



## THE PRESENCE OF *Escherichia coli* and *Enterococcus* IN THE WATER OF THE FIFTH CANAL AND CRNA RIVER NEAR BITOLA

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### Abstract

Water is a natural resource necessary for all living organisms. In our research, the research material is water. As measuring points for sampling analysis are: measuring point 1 (Crna River near the village of Novaci), measuring point 2 (fifth canal exit of Bitola), measuring point 3 (fifth canal near the village of Kravari) measuring point 4 (fifth canal before flowing into the Crna River), measuring point 5 (Crna River before being mixed with water from the fifth canal), measuring point 6 (Crna River after mixing with water from the fifth canal) and measuring point 7 (Crna River near the village of Skocivir). In order to determine the quality of the water from sanitary-hygienic aspect the following microbiological tests have been made: determining the number of *Escherichia coli* with a membrane filtration method made according to the ISO 9308-1:2000 standard for water quality and determination of the number of *Enterococcus* with membrane filtration according to the ISO 7899-2:2000 standard.

The number of types of microorganisms tested indicates an increase in the number of microorganisms in the canal (measuring point 2, 3 and 4) which, at the measuring point 6 (junction of Crna River with the canal), are transmitted in Crna River. And with that, not only the Crna River is getting polluted, but also the entire environment.

All this indicates that the cleaning of the canal and the watercourse should become an obligation before the competent institutions but also the obligation of each individual is to protect his environment.

**Key words:** *microorganisms, contamination, environment, monitoring, wastewater*

### INTRODUCTION

Water is an irreplaceable natural resource. The biggest pollution that occurs in the aquatic ecosystem is as a result of anthropogenic influence (Blazhevskaa, 2021). The quality of water is determined by physico-chemical, microbiological and radiological parameters. Rivers are a vital resource for irrigation, agricultural, hydroelectric, and recreational purpose (Karunanidhi et al., 2021).

The origin of microorganisms in the water is different and that is air, soil, waste water and parts of living organisms. Wastewater management is a demanding, complex and difficult task, because in addition to technical, technological, ecological and economic sustainability, "management" also implies the sustainability of the development of the urban water system, as

well as the urban environment and society as a whole (Rozić, 2022)

The largest right tributary of the Vardar River is Crna River. Moving away from the source part and flowing into the populated areas, the anthropogenic influence on the Crna River is felt more and more. The fifth canal is filled with water from the river Siva Voda, which is relatively clean mountain water with a low water level, and waste water from the industrial sector located in the City of Bitola and its surroundings flows into it. The purpose of the research in this paper is to examine the current state of quality of the water through the number of *Escherichia coli* and *Enterococcus* in the water of the fifth canal and Crna River near Bitola.

## MATERIAL AND METHODS

In this paper, we examined the quality of water in the fifth canal and Crna Reka. Different locations were selected as target measuring points at the fifth canal and Crna River. Measuring points for sampling analysis are: measuring point 1 (Crna River near the village of Novaci), measuring point 2 (fifth canal exit of Bitola), measuring point 3 (fifth canal near the village of Kravari) measuring point 4 (fifth canal before flowing into the Crna River), measuring point 5 (Crna River before being mixed with water from the fifth canal), measuring point 6 (Crna River after mixing with water from the fifth canal) and measuring point 7 (Crna River near the village of

Skocivir).

The samples were taken in different seasons, spring and autumn, in the months of May and November. Laboratory analyses of the samples were performed by using the standard methods.

In order to determine the quality of water from sanitary-hygienic aspect, the following microbiological tests have been made: determining the number of *Escherichia coli*, with a membrane filtration method made according to the ISO 9308-1:2000 standard for water quality and determination of the number of Enterococcus with membrane filtration according to the ISO 7899-2:2000 standard.

## RESULTS AND DISCUSSION

Non-pathogenic bacteria present in faeces are used to indicate the occurrence of faecal contamination of water and hence the possibility that pathogens may be present (Vincent, 2006). Faecal contamination of water continues to be a major public health concern, with new challenges necessitating a renewed urgency in

developing rapid and reliable methods to detect contamination and prevent human exposures. (David A. Holcomb, 2020)

Table 1 shows the representation of *Escherichia coli* in the measuring points of the fifth canal and Crna River in the months of May and November.

**Table No. 1** Presence of *Esherishia coli* in the water from measuring points of the fifth canal and Crna River in the months of May and November.

<i>E. coli</i> (CFU/100 mL)	MM1	MM2	MM3	MM4	MM5	MM6	MM7
May	30 000	300 000	350 000	300 000	200 000	280 000	200 000
November	45 000	200 000	220 000	250 000	140 000	200 000	150 000

In all examined water samples from Crna River, the presence of *Esherishia coli* was found. The highest number of *Esherishia coli* among the examined samples was found at measuring point 6 (280,000 bacteria/100 mL) in the month of May after the inflow of faecal and industrial waste water from the fifth canal. According to the representation of faecal coliform bacteria, according to Kavka (2006), the water in this measuring site is in V class. The temperature of the water during this period is correlated with the number of *Esherishia coli*, which has a stimulating effect on their growth and development. The number of *Escherichia coli* at measuring point 7 is reduced compared to measuring point 6 (150,000 in November and 200,000 bacteria/100 mL in May). The distance of this measuring place from the inhabited places and the lower population density affects the reduction of the number of *E. coli* in the water of Crna River,

however, the additional intake of contaminated water from the fifth canal does not contribute to a significant reduction of the tested bacteria.

Disturbances from various anthropogenic activities have caused entry of more pollutants into the river, resulting in significant spatial heterogeneity in water quality parameters and bacterial communities (Xianbin Zhu, 2022)

In our research, the lowest number of *Esherishia coli* was found in the water from measuring point 1 (30,000 bacteria/100 mL in May and 45,000 bacteria/100 mL in November). It is a measuring point of Crna Reka before it receives the wastewater from the city of Bitola.

In all examined water samples from the fifth canal, the presence of *Esherishia coli* was found. The highest number was determined in measuring point 3 (350,000 bacteria/100 mL) in the month of May. According to the number of *Esherishia coli* in the measuring points of the

canal, the water is in V class. If we compare these values with the given classification for faecal pollution by Kavka (2006), we conclude that the water is extremely polluted. The reason for the high value, apart from the anthropogenic impact, is the presence of livestock farms in the environment and the discharge of animal waste (Cabral et al., 2010; Boron et al., 2015).

Enterococci are Gram-positive, non-sporogenous, catalase-negative, round-shaped bacteria. The optimal growth temperature for most species is between 35 – 37 °C, but there are some that grow at 42 – 45 °C, and even survive at

60°C for 30 minutes (Cabral 2010; Altinoluk (2014). They are facultatively anaerobic microorganisms, but prefer anaerobic conditions. According to Cabral (2010), soil and surface water do not represent an accessible environment for the development of enterococci, although they are found in them. They enter aquatic ecosystems as a result of contact with faecal waters, compost, waste, etc.

Table 2 presents the number of microorganisms of *Enterococcus* in the measuring points of the fifth channel and Crna River in the months of May and November.

**Table 2.** Representation of *Enterococcus* in the measuring points of the fifth channel and Crna River in the months of May and November.

<i>Enterococcus</i> (CFU/100 mL)	MM1	MM2	MM3	MM4	MM5	MM6	MM7
May	30 000	55 000	60 000	60 000	40 000	50 000	30 000
November	42 000	130 000	130 000	140 000	50 000	100 000	150 000

Table 2 shows the representation of the genus *Enterococcus* at all measuring points in Crna Reka water. The highest number of microorganisms of enterococci was observed at measuring point 7 (150,000 bacteria/100 mL), near the village of Skocivir. The high number of enterococci in this measuring point is due to the presence of faecal water originating from septic tanks. The enterococci in measuring point 6 (100,000 bacteria/100 mL) originate from waste and faecal waters coming from the fifth canal. Most of the species of this genus originate from the intestinal flora of mammals, birds, reptiles and man. Some species have been isolated from the urinary tract as well as from infected wounds (Cabral 2010).

The lowest representation of enterococci was found at measuring point 1 (30,000 bacteria/100 mL) in the month of May. Most likely, the higher representation of enterococci in Crna River waters, apart from the inflow of water from the fifth canal, which is loaded with faecal waste water, has an impact they also have atmospheric sediments (rainfall) with the help of which enterococci are brought in from the soil, i.e. the plant material present along the river. According to the Decree on water classification (no. 18/99), the water in measuring points 1, 5, 6 and 7 is in V class.

From Table 2, it can be concluded that streptococci of faecal origin are represented in all measuring points of the fifth canal. Continuous dynamics of increased number of enterococci is observed in almost all measuring points with the exception of the month of May. During this period, lower values of enterococci (55,000 bacteria/100 mL) were found in measuring point 2. Such low values, which are expressed in the month of May compared to the month of November, are most likely the result of the increased amounts of precipitation in May compared to with other periods of the year. The highest values of enterococci were ascertained at measuring points 3 and 4 (60,000 bacteria/100 mL) in November, as a result of the continuous presence of waste and faecal waters in the canal that flow from the settlements, as well as from the livestock farms of the village. The decrease in the representation of enterococci at measuring point 2, where the lowest value of 55,000 bacteria/100 mL was found, apart from increased precipitation, is most likely influenced by the inflow of clean mountain water (Siva Voda) into the beginning of the canal, which although has a small water mass contributes to reducing the number of the examined group of microorganisms.

## CONCLUSIONS

Based on the results obtained during the research, it can be concluded that *Escherichia coli*, as a typical representative of coliform bacteria, is present in the water at all measuring points, in the fifth canal and in Crna River. The highest representation in the waters of the Crna River is at measuring point 6, after the inflow of water from the canal into the river, which is due to the presence of waste and fecal waters flowing in from the canal.

The genus *Enterococcus* is considered as a bioindicator that indicates the contamination of water with pathogenic microorganisms. The

presence of enterococci was ascertained in all measuring points, in the fifth canal and Crna River.

As a general conclusion, it can be stated that water pollution is closely related to anthropogenic impact. Specifically, the fifth canal puts a heavy load on Crna River. This research provides guidance for the immediate installation of a treatment plant that will treat industrial and municipal wastewater entering the canal. Cleaning the canal should be the responsibility of the competent institutions.

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## ПРИСУСТВО НА *Escherichia coli* и *Enterococcus* ВО ВОДАТА НА ПЕТТИОТ КАНАЛ И ЦРНА РЕКА ВО ОКОЛИНАТА НА БИТОЛА

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### Резиме

Водата е незаменлив природен ресурс неопходен за сите живи организми и затоа во нашето истражување како материјал за истражување е водата. Мерни места за земање примерок за анализа се: мерно место 1 (Црна Река кај с. Новаци), мерно место 2 (петти канал на излезот од Битола), мерно место 3 (петти канал кај с. Кравари), мерно место 4 (петти канал пред да се влее во Црна Река), мерно место 5 (Црна Река пред да се измеша со водата од петти канал), мерно место 6 (Црна Река по мешање со водата од петти канал) и мерно место 7 (Црна Река кај с. Скочивир). За да се утврди квалитетот на водата, од санитарно-хигиенски аспект, направени се следниве микробиолошки испитувања: одредување на бројот на *Escherichia coli* со метод на мембранска филтрација изработено според стандардот ISO 9308-1:2000 за квалитетот на водата и одредување на бројот на *Enterococcus* со мембранска филтрација според ISO 7899-2:2000 стандардот.

Бројноста на испитуваните видови микроорганизми укажува на зголемување на бројот на микроорганизмите во каналот (мерното место 2, 3 и 4) кои, пак, во мерното место 6 (спојот на Црна Река со каналот), се пренесуваат во Црна Река. И со тоа не се загадува само Црна Река туку и целата животна средина. Сето ова укажува дека чистењето на каналот и речното корито треба да стане обврска пред сè на надлежните институции, меѓутоа и обврска на секој поединец да ја чува својата животна средина.

**Клучни зборови:** микроорганизми, контаминација, животна средина, мониторинг, отпадна вода.