UNIVERSITY OF NOVA GORICA
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SOURCES OF COLIFORM BACTERIA IN LAKE BOHINJSKO JEZERO

MASTER'S THESIS

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ABBREVIATIONS AND SYMBOLS

AL	Alp Lakes
AL 1	Alp Lake 1, Lake Rjavo Jezero
AL 2	Alp Lake 2, Lake Rjavo Jezero
AL 3	Alp Lake 3, Lake Zeleno Jezero
AL 4	Alp Lake 4, Lake Ledvica
AL 5	Alp Lake 5, Lake Dvojno Jezero (5^{th})
AL 6	Alp Lake 6, Lake Dvojno Jezero (6^{th})
AL 7	Alp Lake 7, Lake Črno Jezero
ARA	Antibiotic Resistance Analysis
ARSO	The Environmental Agency of the Republic of Slovenia
a.s.l.	above sea level
EPA	Environmental Protection Agency
IVZ	Institute of Public Health
LAP	Lactose Andrade Peptone
LB	Lake Bohinjsko Jezero
LB 1	Lake Bohinjsko Jezero, first sampling place
LB 2	Lake Bohinjsko Jezero, second sampling place
LB 3	Lake Bohinjsko Jezero, third sampling place
LB 4	Lake Bohinjsko Jezero, fourth sampling place
LB 5	Lake Bohinjsko Jezero, fifth sampling place
LB 6	Lake Bohinjsko Jezero, sixth sampling place
LB 7	Lake Bohinjsko Jezero, seventh sampling place
MOP	Ministry of the Environment and Spatial Planning
MPN	The Most Probable Number
MST	Microbiological Source Tracking
MTF	Multiple Tube Fermentation
NPDES	National Pollution Discharge Elimination Program
PCR	Polymerase Chain Reaction
S	The River Savica
S 1	The River Savica, first sampling place
S 2	The River Savica, second sampling place
S 3	The RiverSavica, third sampling place
TNP	Triglav National Park
TSS	Total Suspended Solids

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, with a coastline of 10,900 m. The lake was formed by a glacier spreading over a karst landscape. There are several small permanent and temporary streams entering the lake. The main inflow represents River Savica, which emerges from the Komarča cliff as a large karst spring, followed by a waterfall. The Savica's water comes from six karst lakes in the Valley of Dolina Sedmerih Triglavskih Jezer (Triglav National Park) as well as from direct precipitation from a high karst plateau. Lake Bohinjsko Jezero should be among the cleanest lakes in Slovenia due to its short retention time and a lack of permanent inhabitants in the watershed. Nevertheless, biological and chemical indicators show that the inflow of nutrients has been increasing in recent years as a result of tourism. In addition, the presence of faecal coliform bacteria in the lake has also been detected. Faecal coliform bacteria should not be neglected from the perspective of health, because the lake is used for swimming and other water sports. Sources of bacteria include households in the very eastern area of the lake's watershed, as well as hotels, summer houses and alpine cottages in the rest of a catchment of the lake. In 2006, several locations along the lake shore were analyzed for the faecal bacterial contamination of the lake. In 2007, the research was expanded from the lake itself to the affluent of the lake, the River Savica and adjacent high-mountain lakes. "The most probable number" (MPN) was used for the coliform bacteria evaluation method. The analysis of the water has resulted in the confirmed presence of coliform bacteria of faecal source during most of ice-free period. The number of total and faecal bacteria in the water samples varied from 0 to more then 438 per 100 mL sample. Based on the results, we can conclude that part of the faecal coliform bacteria comes from the unsuitable adapted septic tanks of individual houses or households and a smaller part from pastures, meadows and field in the lake area.

Keywords: water quality, coliform bacteria, water pollution, sources of pollution, faecal pollution

1 INTRODUCTION

Water is a key part of the environment, without which there would be no life on Earth. Water quality requirements differ, depending on the purpose and type of water use. Substances harmful to humans (pollutants) have always been present in surface waters, long before the development of first civilizations. 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 (Kolar, 1983; Samec, 2005). Waste waters are those that people use in technological process; they add certain substances or even thermal energy (cooling waters in thermo-electricity power plants; thermal water from spas). Waste waters containing organic and/or inorganic compounds are then returned back to rivers or lakes. They can be partially cleaned before that. Indeed, one of the properties of waters in nature is that they have self-cleaning abilities. Today, as we have the opportunity to clean wastewater before discharge into the environment, this practice is unacceptable. Sewage from households is a kind of waste water mentioned above. In the past, there was less pollution than today, when the self-cleaning ability of rivers is often exceeded.

Coliform bacteria are excreted with faeces, from which they enter waste waters and then proceed, through unsuitably constructed sewage systems, to natural waters. The originating sources are numerous, and may include urban runoff, agricultural/farm runoff, watershed flushing, or dispersed sewer overflows (Nevers & Whitman, 2005). Faecal coliform 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.

All of the above may apply to different lakes in Slovenia and also across the globe. The pollution of surface water is, of course, dependent on the pollution from the environment.

The Lake Bohinjsko Jezero is one of those water bodies in which problems may occur concerning the presence of pathogenic coliform bacteria. In theory, we can expect the bacteria to enter the lake in one of three ways: superficial runoff from nearby land, cesspit leakage from summer houses around the lake, and underground transport through the karstic system from mountain lodges. For natural bathing areas, the Slovenian Ministry of the Environment and Spatial Planning prepares annual reports for the monitoring of bathing waters (The Program, 2005). 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. Faecal contamination may originate from point or nonpoint sources. Point sources include discrete sources such as wastewater treatment outfalls, storm sewers and combined sewers and discharges from large farms. Nonpoint sources include agricultural sources, such as livestock access to streams and riparian zones, land application of livestock manure, domestic or municipal sources, including pet waste, septic systems and the application of manure and biosolids to fields. The third nonpoint source is wildlife sources (Wilhelm & Maluk, 1998).

Tourism is not only an important economic activity, but also a source of environmental pollution, including water. Conversely, water quality is important for the development of tourism. The contribution of tourism to pressures on water environments is significant, but not dominant. Exceptions are impacts of tourism on uninhabited areas, where tourism and recreation are the main sources of pressure on water resources (Cigale, 2007). Alpine tourism has been growing in the Bohinj area, where there are several mountain huts. According to the Alpine Association of Slovenia, more than 1.5 million mountaineers were recorded in the Slovenian mountains in 2005. Furthermore, the number of visitors has increased since then: three million visitors were observed in 2007 (Gruden, 2006). Some huts located in the area of the Lake Bohinjsko Jezero have already established treatment plants (hut Zasavska Koča Na Prehodavcih, hut Dom Na Komni, hut Dom Na Planini Pri Jezeru), but some still contaminate the environment (hut Dom Pri Sedmerih Jezerih, hut Merjasec Na Voglu, hut Kosijev Dom na Vogarju). Since the terrain in this area is karstic and thus very porous, contaminated water from mountains huts can run through underground galleries and cracks towards valleys and pollute Lake Bohinjsko Jezero.

Research on mountain lakes was sparse up until the early 1990s. Since then, several projects have indicated problems related to the lakes in Triglav National Park (Brancelj, 1998). In 1990, Brancelj and his colleagues conducted the first chemical, physical and biological analysis of the lakes in the Valley of Dolina Sedmerih Triglavskih Jezer (Bricelj et al., 1991). The research mentioned above was the start of a comprehensive project focused on the establishment of a catalogue of flora and fauna in the Alp Lakes. In 1996, the national project SLO-Alps aimed to determine the differences among the lakes, focusing on the Alp Lakes, particularly Lake Ledvica, Lake Krnsko Jezero and Lake Jezero na Planini pri Jezeru (Brancelj et al., 1998). In 2004, Slovenia joined three European countries (France, Italy and Austria) in the Alpine Lakes Network project. Brancelj and other experts from the National Institute of Biology joined the European project, since the study also included Lake Bohinjsko Jezero (Magni et al. 2008).

1.1 RESEARCH SUBJECT – LAKE BOHINJSKO JEZERO

At the beginning of our research, Lake Bohinjsko Jezero was set as the principle subject. However, based on preliminary results, research was later also extended to the River Savica and then to the lakes in the Valley of Dolina Sedmerih Triglavskih Jezer, which represent a natural watershed area of the lake.

The lake is situated in Triglav National Park, in the north-western part of Slovenia (Figure 1). The area is part of the Julian Alps biosphere. The lake-bowl was formed by a glacier. The lake is still in a very pristine condition with good water quality (Keršič-Svetel, 2007). It is the largest permanent natural lake in Slovenia: 4,350 meters long, 1,250 m wide and 45 meters deep; the total length of the lake's coastline is 10,900 m.

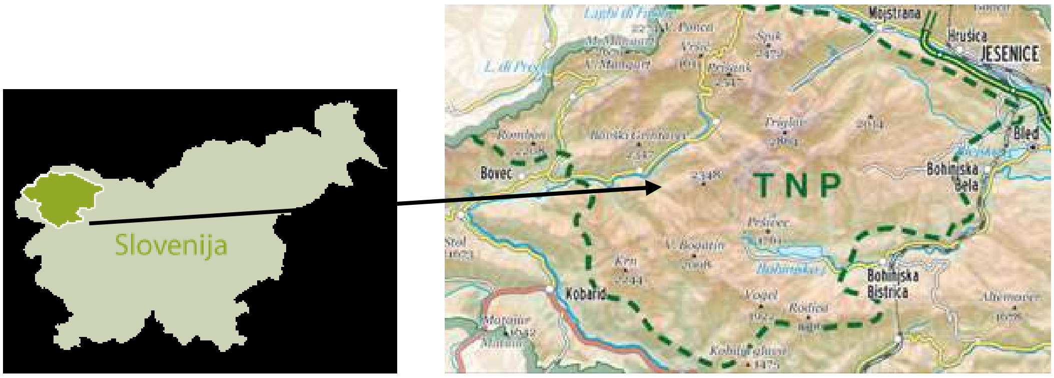

Figure 1: Triglav National Park (Cartography, 2006)

Due to a short retention time and the scarce settlements of the watershed, Lake Bohinjsko Jezero is one of the most pristine lakes in Slovenia. Nevertheless, biological and chemical indicators show that the inflow of nutrients has been increasing in recent years (Hlad & Skoberne, 2001). The catchment area of the lake includes the highland karst area, where the influence of man is limited but with clear indications of increase. Opposite to that, there is intensive tourism activity around the lake, where the urban pressure is growing. As a result, preventive measures, including careful planning with special emphasis on sustainable spatial planning and reasonable development of catchment area, are crucial for the condition of the lake (Remec, 2006).

The main inflow into the lake is the River Savica, which emerges in Komarča as a large waterfall, emerging from a spring cave. The water from the waterfall sinks as precipitation water into the karstic underground about 500 m above the spring on the high-mountain plateau (1,300–2,000 m a.s.l.) and through vertical underground galleries to a horizontal tunnel, finally emerging as a 78 m high waterfall (Burger, 2006).

Many smaller streams flow into the lake (Figure 2; red oval). Along the northern part of the lake, there are several underwater seepages and springs. The best known is the karst spring Govic (Firbas, 2001). The outlet is Mostnica, a short river that flows from Lake Bohinjsko Jezero.

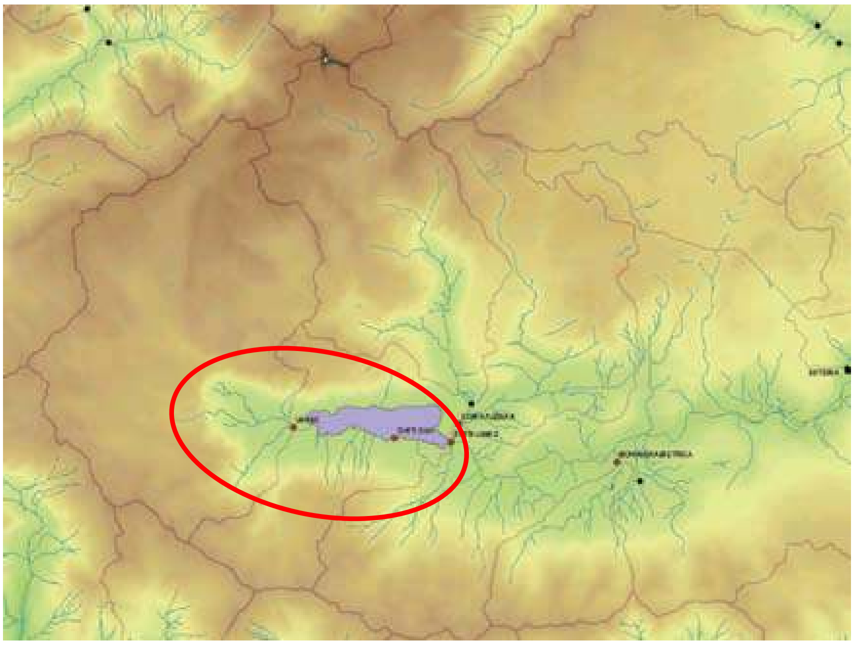

Figure 2: The surface inflows of the Lake Bohinjsko Jezero (Bat, 2007)

The Environmental Agency of the Republic of Slovenia (ARSO) performs hydrological measurements in the Bohinj region at water gauging stations on the Sava Bohinjka, Savica, Bistrica and Mostnica Rivers, and on the Lake Bohinjsko Jezero. Within the water balance analyses, the data on water discharges are harmonized with the data on precipitation and evapo-transpiration. Two water tracing tests were made in the Bohinj region in order to rectify the established discordances. They show that, although in the area of Triglav National Park there is a watershed division between the Black Sea and the Adriatic Sea, as a result of karstic bifurcation, the drainage area of the water gauging stations in the Bohinj region could be treated as one unit with regard to the water balance (Bat, 2007).

1.2 INDICATORS OF FAECAL POLLUTION

Bacteria are prokaryontes and are found everywhere in the environment. Pathogenic bacteria cause disease, but pathogens constitute only a small portion of known bacteria (Bibič & Peterlin, 2002). Some organisms that cause disease in humans originate with the faecal discharges of infected individuals. Others are from the faecal discharge of animals (Davis & Masten, 2004a).

Recreational waters and beaches contaminated with faecal bacteria may contain pathogens that pose a health risk to humans (Dufour, 1984). 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 such water. Some people may become ill, even if there are only a small number of pathogens in the water. Microorganisms, including bacteria, are typically found in colonies or small groups. A lower number of pathogenic units reduces the danger of infection, but in contrast, 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 that use natural water (Moeller, 2005). Widespread disease generally occurs in regions where sanitary disposal of human faeces is not practiced (Cheremisinoff, 2002).

There are two types of bacteria that belong to the indicators of faecal pollution and have been confirmed in our samples: coliform bacteria and enterobacteria.

1.2.1 Coliform bacteria

Coliform bacteria are excreted with faeces. In urban environments they are carried from waste waters to natural waters because of unsuitably constructed sewage systems. Faecal coliform bacteria can be an indicator of water pollution with household sewage. Sewage water also contains waste water from bathrooms (soaps, washing powders) as well as from kitchens (detergents). If, alongside faecal bacteria, pathogenic bacteria are also present, this can cause several diseases in people who were in contact with such water. Faecal bacteria (i.e. faecal coliform: *Escherichia* *coli* and *Enterococcus* spp.) are widely used to monitor the faecal contamination of water bodies (Likar, 2000; Davis & Masten, 2004b).

The coliform group, which includes taxa from the genera *Escherichia, Citrobacter, Enterobacter* and *Klebsiella*, is relatively easy to detect. Specifically, this group includes all aerobic and facultative anaerobic, gram-negative, non-spore-forming and rod-shaped bacteria that produce gas upon lactose fermentation in defined culture media within 48 hours at 35 $^{\circ}$C. The coliform group has been used as a standard for assessing faecal contamination of recreational and drinking water (Gerba, 2000). The faecal coliform assay involves the culturing of bacteria at defined and elevated temperatures, which mimics the conditions encountered by bacteria in the intestinal tracts of warm-blooded animals (Archibald, 2000; Davis et al., 2005).

1.2.2 Enterobacteriaceae

Enterobacteriaceae is a family of Gram-negative bacilli. The family contains more than 100 species of bacteria that normally inhabit the intestines of humans and animals. Enterobacteriaceae are commonly part of the normal intestinal tract flora. Members of the Enterobacteriaceae are small, non-spore forming bacilli. Some are motile, while others are not; some have capsules, others do not. Members are frequently resistant to common antibiotics. They ferment a variety of different carbohydrates. The patterns of the fermentation are used to differentiate and classify them. Some members are found in soil, water, and decaying matter, too. Some pathogenic strains also produce exotoxins, while others produce substances that are called “enterotoxins” because they specifically affect the intestinal tract, causing diarrhoea and loss of body fluids. Various species of the Enterobacteriaceae are able to cause pneumonia and urinary tract infections. They are also recognized as the major cause of wound infections and other nosocomial (i.e. hospital-acquired) infections. Under special conditions, they may also cause bacteraemia and meningitis. They do succumb to relatively low concentrations of common disinfectants, including chlorination; but their susceptibility to antibiotics varies. Some strains are now frequently resistant to antibiotics; furthermore, freezing does not destroy them. Since Enterobacteriaceae are commonly found in the intestinal tract, they are transmitted mostly via the faecal or oral route (Banič, 1994; Rusin et al., 1999; Engelkirk & Engelkirk-Duben, 2008; Fraser et al., 2010). A specific position within the family belongs to *Escherichia coli,* which is intimately, but not exclusively, associated with man.

1.2.3 *Escherichia coli* – as indicator of faecal pollution

E. coli belongs to a family of Enterobacteriaceae. It is potentially an opportunistic pathogen that causes diseases in certain circumstances, especially intestinal infection (Andlovic, 2002). *E. coli* is the most common member of faecal coliform bacteria, indigenous to the intestinal tract of mammals or warm-blooded animals (Dufor, 1977).

The presence of *Escherichia coli* in recreational waters is commonly used as an indicator of recent faecal contamination (Haack et al. 2003; Hansen et al., 2009). Human faecal pollution spreads many dangerous bacterial pathogens, including *E. coli*, but it can be also associated with animal faecal pollution (Field et al., 2003, Jiang et al., 2007). Many studies have attempted to determine the sources of *E. coli* in the environment (McLellan, 2004; Scott et al., 2004; Byappanahalli et al., 2006; Ishii et al., 2007; Vogel et al., 2007). The presence of *E. coli* in lake water also indicates the potential for the presence of pathogenic organisms. The source of *E. coli* contamination in surface water includes municipal waste-water discharges, septic leaching, agricultural or storm runoff, wildlife populations, or nonpoint sources of human and animal waste (An et al., 2002).

1.2.4 Transport of bacteria

Transport of bacteria through soil layers is determined by the size of the pores, the degree of water saturation in the substrate and the adsorption of bacteria on particles of soil. Bacterial cells are negatively charged bio-colloids and bind to particles of the soil. Low concentrations of soluble organic compounds and a low pH increase the adsorption of bacteria. All porous media are three-phase systems consisting of:

a) a solid or mineral inorganic phase that is often associated with organic matter,

b) a liquid or solution phase and
c) a gas phase or atmosphere.

The unique properties of any porous medium are dependent on the specific composition of each of those phases (Newby et al., 2000). All phases interact together, when the system is perturbed and then move toward a new, equilibrium state to create a new environment, which remains fairly constant if left undisturbed. However, the status of porous media determines the speed and direction of bacterial transport.

The transport of microbes through soil and water zones is a complex issue of growing concern. The artificial introduction of microorganisms into an environmental system is a potentially powerful tool for the manipulation of a variety of processes. These include enhanced biodegradation of organic contaminants, remediation of metal-contaminated sites, improvement of soil structure, increased crop production through symbiotic relations, and even biological control of plant-pathogenic organisms. Microbes are not always introduced intentionally and their introduction does not always produce desirable effects (Newby et al., 2000). The transport and fate of microorganisms in porous media was originally the focus of interest because of concern about outbreaks of disease caused by groundwater contaminated with pathogenic microorganisms (Tan & Bond, 1995). Faecal bacteria may be highly concentrated in sediments (Pommepuy et al., 1992).

Each river's ecosystem has four dimensions (Figure 3), in which even a small amount of pollution can cause significant ecological problems even in places that are far from the source of the pollution as a result of specific water pathways in rivers (Report, Alpine convention, 2009)

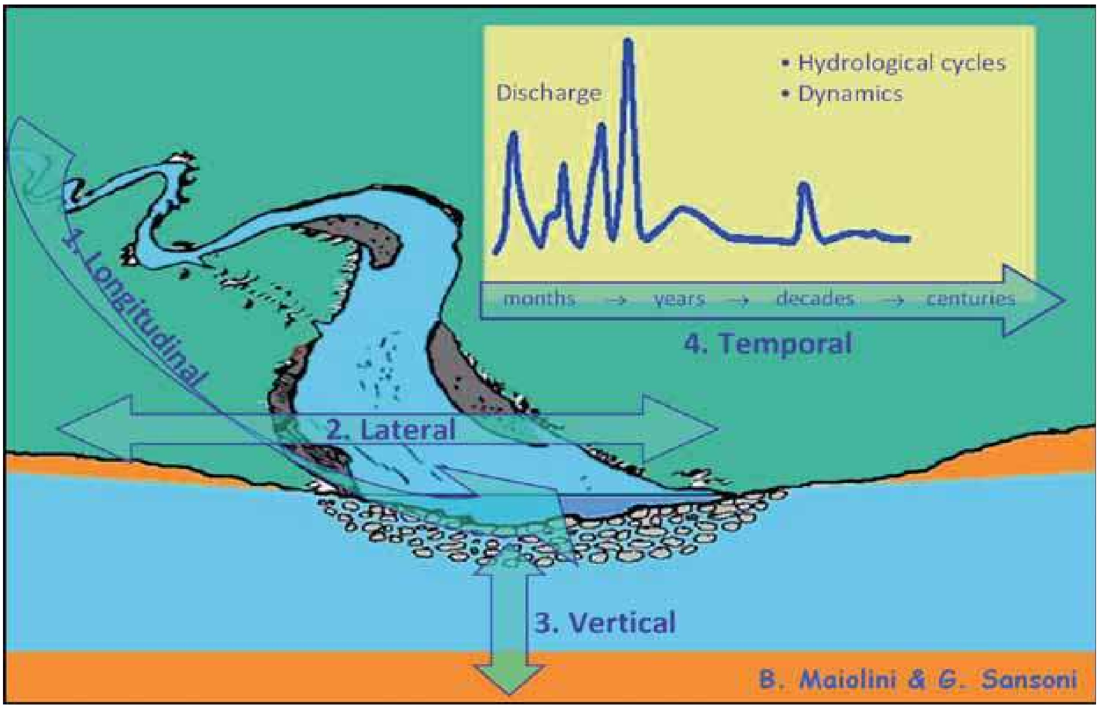

Figure 3: The four dimensions of a river ecosystem – longitudinal, lateral and vertical connectivity and dynamics in time (Report, Alpine convention, 2009)

Bacteria in water bodies of different types (running water, standing water, and groundwater) can travel long distances. Precipitation, especially rain and melt water, ease the bacteria's penetrating deep into the soil and afterwards into groundwater. This is confirmed by observations that the contamination is greater after intensive rainfall. Laboratory tests conducted with columns of soil with added slime, confirm that (pathogenic) bacteria travel faster when the amounts of rain are larger. Conceptual and mathematical models for microbial transport in porous media have been developed as a tool not only for risk evaluation but also for understanding the dispersion pattern observed in pore waters connected with terrestrial, limnetic, riverine and marine environments (Heisel & Gust, 1999).

Šmuc and Rožič (2009) present a study on the impact of litho-structural settings and neotectonic activity in Alpine areas, specifically in the Valley of Dolina Sedmerih Triglavskih Jezer. The study provides an answer to the question why water from the Alps may influence the water quality in Lake Bohinjsko Jezero. The Valley of Dolina Sedmerih Triglavskih Jezer is characterised by a generally asymmetric transverse (E–W) profile: a very steep eastern slope, a relatively flat valley and a relatively gentle western slope. The transverse profile the valley floor (Figure 4) is essentially flat, gently dipping towards the east. In the longitudinal cross-section, however, the valley floor is marked by sharply-defined fault blocks extending in a W–E to NW–SE direction. Additionally, the highest block (elevations ~2,100m) is in the northern part of the valley, the lowest (elevations ~ 1,600 m) in the southern part of the valley. The Valley of Dolina Sedmerih Triglavskih Jezer directly represents the topographic expression of Paleogene–Neogene thrusting and faulting, which show that the valley almost perfectly mimics the wedge-shaped damage zone located between these faults. Due to ground structure in the vicinity of the Valley of Dolina Sedmerih Triglavskih Jezer, water passes from each lake individually into massive rock and finally appeared in spring as the main affluent of Lake Bohinjsko Jezero as the waterfall Savica (Šmuc & Rožič, 2009).

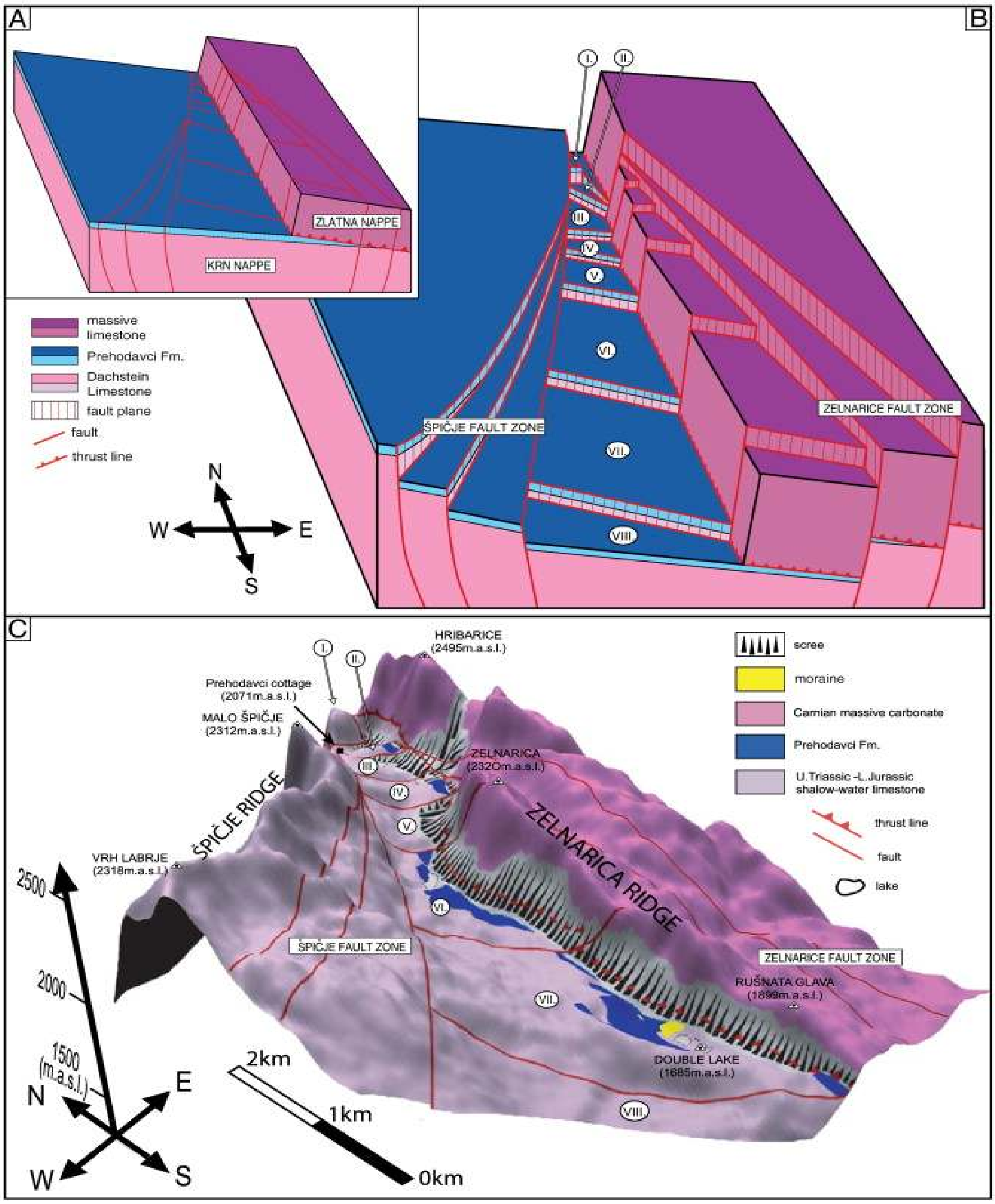

Figure 4: Evolutionary model of the formation of the Valley of Dolina Sedmerih Triglavskih Jezer (Šmuc & Rožič, 2009)

1.3 REVIEW OF PUBLICATIONS

Extensive research has been conducted in many places on different lakes, seas and rivers that examined the presence, dynamics, distribution and survival of coliform bacteria of faecal origin (Noble et al., 2003; Hadas et al., 2004; Chigbu et al., 2005; Yong et al., 2005; Vogel et al., 2007). Some of these authors reported that the number of bacteria of faecal origin increases after a heavy rain (Noble et al., 2003). Researchers observed an increase in the number of faecal coliforms in the Mississippi River after a heavy rain and then recorded a decrease of the bacterial population (Chigbu et al., 2005). Research on Lake Ontario in North America revealed that bird excrement is an important source of faecal pollution (Edge & Hill, 2007). A study in China of the activity of coliform bacteria of faecal source revealed their presence not only in the water column, but also in the top layers of the sediments of three lakes (Yong et al., 2005). Djuikom et al. (2006) assessed the microbiological water quality of the Mfoundi River and counted 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 indicator organisms. They found high concentrations of total coliform and faecal streptococci that varied with the sampling sites and points. The ratio between faecal coliform and faecal streptococci showed that the waters were contaminated by warm-blooded animals rather than humans and, according to the correlation analysis, rainfall was an important contributing factor that enhanced the bacterial numbers detected. The authors concluded and assessed that waters from the Mfoundi River and its tributaries present a great potential risk of infection for its users (Djuikom et al., 2006).

Surbeck et al. (2006) conducted field studies to characterize the concentration versus stream flow relationships of faecal pollution and suspended solids in storm water runoff from the Santa Ana River watershed (California, USA). The results have shown that the concentrations of faecal indicator are small or not dependent on stream flow rates. In contrast, the dependence of the concentrations of total suspended solids (TSS) in the stream flow is strong. The difference between both parameters is the reflection of different sources and transport pathways for those storm water constituents. The independence of the number of faecal bacteria in the stream flow is consistent with the idea that these contaminants are present on the surface of urban landscapes and rapidly enter surface waters when rain starts. Meanwhile, the dependence of the TSS on the stream flow is usually ascribed to the shear-induced erosion of channel bed sediments and/or the expansion of the drainage area contributing to runoff. The faecal bacteria, the very high storm-loading rates of faecal bacteria and the low detection of human adenovirus and entero viruses indicate that faecal pollution in storm water runoff from the Santa Ana River is primarily of nonhuman waste origin. The findings emphasize the point that the reduction of faecal bacteria pollution in storm water is a challenging task, as there are almost unlimited potential sources of contamination, as well as extremely high volumes of storm water runoff that need to be treated in order to eliminate bacteria (Surbeck et al., 2006).

Waste water is commonly known for its potential to create odour nuisances from a variety of sources, including odours escaping from sewer manholes, wastewater treatment facilities, and factory farming lagoons. Agricultural animals can also serve as a vector for important pathogens including *Escherichia coli* (Fujioka, 2002; Moe, 2002). The health risk from diffuse, nonpoint runoff is relatively unknown. Some studies have noted increased reports of illness following swimming near storm water outfalls (O'shea & Field, 1992; Haile et al., 1999). Other researchers found out that most indicator bacteria measurements from lakes are made on surface samples collected at a small number of locations, typically near probable sites of external loading, such as river inflows (Ben-Dan et al., 2001; Jin et al., 2003; Hadas et al., 2004), at beaches (Withman et al., 1999; Haack et al., 2003; , and other pollution sources (An et al., 2002).

The authors Suzan Given, Linwood H. Pendleton and Alexandria B. Boehm (2006) present reports on annual public health impacts (both illnesses and the cost of illness) from excess gastrointestinal illnesses. These illnesses originated from swimming in contaminated coastal waters at beaches in southern California. Beach specific enterococci densities were used as inputs to two epidemiological dose-response models to evaluate the risk of gastrointestinal illness at 28 beaches from Los Angeles to Orange Counties. By estimating the number of illnesses among swimmers and their likely economic impact, the authors used attendance data along with the health cost of gastrointestinal illnesses. The results have shown that there is a possibility that gastrointestinal illnesses and concurrent savings on expenditures on related health care costs decrease if coastal water quality improves. However, removing bacterial contamination from examined coastal waters has its limitations; for example, if people believed that swimming could be connected with several illnesses, they would be discouraged from going to the beach, which would result in less revenue for local businesses. In conclusion, there is a need to bear in mind the authors' statement that future studies that establish dose-relationships medically would improve reports of public health burden and costs (Given et al., 2006).

Every human being reacts differently to microorganisms that may cause disease. Bathing in polluted water can also cause disease (Pote et al., 2008). Surely, besides the individual reaction, the quantity of disease sources we are exposed to is important as well. Alfred P. Dufuor et al. attempted to determine the quantity of water swallowed during a swimming activity. For this study, fifty-three recreational swimmers were asked to actively swim for at least 45 minutes in a public swimming pool disinfected using cyanotic acid-stabilized chlorine and to collect their urine for the next 24 hours. Swimmers were not allowed to swim one day before and after the test swim. After the test swim, the urinary proteins and interfering substances were removed from the collected urine and the results showed that non-adults during swimming activity ingest twice as much water as adults, which is 37 mL while adults ingest 16 mL (Dufuor et al., 2006).

For identifying faecal contamination sources, microbiological source tracking (MST) methods are often used, as they have been subjected to limit comparative testing. John Griffith and 21 other researchers compared 12 different methods. None of the microbiological source tracking methods in this study provided a perfect characterization of the faecal contamination (Griffith et al., 2003).

1.4 AIM OF RESEARCH

The presence of coliform bacteria of faecal origin was proved with previous sampling in Lake Bohinjsko Jezero; thus our aim is to establish their source with further research. Possible sources are households in the area of the lake and alpine cottages in the water-collecting area. The source of microorganisms could be waste water from cesspools since they are often old and outdated or even intentionally made in a way that causes the faeces to leak. In this kind of waste water, pathogenic microbes can be present. Some kinds of microorganisms can survive even a few weeks in nature (for example *Escherichia coli* can do so up to 90 days) (Likar, 2000). Their survival is affected by the temperature of the environment, moisture, pH, organic compounds, the type of bacteria and antagonistic natural flora. Some kinds of bacteria can survive a few months in nature. Microbes can trickle through the ground, but this is conditioned by geo-hydrological, chemical and biological factors. Some of the laboratory tests in the last few years have drawn attention to the fact that the bacteria in natural soil move differently than in columns in the laboratory with the same structure of the soil. That is why tests "in situ" are mostly valid, because packing the soil and sediments in columns destroys secondary structural openings between parts of soil.

In addition to chemicals that are used in agriculture (remains of herbicides, insecticides, fungicides and mineral manures) and are washed away with waters from agrarian surfaces, there are other waste substances washed away in running water and the underground water. Liquid manure often flows directly to meadows or nearby brooks. Liquid manure from modern stables, where the excrement of pigs and cattle are washed away with water also belongs in this group. This can be one of the sources of coliform bacteria that are potentially dangerous to human health. Alongside direct outflows from stables and middens, there are pasturelands next to Lake Bohinjsko Jezero, where cows, horses and sheep graze during summer months. Their excrement is a potential source of lake soiling as well as a source of manure for grassy surfaces. Every time it rains, the water washes off some of the liquid manure that has been brought to meadows and fields (Report, 2002).

We should not neglect the health point of view, because Lake Bohinjsko Jezero is used for swimming and different water sports. The Ministry of Environment publishes a list of swimming waters and the Bay of Fužine in the eastern part of the lake is on the list of swimming waters where there are usually many people bathing, and bathing is not forbidden (Rules, 2008). If there are pathogenic organisms in the water, people who were in contact with the water can fall sick. Every individual has a different level of resistance. There are differences in age and health condition, which is why some people can fall sick even if there is only a small amount of pathogenic agents in the water. It is significant for microorganisms to be found in colonies or small groups in the water, which decreases the danger of infection, but when the colonies come into the human body it is more dangerous than if there was only an individual cell.

1.5 LEGISLATION

In order to protect surface and other waters, Slovenia has adopted many legislative documents based on European directives. A part of Lake Bohinjsko Jezero belongs to natural bathing spots; for this reason; bathing water will be considered in analysing the legislation defining norms.

Some of the most important European directives and Slovenian legislation concerning the field of bathing waters are:

- Council Directive 76/160/EEC concerning the quality of bathing water. This directive concerns the quality of bathing water. The physical, chemical and microbiological parameters applicable to bathing water are indicated in the annex that forms an integral part of the directive. It also determines the way in which to sample bathing water and the methods of analysing water in the laboratory.
- As early as in the introduction, the Directive 2006/7/EC concerning the management of bathing water quality says that water is a scarce natural resource, the quality of which should be protected, defended, managed and treated as such. Surface waters in particular are renewable resources with a limited capacity to recover from adverse impacts from human activities. In general, provisions are established for:
    a) the monitoring and classification of bathing water,
    b) the management of bathing quality,
    c) the provision of information to the public on bathing water quality;

The purpose of this directive is to preserve, protect and improve the quality of the environment and protect human health.

- The basis for every other law is Zakon o varstvu okolja (Environmental Protection Act - Official Gazette RS no. 39/2006).

  The purpose of this law is environmental protection, however its goals are:
    a) prevention and reduction of environmental contamination,
    b) preservation and improvement of environment quality
    c) long-lasting usage of natural sources

d) reduction of environmental contamination, bioremediation of ruined natural balance and re-establishing its regeneration mechanisms.
e) and other goals, not referring to water protection.

- Zakon o vodah (Water Act - Official Gazette RS no. 67/2002) regulates water management and coastal areas. The purpose of this regulation is to achieve appropriate water quality and other ecosystems related to water; ensuring protection from harmful water activity, preservation and regulation of water quantities and encouraging (stimulating) long-lasting water usage, that enables different water usages, while considering long-lasting protection disposable water sources and their quality. According to the Water Act quality criteria, Lake Bohinjsko Jezero is determined a as lake with the first degree of quality.
- Pravilnik o kriterijih za označevanje vodovarstvenega območja in območja kopalnih voda (Rules on criteria for marking a water protection zone and bathing water zone - Official Gazette RS no. 88/2004 and 71/2009) determines technical and formative elements, the manner of execution and the manner of marking (noting) water protection areas and bathing areas.
- Uredba o območjih kopalnih voda ter o monitoringu kakovosti kopalnih voda (Decree on bathing water areas and the monitoring of bathing water quality - Official Gazette RS no. 70/2003, 72/2004 and 25/2008) determines precisely the areas where bathing is not forbidden (prohibited) and the Fužine Bay on Lake Bohinjsko Jezero is on the bathing area list. It also determines the monitoring program:

a) enlistment of parameters
b) annual plan of sampling frequency,
c) determination of sampling places
d) manner of sampling, description of procedures, equipment of sampling,
e) sampling and field measurements,
f) filling out the record of taking samples away,
g) conservation and transportation of samples,
h) laboratory testing of samples,
i) estimation of hygiene suitability.

1.6 WORKING HYPOTHESES

a) Faecal coliform bacteria are present in Lake Bohinjsko Jezero. The main source of pollution originates from tourist activities (hotels on the lake's shore, alpine cottages, individual holiday houses), and to a lesser extent from agriculture (pastures next to the lake seasonally occupied by cattle/horses, manuring meadows).

b) The concentration of bacteria in the water follows, with some time delay, the fluctuation of the number of tourist visits. The number of bacteria is supposed to be smaller or could even be absent out of the main tourist season.

c) Alpine cottages in the hinterlands are also a source of pollution of the lake with bacteria of faecal origin, but with a modest contribution.

2 THE EXPERIMENTAL PART

2.1 STUDY SITE

Lake Bohinjsko Jezero was formed when glaciers reshaped tectonic faults. The catchment area of the lake is not accurately determined because of its karstic nature. The lake is of the through-flow type, with a retention time of 3-4 months. The main tributary is the River Savica, which starts as a karstic spring from Komarča. The lakes in the Valley of Dolina Sedmerih Triglavskih Jezer are high-altitude lakes, with weak or no direct connections between each other (Figure 4). The main connection was confirmed between Lake Ledvica and Savica waterfall (Urbanc & Brancelj, 2000; Brancelj, 2002).

In the Valley of Dolina Sedmerih Triglavskih Jezer, the first four lakes are positioned on rocks in Jurassic period which are impermeable to water. While water from the second, third and fourth lakes goes underground into stony gravel, water from the first, fifth and sixth lakes goes underground into rocky cracks. A larger part of the water that fills the lakes up comes from the screes and snowfields as underground water; thus there are no surface affluences (Firbas, 2001).

Besides the waters, the authors sampled and closely examined the vicinity of the lakes and Savica's affluence. Permeable carbonic soils are found on the limy ground there. The area surrounding Lake Bohinjsko Jezero rises steeply and is overgrown with beech and pine forests. On the north-eastern and eastern parts, directly next to the lake, there are rural pieces of land (pastures and meadows), and on the higher lying slopes the larch grows (Kolbezen, 1998; Lovrenčak, 1998; Skaberne et al., 2009).

2.2 SAMPLING LOCATIONS

We sampled the Lake Bohinjsko Jezero, the River Savica and the Lakes in the Valley of Dolina Sedmerih Triglavskih Jezer. All the water bodies are located in the north-west part of Slovenia, within Triglav National Park (Figure 5). The park is a protected area, where human activities are limited and controlled by the state.

Sampling locations were separated into three groups (Figure 5):

- the Lake Bohinjsko Jezero – sampled in 2005, 2006, 2007
- the River Savica – sampled in 2006, 2007
- high-mountain lakes in the Valley of Dolina Sedmerih Triglavskih Jezer – sampled in 2007

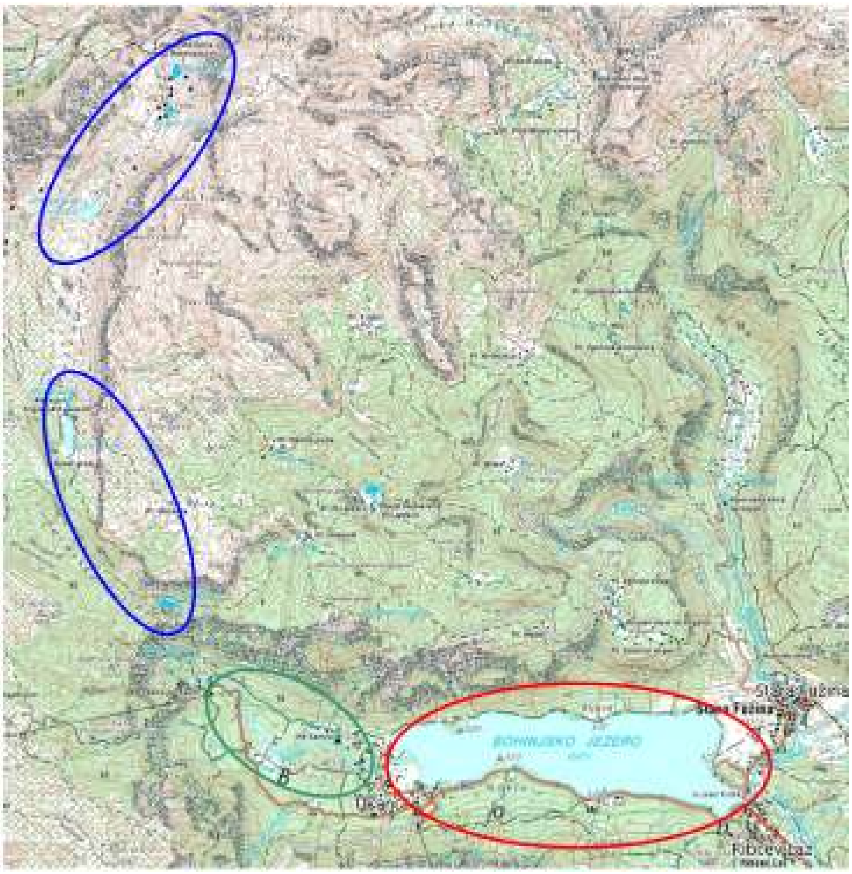

Figure 5: The Lake Bohinjsko Jezero and adjacent water bodies (www.geopedia.si)

Legend:

The Lake Bohinjsko Jezero

The River Savica

The Valley of Dolina Sedmerih Triglavskih Jezer

2.2.1 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 (Figure 6). During the June–August period, sampling locations 1–3 were intensively occupied by swimmers. At sampling location LB 4, there were many boats for most of the year and numerous tourists during the bathing season. There are also several summer houses in the neighbourhood. Sampling locations LB 5 and 6 were the least occupied by swimmers. Sampling locations are located along the northern coast, where there are no houses or holiday camps. Sampling place LB 7 is officially registered as an area with natural baths (Uredba, 2003).

Figure 6: Sampling locations on the Lake Bohinjsko Jezero (Google Earth, 2008)

Legend:

1 – Lake Bohinjsko Jezero, first sampling place (LB 1)

2 – Lake Bohinjsko Jezero, second sampling place (LB 2)

3 – Lake Bohinjsko Jezero, third sampling place (LB 3)

4 – Lake Bohinjsko Jezero, fourth sampling place (LB 4)

5 – Lake Bohinjsko Jezero, fifth sampling place (LB 5)

6 – Lake Bohinjsko Jezero, sixth sampling place (LB 6)

7 – Lake Bohinjsko Jezero, seventh sampling place (LB 7)

Temperature data (ºC) of the Lake Bohinjsko Jezero were obtained from the Environmental Agency of the Republic of Slovenia (ARSO), which performs hydrological measurements in the Bohinj region. The oldest series of data dates from the early 20th century. At the outlet of Jezernica from the Lake Bohinjsko Jezero, temperatures have been recorded since 1939 (ARSO, 2003; Bat, 2007). Long-term average monthly water temperature in the period 1939 – 2005 in Lake Bohinjsko jezero is according to available data (Bat, 2007) similar to our measured data. Water in the lake from April to August gradually warmed from about 5 ºC to about 18 ºC and then begins to decline when, in December reached less than 5 ºC (Bat, 2007).

2.2.2 The River Savica

The River Savica receives most of its water from the high-mountain lakes from the Valley of Dolina Sedmerih Triglavskih Jezer. Water emerging from a water-filled gallery, in the form of a waterfall of the River Savica, sinks about 500 m from higher up on the plateau, where the Valley of Dolina Sedmerih Triglavskih Jezer is located. Through vertical underground channels, water is drained into horizontal channels and finally appears in the form of a waterfall in the middle of a vertical cliff. Three sampling points were selected on the River Savica (Figure 7). Sampling location 1 was right below the waterfall, sampling location 2 approximately 500 m downward from the waterfall, just behind the hut Koča pri Savici and its cesspit. Sampling location 3 was right before the inflow of the River Savica into Lake Bohinjsko Jezero.

Figure 7: Three sampling points on the River Savica (Google Earth, 2008)
Legend:
1 – the River Savica, under the waterfall (S 1)
2 – the River Savica, behind the cottage (S 2)
3 – the River Savica, before Lake Bohinjsko Jezero (S 3)

The temperature of the River Savica is quite constant and low throughout the year. The temperature is at its lowest in January, increasing gradually until July. Water in the Savica starts cooling in late September. On average, the difference between January and July mean temperatures is only 2 ºC. Long-term average monthly water temperature in the period 1951 – 2068 in the River Savica, is according to available data similar to our measured data. Water temperature of the River Savica is in average throughout the year between 4.4 and 6.5 ºC. The highest average temperature is reached in the summer months; in July and August (Bat, 2007).

2.2.3 The High-mountain lakes

Sampling places on the high-mountain lakes were determined for each lake separately. The highest lake, Rjavo Jezero, is located 2,002 m a.s.l. The second and third Triglav Lakes, Rjava Mlaka and Zelena Mlaka, respectively, are positioned not far from the first lake. The next is Lake Jezero v Ledvicah, which is situated 1,830 meters a.s.l. The next two lakes are referred as Lake Dvojno Jezero (lake numbers 5 and 6), which stand close to the mountain hut (1,685 m a.s.l.). The lowest lake, Črno Jezero, is located at 1,319 m a.s.l. (Dobravec & Šiško, 2002).

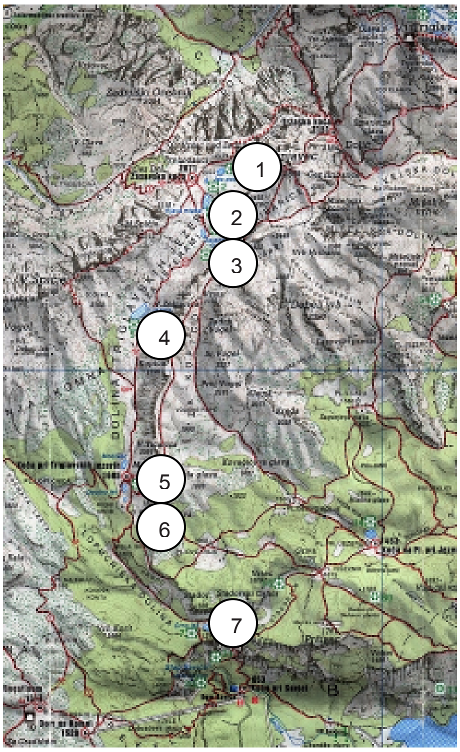

Figure 8: Seven high-mountain lakes in the Valley of Dolina Sedmerih Triglavskih Jezer (Triglav National Park, Slovenia) (Google Earth, 2008)

Legend:

1 – Alp Lake 1, Lake Jezero pod Vršacem (AL 1)

2 – Alp Lake 2, Lake Rjava Mlaka (AL 2)

3 – Alp Lake 3, Lake Zelena Mlaka (AL 3)

4 – Alp Lake 4, Lake Jezero v Ledvicah (AL 4)

5 – Alp Lake 5, Lake Dvojno Jezero (5^{th}) (AL 5)

6 – Alp Lake 6, Lake Dvojno Jezero (6^{th}) (AL 6)

7 – Alp Lake 7, Lake Črno Jezero (AL 7)

2.3 ENVIRONMENTAL PARAMETER MEASUREMENTS

A protocol regarding the parameters to be measured or observed on the field was prepared in advance. It contains information on the place and time of sampling, air temperature, water temperature, water pH and sensory estimation of water. A sensory estimation includes the evaluation of presence of visual pollution and smell. Field measurements and sensory evaluation was conducted on the ground, because we followed the protocol for the implementation of monitoring for natural bathing water required by the Decree on bathing water areas and the monitoring of bathing water quality (Uredba, 2003). Water temperature and water pH were measured on the spot. Temperature was measured by means of the mercury thermometer and pH by means of the TESTO kit.
Regular sampling in the Lake Bohinjsko Jezero was carried out throughout the bathing season (June–September) in 2005, 2006 and 2007, as well as in the late autumn and early spring (one late case: immediately after ice break). Occasionally, usually after rainfall, samples were taken from some permanent tributaries and just below the waterfall Savica. In 2007, the Alp Lakes 1 – 4 were sampled five times (May, Jun, July: two times, September), and Alp Lakes 5 - 7 were sampled seven times (May–October).

Water for microbiological analysis was collected into sterile, 300 mL bottles 1 m from the shore and 15 to 30 cm below the surface, following the protocol from the Rules on the Quality of Bathing Water (Rules, 2003/b). During sampling, single-use plastic gloves were used. Prior to being transported to the laboratory, samples were kept in a cooling bag at +5 °C. The samples were delivered to the laboratory within six hours after collecting them. There was a longer delivery time for water from high mountain lakes, since samples were transported to the valley by foot. The sampling was always conducted before sunrise, i.e. between five and six a.m.

2.4 SAMPLE PREPARATION AND ANALYSES

For the water samples analysis three different methods were used: The Most probable number method, the number of colony-forming units and biochemical tests for determination of microorganisms. Analyses of water samples by the method of

MPN was conducted at the National Institute of Biology, CFU analyses at Faculty of Health Studies, meanwhile bacteria species were determined laboratory at the Institute of Public Health.

2.4.1 Most probable number method - MPN

For the detection of coliform bacteria in water samples we used the most probable number method, following the international standard SIST EN ISO 9308-2 (ISO 9308-2, 1990).

The lake water was analyzed with test tube fermentation, which is used to determine the most probable number (MPN) of bacteria in a sample. This method relies on the dilution of the population down to the level of detection followed by inoculation of 5–10 replicate tubes containing a specific liquid medium with each dilution (Josephson et al., 2000). The method is suitable for more polluted samples, or with high number of bacteria. The principle method is inoculating diluted or undiluted samples with several test tubes with the isolative nutritional medium at the same time. After the incubation, all the test tubes with the positive reaction separate in their confirming medium; only this analysis gives us the right number of bacteria (Rupelj & Majstorović, 2005).

For determining common coliform bacteria, we used agar prepared with a Lactose Andrade Peptone (LAP) prescription (Microbiology Manual, 2000). However for faecal coliform bacteria, we used a prescription by MacConkey (Microbiology Manual, 2000). If a bacterial growth medium is selective, that means that it grows only certain types of microbes while inhibiting the growth of others (Port, 2008).

LAP nutrient medium contains the following ingredients:Tryptane 10 g L^{-1}, Lactose 10 g L^{-1}, Sodium chloride 5 g $L^{-1}$and Acid fuchsin 0.01 g L^{-1}.

MacConkey nutrient medium contains the following ingredients: Pepton from casein 20 g L^{-1}, Lactose 10 g L^{-1}, Ox bile (dried) 5 g L^{-1}, Cromocresol purple 0.01 g L^{-1}.

We prepared nine test tubes for every sample. Into every test tube, we inserted a small Durham's tube. We covered the test tubes with metallic covers and autoclaved them for 15 minutes at 121 °C. After the autoclaving, we added samples of sample water into test tubes; first we put 10 mL of water samples into each of the three test tubes, then 1 mL of water sample into another three test tubes each and finally 0.1 mL of water sample into another three test tubes each. The test tubes with agar (LAP) were put into a water bath for 48 hours at 37 °C. All the positive samples (i.e. producing gas trapped into the Durham's tubes) were afterwards transplanted into the agar medium prepared according to MacConkey. It is a special bacterial growth medium that is selective for Gram-negative bacteria and can differentiate those bacteria that are able to ferment lactose (Port, 2008). Positive samples were those which changed colour and had gas bubbles in the Durham tubes (Figure 9). Bacteria, known as "lactose fermenters", ferment the media's lactose into an acidic end-product that causes the pH indicator, neutral red, to turn pink. With MacConkey's medium, it is not the media that changes colour, but rather the actual colonies of lactose fermenting bacteria that appear pink. Non-lactose fermenting bacteria will be colourless (or, if they have any colour, it will be their natural colour, rather different from pink) (Port, 2008).

Figure 9: Tubes with positive (left) and negative (right) samples

After transfer of positive test tubes onto the agar medium, test tubes with agar were dipped into a water bath and left for an additional 48 hours at 44 °C. After incubation, the tubes are scored as +/- for growth on the basis of factors such as: turbidity, gas production and appearance or disappearance of a substrate. Scoring a tube positive for growth means 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 is 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 (Josephson et al., 2000).

The calculation of the number of bacteria in 100 mL of sample is as follows:

$$MPN/100mL = \frac{\text{no. of positive tubes} \times 100}{\sqrt{(\text{mL of sample in negative tubes}) \times (\text{mL of sample in all tubes})}}$$

(Clesceri et al., 1998)

The data of were compared with maximum / boundary and recommended values regard to the Supplement 2 of the Rules on the Quality of Bathing Water (Pravilnik, 2003), which are shown in Table 1.

Table 1: Hygiene demands for bathing waters in natural baths (Pravilnik, 2003)

PARAMETER	UNIT	RECOMMENDED VALUE	BOUNDARY VALUE
1. Total coliform bacteria	No. per 100 mL	500	2000
2. Faecal coliform bacteria	No. per 100 mL	100	500

2.4.2 The number of colony-forming units (CFU) – Rapid Test

In addition to MPN, in 2007 we performed a series of rapid tests concerning the presence of enterobacteria and Escherichia coli in Lake Bohinjsko Jezero. RIDA®COUNT dry medium plates (produced by R-biopharm) were used to prove the presence of enterobacteria in water samples. The method cannot determine the exact number of bacteria in a water sample, but can determine the number of formed colonies on the nutrient medium. Rida Count dry medium plates were used instead of conventional agar media in preparation petri dishes. At the prepared media Rida Count bacteria grows in the colonies like in the classic media. Therefore results are expressed as the number of colonies in one milliliter of sample (CFU / mL).

RIDA®COUNTs are non-woven membrane culture medium sheets in a ready-to-use test card format. The card is multi-layered, built up from a base followed by a nutrient layer and topped with a layer of inert polymer to absorb the test liquid. It is divided into 20 squares with lines.

A 1mL sample was added onto the test plate. Blue and green colonies, developed after 24 h of incubation at 35 °C, were counted. In case many colonies appeared on the plate, only one square (i.e. subsample) was counted and afterwards re-calculated to the total number using the equation:
$20/n \times c = \text{total count}$

n = number of squares counted
c = colonies counted

2.4.3 Bacterial identification Test Strip API

In 2006, some samples were analysed to identify the type of bacteria with the API 20 E test in the laboratory of the National Public Health Institute (IVZ). Water sampling was conducted in the same manner as the other samples; the only difference was that water was given in one-litre sterile packaging (obtained by IVZ). In the research, they used the MPN method for coliform bacteria and the API 20E test (produced by

Biomereieux) to identify the type. The types of bacteria detected in water samples are presented in Tables 8, 9 and 10.

The API tests consists of a plastic strip of 20 individual, miniaturized tests tubes each containing a different reagent used to determine the metabolic capabilities, and, ultimately, the genus and species of bacteria. The reagents in the cupules are specifically designed to test for the presence of products of bacterial metabolism specific to certain kinds of bacteria. Interpretation of the 20 reactions, in addition to the oxidase reaction, is converted to a seven-digit code, a process that seems much like decoding a message with a super secret spy decoder ring. And can then look up the code in a manual that has the names of bacterial species associated with each seven-digit string of numbers (Port, 2008).

3 RESULTS

3.1 THE RESULTS OF FIELD MEASUREMENTS OF WATER TEMPERATURE

Average water temperatures at individual sampling locations in Lake Bohinjsko Jezero are presented in Table 2. Only the average temperature calculations of sampling locations and years are presented. The average water temperature in 2006 was almost three degrees lower than in 2005 and more than four degrees lower than in 2007. This is due to a large quantity of rain in the summer of 2006 that contributed to water cooling. Detailed information on the temperature at sampling points revealed that the highest measured temperature was at the sampling site LB 7 (registered as a natural swimming area), which can be attributed to several factors. LB 7 is located in a bay on the eastern shore of the lake; probably, the effect of through-flow water is less expressed than at other sampling points. Another reason is possibly the rather shallow depth of the littoral zone of the bay, meaning the water warms up faster.

Table 2: Average temperatures of water in Lake Bohinjsko Jezero in the years 2005, 2006 and 2007 (values as °C)

Sampling location	**LB 1**	**LB 2**	**LB 3**	**LB 4**	**LB 5**	**LB 6**	**LB 7**	**Average**
Year								
2005	15.7	16.1	16.6	16.5	16.8	16.8	17.2	**16.5**
2006	13.3	13.3	13.0	12.7	13.0	13.0	13.2	**13.1**
2007	16.9	17.6	16.7	17.7	17	17.3	17.9	**17.3**

Legend:

(for details on sampling locations see Figure 6)

The average temperatures for three sampling location in the River Savica in 2007 were calculated (Table 3). For 2006, the only available data are for sampling point S 3.

The temperature of the Savica was relatively constant; it only oscillated between 1 to 2 °C (Table 3) in the period from May to October. During summer months, the water temperature in the Savica was 10–12 °C lower than the water temperature in Lake Bohinjsko Jezero. In 2007, the average temperature of the lake was 17.3 °C (Table 2), the average temperature of Savica S 1 and S 2 was 5.9 °C and the average temperature of sampling place S 3 right before the lake was 6.9 °C.

Table 3: The average temperatures of water in the River Savica in years 2006 and 2007 (values as °C)

Sampling location / **Year**	**S 1**	**S 2**	**S 3**
2006	/	/	6.3
2007	5.9	5.9	6.9

Legend:
(for details on sampling locations, see Figure 7)

In Table 4, the calculated average temperatures for the lakes in the Valley of Dolina Sedmerih Triglavskih Jezer are presented. The temperatures were measured in 2007 from May to the end of October.

Regarding the temperatures of the Alp Lakes, they do not differ much from the temperature in the River Savica. The temperature of Alp Lakes decreases with the height above the sea level; Table 4 shows the difference in the average temperature between the first and the seventh lake is 2.2 °C for the summer period. The average water temperature decreases with altitude.

Table 4: Average temperature of surface water in the lakes in the Valley of Dolina Sedmerih Triglavskih Jezer in summer 2007 (values as °C)

Sampling location	**AL 1**	**AL 2**	**AL 3**	**AL 4**	**AL 5**	**AL 6**	**AL 7**
Year							
2007	4.2	4.4	5.2	5.6	6.3	6.3	6.4

Legend:

(for details on sampling locations see Figure 8)

3.2 BACTERIA IN WATER SAMPLES

In 2005, 2006 and 2007, we collected more than 200 water samples from Lake Bohinjsko Jezero, from the River Savica and from the high mountain lakes. The results on number of total coliform bacteria and faecal coliformn bacteria in the samples are shown in Figures 10, 11 and 12.

The results of the Lake Bohinjsko jezero are presented in Figure 10. On the figure we can see that in all three years the least contaminated sampling location is LB 5, located on the north shore of the lake. The low number of total coliform and faecal coliform bacteria may be due to uninhabited areas of the lake (forest, clifs), as well as a small number of bathers in the summer season, due to difficult accessibility. LB 5 is not reachable by car, but only on foot. Most swimmers can be found on the part of the lake where the sampling locations LB 1 and LB 7 are located. As seen in Figure 10 these are the sampling points where faecal coliform bacteria exceed 400 per 100ml bacteria.

Results for each year show that the minimum number of bacteria in water samples in 2006. This year it was pretty rainy days, and if we look at the average water temperature (Table 2), we can see that this year the average temperature of the water is for more than 3 ºC lower than in 2005 and slightly more than in 2007.

Figure 10: The number of total coliform bacteria (TC) and number of faecal coliform bacteria (FC) in samples from Lake Bohinjsko Jezero in 2005, 2006 and 2007; (for details on sampling location see Figure 6)

The results of water analysis from Lake Bohinj's main affluence, the River Savica, are presented in Figure 11. The first samplings in the River Savica were performed in 2005 at the Savica's outflow to Lake Bohinjsko Jezero, where we confirmed the presence of faecal coliform bacteria right under the waterfall Savica. Faecal coliform bacteria were present in most of the samples (32 out of 37). Due to the presence of faecal bacteria on the sampling place S 1, the lakes in the Valley of Dolina Sedmerih Triglavskih Jezer (which are linked to the Savica waterfall) also appeared as a possible source of bacteria. The number of bacteria in the water samples from S 1 and S 2 is the greatest during the summer months. However, the sampling point S 3, it was already the largest in June.

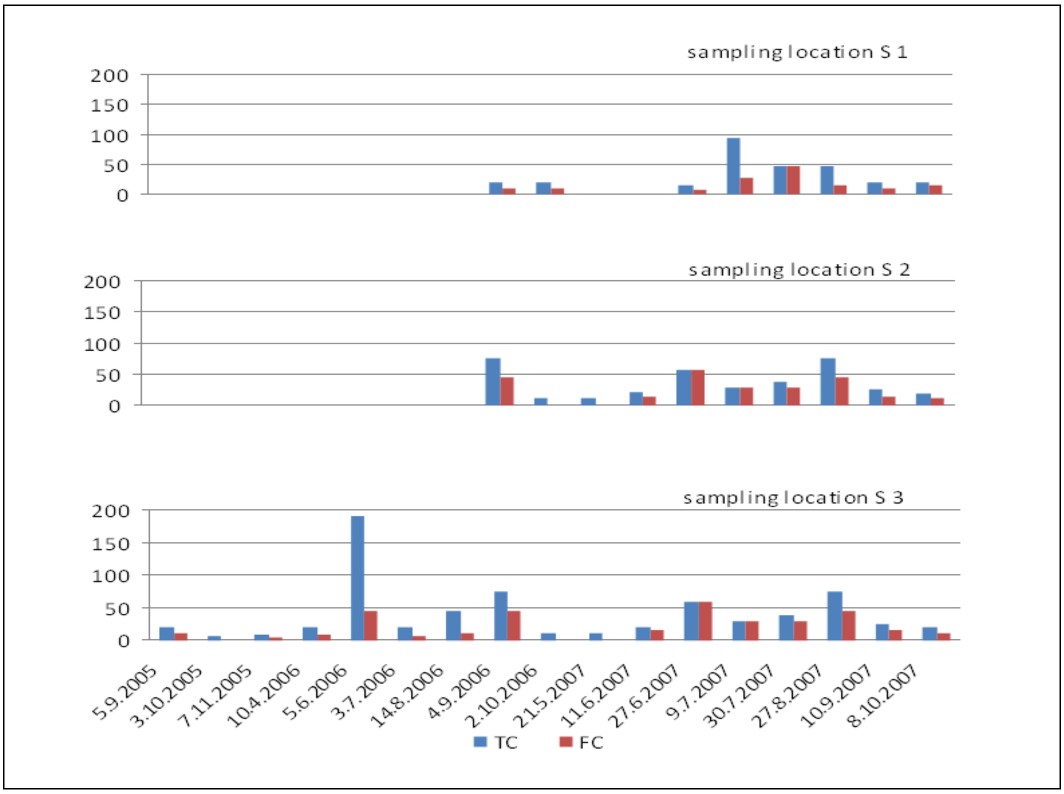

Figure 11: The number of total coliform bacteria (TC) and number of faecal coliform bacteria (FC) in samples of the River Savica in the years 2005, 2006 and 2007; (for details on sampling location see Figure 7)

The results of bacteria in water samples from high mountain lakes that were collected in 2007 are shown in Figure 12. The decision on the sampling frequency was done on the basis of the distance from and the difficulty accessing the first four lakes and the fact that all the samples collected by the end of July were negative. The faecal coliform bacteria were present in all samples from the lakes AL 5, AL 6 and AL 7 from July to October.

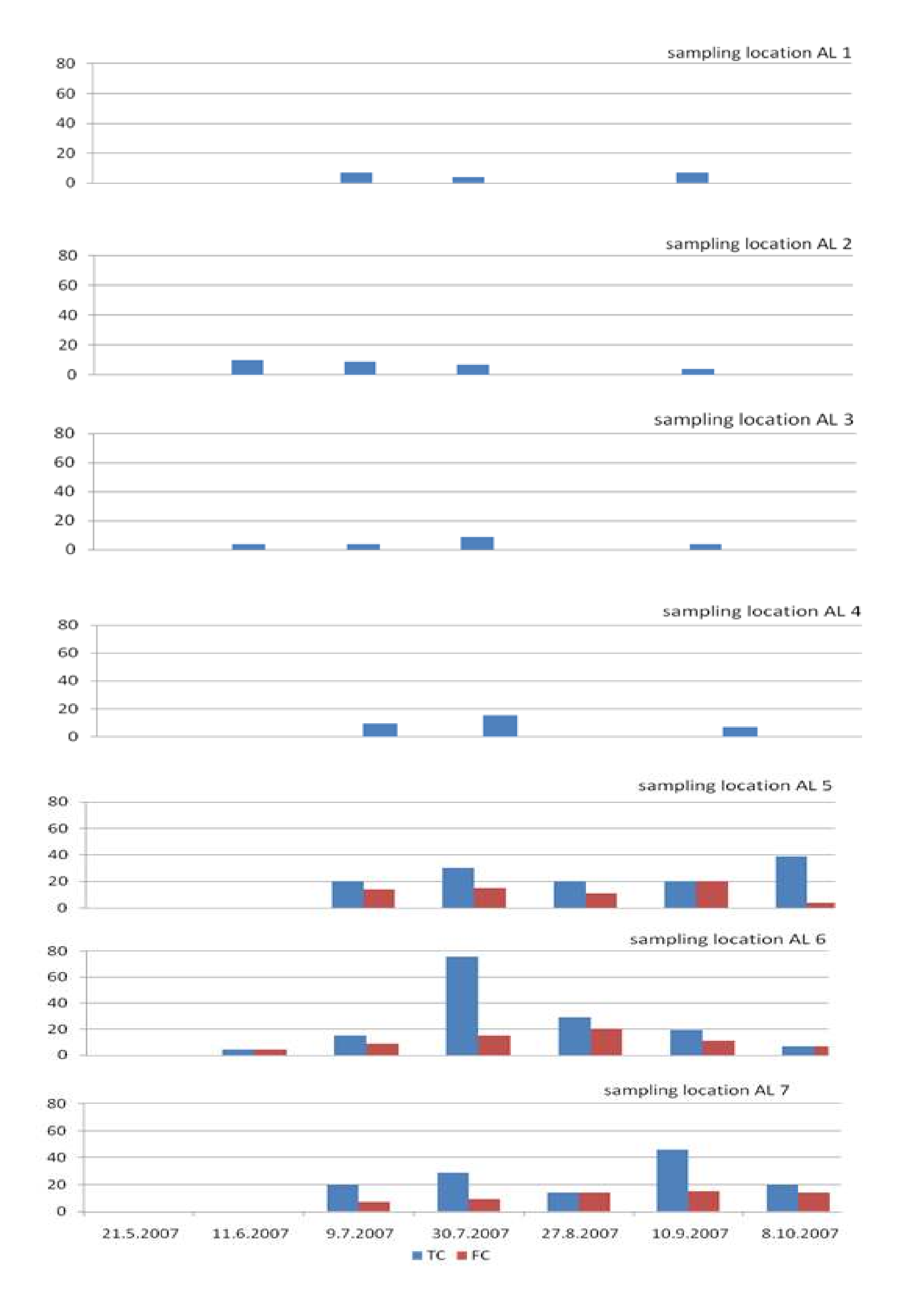

Figure 12: The number of total coliform bacteria (TC) and number of faecal coliform bacteria (FC) in a sample of Alp Lake water in the year 2007; (for details on sampling locations see Figure 8)

3.3 BACTERIAL COMMUNITIES IN THE WATER – TYPE IDENTIFICATION

In 2006, twelve water samples were analyzed in the laboratory IVZ Ljubljana, where the identification of bacterial species was made. Table 5 shows the results from Lake Bohinjsko Jezero and the River Savica. In samples collected from Lake Bohinjsko Jezero (10th July, 14th August, 4th September 2006) and from the River Savica (4th September 2006), several types of Enterobacteriaceae were detected. As can be seen at all sampling sites where *Escherichia coli* occur, it is an indicator of faecal pollution. In addition to *E. Coli*, in the majority of the samples bacterial strains *Enterobacter*, and *Citrobacter* and *Klebsiella* were identified. The sampling site S 3 (River Savica) was only confirmed with *Escherichia coli*. The results of the analysis-by-day and the number of bacteria in 100 mL of water can be seen in Annex 2, where reports are collected on the microbiological analysis of water.

Table 5: The types of coliform bacteria in 100 mL water samples of Lake Bohinjsko Jezero (10th July, 14th August, 4th September 2006) and River Savica (4th of September)

Sampling location	Type of bacteria
LB 2	*Escherichia coli* *Enterobacter* sp. *Klebsiella* spp. *Citrobacter youngae* *Citrobacter* sp.
LB 4	*Escherichia coli* *Enterobacter* sp. *Klebsiella pneumoniae* *Enterobacter cloacea* *Citrobacter* spp. *Klebsiella oxytoca* *Citrobacter freundii*
LB 6	*Escherichia coli* *Klebsiella oxytoca* *Klebsiella ornithinolytica*
LB 7	*Escherichia coli* *Enterobacter* sp. *Klebsiella pneumoniae* *Klebsiella oxytoca* *Klebsiella ornitholytica* *Citrobacter* sp. *Pantoea* sp.
S 1	*Escherichia coli*

Legend: (for details on sampling locations see Figure 6 and 7)

3.4 THE RESULTS OF RAPID TESTS – ENTEROBACTERIACEAE AND *ESCHERICHIA COLI*

In 2007, we performed an analysis of water for presence of Enterobacteriaceae and *Escherichia coli*. All twenty-one samples collected in 2007 contained Enterobacteriaceae (Table 6), with 9 to 146 colonies per sample. Comparing the results of sampling in July and August, sampling places LB 1, LB 2 and LB 3 contained a smaller number of colonies in July than in August, whereas sampling places LB 4, LB 5, LB 6 and LB 7 had bigger numbers of colonies in July, but decreasing in August and October. The presence of *Escherichia coli* was confirmed in 16 samples out of 21. The number of CFU on the nutrient medium was lower than the number of Enterobacteriaceae. The largest number of CFU was in all three samplings on the sampling place LB 7 (8–17 colonies) and in sampling place LB 4 (2–6 colonies).

Table 6: The number of colony-forming units (CFU) Enterobacteriaceae *and Escherichia coli* in the water samples from Lake Bohinjsko Jezero in 2007

Date of sampling and CFU	**CFU Enterobacteriaceae per 1mL sample**			**CFU *Escherichia coli* per 1mL sample**		
	9.7.2007	**27.8.2007**	**8.10.2007**	**9.7.2007**	**27.8.2007**	**8.10.2007**
Sampling location						
LB 1	16	49	9	0	1	0
LB 2	14	15	13	3	1	0
LB 3	16	35	13	3	3	1
LB 4	108	81	39	6	5	2
LB 5	37	12	8	0	1	0
LB 6	54	16	11	2	1	1
LB 7	146	97	45	8	17	15

Legend: (for details on sampling locations see Figure 6)

Analyses of water for the presence of Enterobacteriaceae and *Escherichia coli* were also carried out in the River Savica. The results of the number of colonies in one millilitre of the sample are shown in Table 7. The number of colonies in all three samples was the lowest at sampling place S 1. Comparing sampling places S 2 and S 3, the number of colonies in July was the same as August and October. Thus, due to the low number of colonies of *Enterobacteriaceae* in one millilitre (2–4 colonies), statistically there were no differences. In all of the sampling places on the River

Savica, the presence of *Escherichia coli* was also confirmed. The number of colonies is smaller than the number of Enterobacteriaceae colonies, but they are present at all three sampling places; including sampling place S 1, which is next to the waterfall.

Table 7: The number of colony-forming units (CFU) of Enterobacteriaceae *and Escherichia coli* in water samples of the River Savica in 2007

Date of sampling and CFU	**CFU Enterobacteriaceae per 1mL sample**			**CFU *Escherichia coli* per 1mL sample**		
	9.7.2007	**27.8.2007**	**8.10.2007**	**9.7.2007**	**27.8.2007**	**8.10.2007**
Sampling location						
S 1	2	3	2	1	1	0
S 2	4	4	3	1	1	2
S 3	4	3	4	1	3	2

Legend: (for details on sampling locations see Figure 7)

The results of analyses of water samples from three Alp Lakes are presented in Table 8. Analyses on the presence of Enterobacteriaceae *and Escherichia coli* were performed in three Alp Lakes (AL 5, AL 6, and AL 7). In Lake Dvojno Jezero (AL 5), the number of colonies in October was higher than in July, although a third sample was collected after the nearby hut was out of use. Between July and October, the number of colonies in Lake Dvojno Jezero (AL 6) decreased, while in the third case – Lake Črno Jezero (AL 7) – the number of growing colonies remained the same during all three months.

Table 8: The number of colony-forming units (CFU) of Enterobacteriaceae and *Escherichia coli* in samples from the Alp Lakes 5, 6 and 7 in 2007

Date of sampling and CFU	**CFU Enterobacteriaceae per 1mL sample**			**CFU *Escherichia coli* per 1mL sample**		
	9.7.2007	**27.8.2007**	**8.10.2007**	**9.7.2007**	**27.8.2007**	**8.10.2007**
Sampling location						
AL 5	4	4	6	2	4	1
AL 6	6	5	4	2	2	2
AL 7	4	4	4	0	2	2

Legend: (for details on sampling locations see Figure 8)

3.5 COMPARISON BETWEEN WATER AND AIR TEMPERATURE AND THE NUMBER OF BACTERIA

The comparison between water temperature and air temperature in relation to the number of faecal coliform bacteria are presented in Figures 13 through 17. Only the most representative sampling places are presented (i.e. LB 1, LB 2, LB 7, S 3 an AL 5). There was no clear relation between the number of bacteria and ambient temperature (air and water temperature (Figure 13)). The maximum number of bacteria was observed in July 2007, although the water was warmer in August 2007.

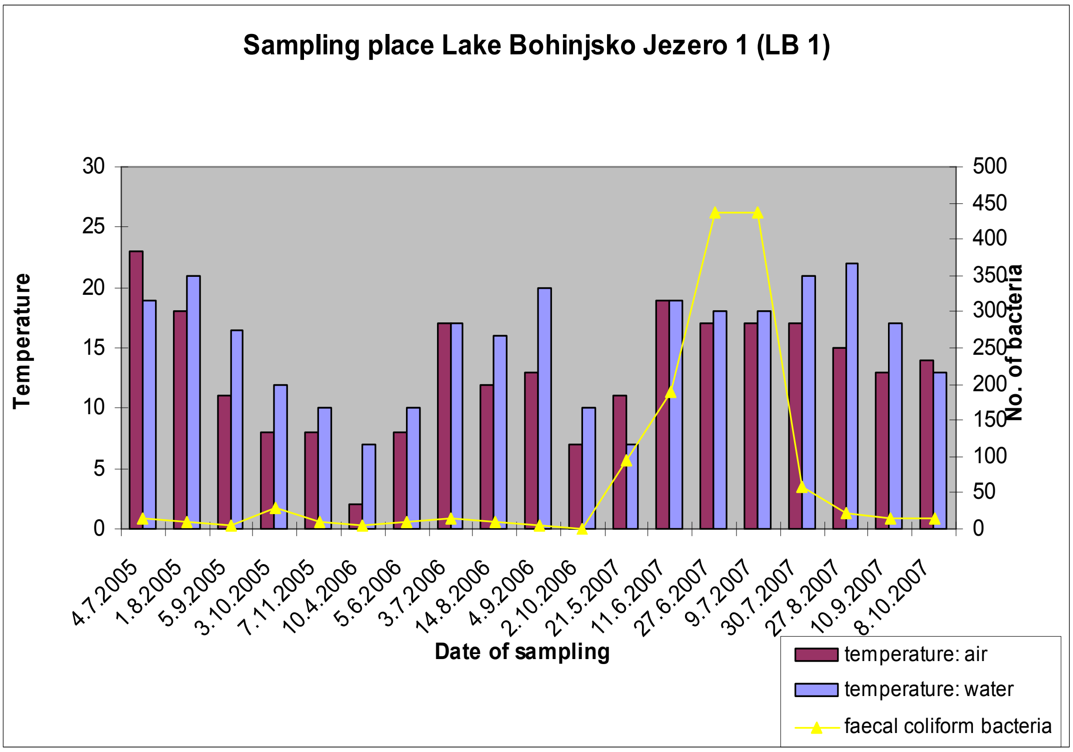

Figure 13: The comparison between water temperature (as °C), air temperature (as °C) and the number of faecal coliform bacteria (as No. per 100 mL) at the sampling place Lake Bohinjsko Jezero LB 1 in the period between 2005 and 2007

The air and water temperatures and the number of bacteria in the sampling place LB 2 in the years 2005 to 2007 are presented in Figure 14. The number of bacteria in sampling place LB 2 was the highest in the first sampling period of 2007 (May 2007), although the water was only 15 °C (Figure 14). In the months when the water temperature exceeded 20 °C, no increase in the number of bacteria in water samples was detected. Even at the sampling place LB 2, there was no correlation between water temperature and the number of bacteria.

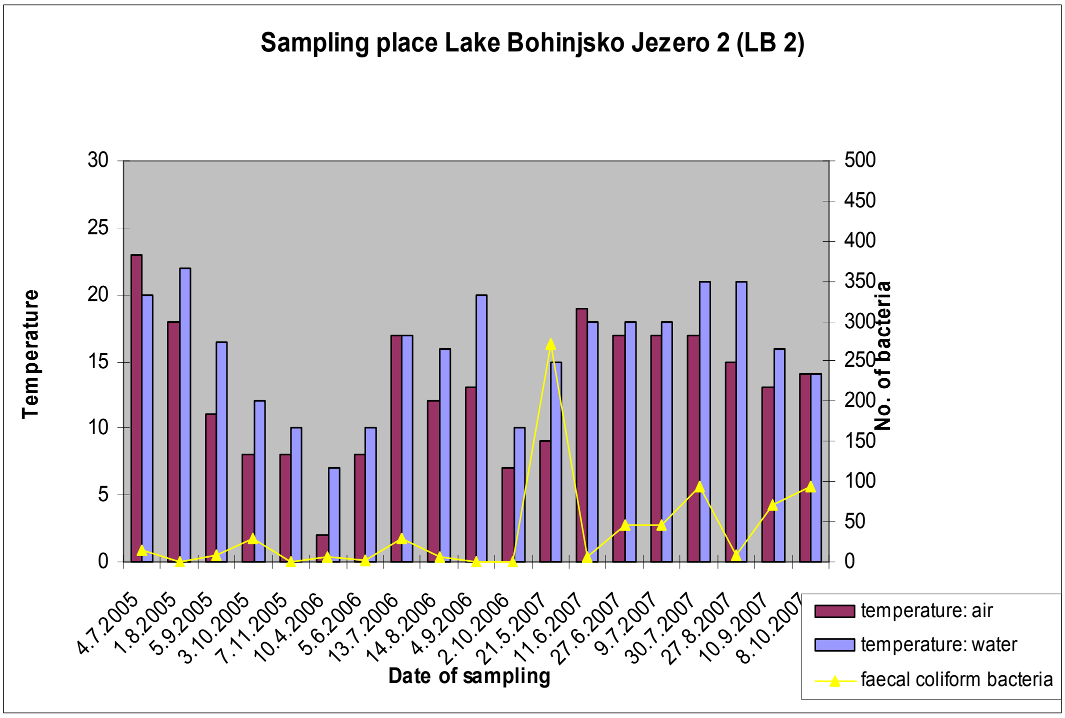

Figure 14: The comparison between water temperature (as °C), air temperature (as °C) and the number of faecal coliform bacteria (as No. per 100 mL) at the sampling place Lake Bohinjsko Jezero LB 2, between the years 2005 to 2007

Figure 15 shows the results of measurements of temperature air and water and the number of bacteria in 100 mL of water at sampling place LB 7 from the years 2005 to 2007. According to the results, we cannot conclude that there is a correlation between water temperature and the number of bacteria in water samples. As can be seen in the figure below, the numbers of bacteria in August 2005 were lower than in other months, although at that time the highest temperature of the water was measured. The highest number of bacteria in the 100 mL water sample was confirmed in June 2007, although the highest measured temperature was in August.

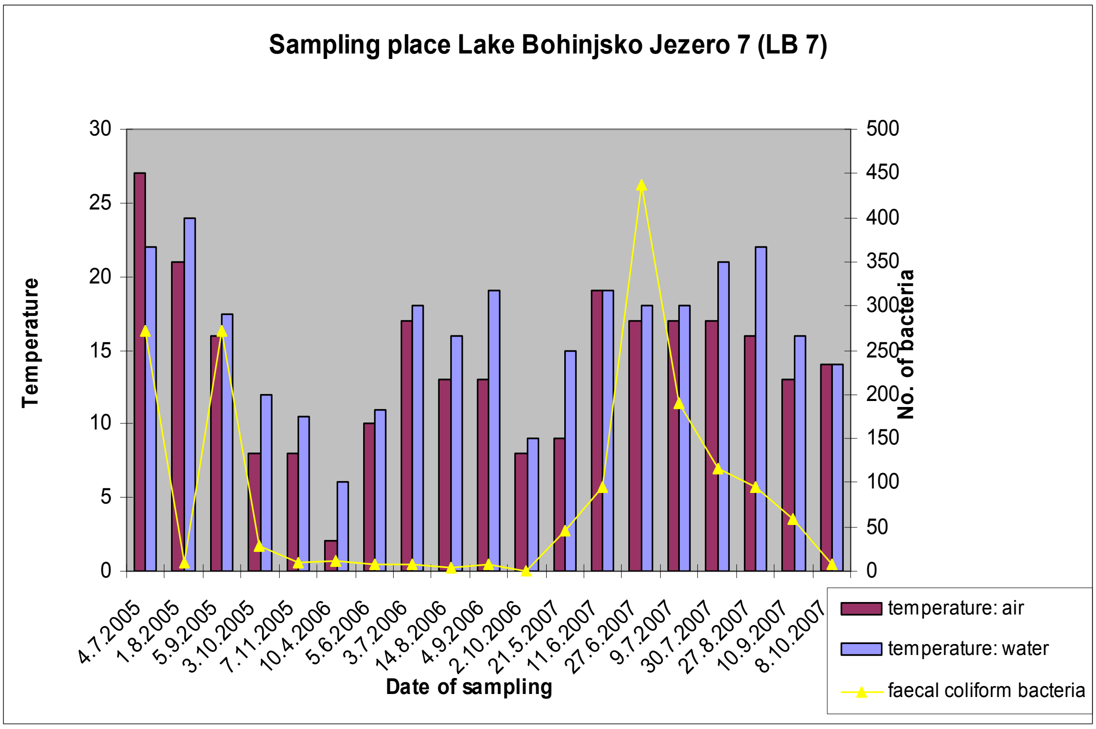

Figure 15: The comparison between water temperature (as °C), air temperature (as °C) and the number of faecal coliform bacteria (as No. per 100 mL) at the sampling place Lake Bohinjsko Jezero LB 7 between 2005 and 2007

The comparison between water and air temperature and the number of coliform bacteria in the River Savica at sampling point S 3 was analyzed (Figure 16). There is no relation between water temperature and the number of bacteria in water samples. The number of bacteria was higher in June 2006 despite the lower temperature, and not a month later when the water temperature was higher by 3 °C but the number of bacteria was lower (Figure 16).

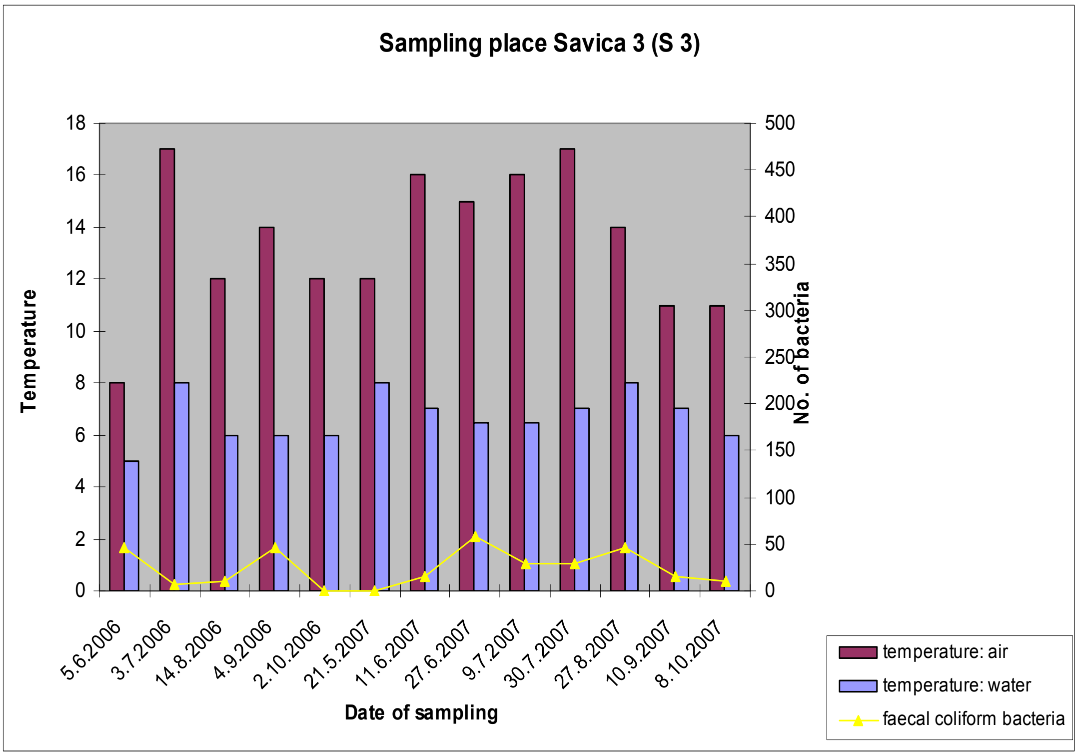

Figure 16: The comparison between water temperature (as °C), air temperature (as °C) and the number of faecal coliform bacteria (as No. per 100 mL) at the sampling place Savica 3 in 2006 and 2007

At the sampling place AL 6, Lake Dvojno Jezero, one of the three high mountain lakes, the faecal coliform bacteria were detected (Figure 17). There was no comparison between air and water temperatures and the number of bacteria in samples of water from the lake. Although the measurements of water temperature taken at the shore (approximately 0.5 m) of the lake were constant throughout the year (varying by 1–2 °C), the amount of faecal coliform bacteria increases during the summer months. This partly confirms our assumptions that the cause of the presence of faecal coliform bacteria is the effect of the mountain hut that lies less than 100 m from the fifth Triglav Lake (AL 5). In May and June 2007, our analysis did not confirm the presence of faecal coliform bacteria, while in July (when the hut has been opened already for one month) the presence of bacteria was confirmed in water from Lake Dvojno Jezero (AL 6). However, the number of bacteria throughout the summer season fluctuates minimally. In October, about a month after the closure of the cottage, the number decreased again.

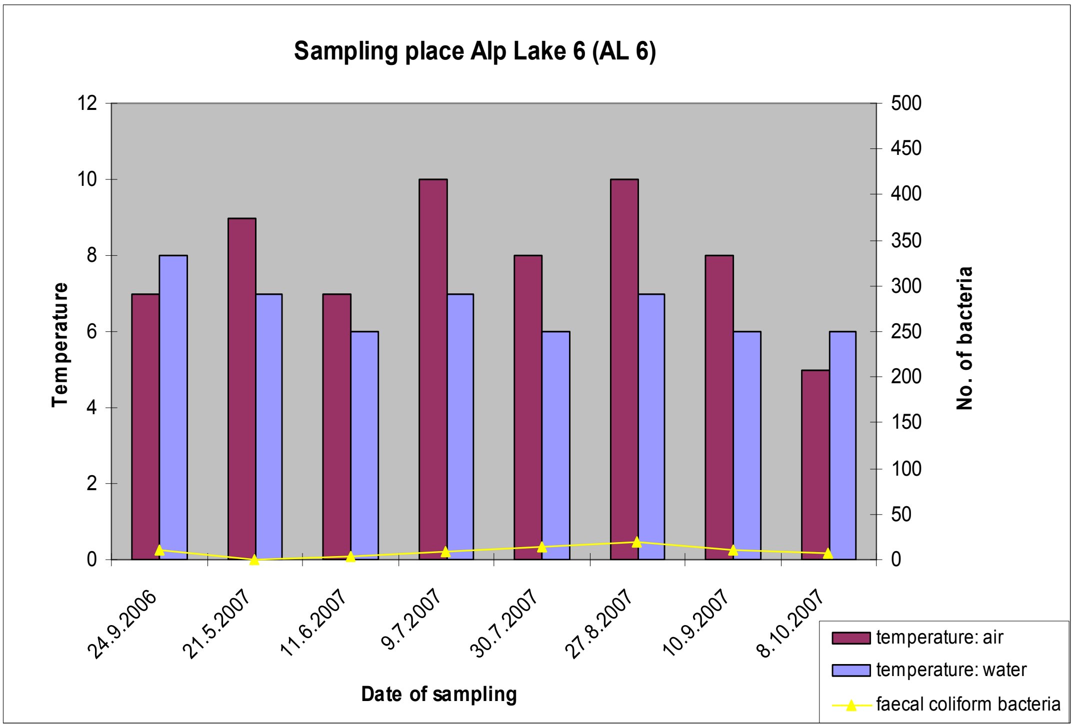

Figure 17: The comparison between water temperature (as °C), air temperature (as °C) and the number of faecal coliform bacteria (as No. per 100 mL) at the sampling place Lake Dvojno Jezero AL 6 between 2006 and 2007

4 DISCUSSION

Lake Bohinjsko Jezero is a complex eco-system, about which much research has been done (Brancelj et al. 1997; Brancelj et al., 1998; Urbanc & Brancelj, 2000; Brancelj, 2002). In addition to Lake Bohinjsko Jezero, numerous studies have been performed on the lakes in the Valley of Dolina Sedmerih Triglavskih Jezer (Brancelj, 1998). Researchers note that the mountain lake has experienced qualitative and quantitative changes due to the amount of human activities. Based on this research, the presence of faecal coliform bacteria in selected lakes was confirmed, and the knowledge of the negative impact of humans on the lakes from the microbiological point of view was enhanced.

Since part of Lake Bohinjsko Jezero is also registered as a natural swimming area, the results were interpreted according to the current "Rules on the Minimum Health and Other Requirements for Bathing Water" (Tabel 1). The annex referred to limits and guide values for total coliform and faecal coliform bacteria. The presence of bacteria of faecal origin in the lake may mean that its source is an unregulated sewage system (human resource) or natural origin. With the analysis done so far, the exact origin of bacteria (whether human or other origin) cannot be determined. To prove this, additional (and complex) analysis of bacterial community in water would have to be done.

Infection originating from polluted bathing water is dependent on several factors. How much water enters in our body while bathing? During 2006, Dufour et al. conducted a pilot study on the amount of water swallowed while swimming. This study on the health effects of Chloroisocyanurates among swimmers also contains information about the amount of water consumed by people while swimming. According to the findings of this study, adults swallow 16 mL of water during 45 minutes of swimming. Children are more vulnerable as a group, and swallow about 37 mL of water, which may increase the risk of infection (Dufour et al., 2006). According to the results of this study, the average value of bacteria in Lake Bohinjsko Jezero on sampling place LB 7 (public baths) in the summer period was 209 per 100 mL. Therefore, adult swimmers on average swallow 32 bacteria during

45 minutes of swimming. Thus, there is a possibility that swimmers, if the water is contaminated with pathogenic bacteria, may have health problems. Nevertheless, health problems depend on pathogenicity, human immunity and age.

Field measurements of water temperature

The Lake Bohinjsko Jezero temperature pattern, as well as that of the Alp Lakes and the River Savica is important to research because of a possible correlation between water temperature and the number of bacteria in the water samples. The bacteria levels are expected to rise in warmer water. In Lake Bohinjsko Jezero, a rise of water temperatures in summer months in all three years was detected. Similar results indicate that this is also true over a longer time period, since the average temperature in winter varies from 2 to 4 °C, while in summer increases over 20 °C can be observed (ARSO, 2000; Bat, 2007; Piston, 2007). Our measurements were carried out only during the sampling period; therefore, results from the winter period are not available. Otherwise, minor fluctuations within individual years were detected; however, it depends on the weather during the summer months. Thus, the water temperature in 2006 was on average 3 °C lower than the previous year or the following year.

In contrast to Lake Bohinjsko Jezero, the Alp Lakes and the River Savica have fairly constant water temperatures. During the summer period, rises of only 2 to 4 °C can be observed; therefore, no significant weather effects can be considered.

Coliform bacteria in water samples

Various experts around the world monitor the quality of bathing water using indicator microorganisms (Dufour, 1984; Field et al., 2003; Haack et al., 2003; Davis et al., 2004a; Jiang et al., 2007; Hansen et al., 2009). With similar methods, we also observed the presence of faecal coliform in the water of Lake Bohinjsko Jezero, as well as the lakes in the Valley of Dolina Sedmerih Triglavskih Jezer and the River Savica. In choosing methods for determining the presence of faecal coliform bacteria, the then-current “Rules on the Minimum Health and Other Requirements for Bathing Waters” (Uredba, 2003; see Annex 1) were consulted; the MPN method is recommended as a possible method. Today in Slovenia, there is a new regulation on the management of bathing water, which considers only two parameters regarding microbiological analysis of bathing water: intestinal enterococci and *Escherichia coli*. The European Union also has Directive 76/160/EEC on bathing water quality. The New Bathing Water Directive puts great emphasis on protecting the health of bathers as well as protecting the environment.

Researchers have conducted a similar survey along the east coast of North America (Given et al., 2006). Sampling points were evenly distributed from north to south, which led us to the idea that we make our sampling points evenly along the entire shore of Lake Bohinjsko Jezero. In mountain lakes, we decided on only one sampling location in each lake, due to the small surfaces of lakes.

In the period between 2005 and 2007, more than 130 samples of water from Lake Bohinjsko Jezero were collected. Of these, in only the 14 samples were faecal coliform not present; in 15 samples, their number was greater than 90 bacteria per 100 mL. With these numbers, the question arises whether the presence of bacteria can cause problems in humans. On this issue, unfortunately we cannot provide an answer, since it depends on several factors. The first is certainly the susceptibility or sensitivity of human beings. Secondly, it also requires a significant amount of micro-organisms or degree of pathogenicity. Even in very small numbers, some microorganisms can cause disease, while others need a few million to cause any ill effect in people (Radšel-Medvešček, 2002). In 1968, the Alliance for Environmental Protection and Water announced a decree stating that recreational waters must not contain more than 200 faecal coliform per 100 mL of water (Likar, 2000). Therefore, if we apply this now outdated decree, the samples of water from the Lake Bohinjsko Jezero were inadequate six times in three years. During our research, legislation was changed, but for interpretation of results the old rule was used, since the new rule does not define values for coliforms. The recommended value of coliform bacteria is 100 organisms per 100 mL of sample water; the maximum value is 500 organisms per 100 mL of water (Tabel 1). Therefore, if we take into account the recommended value, 15 samples in three years would be inappropriate because the threshold is quite high. All 131 samples of water from the Lake Bohinjsko Jezero would have to be appropriate. The same results were found by ZZV Kranj, which was authorized to carry out the monitoring of bathing water at Lake Bohinjsko Jezero. The analysed results are shown in annual reports of ARSO and IVZ, where we can see that from 2005 to 2007 only one sample is inappropriate, and this is when compared with the recommended value and not the maximum values (Report ARSO and IVZ 2005, 2006, 2007).

According to the situation in the River Savica, we can see that almost all samples contain coliform faecal bacteria. When comparing only the first two sampling points, S 1 and S 2, the highest number of faecal bacteria were analysed in S 2, although the sampling points are only some 10 meters apart. Based on the results, the question arises as to whether the septic tank at the Savica hut is properly sealed. It was impossible to receive a formal response regarding this, but we had our suspicions confirmed after an interview with the manager of the Savica hut.

The results of the analysis of water from the Lake Sedmera Triglavska jezera in part answer the question as to who (or what) is the main cause of water pollution by faecal bacteria. Samples of water from the first four lakes do not contain faecal coliform bacteria (all results were negative). In all lakes that lie below the Triglav lakes hut, the presence faecal bacteria in all samples between July and October 2007 can be confirmed. The first water samples (May and June 2007) were negative, which means that bacteria occur only after the opening of the hut. Of course, there are also natural sources of possible coliform bacteria and their presence in the Alp Lakes. As outlined in the various research papers, sources of coliform bacteria can also be birds (Edge & Hill, 2007) and larger mammals (Djuikom et al., 2006). Of course, animals are also present near the lakes that lie above the hut at Lakes Triglavska Jezera, but our research found no presence of faecal coliform bacteria in lakes AL 1–4.

The types of bacteria in the water - identifying species

More than ten types of bacteria (*Escherichia coli, Citrobacter sp., Klebsiella oxytoca, Citrobacter freundii, Klebsiella ornitholytica, Pantoea sp., Enterobacter sp*.) were present in the samples of water from the Lake Bohinjsko Jezero that were collected in July, August and September 2006 (Table 5). Their numbers (as No. of cells volume^{-1}) increased from July to August. The sampling places that had the lowest levels of bacteria per sample in July had the highest levels of bacteria in August (LB 4 and LB 7 240 per 100 mL). From the species that were isolated, *Escherichia coli* and various types of *Klebsiella* and additional types of *Enterobacter* were frequently present. All of these types belong to a group of Enterobacteriaceae. These represent the family of a bacillus that contains more than 100 species that are normal inhabitants of human and animal intestines. However, they are harmful if they are reproduced in other tissues or they become dominant in intestine. They are located in soil and water. Some strains form exotoxines, some of them form enterotoxins, which affect the digestive tract, causing diarrhoea and loss of fluids (Banič, 1994; Guidelines, 2005).

Previously, little research on risk to humans had been done. The impact on health is known primarily, if present in drinking water or food. It is also known that people accidentally ingest bacteria while swimming (Moeller, 2005; Dufour et al., 2006), but it is difficult determine what levels of micro-organisms cause infection. The theoretical infective dose of *Escherichia coli* is at 10^8 (Radšel-Medveščček, 2002). Whether we become ill or not, depends not only on the infectious dose or the number of microorganisms that get into the body, but also on the age of the swimmers and their levels of resistance.

Furthermore, water analysis carried out by rapid tests indicated that the most loaded sampling point is LB 7, followed by LB 4, where the maximum number of bathers is in the summer season. The number of colonies is reduced in October, when the bathing season ends. Enterobacteriaceae were present in all 21 samples taken; the presence of *Escherichia coli* was confirmed in 16 out of 21 samples.

Comparison between the temperature and the number of bacteria in water

Based on water analysis of the Lake Bohinjsko Jezero, the lakes in the valley Dolina Sedmerih Triglavskih Jezer and the River Savica, we found that there are numbers fluctuations in the levels of faecal coliform over time and space. Differences occur between sampling points, as well between individual sampling points within one season, but the cause of these fluctuations is not yet clear. During the change in Lake

Bohinjsko Jezero’s water temperature, we checked whether the rising temperatures may also increase in bacteria. A possible correlation between the number of bacteria and the water temperature was evaluated at three sampling sites on the Lake Bohinjsko Jezero: LB 1, LB 2 and LB 7.

At the sample site LB 1, there was no correlation between water temperature and the number of bacteria, because the bacterial count does not increase with increasing water temperatures. The maximum number of bacteria was in July 2007, but the water was warmest in August (Figure 13). Related results were also at site LB 2, since the number of bacteria was highest at the first sample in 2007 (June 2007), although the water was then only 15 ºC (Figure 14). The correlation between water temperature and the number of bacteria was monitored at the sampling site LB 7, which (according to our records) is the most affected by faecal coliform bacteria, but it also has the hottest water of all sampling points on the lake. Based on the results of 2005, in August when water temperature was highest, the number of faecal coliform bacteria was less than 10. A month earlier and a month later, the number of bacteria was significantly higher (271 per 100 mL), although the water temperature was lower than in August.

Even in the case of the River Savica, we did not find a correlation between water temperature and the number of bacteria. The water temperature is fairly constant throughout the year and an increase in bacteria in the water occurs regardless of water temperature. Similar as the water in the Savica, the water in the seven lakes had constant temperature (about 1-2 °C) at the time of sampling. The numbers of faecal coliform bacteria, however, increased during the summer months. This is consistent with our hypothesis that the cause of the presence of faecal coliform bacteria is in the Triglav Lake huts, which lie less than 100 m from the fifth Triglav Lake (AL 5). In May and June 2007, our research confirmed the presence of faecal coliform bacteria, while in July, when the hut was open, faecal coliform bacteria in the water appeared. In October, about a month after the closure of the hut, the number of bacteria decreased again. Nevertheless beside mountain huts, that are sources of coliform contamination, “wild toilets” around lakes and animal faeces can represent another potential source of coliform contamination.

5 CONCLUSIONS

Analysis of water from the Lake Bohinjsko Jezero and the River Savica and lakes in the Valley of Dolina Sedmerih Triglavskih Jezer has confirmed the presence of faecal coliform bacteria in almost all water bodies, with the exception of the first four lakes in the Valley of Dolina Sedmerih Triglavskih Jezer. To interpret the data from the study, we used the minimum hygiene rules and other requirements for bathing water, although in 2008 new rules came into force, which no longer include the parameters of total coliform and coliform bacteria from faecal origin. Nevertheless, our primarily interest was the presence and origin of coliform bacteria in the waters of the Lake Bohinjsko Jezero and its hinterland.

Differences between individual sampling points by the presence of faecal coliform bacteria were found. Possible sources are unregulated sewage systems and inadequate septic tanks. The analytical results showed that in the Lake Bohinjsko Jezero, the most important faecal coliform burden is sampling point LB 6, which lies just below the pasture. In this part, we refute the hypothesis that pasture and meadows along the lake can also be a source of faeces. The maximum number of bacteria was at sampling sites LB 1 and 4, and LB 7, where the largest number of summer visitors and swimmers are present. Despite some increase in micro-organisms in the water of the Lake Bohinjsko Jezero, limits on the “Rules on the Minimum Health and other Requirements for Bathing Water” have not been exceeded.

Analyses of water from the Alp Lakes have confirmed the presence of faecal coliform bacteria in three lakes. Although present in very small numbers, it is interesting that this occurred only in lakes that lie below the Triglav lakes hut and only when the hut is open.

The results of water analysis showed the presence of faecal coliform bacteria in the River Savica, which of course is not surprising, since bacteria were found in three lakes in the Valley of Dolina Sedmerih Triglavskih Jezer. In early 2007, when we started to sample water at locations within the Savica, we expected that the

maximum number of faecal bacteria would be at site S 3, but this was not confirmed. Between the sampling sites S 2 and S 3 are located houses of the village of Ukanc. Although the sewage system in Ukanc was built in 2006, we were informed that the holiday house in Ukanc had not yet been connected to a sewerage system in 2007.
Although the bacteria levels increase during the summer months, we have shown that water temperature does not affect the growth of microorganisms. Consequently, the bacteria levels are associated with an increase of visitors of Bohinj, as well as the highlands. The occurrence of bacteria in the three lakes that lie below the Triglav lakes hut, when the cottage is open, confirm the hypothesis that an increase in the number of visitors to the mountains is one of the main reasons for faecal contamination. Probably an unregulated cottage septic tank is not the only reason; there is also the case of "wild toilets" in the vicinity of the hut, as well as natural sources (animal). The analytical methods that were used to determine the bacteria do not confirm whether the bacteria are human or animal origin. However, there are many indicators that the presence of bacteria is associated with human activities, both in the lakes in the Valley of Dolina Sedmerih Triglavskih Jezer as well as Lake Bohinjsko Jezero. In the future, more attention should be directed at specific risk analysis, which could establish whether the bacteria are of human origin, domestic animals or wildlife.

6 SUMMARY

The Lake Bohinjsko Jezero is valuable habitat (fauna, macrophytes) and economically important for the people of the Bohinj valley. The lake is popular for swimming and other water sports in the summer season. There are several small streams entering the lake, both permanent and temporary. The main inflow is the River Savica, which emerges from Komarča cliff as a large karst spring, followed by a waterfall. The Savica receives water from six karst lakes in the Valley of Dolina Sedmerih Triglavskih Jezer (Triglav National Park). The Lake Bohinjsko Jezero should be among the cleanest lakes in Slovenia. Different indicators show that the inflow of nutrients has been increasing in recent years as a result of tourism. Pollution in its catchment area can be categorized as point-source and nonpoint-source pollution. Sources of bacteria include households, summer houses and alpine cottages in a catchment of the lake.

Sampling and analysis of water from the Lake Bohinjsko Jezero started in 2005; several locations along the lake shore were monitored before, during and after the swimming season to investigate the total and faecal coliform bacterial contamination of the lake. At the first samplings of water from the Lake Bohinjsko Jezero, we confirmed that faecal coliform bacteria were present in the lake. Experimental sampling of the River Savica right before outflow into the lake (September 2005) showed that some of coliform bacteria come to the lake by the same inflow (affluent). In 2006, the research was expanded from the lake itself to the affluent of the lake, the River Savica and, in 2007, to the high-mountain lakes.

The results have shown that all the faecal pollution in the lake did not come from the lower part of the Valley of the Lake Bohinjsko Jezero, since the bacteria were also present in Savica waterfall, which is connected to seven lakes in the Valley of Dolina Sedmerih Triglavskih Jezer. Altogether, there were more than 209 samples collected.

The evaluation of coliform bacteria used “the most probable number” (MPN) method. The MPN technique is sometimes used instead of the standard plate count

method to estimate microbial populations in the environment. Results for the analysis of water from the Lake Bohinjsko Jezero showed that total coliform bacteria are present during all sampling periods (May to October). The number of total and faecal bacteria in water samples varied from 0 to more than 438 per 100 mL sample. Comparison values with demands for bathing water in natural environment (Rules on the Quality of Bathing Water) shows that values greater than 438 at the sampling places of the Lake Bohinjsko Jezero LB 1, LB 3 and LB 7 are close to the recommended level for total coliform bacteria. 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. Our hypothesis was 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. However, it was confirmed that some faecal coliform bacteria are present in the Lake Dvojno Jezero (Alp Lakes 5 and 6), which drains into the Lake Bohinjsko Jezero. Faecal coliform bacteria were present in three (AL 5–7) of the seven Alp Lakes. The number of total coliform bacteria per 100 mL samples from these three lakes varied from 0 to 76 bacteria. Common total coliform bacteria were present (0–15 bacteria per 100 mL sample) in the first four lakes, but we could not confirm the presence of faecal coliform bacteria in these samples. The hut near Lake Dvojno Jezero (Alp Lakes 5 and 6) does not have a waste-water treatment plant, and analysis showed that the appearance of faecal coliform bacteria in water samples from Alp Lakes AL 5–7 is linked to the opening of the alpine hut, as samples in May and June were negative (i.e. in a period when the hut was closed). Water from toilets and kitchens is discharged into the surrounding environment only about 100 metres from the lake. We hypothesise that faecal bacteria found in the Savica waterfall arrive from the mountain hut near the Lake Dvojno Jezero because such a direct (although weak) connection has already been confirmed (Urbanc & Brancelj, 2002).

Results for the sampling location of the River Savica (S 2), which is about one kilometre from the waterfall, indicated a higher number of faecal colform bacteria (4–95 bacteria per 100 mL sample) in comparison to sampling location S 1 (0–46). The source of additional bacteria is Koča pri Savici and its septic tank, which is only 5 meters from the River Savica. Results for the sampling location of Savica 3 (S 3) (near the inflow to the Lake Bohinjsko Jezero) indicated that faecal bacteria were found regularly, except 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 sewage system.

Results for the analysis of water from the Lake Bohinjsko Jezero showed that faecal coliform bacteria are present during all sampling periods (May to October). Their numbers in a 100 mL sample were lowest in sampling places LB 5 (0–58 bacteria) and LB 6 (0–71 bacteria) on the northern side of the lake. There are neither contaminators nor 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 LB 1 and LB 7 are the most contaminated (from 0 to more than 438 faecal coliform bacteria per 100 mL sample). These are areas with the highest number of swimmers.

The results of this study confirmed assumptions that most of the faecal coliform 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. Our hypothesis was 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 LB 6). However, it was confirmed that some faecal coliform bacteria are present in Lake Dvojno Jezero (Alp Lakes 5 and 6) and Alp Lake Črno Jezero (AL 7), which drains into the Lake Bohinjsko Jezero. They were detected in a sample from the spring of the River Savica (S 1), which connects directly with the aforementioned lake. Some increase in faecal coliform bacteria was detected in the lower reach of the River Savica, indicating local pollution along the river itself. The effects of human activities on the Lake Bohinjsko Jezero and the Alp Lakes have always been present, but in recent years, due to the impacts of tourism, they are becoming increasingly burdensome. Easier and better access to remote places means more waste and waste water, which largely mirrored in nature. Despite this, with appropriate measures, nature can be preserved. Otherwise, it may happen that life will no longer be present in the Lakes Sedmera Triglavska Jezera or the Lake Bohinjsko Jezero will not be suitable for swimming and other water sports.

7 POVZETEK

Bohinjsko jezero predstavlja pomembno vlogo z vidika naravnih danosti in gospodarskega razvoja, saj je jezero priljubljena točka turistov, zlasti plavalcev in drugih športnikov. Več manjših stalnih ali začasnih pritokov napaja Bohinjsko jezero. Glavni pritok je reka Savica, ki zbira vodo iz šestih kraških jezer v Dolini sedmerih triglavskih jezer. Bohinjsko jezero bi moralo biti eno izmed najbolj čistih v Sloveniji, toda različni okoljski kazalci kažejo na povečano prisotnost hranil v vodi, predvsem zaradi turizma. Onesnaževanje Bohinjskega jezera lahko opredelimo kot točkovni in netočkovni vir onesnaževanja. Vire bakterijskega onesnaževanja pa vključujejo gospodinjstva, počitniške hiše in alpske koče v zaledju Bohinjskega jezera.

Prvo vzorčenje in analiza vode iz Bohinjskega jezera je bila izvedena leta 2005, ko smo ob obali Bohinjskega jezera na večih lokacijah analizirali onesnaženje jezera na bakterije fekalnega izvora. Že prvo vzorčenje vode je potrdilo prisotnost fekalnih bakterij v jezeru. Poskusno vzorčenje vode iz reke Savice tik pred izlivom v jezero smo izvedli septembra 2005. Vendar je tudi ta analiza pokazala prisotnost nekaj bakterij fekalnega izvora. Na podlagi rezultatov smo v letu 2006 raziskavo razširili z jezera tudi na pritok reko Savico in v letu 2007 še na visokogorska jezera.

Rezultati so pokazali, da fekalno onesnaženje jezera ne izvira le iz doline v okolici Bohinjskega jezera, saj so bile bakterije prisotne tudi v slapu Savica, ki je povezan s Sedmerimi jezeri v Dolini sedmerih triglavskih jezer. V celotnem poteku raziskave je bilo zbranih 209 vzorcev, med katerimi je bilo 131 vzorcev odvzetih iz Bohinjskega jezera, 37 vzorcev iz reke Savica ter 41 vzorcev iz visokogorskih jezer.

Za dokazovanje prisotnosti koliformnih bakterij je bila uporabljena metoda najverjetnejšega števila – metoda MPN. MPN metoda je včasih uporabljena namesto standardne metode (CFU) za oceno mikrobnih populacij v okolju. Rezultati analize vode iz Bohinjskega jezera so pokazali prisotnost koliformnih bakterij v vseh vzorčevalnih obdobjih (od maja do oktobra). Število bakterij v vzorcih vode je nihalo med 0 do več kot 438 na 100 mL vzorca. Primerjava vrednosti z zahtevami kopalnih vod v naravnem okolju (Pravilnik o minimalnih higienskih in drugih zahtevah za kopalne vode) kaže, da so vrednosti, večje od 438 na vzorčevalnih mestih Bohinjskega jezera LB 1, LB 3, LB 7, blizu priporočeni vrednosti za skupne koliformne bakterije. Rezultati potrjujejo domnevo, da del fekalnih koliformnih bakterij izvira iz greznic stanovanjskih hiš in drugih bivališčih; manjši del pa s pašnikov, travnikov in obdelovalnih površin v neposrednem zaledju jezera. Naša hipoteza je bila, da so glavni onesnaževalci živinoreja in kmetijstvo, vendar se zdi, da je na območjih, kjer prevladujejo pašniki in obdelovalne površine, onesnaževanje jezera manjše. So pa rezultati raziskave potrdili prisotnost koliformnih bakterij tudi v Dvojnem jezeru (Alpska jezera 5 in 6), ki se posredno, preko Savice, izliva v Bohinjsko jezero.

Fekalne koliformne bakterije so bile prisotne v treh od sedmih visokogorskih jezer. Število koliformnih bakterij v 100 mL vzorca iz omenjenih treh jezer je nihalo med 0 in 76 bakterij. Skupne koliformne bakterije so bile prisotne (0 – 15 bakterij na 100 mL vzorec) v prvih štirih jezerih, vendar nismo mogli potrditi prisotnosti fekalnih koliformnih bakterij v teh vzorcih. Koča v bližini Dvojnega jezera (AL 5 in 6) nima čistilne naprave in analiza je pokazala, da pojav fekalnih bakterij v vzorcih vode iz visokogorskih jezer (AL 5 – 7) sovpada z odprtjem gorske koče, saj so bili vzorci v maju in juniju, ko je koča še zaprta, negativni. Voda iz stranišč in kuhinj odteka v okolico le približno 300 metrov od jezera. Domnevali smo, da tiste fekalne bakterije, ki jih najdemo v Slapu Savica, pridejo iz gorske koče blizu Dvojnega jezera, saj je neposredna povezava (čeprav šibka) že bila potrjena (Urbanc & Brancelj, 2002).

Rezultati vzorčevalnega mesta Slapa Savica (S 2), ki je približno kilometer oddaljen od slapa, nakazujejo višje število fekalnih bakterij (4 – 95 bakterij na 100 mL vzorca) v primerjavi z vzorčevalnim mestom S 1 (0 – 46). Vir dodatnih bakterij je Koča pri Savici in njena greznica, ki je samo pet metrov oddaljena od reke Savica. Rezultati vzorčevalnega mesta Savica 3 (S 3) (blizu pritoka v Bohinjsko jezera) kažejo, da so bile fekalne baterije najdene v vseh vzorcih, razen tistih, odvzetih v maju 2007. Viri bakterij na tem mestu so najverjetneje počitniške hiše in stanovanja v Ukancu, ker še niso vse priključene na obstoječi kanalizacijski sistem.

Rezultati analize vode iz Bohinjskega jezera so pokazali, da so koliformne bakterije prisotne skozi vsa vzorčevalna obdobja (od maja do oktobra). Število bakterij na 100 mL vzorca je bilo najnižje na vzorčevalnih mestih LB 5 (0 – 38) in LB 6 (7 – 71 bakterij), ki ležita na severni strani jezera. Na tem območju ni onesnaževalcev niti stalnih pritokov. Druga vzorčevalna mesta so pokazala nihanja v številu bakterij v vzorcih vode skozi sezono. Najbolj onesnaženi sta bili vzorčevalni mesti LB 1 in LB 7 (od 7 do več kot 438 bakterij na 100 mL vzorca). To so hkrati tudi območja z največjim številom kopalcev v poletni sezoni.

Rezultati te študije so potrdili domneve, da večina fekalnih bakterij prispe v jezero preko neurejenih greznic v okolici Bohinjskega jezera. Nekaj fekalnih bakterij prispevajo tudi pašniki, obdelovalne površine in travniki, ki so v neposredni bližini jezera. Naša hipoteza je bila, da so glavni onesnaževalci živinoreja in kmetijstvo, vendar se je pokazalo, da je na območjih, kjer prevladujejo pašniki in obdelovalne površine, jezero manj onesnaženo (vzorčevalno mesto LB 6). Vendar je bilo potrjeno, da je nekaj koliformnih bakterij prisotnih v Dvojnem jezeru (AL 5 in 6) in Črnem jezeru (AL 7), iz katerih voda odteka po podzemnih kanalih v Bohinjsko jezero. Bakterije smo zasledili v vzorcu iz izvira Slapa Savice (S 1), ki je neposredno povezan z zgoraj omenjenima jezeroma. Porast koliformnih bakterij smo odkrili v spodnjem toku reke Savice, ki nakazuje na lokalno onesnaževanje vzdolž same reke.

Vplivi človekove dejavnosti na Bohinjsko jezero in visokogorska jezera je prisoten že od nekdaj, vendar pa v zadnjih letih zaradi turizma vplivi postajajo vse bolj obremenjujoči. Lažja in boljša dostopnost odročnejših krajev pomeni več odpadkov in odpadnih voda, kar se v največji meri zrcali v naravi. S primernimi ukrepi lahko kljub razvoju zaščitimo naravo in jo ohranimo takšno, kot je. V nasprotnem primeru se lahko zgodi, da v Sedmerih jezerih čez nekaj let ne bo več življenja, Bohinjsko jezero pa ne bo več primerno za kopanje in druge vodne športe.

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Martina Oder

ANNEXES

Annex 1: Field protocol

TERENSKI LIST

Odvzem vzorca površinske kopalne vode

Mesto vzorčenja:

Datum odvzema: ***Čas odvzema (ura in min):***

Terenske meritve:

Parameter	***Izmerjena vrednost***	***Enota***
Temperatura vode		***^oC***
Temperatura zraka		***^oC***
pH		

Organoleptična opažanja	***Da/Ne***	***Opombe***
Barva		
Vidne nečistoče		
Mineralna olja (oljni film na površini)		

Kontrola temperature hladilne komore med transportom:

Pred prvim vzorčenjem: _____ °C ***Pred oddajo vzorca: _____ °C***

OPOMBE:

Annex 2: Reports on microbiological testing of water

INŠTITUT ZA VAROVANJE ZDRAVJA REPUBLIKE SLOVENIJE
INSTITUTE OF PUBLIC HEALTH OF THE REPUBLIC OF SLOVENIA
LJUBLJANA, Trubarjeva 2 p.p. 260, tel. 01 24 41 400, fax. 01 24 41 447, SI 10007989
IVZ RS **Oddelek za sanitarno mikrobiologijo,** Grablovičeva 44, Ljubljana, tel. 01 520 57 29, fax. 01 520 57 30

Protokol: **PV/2020**
Status: **Končni**, Verzija: **1.00**

POROČILO O MIKROBIOLOŠKEM PRESKUSU - VODE

Pošiljatelj, naročnik, plačnik, lastnik: **Nacionalni inštitut za biologijo**, Večna pot 111, 1000 LJUBLJANA

Sprejem: **10.07.2006** ob **09:14**, Preiskano do: **24.07.2006**
Namen odvzema: **Lastnikova zahteva** Vzorce prinesel: **naročnik**

Vzorec: **Površinska voda- stoječa - iz jezera št. 2**

Odvzel: **Milič Martina**
Datum odvzema: **10.07.2006**
Stanje ob prevzemu: **ustrezno**

Preiskava: **Vode- koliformne bakterije MPN (15)**
Zahtevek: Obseg preiskav in kriteriji so določeni v skladu z internimi zahtevami naročnika.

PARAMETER	METODA	PREISKANA KOLIČINA	KRITERIJ	REZULTAT	ENOTA	OCENA	ZAČETEK KONEC
Koliformne bakterije	ISO 9308-2	100 ml	0	30	MPN/100 ml	/	10.07.2006 19.07.2006

OCENA:

OPOMBA: **V vzorcu smo s testom API 20E (BIOMERIEUX) potrdili prisotnost enterobakterij *(Escherichia coli, Klebsiella* spp. in *Citrobacter youngae).***

INŠTITUT ZA VAROVANJE REPUBLIKE SLOVENIJE 13

Vodja oddelka:
Tatjana Rupel, univ.dipl.biol.,
spec.med.mikrobiol.

Rezultati preskusa se nanašajo izključno na preskušani vzorec. Ocena velja, če je bilo vzorčenje izvedeno v skladu z veljavnimi predpisi.
Poročilo se brez pisnega pristanka preskusnega laboratorija ne sme reproducirati, razen v celoti in se ne sme uporabljati v reklamne namene.

V Ljubljani, 28.07.2006
Stran: 1 od 1

INŠTITUT ZA VAROVANJE ZDRAVJA REPUBLIKE SLOVENIJE
INSTITUTE OF PUBLIC HEALTH OF THE REPUBLIC OF SLOVENIA
LJUBLJANA, Trubarjeva 2 p.p. 260, tel. 01 24 41 400, fax. 01 24 41 447, SI 10007989
IVZ RS **Oddelek za sanitarno mikrobiologijo**, Grablovičeva 44, Ljubljana, tel. 01 520 57 29, fax. 01 520 57 30

Protokol: **PV/2022**
Status: **Končni**, Verzija: **1.00**

POROČILO O MIKROBIOLOŠKEM PRESKUSU - VODE

Pošiljatelj, naročnik, plačnik, lastnik: **Nacionalni inštitut za biologijo**, Večna pot 111, 1000 LJUBLJANA

Sprejem: **10.07.2006** ob **09:14**, Preiskano do: **24.07.2006**
Namen odvzema: **Lastnikova zahteva** Vzorce prinesel: **Milič Martina**

Vzorec: **Površinska voda- stoječa - iz jezera št. 6**

Odvzel: **Milič Martina**
Datum odvzema: **10.07.2006**
Stanje ob prevzemu: **ustrezno**

Preiskava: **Vode- koliformne bakterije MPN (15)**
Zahtevek: Obseg preiskav in kriteriji so določeni v skladu z internimi zahtevami naročnika.

PARAMETER	METODA	PREISKANA KOLIČINA	KRITERIJ	REZULTAT	ENOTA	OCENA	ZAČETEK KONEC
Koliformne bakterije	ISO 9308-2	100 ml	0	11	MPN/100 ml	/	10.07.2006 19.07.2006

OCENA:

OPOMBA: V vzorcu smo s testom API 20E (BIOMERIEUX) potrdili prisotnost enterobakterij- *(Escherichia coli in Klebsiella oxytoca).*

Vodja oddelka:
Taljana Rupel, univ.dipl.biol.
spec.med.mikrobiol.

Rezultati preskusa se nanašajo izključno na preskušani vzorec. Ocena velja, če je bilo vzorčenje izvedeno v skladu z veljavnimi predpisi.
Poročilo se brez pisnega pristanka preskusnega laboratorija ne sme reproducirati, razen v celoti in se ne sme uporabljati v reklamne namene.

V Ljubljani, 28.07.2006
Stran: 1 od 1

INŠTITUT ZA VAROVANJE ZDRAVJA REPUBLIKE SLOVENIJE
INSTITUTE OF PUBLIC HEALTH OF THE REPUBLIC OF SLOVENIA
LJUBLJANA, Trubarjeva 2 p.p. 260, tel. 01 24 41 400, fax. 01 24 41 447, SI 10007989
IVZ RS Oddelek za sanitarno mikrobiologijo, Grablovičeva 44, Ljubljana, tel. 01 520 57 29, fax. 01 520 57 30

Protokol: **PV/2021**
Status: **Končni**, Verzija: **1.00**

POROČILO O MIKROBIOLOŠKEM PRESKUSU - VODE

Pošiljatelj, naročnik, plačnik, lastnik: **Nacionalni inštitut za biologijo**, Večna pot 111, 1000 LJUBLJANA

Sprejem: **10.07.2006** ob **09:14**, Preiskano do: **24.07.2006**
Namen odvzema: **Lastnikova zahteva** Vzorce prinesel: **Milič Martina**

Vzorec: **Površinska voda- stoječa - iz jezera št. 4**

Odvzel: **Milič Martina**
Datum odvzema: **10.07.2006**
Stanje ob prevzemu: **ustrezno**

Preiskava: **Vode- koliformne bakterije MPN (15)**
Zahtevek: Obseg preiskav in kriteriji so določeni v skladu z internimi zahtevami naročnika.

PARAMETER	METODA	PREISKANA KOLIČINA	KRITERIJ	REZULTAT	ENOTA	OCENA	ZAČETEK KONEC
Koliformne bakterije	ISO 9308-2	100 ml	0	8	MPN/100 ml	/	10.07.2006 19.07.2006

OCENA:

OPOMBA: **V vzorcu smo s testom API 20E (BIOMERIEUX) potrdili prisotnost enterobakterij *(Escherichia coli, Enterobacter cloacae* in *Citrobacter* spp.).**

Vodja oddelka:
Tatjana Rupel, univ.dipl.biol.,
spec.med.mikrobiol.

Rezultati preskusa se nanašajo izključno na preskušani vzorec. Ocena velja, če je bilo vzorčenje izvedeno v skladu z veljavnimi predpisi.
Poročilo se brez pisnega pristanka preskusnega laboratorija ne sme reproducirati, razen v celoti in se ne sme uporabljati v reklamne namene.

V Ljubljani, 28.07.2006
Stran: 1 od 1

INŠTITUT ZA VAROVANJE ZDRAVJA REPUBLIKE SLOVENIJE
INSTITUTE OF PUBLIC HEALTH OF THE REPUBLIC OF SLOVENIA
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IVZ RS Oddelek za sanitarno mikrobiologijo, Grablovičeva 44, Ljubljana, tel. 01 520 57 29, fax. 01 520 57 30

Protokol: **PV/2023**
Status: **Končni**, Verzija: **1.00**

POROČILO O MIKROBIOLOŠKEM PRESKUSU - VODE

Pošiljatelj, naročnik, plačnik, lastnik: **Nacionalni inštitut za biologijo**, Večna pot 111, 1000 LJUBLJANA

Sprejem: **10.07.2006** ob **09:14**, Preiskano do: **24.07.2006**
Namen odvzema: **Lastnikova zahteva** Vzorce prinesel: **Milič Martina**

Vzorec: **Površinska voda- stoječa - iz jezera št. 7**

Odvzel: **Milič Martina**
Datum odvzema: **10.07.2006**
Stanje ob prevzemu: **ustrezno**

Preiskava: **Vode- koliformne bakterije MPN (15)**
Zahtevek: Obseg preiskav in kriteriji so določeni v skladu z internimi zahtevami naročnika.

PARAMETER	METODA	PREISKANA KOLIČINA	KRITERIJ	REZULTAT	ENOTA	OCENA	ZAČETEK KONEC
Koliformne bakterije	ISO 9308-2	100 ml	0	8	MPN/100 ml	/	10.07.2006 19.07.2006

OCENA: /

OPOMBA: V vzorcu smo s testom API 20E (BIOMERIEUX) potrdili prisotnost enterobakterij (*Escherichia coli* in *Klebsiella oxytoca*.

INŠTITUT ZA VAROVANJE ZDRAVJA REPUBLIKE SLOVENIJE 13

Vodja oddelka:
Tatjana Rupel, univ.dipl.biol.,
spec.med.mikrobiol.

Rezultati preskusa se nanašajo izključno na preskušani vzorec. Ocena velja, če je bilo vzorčenje izvedeno v skladu z veljavnimi predpisi.
Poročilo se brez pisnega pristanka preskusnega laboratorija ne sme reproducirati, razen v celoti in se ne sme uporabljati v reklamne namene.

V Ljubljani, 28.07.2006
Stran: 1 od 1

INŠTITUT ZA VAROVANJE ZDRAVJA REPUBLIKE SLOVENIJE
INSTITUTE OF PUBLIC HEALTH OF THE REPUBLIC OF SLOVENIA
LJUBLJANA, Trubarjeva 2 p.p. 260, tel. 01 24 41 400, fax. 01 24 41 447, SI 10007989
IVZ RS **Oddelek za sanitarno mikrobiologijo,** Grablovičeva 44, Ljubljana, tel. 01 520 57 29, fax. 01 520 57 30

Protokol: **PV/2525**
Status: **Končni**, Verzija: **1.00**

POROČILO O MIKROBIOLOŠKEM PRESKUSU - VODE

Pošiljatelj, naročnik, plačnik, lastnik: **Nacionalni inštitut za biologijo**, Večna pot 111, 1000 LJUBLJANA

Sprejem: **14.08.2006** ob **10:30**, Preiskano do: **25.08.2006**
Namen odvzema: **Lastnikova zahteva** Vzorce prinesel: **naročnik**

Vzorec: **Iz jezera št. 1**

Odvzel: **Milič Martina**
Datum odvzema: **14.08.2006**
Stanje ob prevzemu: **ustrezno**

Preiskava: **Vode - koliformne bakterije MPN (15)**
Zahtevek: Obseg preiskav in kriteriji so določeni v skladu z internimi zahtevami naročnika.

PARAMETER	METODA	PREISKANA KOLIČINA	KRITERIJ	REZULTAT	ENOTA	OCENA	ZAČETEK KONEC
Koliformne bakterije	ISO 9308-2	100 ml	0	80	MPN/100 ml	/	14.08.2006 25.08.2006

OCENA:

OPOMBA: V vzorcu smo s testom API-20E (BioMerieux) potrdili prisotnost bakterij *Escherichia coli* in *Enterobacter sp.*

INŠTITUT ZA VAROVANJE ZDRAVJA REPUBLIKE SLOVENIJE 13

Za Vodja oddelka:
Tatjana Rupel, univ.dipl.biol.,
spec.med.mikrobiol.

Rezultati preskusa se nanašajo izključno na preskušani vzorec. Ocena velja, če je bilo vzorčenje izvedeno v skladu z veljavnimi predpisi.
Poročilo se brez pisnega pristanka preskusnega laboratorija ne sme reproducirati, razen v celoti in se ne sme uporabljati v reklamne namene.

V Ljubljani, 25.08.2006
Stran: 1 od 1

INŠTITUT ZA VAROVANJE ZDRAVJA REPUBLIKE SLOVENIJE
INSTITUTE OF PUBLIC HEALTH OF THE REPUBLIC OF SLOVENIA

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IVZ RS **Oddelek za sanitarno mikrobiologijo,** Grabloviceva 44, Ljubljana, tel. 01 520 57 29, fax. 01 520 57 30

Protokol: **PV/2526**
Status: **Končni**, Verzija: **1.00**

POROČILO O MIKROBIOLOŠKEM PRESKUSU - VODE

Pošiljatelj, naročnik, plačnik, lastnik: **Nacionalni inštitut za biologijo**, Večna pot 111, 1000 LJUBLJANA

Sprejem: **14.08.2006** ob **10:30**, Preiskano do: **25.08.2006**
Namen odvzema: **Lastnikova zahteva** Vzorce prinesel: **naročnik**

Vzorec: **Iz jezera št.2**

Odvzel: **Milič Martina**
Datum odvzema: **14.08.2006**
Stanje ob prevzemu: **ustrezno**

Preiskava: **Vode - koliformne bakterije MPN (15)**
Zahtevek: Obseg preiskav in kriteriji so določeni v skladu z internimi zahtevami naročnika.

PARAMETER	METODA	PREISKANA KOLIČINA	KRITERIJ	REZULTAT	ENOTA	OCENA	ZAČETEK KONEC
Koliformne bakterije	ISO 9308-2	100 ml	0	240	MPN/100 ml	/	14.08.2006 25.08.2006

OCENA:

OPOMBA: V vzorcu smo s testom API-E (BioMerieux) potrdili prisotnost bakterij *Escherichia coli, Klebsiella pneumoniae* in *Enterobacter sp.*

INŠTITUT ZA VAROVANJE ZDRAVJA REPUBLIKE SLOVENIJE 13

29 Vodja oddelka:
Tatjana Rupel, univ.dipl.biol.,
spec.med.mikrobiol.

V Ljubljani, 25.08.2006
Stran: 1 od 1

INŠTITUT ZA VAROVANJE ZDRAVJA REPUBLIKE SLOVENIJE
INSTITUTE OF PUBLIC HEALTH OF THE REPUBLIC OF SLOVENIA
LJUBLJANA, Trubarjeva 2 p.p. 260, tel. 01 24 41 400, fax. 01 24 41 447, SI 10007989
IVZ RS Oddelek za sanitarno mikrobiologijo, Grablovičeva 44, Ljubljana, tel. 01 520 57 29, fax. 01 520 57 30

Protokol: **PV/2527**
Status: **Končni**, Verzija: **1.00**

POROČILO O MIKROBIOLOŠKEM PRESKUSU - VODE

Pošiljatelj, naročnik, plačnik, lastnik: **Nacionalni inštitut za biologijo**, Večna pot 111, 1000 LJUBLJANA

Sprejem: **14.08.2006** ob **10:30**, Preiskano do: **25.08.2006**
Namen odvzema: **Lastnikova zahteva** Vzorce prinesel: **naročnik**

Vzorec: **Iz jezera št. 6**

Odvzel: **Milič Martina**
Datum odvzema: **14.08.2006**
Stanje ob prevzemu: **ustrezno**

Preiskava: **Vode - koliformne bakterije MPN (15)**
Zahtevek: Obseg preiskav in kriteriji so določeni v skladu z internimi zahtevami naročnika.

PARAMETER	METODA	PREISKANA KOLIČINA	KRITERIJ	REZULTAT	ENOTA	OCENA	ZAČETEK KONEC
Koliformne bakterije	ISO 9308-2	100 ml	0	130	MPN/100 ml	/	14.08.2006 25.08.2006

OCENA:

OPOMBA: V vzorcu smo s testom API-E (BioMerieux) potrdili prisotnost bakterij *Escherichia coli* in *Klebsiella ornithinolytica*.

INŠTITUT ZA VAROVANJE ZDRAVJA REPUBLIKE SLOVENIJE 13

za Vodja oddelka:
Tatjana Rupel, univ.dipl.biol.,
spec.med.mikrobiol.

Rezultati preskusa se nanašajo izključno na preskušani vzorec. Ocena velja, če je bilo vzorčenje izvedeno v skladu z veljavnimi predpisi.
Poročilo se brez pisnega pristanka preskusnega laboratorija ne sme reproducirati, razen v celoti in se ne sme uporabljati v reklamne namene.

V Ljubljani, 25.08.2006
Stran: 1 od 1

INŠTITUT ZA VAROVANJE ZDRAVJA REPUBLIKE SLOVENIJE
INSTITUTE OF PUBLIC HEALTH OF THE REPUBLIC OF SLOVENIA
LJUBLJANA, Trubarjeva 2 p.p. 260, tel. 01 24 41 400, fax. 01 24 41 447, SI 10007989
IVZ RS **Oddelek za sanitarno mikrobiologijo,** Grablovičeva 44, Ljubljana, tel. 01 520 57 29, fax. 01 520 57 30

Protokol: **PV/2528**
Status: **Končni**, Verzija: **1.00**

POROČILO O MIKROBIOLOŠKEM PRESKUSU - VODE

Pošiljatelj, naročnik, plačnik, lastnik: **Nacionalni inštitut za biologijo**, Večna pot 111, 1000 LJUBLJANA

Sprejem: **14.08.2006** ob **10:30**, Preiskano do: **25.08.2006**
Namen odvzema: **Lastnikova zahteva** Vzorce prinesel: **naročnik**

Vzorec: **Iz jezera št. 7**

Odvzel: **Milič Martina**
Datum odvzema: **14.08.2006**
Stanje ob prevzemu: **ustrezno**

Preiskava: **Vode - koliformne bakterije MPN (15)**
Zahtevek: Obseg preiskav in kriteriji so določeni v skladu z internimi zahtevami naročnika.

PARAMETER	METODA	PREISKANA KOLIČINA	KRITERIJ	REZULTAT	ENOTA	OCENA	ZAČETEK KONEC
Koliformne bakterije	ISO 9308-2	100 ml	0	240	MPN/100 ml	/	14.08.2006 25.08.2006

OCENA:

OPOMBA: V vzorcu smo s testom API-E (BioMerieux) potrdili prisotnost bakterij *Escherichia coli*, *Klebsiella pneumoniae* in *Enterobacter sp.*

ZA VAROVANJE ZDRAVJA REPUBLIKE SLOVENIJE INŠTITUT 13

za Vodja oddelka:
Tatjana Rupel, univ.dipl.biol.,
spec.med.mikrobiol.

Rezultati preskusa se nanašajo izključno na preskušani vzorec. Ocena velja, če je bilo vzorčenje izvedeno v skladu z veljavnimi predpisi.
Poročilo se brez pisnega pristanka preskusnega laboratorija ne sme reproducirati, razen v celoti in se ne sme uporabljati v reklamne namene.

V Ljubljani, 25.08.2006
Stran: 1 od 1

INŠTITUT ZA VAROVANJE ZDRAVJA REPUBLIKE SLOVENIJE
INSTITUTE OF PUBLIC HEALTH OF THE REPUBLIC OF SLOVENIA
LJUBLJANA, Trubarjeva 2 p.p. 260, tel. 01 24 41 400, fax. 01 24 41 447, SI 10007989
IVZ RS Oddelek za sanitarno mikrobiologijo, Grablovičeva 44, Ljubljana, tel. 01 520 57 29, fax. 01 520 57 30

Protokol: **PV/2780**
Status: **Končni**, Verzija: **1.00**

POROČILO O MIKROBIOLOŠKEM PRESKUSU - VODE

Pošiljatelj, naročnik, plačnik, lastnik: **Nacionalni inštitut za biologijo**, Večna pot 111, 1000 LJUBLJANA

Sprejem: **04.09.2006** ob **11:00**, Preiskano do: **11.09.2006**
Namen odvzema: **Lastnikova zahteva** Vzorce prinesel: **naročnik**

Vzorec: **Površinska voda- stoječa - iz jezera - št. 8 (slap Savica)**

Odvzel: **Milič Martina**
Datum odvzema: **04.09.2006**
Stanje ob prevzemu: **ustrezno**

Preiskava: **Vode - koliformne bakterije MPN (15)**
Zahtevek: Obseg preiskav in kriteriji so določeni v skladu z internimi zahtevami naročnika.

PARAMETER	METODA	PREISKANA KOLIČINA	KRITERIJ	REZULTAT	ENOTA	OCENA	ZAČETEK KONEC
Koliformne bakterije	ISO 9308-2	100 ml	0	30	MPN/100 ml	/	04.09.2006 11.09.2006

OCENA:

OPOMBA: V vzorcu smo potrdili prisotnost bakterij vrste *Escherichia coli* v številu MPN 30/100ml.

INŠTITUT ZA VAROVANJE ZDRAVJA REPUBLIKE SLOVENIJE 13

Vodja oddelka:
Tatjana Rupel, univ.dipl.biol.,
spec.med.mikrobiol.

V Ljubljani, 15.09.2006
Stran: 1 od 1

INŠTITUT ZA VAROVANJE ZDRAVJA REPUBLIKE SLOVENIJE
INSTITUTE OF PUBLIC HEALTH OF THE REPUBLIC OF SLOVENIA
LJUBLJANA, Trubarjeva 2 p.p. 260, tel. 01 24 41 400, fax. 01 24 41 447, SI 10007989
IVZ RS **Oddelek za sanitarno mikrobiologijo,** Grablovičeva 44, Ljubljana, tel. 01 520 57 29, fax. 01 520 57 30

Protokol: **PV/2781**
Status: **Končni**, Verzija: **1.00**

POROČILO O MIKROBIOLOŠKEM PRESKUSU - VODE

Pošiljatelj, naročnik, plačnik, lastnik: **Nacionalni inštitut za biologijo**, Večna pot 111, 1000 LJUBLJANA

Sprejem: **04.09.2006** ob **11:00**, Preiskano do: **15.09.2006**
Namen odvzema: **Lastnikova zahteva** Vzorce prinesel: **naročnik**

Vzorec: **Površinska voda- stoječa - iz jezera št. 7**

Odvzel: **Milič Martina**
Datum odvzema: **04.09.2006**
Stanje ob prevzemu: **ustrezno**

Preiskava: **Vode - koliformne bakterije MPN (15)**
Zahtevek: Obseg preiskav in kriteriji so določeni v skladu z internimi zahtevami naročnika.

PARAMETER	METODA	PREISKANA KOLIČINA	KRITERIJ	REZULTAT	ENOTA	OCENA	ZAČETEK KONEC
Koliformne bakterije	ISO 9308-2	100 ml	0	70	MPN/100 ml	/	04.09.2006 15.09.2006

OCENA:

OPOMBA: V vzorcu smo s testom API-E (BioMerieux) potrdili prisotnost bakterij *Klebsiella ornithinolytica*, *Citrobacter sp.*, *Pantoea sp.* in *Enterobacter sp.*

INŠTITUT ZA VAROVANJE ZDRAVJA REPUBLIKE SLOVENIJE 13

za Vodja oddelka:
Tatjana Rupel, univ.dipl.biol.,
spec.med.mikrobiol.

Rezultati preskusa se nanašajo izključno na preskušani vzorec. Ocena velja, če je bilo vzorčenje izvedeno v skladu z veljavnimi predpisi.
Poročilo se brez pisnega pristanka preskusnega laboratorija ne sme reproducirati, razen v celoti in se ne sme uporabljati v reklamne namene.

V Ljubljani, 15.09.2006
Stran: 1 od 1

INŠTITUT ZA VAROVANJE ZDRAVJA REPUBLIKE SLOVENIJE
INSTITUTE OF PUBLIC HEALTH OF THE REPUBLIC OF SLOVENIA
LJUBLJANA, Trubarjeva 2 p.p. 260, tel. 01 24 41 400, fax. 01 24 41 447, SI 10007989
IVZ RS **Oddelek za sanitarno mikrobiologijo,** Grablovičeva 44, Ljubljana, tel. 01 520 57 29, fax. 01 520 57 30

Protokol: **PV/2782**
Status: **Končni**, Verzija: **1.00**

POROČILO O MIKROBIOLOŠKEM PRESKUSU - VODE

Pošiljatelj, naročnik, plačnik, lastnik: **Nacionalni inštitut za biologijo**, Večna pot 111, 1000 LJUBLJANA

Sprejem: **04.09.2006** ob **11:00**, Preiskano do: **15.09.2006**
Namen odvzema: **Lastnikova zahteva** Vzorce prinesel: **naročnik**

Vzorec: **Površinska voda- stoječa - iz jezera št. 4**

Odvzel: **Milič Martina**
Datum odvzema: **04.09.2006**
Stanje ob prevzemu: **ustrezno**

Preiskava: **Vode - koliformne bakterije MPN (15)**
Zahtevek: Obseg preiskav in kriteriji so določeni v skladu z internimi zahtevami naročnika.

PARAMETER	METODA	PREISKANA KOLIČINA	KRITERIJ	REZULTAT	ENOTA	OCENA	ZAČETEK KONEC
Koliformne bakterije	ISO 9308-2	100 ml	0	11	MPN/100 ml	/	04.09.2006 15.09.2006

OCENA:

OPOMBA: V vzorcu smo s testom API-E (BioMerieux) potrdili prisotnost bakterij *Escherichia coli*, *Klebsiella oxytoca* in *Citrobacter freundii*.

za Vodja oddelka:
Tatjana Rupel, univ.dipl.biol.,
spec.med.mikrobiol.

Rezultati preskusa se nanašajo izključno na preskušani vzorec. Ocena velja, če je bilo vzorčenje izvedeno v skladu z veljavnimi predpisi.
Poročilo se brez pisnega pristanka preskusnega laboratorija ne sme reproducirati, razen v celoti in se ne sme uporabljati v reklamne namene.

V Ljubljani, 15.09.2006
Stran: 1 od 1

IVZ RS

INŠTITUT ZA VAROVANJE ZDRAVJA REPUBLIKE SLOVENIJE
INSTITUTE OF PUBLIC HEALTH OF THE REPUBLIC OF SLOVENIA
LJUBLJANA, Trubarjeva 2 p.p. 260, tel. 01 24 41 400, fax. 01 24 41 447, SI 10007989
Oddelek za sanitarno mikrobiologijo, Grablovičeva 44, Ljubljana, tel. 01 520 57 29, fax: 01 520 57 30

Protokol: **PV/2783**
Status: **Končni**, Verzija: **1.00**

POROČILO O MIKROBIOLOŠKEM PRESKUSU - VODE

Pošiljatelj, naročnik, plačnik, lastnik: **Nacionalni inštitut za biologijo**, Večna pot 111, 1000 LJUBLJANA

Sprejem: **04.09.2006** ob **11:00**, Preiskano do: **15.09.2006**
Namen odvzema: **Lastnikova zahteva** Vzorce prinesel: **naročnik**

Vzorec: **Površinska voda- stoječa - iz jezera št. 1**

Odvzel: **Milić Martina**
Datum odvzema: **04.09.2006**
Stanje ob prevzemu: **ustrezno**

Preiskava: **Vode - koliformne bakterije MPN (15)**
Zahtevek: Obseg preiskav in kriteriji so določeni v skladu z internimi zahtevami naročnika.

PARAMETER	METODA	PREISKANA KOLIČINA	KRITERIJ	REZULTAT	ENOTA	OCENA	ZAČETEK KONEC
Koliformne bakterije	ISO 9308-2	100 ml	0	23	MPN/100 ml	/	04.09.2006 15.09.2006

OCENA:

OPOMBA: V vzorcu smo s testom API-E (BioMerieux) potrdili prisotnost bakterij *Escherichia coli* in *Citrobacter sp.*

REPUBLIKE SLOVENIJE 13

Vodja oddelka:
Tatjana Rupel, univ.dipl.biol.,
spec.med.mikrobiol.

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V Ljubljani, 15.09.2006
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