# LAKE LUDAS SPECIAL NATURE RESERVE – BACTERIOLOGICAL POINT OF VIEW

ŽELJKA RUDIĆ[1], BOJANA VUJOVIĆ[2], MILE BOŽIĆ[1], VERA RAIČEVIĆ[2]
[1]*Institute for the Development of Water Resources „Jaroslav Černi", Jaroslava Černog 80, Belgrade, Serbia,*
[2]*University of Belgrade, Faculty of Agriculture, Nemanjina 6, Zemun-Belgrade, Serbia*

## STANJE SPECIJALNOG REZERVATA PRIRODE "LUDAŠKO JEZERO" SA BAKTERIOLOŠKOG ASPEKTA

*Apstrakt*

U ovom radu su prikazani rezultati bakteriološkog ispitivanja vode i sedimenta severnog i južnog dela jezera Ludaš. Jezero Ludaš je deo parka prirode Palić-Ludaš, a zbog velikog značaja u očuvanju ornitološkog diverziteta ovog područja od 1977. godine je zaštićeno i Ramsarskom konvencijom. Uzorci su sakupljani od novembra 2013. do marta 2015. godine i ukupno je analizirano 10 uzoraka vode i sedimenta. Kvalitet vode je ocenjen na osnovu ukupnog broja heterotrofnih bakterija (mezofila i psihrofila), fakultativnih oligotrofa, ukupnog broja koliformnih i fekalnih koliformnih bakterija, broja *E.coli* i fekalnih enterokoka. Ocenjivanje je izvršeno prema ekološkom i sanitarnom kriterijumu koristeći kriterijume Uredbe o kvalitetu površinskih voda i sedimenta Republike Srbije. Rezultati ukazuju na veliko variranje mikrobnog diverziteta tokom ispitivanog perioda. Na osnovu ukupnog broja bakterija, voda se može svrstati u II - IV klasu voda na severnoj strani, dok je na južnom delu voda u kategoriji III - V klase kvaliteta. Međutim, na osnovu indikatora fekalnog zagađenja, voda jezera na južnoj strani je značajno boljeg kvaliteta i u većini merenja odgovara kvalitetu I i II klase vodotoka. Razlike u sastavu saprofitne i potencijalno patogene grupe mikroorganizama zastupljene u različitim delovima jezera se mogu objasniti geografskim karakteristikama i vodi koja se kanalom Palić-Ludaš uliva u severni deo jezera, što utiče na broj bakterija indikatora fekalnog zagađenja. Veliko organsko opterećenje u južnom delu jezera se ogleda u značajno većem broju saprofitnih heterotrofa, a manjem autohtonih oligotrofa čime se smanjuje sposobnost autopurifikacije vode i ukazuje na ekološku ugroženost i ubrzan process eutrofikacije jezera Ludaš.

*Ključne reči: jezero Ludaš, heterotrofne bakterije, indikatori fekalnog zagađenja, autopurifikacija vode*
*Key words: Lake Ludas, bacterial density, indicator bacteria, autopurification*

## INTRODUCTION

Lakes provide water for numerous uses ranging from recreation and fisheries to power generation, industry and waste disposal. As a result of the last two uses, most lakes suffer from water quality degradation to some extent. The primary targets representing fecal contamination in temperate waters are now considered to be *E. coli* and enterococci (Ashbolt et al., 2001). Coliform bacteria originate from different sources (e.g. birds, boats, recreational swimmers, etc.), whose participation in pollution is difficult to assess, especially in urban areas where additional sources may exist, as industrial or urban wastewater (Canale et al., 1973). Activities like disposal of biological waste, including fertilization with manure, may, at the same time, increase the load of phosphorous, nitrogen and coliform bacteria in surface waters (Hofmann & Beaulieu, 2001). Both, suspended sediment at the bottom of the lake and the sediment of the tributaries that recharge the lake represent a source of *E. coli* (Struffolino, 2010). Prolonged survival of fecal coliforms and *E. coli* in freshwaters, as well as survival of *E. coli* in sediments and soils over extended periods of time has already been indicated in several studies (Ksoll et al., 2007). Pathogens that reach the watercourses as a consequence of wastewater discharge, can also influence aquatic organisms (Harvell et al., 2004), as well as birds that live in the vicinity (Anza et al., 2014), which is especially important for protected areas, such as nature reserves. The Lake Ludas is a shallow lake in the province of Vojvodina in northern Serbia. In 1977 this lake was added on a list of Ramsar sites, while it is a centre of biological diversity, where numerous rare, endemic and relict species exist. The aim of the paper is to highlight the current state of this nature reserve from the bacteriological and ecological point of view, considering both water and sediment quality of the central part of the reserve, the Lake Ludas.

## MATHERIAL AND METHODS

The Lake Ludas lies on a sandy terrain between the Danube and the Tisza River. It covers area of 3.17 $km^2$, with the depth up to 2 m (average 0.9 m). The canal "Palic-Ludas", which carries a mixture of atmospheric water collected by open-canal network, wastewater and water from Lake Palic, represents an important water source for Lake Ludas.

Water and sediment samples were collected in the period November 2013 - March 2015 (10 samples of each). Sampling was performed at the South and North side of the Lake (near the school and near Visitor's centre). Water samples were collected at 20-50 cm beneath the water surface and the sediment from the surface layer of sediments. Samples were transported in cool containers at 4°C and tested within 12-24 hours of collection.

Detection of fecal contamination indicators (total coliforms, fecal coliforms, *Escherichia coli* and *Enterococcus*) was done by the most probable number (MPN) method using three tubes in each dilution. Presumptive test for total and fecal coliforms were done using the Mac Conkey (lactose) broth with inverted Durham tubes at 37°C/48 h. Detection of *Escherichia coli* and other fecal coliform strains were done by inoculation Endo agar from positive tubes and incubation at 44°C/24h. Presumptive test of *Enterococcus* were done by using azide–dextroze broth (37°C/48h), and the confirmation by inoculation on the Bile Esculin Agar (37°C/24h). Results are reported as MPN per 100 mL of water, or 1 g dw sediment.

Total number of heterotrophic bacteria was estimated by Pour Plate method, using Nutrient agar; incubation of mesophile was ensured at 37°C/48h and psychrophile at 20°C/3-5 days. Assessing facultative oligotrophs were done using diluted Nutrient agar (1:10), 20°C/3-5 days. Present results demonstrate total number of viable bacteria cells as counted colonies (CFU) per 1 mL. Petrović et al. (1998) suggest using density ratio of facultative oligotrophs and heterotrophic bacteria (FO/H) to obtain more in-depth ecological information about water quality: FO/H <1 pour, ≥1 satisfactory and >10 is a sign of good autopurification efficiency.

## RESULTS AND DISCUSION

Nation Regulation of surface water quality (Official gazette RS 50/2012) differs five water categories regarding bacterial density, from I category - excellent ecological status, to V category – very polluted. Qualitative and quantitative composition of bacterial population in the lake reflects all seasonal and physico-chemical changes. High eutrophication level and intensive agricultural and anthropogenic impact to the lake intensify bacterial activity in numerous natural decomposition processes. These conditions are favorable to many human, animal and plant pathogens or potentially pathogen microorganisms.

During investigated period, high fluctuation of bacteria number was noticed. Total number of mesophile heterotrophs at South varied between $30x10^1$ and $77x10^4$ cfu·mL$^{-1}$, while the number of psychrophiles ranged from $3x10^3$ to $7.2x10^6$ cfu·mL$^{-1}$. At North mesophiles ranged from $32x10^1$ to $11.1x10^4$ cfu·mL$^{-1}$, and psychrophiles from $1x10^3$ to $19.2x10^4$ cfu·mL$^{-1}$ (Fig.1). High number of psychrophile heterotrophs (August) indicates high organic load. Nevertheless, the autopurification process was intensive (FO/H ≥1). Water is mainly categorized in II-IV category at North and III-V in August at South (Fig.1).

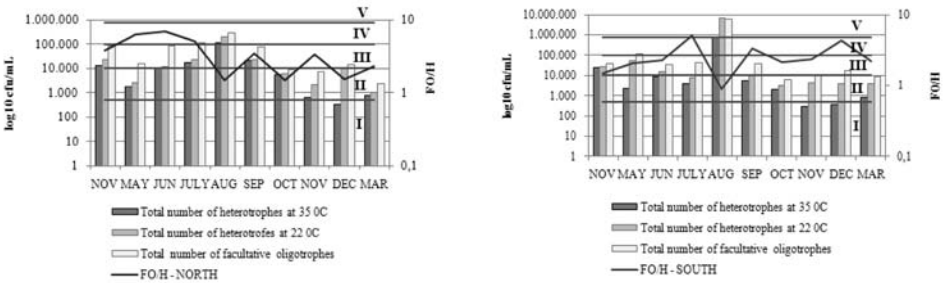


**Figure 1**. Total number of bacteria and autopurification capacity: South (left) and North (right)

Number of total coliforms was in 60% (South) and 50% (North) samples within I category (Fig.2). MPN of fecal coliforms was one log10 lower then coliforms. Maximal MPN was in July 29.870/100 mL at South and 20.140/100 mL in September at North. The lowest levels of fecal coliform contamination were detected in November and March. Number of *E.coli* ranged from $3x10^0$ to $3x10^3$ at North and notably decreased at South ($3x10^0$ - $3x10^2$/100 mL). Number of *E.coli* was one log10 lower than fecal coliforms, respectively (Fig.2c). The content of *Enterococcus* mostly refer to moderate lake pollution and water belongs to I category according to this parameter (Fig.2d).

Content of coliforms was significantly higher in sediment of northern spot, with extremes in July, December and March (Fig.3a). MPN value of fecal coliforms was one log 10 higher than in water and ranged between $18x10^1$ and $1.8x10^3$/1g dw at North. At South, in almost 50% of investigated samples MPN<10/1g dw (Fig.3b). During the investigated period, the MPN of *E. coli* was obviously higher at North, ranging from 18 to 1860/1g dw (Fig.3c). The MPN values of *Enterococcus* varied from 2 to 979/1g dw at the South and from 15 to 544/1g dw at the North. Occasional absence of Enterococci was registered in October, August and November.

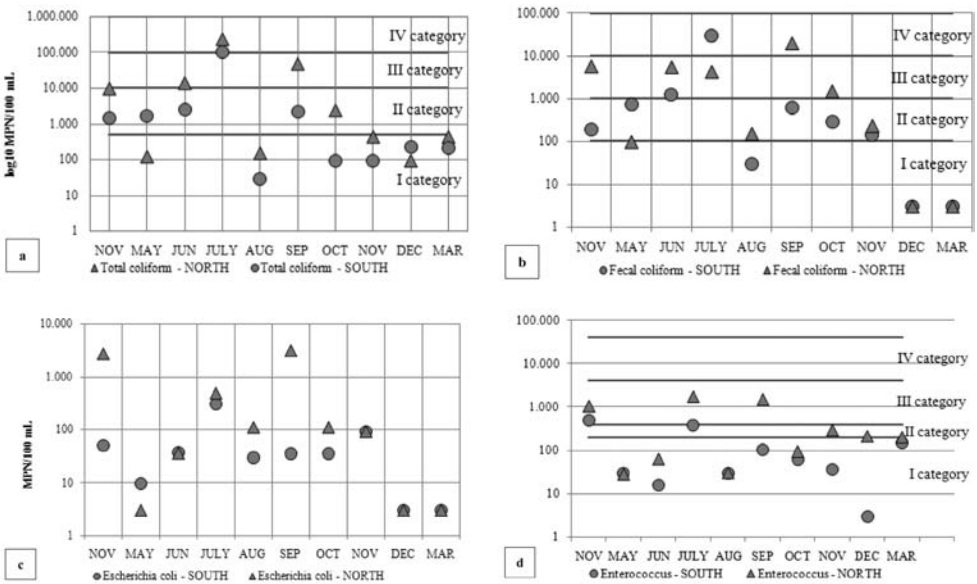


**Figure 2**. Water quality: a. total coliforms, b. fecal coliforms, c. *E. coli* and d. *Enterococcus*

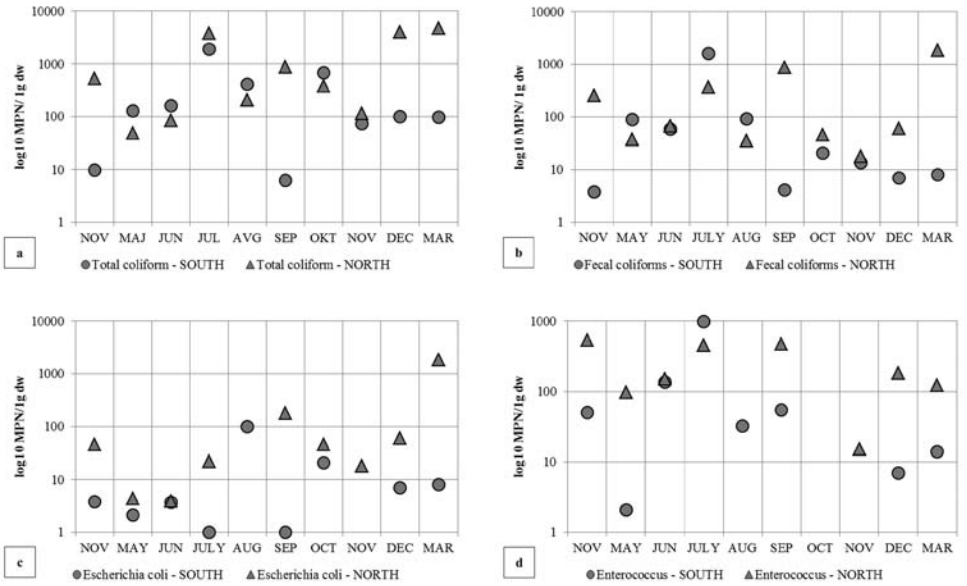


**Figure 3.** Sediment quality: a. total coliforms, b. fecal coliforms, c. *E. coli* and d. *Enterococcus*

## CONCLUSIONS

Obvious differences in water and sediment quality exist between southern and northern parts of the Ludas Lake. Water and bottom sediments at the South were less bacteriologically polluted, when considering average values of indicator bacteria. But content of heterotrophic, saprophyte bacteria in water were higher as a result of higher organic water pollution and poor autopurification capacity in this part of the lake. Content of fecal indicators in most samples of water and sediment taken at the North were significantly higher. This indicates that water from the canal "Palic-Ludas" has a considerable impact on the sanitary quality of water and bottom sediment of Lake Ludas, since the confluence of the canal is approximately 800 m away from the northern sampling point.

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