

## WATER QUALITY OF KOSELSKA RIVER AND OHRID LAKE IN THE DALJAN REGION

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### Abstract

The aim of this paper is to examine the state and quality of the water from Koselska river, as well as its influence on the quality of water in the coastal zone, more precisely where the mouth of the river flows into the lake. Also, the goal of this research was to look at the nature and degree of possible water pollution, due to anthropogenic influence, based on the results obtained from the testing of microbiological and physio-chemical parameters, which would make it possible to predict and recommend remedial measures and minimize pollution. To correctly interpret the results of the microbiological analysis of water, physical and chemical tests of water temperature and BOD<sub>5</sub> were also performed.

The microbiological examination included testing the composition and dynamics of heterotrophic, psychrophilic and mesophilic bacteria, determining the index of mesophilic and psychrophilic bacteria (M/P), the index of facultative oligotrophic and heterotrophic bacteria (FO/X), as well as the total number of coliform bacteria. It was determined that the representation and abundance of all types of microorganisms is higher in the river water than in the lake water. The total number of bacteria from Daljan region is greater in the summer period compared to other periods of the year. Likewise, consistently high values of the number of coliform bacteria were determined in the two types of water. Based on the results obtained in this research, it was determined that the water from the river affects the quality, health and safety of the water in Ohrid Lake.

**Key words:** *microbiological properties, bacteria, pollution.*

### INTRODUCTION

Ohrid Lake is the largest potable water basin on European soil because of its clean water. Also, Ohrid Lake is a unique ecosystem in which relict plant and animal species live. The lake is important in relation to tourism, fishing, and the economy. Ohrid Lake has an oligotrophic character but feels the burden of anthropogenic influence. It is threatened by intense mineral and organic pollution that would cause a change in the water quality.

The need for continuous monitoring and research, to assess the water quality, is imperative in the protection of the lake and the maintaining of the water quality at the level of safe drinking water. Moreover, the research on the water quality of the Koselska River, as one of the biggest tributaries of the lake, is one of the important indicators of the condition of the water and the direction for further action in the protection of the lake.

Microorganisms are constantly present in aquatic ecosystems and actively participate in the transformation of organic matter and biochemical processes in the water, and thus in the functioning of the ecosystem as a whole. Microorganisms are the first indicators of eutrophication (Najdenovska & Čolo, 2012; Matzinger et al., 2007).

The microbiological research and categorization of the water in this paper indicates possible organic and faecal pollution. The assessment of the water quality, as well as the physiochemical parameters, gives a complete picture of the condition of the defined aquatic ecosystems and will enable the assessment of the anthropogenic influence on the Koselska River and Ohrid Lake in Daljan region, near the mouth of the river into the lake.

Similar tests related to water ecology in our country were carried out on parts of Black River, near Bitola, for determination of *Escherichia coli* and *Enterococcus* (Blazhevaska et al., 2024). Also, the investigation conducted in the Strumica valley, located in the south-eastern part of North Macedonia, where the content of arsenic, iron, manganese and some other pollutants were investigated in Strumica River by group of authors (Kovacevic et al., 2021).

The purpose of this research is to evaluate the condition and quality of the water in the Koselska River through microbiological and physiochemical indicators and its influence on the quality of the water in Ohrid Lake in Daljan region, near the river mouth.

## **MATERIAL AND METHODS**

The scope of this paper was determination of water quality of the Koselska River and Ohrid Lake from Daljan region, at the mouth of the river into the lake, where on the riverbed of Koselska River before the lake and the coastal zone of Ohrid Lake in the immediate vicinity of the river's mouth. The examination of water quality in the paper included testing the number and dynamics of bioindicator microorganisms from a sanitary and ecological aspect, as well as testing the physiochemical parameters of the water, such as water temperature and BOD<sub>5</sub>. (Strickland & Parsons, 1972). Microbiological analyses of the water included testing of heterotrophic (saprophytes and organotrophs) and oligotrophic bacteria and their ratio (FO/X), mesophilic and psychrophilic bacteria and their ratio (M/P), and total coliform bacteria (Govedarica & Jarak, 2003).

### *Physico-chemical analysis*

For the physico-chemical parameters, the classification was carried out according to the Decree on the categorization of waters (Official Gazette of Macedonia No. 18/99) and the legal regulations on the categorization of surface waters. An assessment of the quantitative-trophic state is calculated according to the trophic state index (Carlson, 1977) while the classification is performed according to the trophic scale (Aizaki et al., 1981).

Sampling for physico-chemical and microbiological research from the coastal zone was carried out based on standard limnological methods, using a Routier bottle (APHA-AWWA-WPCF 1980, 2005). The samples were taken in spring, summer, autumn and winter periods.

Two physico-chemical parameters in Koselska River were examined: water temperature (measured with a reversible depth thermometer Welch, 1948), and biochemical oxygen demand (BOD<sub>5</sub>), determined according to the Winkler's method (Bether 1953, APHA-AWWA-WPCF 1980).

### *Microbiological analyses*

Microbiological analyses were carried out at the Department of Microbiology at Hydrobiological Institute - Ohrid. Liquid and solid media were used to demonstrate the number of microorganisms, general and selective media according to standard methods: APHA-AWWA-WPCF (2005).

The M/P index has been used as an indicator of bacterial contamination from a hygienic point of view. Facultatively oligotrophic bacteria are identified on 10% MPA medium (mesopeptone agar diluted 10 times) and incubated for 5-7 days at 22 °C.

The FO/X index (facultative oligotrophs/heterotrophs) has been used as an indicator of the state and quality of water from a broader ecological perspective.

Heterotrophic (saprophytes, organophytes) bacteria are determined on a standard substrate of mesopeptone agar (MPA) by incubation for 48 hours at 35-37 °C for mesophilic bacteria (M) and 5-7 days at 20-22 °C for psychrophilic bacteria (P).

Total coliform bacteria are determined by the petri plate method (with the dilution method) on a selective chromogenic medium.

## RESULTS AND DISCUSSION

The results obtained from the research of the water quality from the Koselska River and the mouth of the river in Ohrid Lake from Daljan region are presented in this paper. For this paper, microbiological analyses of the examined water from the Koselska River and Ohrid Lake were conducted. Microbiological parameters refer to the microbiological characteristics of the examined water.

Heterotrophic bacteria are important for the biological decomposition of organic substances that play a key role in the release and recycling of nutrients as well as in the processes of natural water self-purification (Najdenovska & Čolo, 2012).

The high value of the total number of heterotrophic bacteria indicates water rich in organic substances, susceptible to bacterial decomposition (Najdenovska & Čolo, 2012). In these researches, according to the obtained results (Figure 1), it was established that there is a constant presence of heterotrophic mesophilic bacteria in the water from the two investigated localities (Koselska River and Ohrid Lake, near Daljan region) and that the number of these bacteria is higher in the water from the river than the lake near Daljan, in all four annual periods. The number of bacteria in the Koselska River is highest in the autumn period (1592 bact./mL) and in the summer period (1249 bact./mL). In Daljan region, the number is relatively low, with the lowest value being determined in the spring period (52 bact./mL), and the highest in the summer season (1104 bact./mL). This situation indicates the strong influence of the river on the lake coastline, especially in the summer period (Figure 1). A similar situation was noted in the research by Lokoska (2015) during the summer season in the localities of the coastline zone of Ohrid Lake. The increased number of mesophilic bacteria in the water in the summer and autumn period is due to the increased water temperatures in the aquatic ecosystems and the increased content of soluble organic substances, as pointed out by Sipkoska Gashtarova et al. (2008) in the research conducted in the Streževo reservoir.

Psychrophilic heterotrophic bacteria, which grow at temperatures up to 220 °C are considered natural aquatic bacteria and their numbers are a good indicator of nutrients available for bacterial nutrition (Malecka & Donderski, 2006).

Figure 2 presents the results for the number of heterotrophic psychrophilic bacteria in the water samples from Koselska River and the water from the coastal zone near the river mouth in the Daljan region.

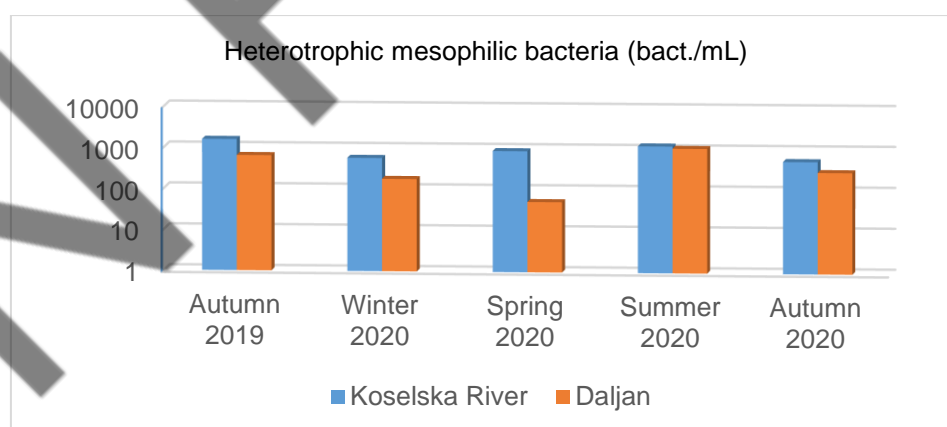


Figure 1. Heterotrophic mesophilic bacteria.

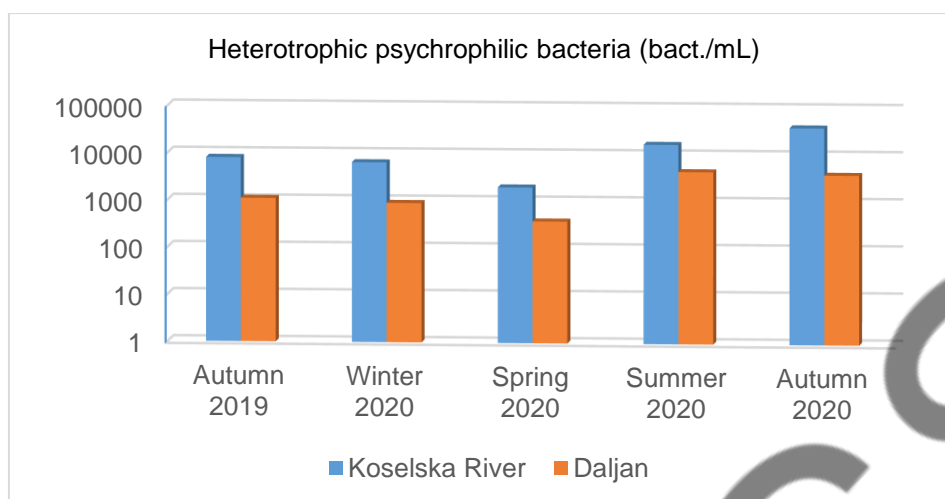


Figure 2. Heterotrophic psychrophilic bacteria.

It can be noted that the psychrophilic heterotrophic bacteria in the Koselska River have higher values in the summer and autumn periods (Figure 2). The highest number was observed in autumn (39840 bact./mL). The same dynamics of the number of bacteria in relation to the season were also determined in the water from the Daljan coastline, with maximum values in the autumn period (3980 bact./mL), and minimum values in the spring (384 bact./mL). In general, it is observed that the values of the number of psychrophilic bacteria in the water from Koselska River are significantly higher than in the water from the coast near the river mouth in Daljan region.

Significantly higher values of psychrophilic bacteria in the research by Lokoska, (2015) were recorded in the water samples from the tributaries of Ohrid Lake than in the lake coastline, specifically in the summer period. The greater representation of psychrophilic bacteria in water in the summer period is due to the decrease in precipitation and the increase in the concentration of organic substances in the water ecosystem (Stojanova, 2012).

The values of the M/P index as an indicator of bacterial pollution from a hygienic point of view, from Koselska River in all examined periods of the year, are presented in Table 1. M/P values below 0.3 are considered favorable, while elevated values indicate a pathogenic effect, that is, of a dominantly higher representation of mesophilic heterotrophic bacteria that are potentially pathogenic for humans and animals (Petrović et al., 1998).

Table 1. M/P index in water from the Koselska River.

Koselska River	Autumn 2022	Winter 2023	Spring 2023	Summer 2023	Autumn 2023
Mesophilic bacteria (bact./mL)	1592	496	1056	1249	560
Psychrophilic bacteria (bact./mL)	6240	5040	2432	16900	39840
M/P index	0.255	0.091	0.434	0.074	0.014

The M/P index for the Koselska River has the highest value for the spring of 2023 (0.434), a period of intense rains and a greater inflow of water into the river bed from melting snow and drainage water. Similar research was done by Blazevska (2016) in the water of the Crna River, into which the Fifth Canal flows. At the junction of the canal and the Crna River, the M/P index was 5.154, which indicates poor water quality at the point where the Fifth Canal flows into the river. The author explains the reason for this situation with the transfer of waste materials from the canal directly into the river. Table 2 shows the value of the M/P index for the water from the coastline near Daljan.

Table 2. Index of M/P in water from Daljan coastline.

Littoral Daljan	Autumn 2022	Winter 2023	Spring 2023	Summer 2023	Autumn 2023
Mesophilic bacteria (bact./mL)	624	227	52	1104	320
Psychrophilic bacteria (bact./mL)	1024	794	384	3920	3980
M/P index	0.609	0.286	0.135	0.282	0.080

The values of M/P in the water from the Daljan coastline are lower than 0.3 in all investigated seasons, which indicates clean coastal waters, where psychrophilic heterotrophic bacteria dominate over mesophilic ones. An exception to this situation was observed in autumn 2022 (Table 2), when the index has the highest value, i.e. 0.609, but also the number of mesophilic heterotrophic bacteria in this period is significantly higher compared to the other seasons in the samples taken from Daljan.

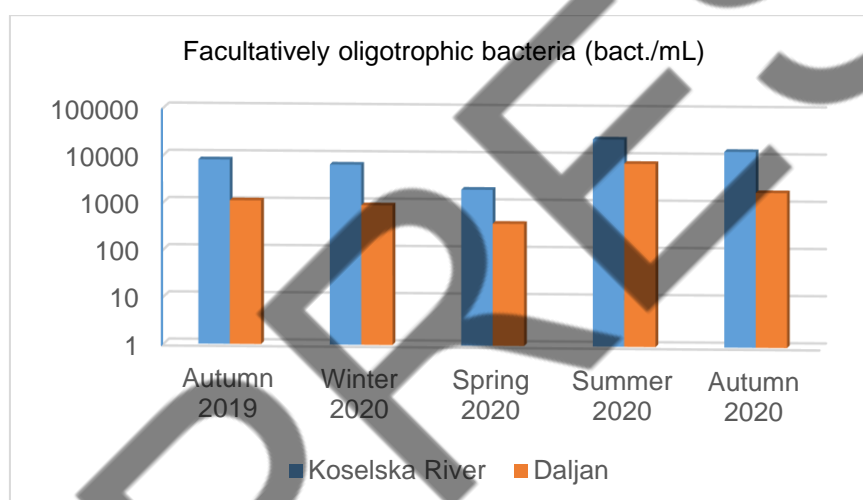


Figure 3. Facultatively oligotrophic bacteria.

In this paper, the representation of facultative oligotrophic bacteria that can live in environments rich in organic matter, but also in an environment with a low concentration of organic matter, was examined. The dominance of these bacteria in water is characteristic of unpolluted water, water with a low concentration of nutrients, that is, water with an oligotrophic character, and water in which self-purification processes are expressed.

Figure 3 shows the values for the number of facultative oligotrophic bacteria in the Koselska River and the Ohrid Lake from Daljan region. The graph presented on Figure 3 shows a higher presence of the examined bacteria in the water from the Koselska River than in the water from the Daljan region. The highest values in the river were determined in the summer of 2023 (24320 bact./mL) and the fall of 2023 (13920 bact./mL) and the lowest value in the spring of 2023 (1692 bact./mL).

The water quality from Daljan region follows the values of the number of facultative oligotrophic bacteria in the river. Hence, for this locality, higher values were measured during the summer of 2022 (7488 bact./mL) and the fall of 2022 (1890 bact./mL). The lowest value of these bacteria was determined in the spring of 2022 (304 bact./mL) both in the water from the Koselska River and from Daljan region.

Veljanoska Sarafiloska et al. (2019), found in their research that in the coastline zone of Ohrid Lake and Prespa Lake, a higher number of facultative oligotrophic bacteria was recorded during the summer period, which is in direct correlation with the increase in nutrients (total nitrogen and phosphorus) and the organic matter in the water. These groups of bacteria

are more prevalent in the water from the rivers in the catchment area of Ohrid Lake (Velgoška, Čerava, Koselska and Sateska) than in the lake coastline near their mouths (Lokoska, 2019). The relationship between facultative oligotrophic bacteria and heterotrophic bacteria (FO/X index) is an indicator of the state and quality of water from a wider ecological perspective. The data obtained from this parameter indicate a greater or lesser ability of self-purification of water ecosystems in the Koselska River (Table 3) and Daljan region (Table 4).

Table 5 presents a proposed characterization for self-purification of water depending on the numerical values obtained for FO/X, based on which the results of the research in this paper will be interpreted.

Table 3. FO/X index for water from the Koselska River.

Koselska River	Autumn 2022	Winter 2023	Spring 2023	Summer 2023	Autumn 2023
Facultative oligotrophic bacteria (bact./mL)	6752	3648	1692	24320	13920
Heterotrophic bacteria (bact./mL)	6240	5040	2432	16900	39840
Index FO/X	1.08	0.72	0.70	1.44	0.35

Table 4. FO/X index for water from Daljan region.

Daljan	Autumn 2022	Winter 2023	Spring 2023	Summer 2023	Autumn 2023
Facultative oligotrophic bacteria (bact./mL)	1038	897	304	7488	1890
Heterotrophic bacteria (bact./mL)	1024	794	384	3920	3980
Index FO/X	1.02	1.13	0.79	1.91	0.48

According to the obtained numerical values for the FO/X - index (Table 5), in this paper, it was established that the values of the index are higher than 1 in Koselska River in autumn 2022 and summer 2023, which according to the proposed classification indicate a satisfactory ability of self-purification of the ecosystem. In the other seasons, the value of the index - FO/X is lower than 1, which means that in those seasons, the studied aquatic ecosystems have a weak self-purification ability.

Table 5. Proposed characterization of the self-purification ability of water depending on the FO/X index (according to Petrovic et al., 1998).

FO/X index value	Self-purification ability of water
<1	Weak
>1	Satisfactory
>1	Good

Coliform bacteria as indicators of faecal water pollution were also investigated in this paper. The value of coliform bacteria in the water from Koselska River and Ohrid Lake was recorded as constantly high. The total number of coliform bacteria in the water from the Koselska River ranges from 18000 bacteria/mL in autumn 2022 to 24100 bacteria/mL in all other seasons.

In lake water from the coastline near Daljan region, the total number of coliform bacteria is significantly lower compared to the river water. The maximum value was recorded in the summer of 2023 (9000 bacteria/mL), and the minimum in the autumn of 2022 (2000 bacteria/mL).

According to Lokoska (2015), faecal pollution is especially pronounced in the lake littoral and near the river mouths, in the summer period. This is due to the introduction of municipal and industrial wastewater rich in pollutants, organic substances, and bacteria into the rivers.

According to the results of the research in this paper on the values of the temperature of the water in Koselska River, it was determined that it is constantly lower than the lake water. The temperature difference between the maximum and minimum values in the Koselska River is lower (9.3 °C), compared to the lake water (12.3 °C).

Also, it was stated that in the summer season, the BOD<sub>5</sub> was recorded, for both localities in the research. The highest value for Koselska River is 3.19 mg/L, while for the Daljan region, it is 3.20 mg/L. This condition corresponds to the intensive processes of mineralization of the organic matter, for which the high temperatures in the summer period represent a stimulating factor.

## **CONCLUDING REMARKS**

Based on the research results obtained in this paper, it has been established that The increased number of heterotrophic bacteria (mesophilic and psychrophilic) during the summer period is in direct correlation with the warming of aquatic ecosystems and the increase of pollutants of organic origin under the influence of people, especially in the summer months, in the peak of the tourist season, when the load is even more pronounced. The quality of water from Koselska River is generally II class, except in summer and autumn 2023 when it is III class. The water from the coastline at Daljan during the research period indicates I class water, and in spring 2023 the water belongs to I class.

According to the value of the M/P index for the Koselska River and the littoral near Daljan, in all the studied seasons it is low, i.e. all below 0.3, which indicates clean waters that are not dominated by mesophilic heterotrophic bacteria. The values for the FO/X index in the water in the lake littoral, during the investigated seasons, have a higher value than the river and according to the proposed classification, generally indicate a satisfactory ability of self-purification of the lake water. In the water of the Koselska River, the number of coliform bacteria is significantly higher compared to the lake water from Daljan region, which indicates an obvious strong anthropogenic influence on the river water, as well as on the water from the coastal zone of the lake. Temperature is the main driver of the intensity and dynamics of the basic processes of circulation of matter in nature and as such affects the biological activity of living organisms.

The water temperature in Koselska River and the lake near Daljan during the research period in this paper is directly proportional to the climatic characteristics of the area, although the water temperature of the Koselska River is lower (9.3 °C) than the lake water (12.3 °C). That is why two periods are differentiated: a period of heating of aquatic ecosystems (spring-summer) and a period of cooling (autumn-winter). The highest biochemical oxygen consumption for five days in the two studied localities was determined during the summer season (3.19 mg/L), and the lowest values for both localities were recorded during the winter period (0.906 mg/L).

In general, it was found that during the researched period, the number of the tested bacteria was higher in the water from Koselska River than in the coastal zone near the Daljan region and in summer period compared to other seasons, which is due to the increase in the temperature of the water in the river and lake ecosystem and directly affects the processes of mineralization of organic substances in water systems.

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## КВАЛИТЕТ НА ВОДАТА ОД КОСЕЛСКА РЕКА И ОХРИДСКО ЕЗЕРО ВО РЕГИОНОТ НА ДАЉАН

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### **Резиме**

Целта на овој труд е да се испита состојбата и квалитетот на водата во Коселска Река, како и нејзиното влијание врз квалитетот на водата во крајбрежниот појас, поточно каде устието на реката се влева во езерото. Исто така, целта на ова истражување беше да се погледне природата и степенот на можното загадување на водата поради антропогено влијание, врз основа на резултатите добиени од тестирањето на микробиолошките и физичко-хемиските параметри, со што би се овозможило да се предвидат и препорачаат корективни мерки и минимизирање на загадувањето.

За правилно толкување на резултатите од микробиолошката анализа на водата, извршени се и физичко-хемиски испитувања на температурата на водата и БПК<sub>5</sub>. Микробиолошкото испитување опфати тестирање на составот и динамиката на хетеротрофните, психрофилните и мезофилните бактерии, одредување на индексот на мезофилни и психрофилни бактерии (М/П), индексот на факултативни олиготрофни и хетеротрофни бактерии (FO/X), како и вкупниот број на колиформни бактерии. Утврдено е дека застапеноста и изобилството на сите видови микроорганизми е поголемо во речната вода отколку во езерската вода. Вкупниот број на бактерии е поголем во летниот период во однос на другите периоди од годината. Исто така, во двата вида вода беа утврдени постојано високи вредности на бројот на колиформни бактерии.

Врз основа на резултатите добиени во ова истражување, беше утврдено дека водата од реката влијае на квалитетот, здравјето и безбедноста на водата во Охридското Езеро.

**Клучни зборови:** микробиолошки карактеристики, бактерии, загадување.