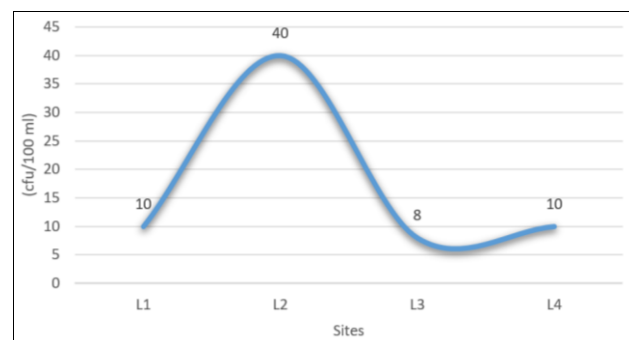
**Graph 3:** Concentration of *Echerichia coli* in the river Buna

Graph 3 shows the results of the analysis of the presence of *Echerichia coli* in the samples. It is possible to notice that its presence at L1 (source) is quite different from other sites. At this site, its highest abundance was recorded (210 cfu/100 ml), while at other sites its concentration was much lower. The lowest value of *E. coli* was recorded at L4 (50 cfu/100 ml).



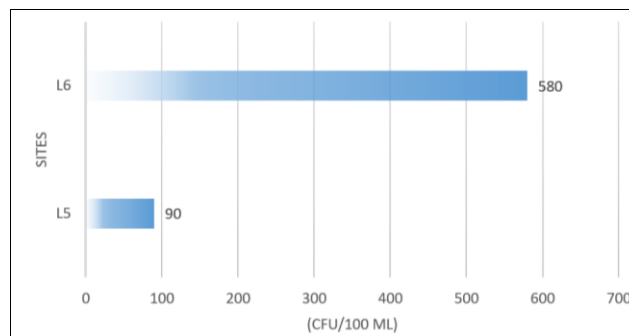
**Graph 4:** Concentration of *Escherichia coli* at river Neretva

The concentration of *Escherichia coli* is much higher on the Neretva River in contrast to the Buna River, although its concentration at the source of the Buna was greater. At L5 it is 190 (cfu/100 ml), and at L6 150 (cfu/100 ml).



**Graph 5:** Concentration of fecal streptococci at river Buna

The lowest concentration values of faecal streptococci were recorded at L3 with a total of 8 (cfu/100 ml). At sites L1 and L4, 10 (cfu/100 ml) of faecal streptococci was recorded. The largest value was noted at L2, 40 (cfu/100 ml).

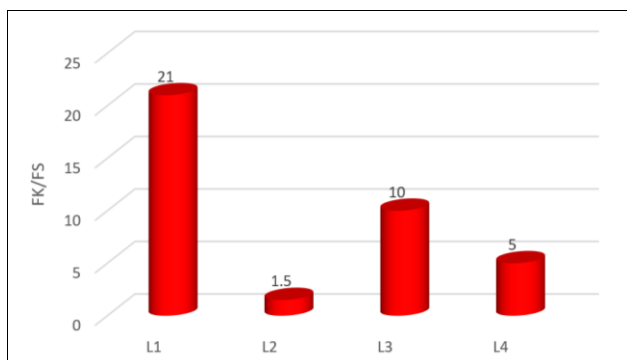


**Graph 6:** Concentration of fecal streptococci at river Neretva

The concentration of fecal streptococci on the Neretva River is drastically different from their concentration on the Buna River. At L5 (Boškovi Kanali) their values are 90 (number/100 ml), while at L6 (Bačevići) they are 580 (number/100 ml).

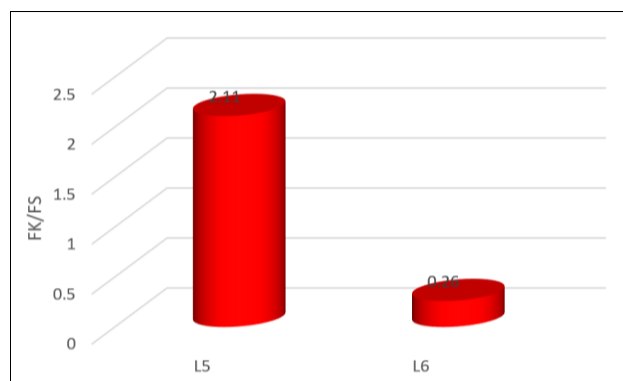
Ratio of faecal coliforms and fecal streptococci  
The correlation between faecal coliforms and fecal streptococci indicates the source of the pollution, i.e. suggests whether the pollution is of animal or human origin.

It is expressed by the quantitative value of fecal coliforms/fecal streptococci. If the obtained values are greater than 4, the pollution is of human origin. Values less than 0.7 are attributed to animal origin, while values between these results are a gray zone of uncertain interpretation (Ridanovic & Ridanovic, 2017) [3].



**Graph 7:** Ratio of fecal coliforms and fecal streptococci at river Buna

Graph 7 shows the values of the ratio between fecal coliforms and fecal streptococci on the Buna River. It can be noted that the results obtained at the locations L1, L3 and L4 show pollution of human origin. At the L2 site, the pollution is of uncertain interpretation.



**Graph 8:** Ratio of fecal coliforms and faecal streptococci at river Neretva

Graph 8 shows the values of the ratio between fecal coliforms and fecal streptococci on the Neretva River. It can be noted that the pollution at the L5 location is of uncertain interpretation, while at the L6 location the pollution is of animal origin.

As microbiological indicators of water quality, the numbers of total coliform bacteria in 100 ml of water, *Escherichia coli* in 100 ml of water and fecal streptococci in 100 ml of water were analysed. The results show that the concentration of total coliform bacteria is quite high, and at certain sites above the permitted limit. However, the lowest concentration of total coliform bacteria (320 cfu/100 ml) was measured at site L1, which is located at the very source of the Buna River. At downstream sites there was an increase in the concentration of total coliform bacteria (900 cfu/100 ml). This increase can be explained by the proximity of the fish farm. Surplus food, which the fish fail to consume, goes beyond the borders of the pond, overflowing into the river's watercourse, which makes a suitable ground for the development of colonies of various

microorganisms. Also, the dead fish is a substrate for the development of microorganisms that accumulate in the area downstream from the pond. The concentration of total coliforms continued to rise. At L3 their concentration was 1200 cfu/100 ml, and at L4 (estuary) 1400 cfu/100 ml.

At the two sites on the Neretva River, the values are also different. At L5, the results of the analysis show 700 cfu/100 ml, which is slightly above the limit of class I watercourse, which is 500 cfu/100 ml. Based on the results, site 6 has the highest concentration of total coliform bacteria (1800 cfu/100 ml).

The concentration values of another microbiological parameter, *Escherichia coli*, are also quite high. Their highest concentration (210 cfu/100 ml) was recorded at the source itself, primarily due to the multitude of catering establishments located in this locality. It can be suspected that wastewater is discharged directly into the river bed. This is supported by the result of the FK/FS correlation ratio, which is 21. A fairly high score indicates that the fecal pollution in this area is of human origin. In the middle and lower reaches of the Buna River, a decrease in the concentration of *E. coli* was observed, compared to the first site. At L2 (below the pond) the concentration was 60 cfu/100 ml, suggesting that the upper course of the Buna River has greater bacteriological load compared to the lower course. At the estuary L4, the lowest concentration of *E. coli* was recorded at 50 cfu/100 ml. A sharp increase in concentration is observed at sites on Neretva river. At site L5 the concentration of *E. coli* was 190 cfu/100 ml, while at L6 it was 150 cfu/100 ml. This effect of an increase can be explained by the proximity of residential buildings of the local population to the localities chosen for the research.

The total concentration of *E. coli* and faecal streptococci is almost identical. However, there is a difference in their distribution across sites. Fecal streptococci are present in fairly small amounts at almost all sites on the Buna River. A sudden increase in concentration was recorded at sites on the Neretva River. The highest concentration on the Buna River was recorded at L2 (40 cfu/100 ml), which can be explained by the immediate proximity of the fish farm's pond. It is known that animal feces contain 1.4 times more faecal streptococci than faecal coliforms. At other sites on the Buna river, the maximum values were 10 cfu/100 ml, recorded at L1 and L4. There is a noticeable increase in the concentration of faecal streptococci downstream of the estuary. In this area, there are many plots of arable land as well as meadows that are used for livestock grazing. The feces of these animals break down during rainy periods and thus indirectly end up in the river. This is supported by the result of the FK/FS correlation ratio, which is 0.26. The result shows that there is pollution of animal origin at this site.

#### 4. Conclusion

Bacteriological parameters show increased levels of indicator bacteria at almost all sites, with an increase along the longitudinal profile. Based on the correlation between faecal coliforms and faecal streptococci it can be noted that at almost all sites at the Buna River, faecal pollution is of human origin. The correlation ratio FK/FS on the Neretva River showed much lower results. So, according to this ratio, the pollution at L6 is of animal origin, and at L5 the interpretation is uncertain.

## 5. Acknowledgement

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