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Sources of the coliform bacteria in the lake Bohinjsko jezero

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ABSTRACT

The lake Bohinjsko jezero is the largest natural lake in Slovenia. It is 4.350 m long, 1.250 m wide and 45 m deep. Nevertheless, biological and chemical indicators show that the inflow of nutrients has been increasing in recent years as a result of tourism. Furthermore, the presence in the lake of coliform bacteria of faecal origin has been detected. In 2006, several locations along the lake shore were controlled to investigate the faecal bacterial contamination of the lake. In 2007, the research was expanded from the lake itself to the affluent of the lake, the Savica river and adjacent high-mountain lakes. The evaluation of coliform bacteria used "the most probable number" (MPN) method. The number of bacteria in water samples varied from 0 to more than 438 per 100-mL sample. The results support the conclusion that part of the faecal coliform bacterial population originates from septic tanks in houses and other dwellings, and a smaller part from pastures, meadows and fields in the lake area.

KEY WORDS:

Coliform bacteria, Water pollution, Sources of pollution, Faecal pollution.

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INTRODUCTION

Each river, brook or lake can accept only a limited amount of substances in waste waters without evident consequences. The self-purification mechanism of natural water is accomplished through physical, chemical and biological processes [1]. Coliform bacteria are excreted with faeces, from which they enter to waste waters and then proceed, through unsuitably organized sewage systems, to natural waters. Faecal bacteria can therefore be an indicator that a water body is polluted with sewage from households, which usually contain waste water from toilets and bathrooms.

The presence of pathogenic bacteria in surface water bodies can represent a serious threat to human health because it can cause illness to those who have been in contact with this water. Some people may become ill even if there are a small number of pathogens in the water. Microorganisms, including bacteria, are typical found in colonies or small groups. A lower number of pathogenic units decrease the danger of infection, but on the other hand, a colony of bacteria represents a bigger threat than a solitary cell if it passes into the body. A human may become infected by the consumption of contaminated water, or by contact during water sports using natural water [2].

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E. Djuikom and co-workers assessed the microbiological water quality of the Mfoundi River with establishing the concentration of the total coliform, faecal coliform, and faecal streptococci. They conducted sampling with the goal of examining the potential origin of faecal contamination and the effect of rainfall on the measured concentrations of indicators organisms. They found high concentrations of total coliform, fecal streptococci, which varied according to the sampling sites and points. The ratio between faecal coliform and faecal streptoccoci shown that waters were contaminated more from warm-blooded animals than humans and according to the correlation analysis the rainfall is a contributing factor, which enhanced the bacterial numbers detected. The authors concluded that water from the Mfoundi River and its tributaries present a great potential risk of the infection for its users [3].

Extensive research has been conducted in many different lakes, seas and rivers that examined the presence, dynamics, distribution and survival of coliform bacteria of the faecal sources [4-7]. Some of these authors reported that the number of bacteria of faecal source increases after a heavy rain [5]. Researchers observed an increase in the number of the faecal coliforms in the Mississippi River after a heavy rain and then recorded a decrease of the bacterial population [6]. Research on Lake Ontario in North America revealed that the excrements of birds are an important source of faecal pollution [8]. A study in China of the activity of the coliform bacteria of the faecal sources revealed their presence not only in the water column, but also in the top layers of the sediments of the three lakes [7].

The lake Bohinjsko jezero is among those water bodies in which problems may occur concerning the presence of the pathogenic coliform

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bacteria. In theory, three ways can be expected how the bacteria may enter the lake: superficial run-off from nearby land, leaking of the cess-pits of summer houses around the lake, and underground transport through the karstic system from mountain lodges. For natural baths, the Slovenian Ministry of the Environment and Spatial Planning regularly prepares annual reports for execution of the monitoring of bathing waters [9]. This program is only executed in locations that are designated as official places for swimming. Other locations along the shores of rivers or lakes are not controlled [10].

The aim of this study is to confirm the presence of the faecal coliform bacteria in Lake Bohinj and determine the potential sources of contamination. The hypothesis is that the main source of pollution is tourism (hotels, alpine cottages, individual holiday houses), and to a less extent agriculture (pastures next to the lake, manuring meadows and fields).

METHODS

Sampling locations

Sampling locations were separated into three groups (Figure 1):

a) The lake Bohinjsko jezero

Seven permanent sampling locations were designated in advance along the lake, which were positioned equidistant and irrespective of tributaries and potential sources of pollution. During June-August, sampling points 1-3 were intensively occupied by swimmers. At the sampling

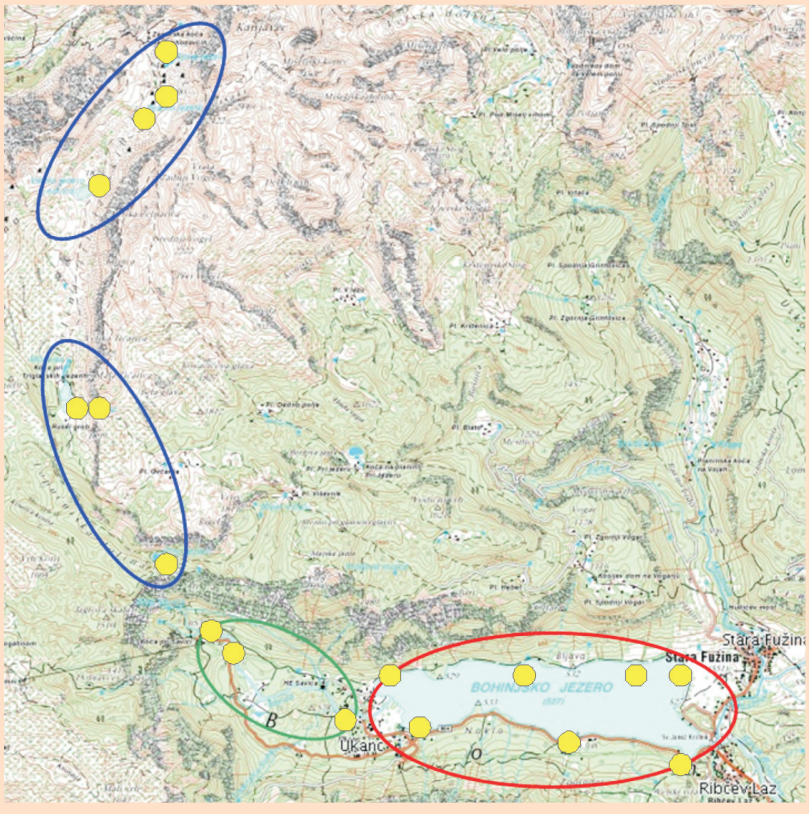

Figure 1:
The lake Bohinjsko Jezero with surroundings [12].

Legend:
- The lake Bohinjsko jezero
- The Savica river
- The Valley of Seven lakes
- Sampling point

point 4, there are many boats for most of the year and numerous tourists during the bathing season. There are also several summer houses in the neighborhood. Sampling points 5 and 6 were the least occupied by the swimmers. The sampling point 7 is officially registered as an area with natural baths [10].

b) The Savica river

Three sampling points were selected on the Savica river. Sampling point 1 was right below the waterfall, and sampling point 2 was located approximately 500 m downward from the waterfall, just behind the cottage Koča pri Savici and its cesspit that had never been officially emptied (according to the householder). Sampling point 3 was right before the outflow of the Savica river into the lake Bohinjsko jezero.

c) The high-mountain lakes

Sampling places on the high-mountain lakes were assigned for each lake separately. They are located in the Valley of Seven Triglav lakes, which is approximately 8 kilometers long. The lakes are referred to as the alp lake No. 1–7, starting from the most distal lake. There is no connection between the lakes in regard to water flow (either surface or subsurface). Water from all the lakes is collected deep in the masiff in a common channel, which opens as a spring of the Savica river. The highest lake (Rjavo jezero) is situated at an elevation of 2.002 m a.s.l. and the lowest lake (Črno jezero) is located at 1.319 m a.s.l. [11]. Right next to the lake Dvojno jezero (lake numbers 5 and 6) stands a mountain lodge (1.685 m a.s.l.), which is open only during the summer season when several thousands of visitors pass by or stay overnight.

Sampling procedure

Regular sampling in the lake Bohinjsko jezero was carried out throughout the bathing season (from June to September) in 2005, 2006 and 2007, and in the late autumn and the early spring, immediately after ice break. Occasionally, usually after rain, samples were also taken from some permanent tributaries and just below the Savica waterfall. The bathing season for continental from surface waters in Slovenia lasts from June 15 to August 31 [13]. Water was sampled in the distal alp lakes (No. 1–4) five times (in May, in June, twice in July, in September), while the remaining alp lakes (No. 5–7) were sampled seven times (May to October) in 2007. Details of air and water temperature, pH, visible pollution and weather were recorded for each sampling location. Water samples for microbiological analysis were collected in sterile bottles (volume of 300 mL) 1 m from the shore and 15 cm – 30 cm below the surface, following the protocol outlined in the Rules on the quality of bathing water [14]. Plastic gloves designed for single use were used during sampling. Samples were transported to the laboratory in a cooling bag at the temperature of +5 °C. Samples were delivered to the laboratory at least six hours after they were collected.

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The MPN method is recommended by the National decree for bathing water areas and the monitoring of bathing water quality.

Sample preparation and analyses

The lake water was analyzed with test-tube fermentation, which is used to determine the total number of the coliform bacteria and the number of the faecal coliform bacteria using the MPN method. Nine test tubes were prepared for each sample. The agar to determine the common coliform bacteria, prepared by LAP prescription, and a prescription by Mac Conkey for the faecal coliform bacteria was used. After incubation, the tubes were scored as +/- for growth on the basis of such factors as turbidity, gas production and appearance or disappearance of a substrate. Scoring a tube positive for growth indicated that at least one culturable organism was present in the dilution used for its inoculation. The number of positive and negative tubes at each dilution was used to calculate the number present in the original sample through the use of published statistical MPN tables or computer programs designed to simplify the analysis.

The MPN technique is very labor intensive and results are usually less precise than those obtained with direct plating methods [15].

However, the MPN method is recommended by the National decree for bathing water areas and the monitoring of bathing water quality. The value resulting from the MPN method was determined from the combination of positive and negative results obtained from a series of fermentation tubes used in a particular laboratory test [14]. The number of positive and negative tubes at each dilution was used to calculate the number present in the original sample through the use of published statistical MPN tables or computer programs designed to simplify the analysis. The MPN technique could be replaced by direct plating methods [15].

Calculation of the number of bacteria in a 100-mL sample was calculated by the equation 1 [16]:

$$C_{MPN} = \frac{n_{pt} \cdot 100}{\sqrt{V_{snt} \cdot V_{sat}}} \qquad (1)$$

C_{MPN} Number of bacteria in a 100 mL sample (mL^{-1}).
n_{pt} Number of positive tubes (1).
V_{snt} Volume of the samples in the negative tubes (mL).
V_{sat} Volume of samples in the all tubes (mL).

Data can be compared with maximum and recommended values as contained in the Supplement 2 of the Rules on the quality of bathing water [14]. Values in Table 1 are those that should not be exceeded in natural baths.

Table 1:
Hygiene demands for bathing waters in natural baths [14].

PARAMETER	RECOMMENDED VALUE	BOUNDARY VALUE
1. Total coliform bacteria (mL^{-1})	500	2.000
2. Faecal coliform bacteria (mL^{-1})	100	500

RESULTS AND DISCUSSION

The preliminary results of the assessment of the lake Bohinjsko jezero (in 2005 and 2006) shows the confirmation presence of the coliform bacteria of the faecal sources. In this 2-year period, 77 samples were taken from the lake Bohinjsko jezero and 15 from the main affluents. The number of bacteria in 100-mL samples from the lake Bohinjsko jezero varied from 0 to 438. On average, the lowest number of bacteria in 2005 was in April and in May, and in October in 2006. In regard to bacterial contamination, the most polluted places were number 1 and 7 [17].

Water analysis of the main affluent of the Savica river in 2006 showed that the faecal bacteria has already been present in the water under the waterfall, which indicates that some of the pollution comes from the high-mountain lakes.

The number of bacteria in 100-mL samples from the lake Bohinjsko jezero varied from 0 to 438.

The results of the analyses of water quality from 2007 are presented in Tables 2, 3 and 4. 49 samples were taken from the lake Bohinjsko jezero, 24 samples from the Savica river, and 41 samples from the high-mountain lakes.

Results for the analysis of water from the lake Bohinjsko Jezero showed (Table 2) that the coliform bacteria are present during all sampling periods (from May to October). Their numbers in a 100-mL sample were lowest in sampling places 5 (0–38 bacteria) and 6 (7–71 bacteria) on the north side of the lake. There are no contaminators or a constant affluent in this region. Other sampling places showed oscillations in the number of bacteria in water samples during the whole season. Sampling places 1 and 7 in the lake Bohinjsko jezero are the most contaminated (from 7 to more than 438 bacteria in a 100-mL sample) in areas with the highest number of swimmers.

The results for the sampling location of the Savica river 2 (Table 3), which is about 1 km from the waterfall indicates a higher number of the faecal bacteria (4–95 bacteria in a 100-mL sample) in comparison with sampling location 1 (0–46). The source of additional bacteria is Koča pri Savici and its septic tank, which is only 5 meters from Savica River. Results for the sampling location of the Savica 3 (near the inflow into the lake Bohinjsko jezero) indicated that faecal bacteria were found regularly, but not in May of 2007. The sources of bacteria for this location include summer houses and apartments in Ukanc, which are not all connected to the sewerage system. Located opposite these dwellings is a hotel, which is connected to the sewerage system.

The faecal coliform bacteria were present in three (No. 5–7) of the seven alpine lakes (Table 4). The number of the coliform bacteria in 100-mL samples from these three lakes varied from 0 to 76 bacteria. Common coliform bacteria were present (0–15 bacteria in a 100-mL sample) in the first four lakes, but it could not be confirmed the presence of the faecal coliform bacteria in these samples. The cottage near the lake Dvojno jezero (Alp lakes No. 5–6) does not have a waste-water treatment plant, and results of the analysis showed that the appearance

Table 2:
Number of the total coliform bacteria (bold) and the faecal coliform bacteria (in brackets) from the lake Bohinjsko jezero in 2007, (/) = not sampled.

Time of sampling / Place of sampling	21. May 2007	11. Jun. 2007	27. Jun. 2007	9. Jul. 2007	30. Jul. 2007	27. Aug. 2007	10. Sep. 2007	8. Oct. 2007
Lake Bohinj 1	**115** (95)	**190** (190)	**>438** (>438)	**>438** (>438)	**95** (58)	**271** (21)	**72** (15)	**14** (14)
Lake Bohinj 2	**271** (271)	**21** (7)	**58** (46)	**76** (46)	**95** (95)	**76** (7)	**20** (9)	**11** (11)
Lake Bohinj 3	**95** (0)	29 (0)	/ (/)	**>438** (139)	/ (/)	**29** (29)	**29** (4)	**19** (7)
Lake Bohinj 4	**4** (04)	**58** (20)	/ (/)	**46** (29)	**271** (21)	**438** (72)	**271** (20)	**190** (21)
Lake Bohinj 5	**15** (0)	**15** (9)	/ (/)	**38** (29)	/ (/)	**21** (15)	**29** (20)	**19** (11)
Lake Bohinj 6	**9** (6)	**190** (71)	/ (/)	**76** (46)	/ (/)	**29** (29)	**58** (20)	**19** (7)
Lake Bohinj 7	**190** (46)	**116** (95)	**>438** (438)	**190** (190)	**116** (116)	**271** (95)	**139** (58)	**58** (7)

Table 3:
Number of the total coliform bacteria (bold) and the faecal coliform bacteria (in brackets) from the affluent the Savica river in 2007.

Time of sampling / Place of sampling	21. May 2007	11. Jun. 2007	27. Jun. 2007	9. Jul. 2007	30. Jul. 2007	27. Aug. 2007	10. Sep. 2007	8. Oct. 2007
Stream Savica 1	**0** (0)	**0** (0)	**15** (7)	**95** (26)	**46** (46)	**46** (15)	**19** (11)	**20** (15)
Stream Savica 2	**9** (9)	**15** (7)	**9** (4)	**95** (95)	**95** (58)	**76** (21)	**15** (11)	**7** (7)
Stream Savica 3	**11** (0)	**21** (15)	**58** (58)	**29** (29)	**39** (29)	**76** (46)	**26** (15)	**20** (11)

Table 4:
Number of the total coliform bacteria (bolt) and number of the faecal coliform bacteria (bracket) in the sample of Lake Bohinj in the year 2007, (/) = not sampled.

Time of sampling / Place of sampling	21. May 2007	11. Jun. 2007	9. Jul. 2007	30. Jul. 2007	27. Aug. 2007	10. Sep. 2007	8. Oct. 2007
Alp Lake 7	**0** (0)	**0** (0)	**20** (7)	**29** (9)	**14** (14)	**46** (15)	**20** (14)
Alp Lake 6	**0** (0)	**4** (4)	**15** (9)	**76** (15)	**29** (20)	**19** (11)	**7** (7)
Alp Lake 5	**0** (0)	**0** (0)	**20** (14)	**31** (15)	**20** (11)	**20** (20)	**39** (4)
Alp Lake 4	**0** (0)	**0** (0)	**9** (0)	**15** (0)	/ (/)	**7** (0)	/ (/)
Alp Lake 3	**0** (0)	**4** (0)	**4** (0)	**9** (0)	/ (/)	**4** (0)	/ (/)
Alp Lake 2	**0** (0)	**10** (0)	**9** (0)	**7** (0)	/ (/)	**4** (0)	/ (/)
Alp Lake 1	**0** (0)	**0** (0)	**7** (0)	**4** (0)	/ (/)	**7** (0)	/ (/)

of the faecal bacteria in the water samples from alp lakes No. 5–7 is linked to the opening of the alpine cottage (samples in May and in June were negative). Water from toilets and kitchens is discharged into the surrounding environment only about 500 meters from the lake. The conclusion in that the faecal bacteria found in the Savica waterfall arrive from the mountain lodge by the lake Dvojno jezero because such a direct connection has already been established [18].

Water from toilets and kitchens is discharged into the surrounding environment only about 500 meters from the lake.

A comparison of Tables 1 with Table 2, 3 and 4 shows that values > 438 at the sampling places of the Lake Bohinj 1, 3 and 7 are close to the recommended level for the total coliform bacteria. If there is a need to obtain a more accurate number of the coliform bacteria, the accurate analysis should be used. Recommended values for the faecal coliform bacteria were exceeded three times in the sampling places of the Lake Bohinj 1 and 7, and once in the Lake Bohinj 2 and 3. None of the values in Table 1, Table 2 and Table 3 exceeded those for samples of the afflux Savica or the alp lakes either for the total coliform bacteria or the faecal coliform bacteria.

CONCLUSIONS

The lake Bohinjsko jezero is among the less polluted in Slovenia, but the results of a long-term monitoring program showed a slight trend towards increased pollution [19]. Water that enters the lake from the Alps is in the most cases clean and of good quality, but it is locally affected by the intensive alpine tourism. There are two main sources of pollution: contamination of high-mountain lakes as a result of the faecal water from alpine cottages, and contamination with water from toilets (and kitchens) that is properly treated.

The most of the faecal bacteria enter the lake through septic tanks within the lake's catchment area.

The results of this study show that most of the faecal bacteria enter the lake through septic tanks within the lake's catchment area. An additional small contribution comes from pastures, meadows and fields in the area neighbouring the lake. The hypothesis claimed that the main contaminators are stock-farming and agriculture, but it appears that in areas with a predominance of pastures and fields, lake contamination is low (sampling place Lake Bohinj 6). On the other hand, it was confirmed that some coliform bacteria are present in the lake Dvojno jezero (alp lakes 5 and 6), which drains into the lake Bohinjsko jezero. They were detected in a sample from the spring of the Savica river (the stream Savica 1), which connects directly with the aforementioned lake. Some increase in the concentration of the coliform bacteria was detected in the lower reach of the Savica river, indicating local pollution along the river itself.

The Bohinj community has a short-term and long-term plan for building a sewerage system connecting the whole region into the central cleaning mechanism.

The Bohinj community has a short-term and long-term plan for building a sewerage system connecting the whole region into the central cleaning mechanism. Due to technical problems, alpine cottages are not in that plan, and so it is necessary to find an *in situ* solution for the contamination problems imposed by these cottages. The reduction and control of pollution of the environment must be performed in all areas in which a human presence is either permanent or periodical.

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