Use of Microbiological Parameters to Evaluate Water Quality in 5 Levels of Gjakova Watershed

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Abstract

A reliable assessment of microbial indicators of fecal pollution (total coliform, *Escherichia coli*, and faecal streptococci) is very important especially after the rainfalls. Therefore, we investigated the concentration of coliform bacteria, faecal streptococci, aerobic mesophilic bacteria in five levels of Gjakova watershed - Lake "Radoniqi". The aim of this study was to quantify the microbial loads in five levels of watershed after the rainfalls and to compare this loads occurring during regular conditions. During the monitoring period we investigated the microbial loads in five levels of lake "Radoniqi". Water samples were taken and analyzed to determine bacterial parameters as: total coliform bacteria, faecal coliform bacteria, *Streptococcus faecalis* and aerobic mesophilic bacteria. Enumeration of bacteria is made by membrane filtration method and by counting colonies on plates with RBA, M-Endo Agar less, Nutrient agar and BEA agar.
The results show high microbial loads in first, second and fifth level of the watershed as result of rainfalls and other environmental factors.

KEYWORDS: Coliform bacteria, *Streptococcus faecalis*, aerobic mesophilic bacteria, lake, rainfalls

1. INTRODUCTION

Water is essential to sustain life and a satisfactory supply of drinking water which must be available to all consumers [1]. The risk of contamination of drinking water supplies with microbial pathogens is minimized by modern approaches of water management, but continues to be of the major public health concern.
Microbiological data for *total coliforms, faecal coliforms* (representing *Escherichia coli* as predominant species) and intestinal enterococci (*faecal streptococci*) were collected in 5 levels of the Lake "Radoniqi". Data were used for the assessment of microbial pollution along the levels of this lake and to establish a microbiological water quality of the investigated lake.
Faecal indicator bacteria like total coliforms, faecal coliforms (thermotolerant coliforms), *E. coli* and intestinal enterococci (*faecal streptococci*) are excreted by humans and warm blooded animals, pass sewage treatment plants to a great amount and survive for a certain time in the aquatic environment [2].
The main objective of this study was the evaluation of water quality in five levels of Lake "Radoniqi" after the rainfalls and to compare this loads occurring during regular conditions. Traditionally, the presence of coliform bacteria in drinking water

has been seen as an indicator of fecal contamination through cross connection, inadequate treatment, or an inability to maintain a disinfectant residual in the water distribution system.[3].The total coliform group belongs to the family *Enterobacteriaceae* and includes the aerobic and facultative anaerobic, gram-negative, nonspore - forming, rod-shaped bacteria that ferment lactose with gas production within 48 hours at 35ºC [8]. Coliform bacteria are regarded as belonging to the genera *Escherichia, Citrobacter, Enterobacter,* and *Klebsiella*. Object of our study are 5 levels of Gjakova watershed - Lake "Radoniqi". The lake has a voluminous capacity of 117.8 million m³ of water, maximum length 5.2 km, maximum width 2.5 km and maximum depth 52 m.

1) Lake "Radoniqi

2. MATERIALS AND METHODS

2.1 Sample collection

A total of five sampling levels were selected for the evaluation of water quality in Gjakova watershed Lake "Radoniqi". Water samples were collected on monthly basis during the one year period, from five levels – H1 (6 m); H2 (10 m); H3 (17 m); H4 (27 m) and H5 (29 m) of Lake "Radoniqi".Samples were taken at different depth (5 levels) through the bottle Ruttner (Hydro-Bios) volume 2 dm^3, equipped with a thermometer to measure the temperature based on with standard methods of examination under Directive 98/83 CE of water.Sampling of water for laboratory analysis is done carefully, respecting the rules. The enumeration of total coliforms (TC) and fecal coliforms (FC) in surface water samples was conducted using membrane filtration according to standard method [4]. Sampling was conducted between September 2015 and August 2016.In all bottles was marked the place of receipt, time, water temperature, and the name of the person who took the sample for analysisand all of the samplings was conducted during 9:30–11:30 am. Water pH and temperature were determined. Water samples were transported in cool box within the same day to the Laboratory of Microbiology in Hydro system "Radoniqi". Microbiological analysis of water samples was started as soon as possible after collection to avoid unpredictable changes in the microbial population [5]. The

sampling and the tests were performed in accordance with the International Standard Methods ISO 9308-1:2003; ISO 7899-2:2000 and ISO 6222:1999.
This test involves taking a specific volume of the sample (usually 100 milliliters) and filtering it through a sterilized filter membrane. The pore size of the membrane is 0.45 µ (microns) so it is small enough to collect any bacteria that may be present. The filter is placed into a
petri dish containing an appropriate growth medium Chromogenic Coliform Agar (CCA) for coliforms and faecal coliforms to assist bacteria growth in incubation at temperature 37° for 24 h.A group of Gram-positive coccoid bacteria known as faecal *streptococci* (FS) were being investigated as important pollution indicator bacteria [6], on plates with Bile aesculin azide agar. Nutrient agar (NA) was used for determination of number of aerobic *mesophyl* bacteria.Colonies showing a pink to dark-red color with a metallic surface sheen were defined as typical coliforms, while the dark red, mucoid, or nucleated without sheen are considered as atypical coliforms.

2.2.1 Determination of coliform and faecal coliform baceria

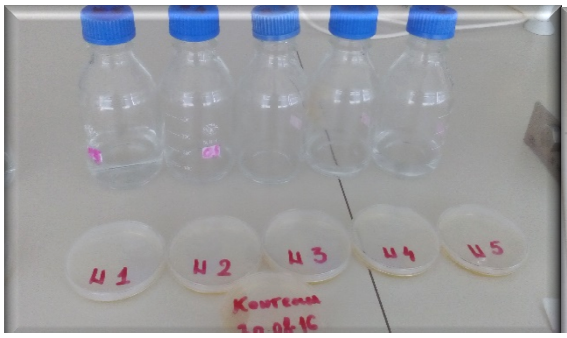
The enumeration of total coliforms (TC) and fecal coliforms (FC) in surface water samples was conducted using membrane filtration according to standard method [4]. In brief, water samples (100 ml water of the sample) was filtered through 0.45 _mpore-sized filters. For TC measurement, after filtration membrane filters were directly placed onto plate with Chromogenic coliform agar (CCA)and the inverted dishes were incubated for (21±3) h at (36 ±2)°C. Colonies showing a pink to red color were defined as typical coliforms, while the dark-blue to violet colonies are considered asE. Coli. To confirm the presumptive coliform bacteria that are not E. Coli, an oxidase test is performed (ISO 9308-1:2004 (E)).

2.2.2 Determination of Faecal streptococci

Fecal streptococci were analyzed similarly to fecal coliforms, however, the membrane filters were transferred to BEA agar. Plates were incubated at 37 °C for 24 h. Red, maroon, or pink colonies were counted as presumptive fecal streptococci. They possess the Lancefield group D antigen. Faecal streptococci are more resistant to stress and chlorination than *E.coli* and other coliform bacteria [7].

2.2.3 Determination of aerobic mesophilic bacteria

Total aerobic mesophilic bacteria were among the first parameters used to monitor the safety of finished drinking water. However, presently they have become an indicator of general water quality within distribution systems [8,9,10]. It is considered that the bacteriological content of drinking-water leaving treatment plants should contain only very low levels of heterotrophic and aerobic spore-forming microorganisms [9]. This parameter, evaluated both at 22° and 37 °C.Members of the genus *Pseudomonas* are possibly the microorganisms most often isolated from bodies of water.



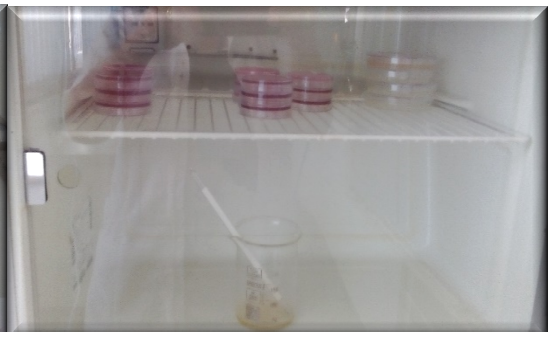


Figure 4.Samples from 5 levels of lake Figure 5. Plates with agar in incubator

3. RESULTS AND DISCUSSIONS

During the period of investigation we analyzed 60 water samples that were taken in five levels of Gjakova watershed – lake "Radoniqi". The highest number of water samples contaminated by total coliform bacteria, faecal coliform bacteria was found in level one (H1 with a maximum 268 CFU/100ml), level two (H2 (with a maximum 254 CFU/100ml) and level five (H5 (with a maximum 236 CFU/100ml), due to the effect of rainfall, especially during the spring and autumn season. This climate changes and increased rainfall and human activity had an impact on the creation of sediment at the bottom of the lake as an important factor in the quality of water. Water intended for human consummation should contain no faecal indicator organisms (11;12;13;14). According to WHO or EPA the guideline level of faecal indicators in drinking water is zero [15;16;13].

In **chart 1** we can see the positive samples of total coliform bacteria in 5 levels of the Gjakova watershed – lake "Radoniqi" during the one year period September 2015 – August 2016. The maximal number of total coliform bacteria was detected in water samples taken from the level H1, H2 and H3 of the lake. In the first level H1 (6m) the maximal number of total coliform bacteria was 254 CFU/ml and the lowest number 142 CFU/100 ml. The maximal number of TC bacteria was found during the period and October–November 2015 and February – April 2016 as a result of rainfall.

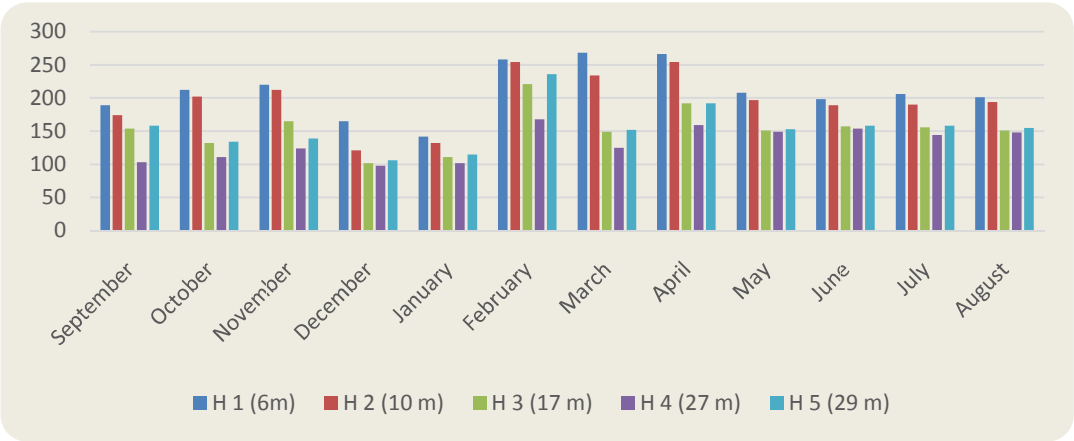


Chart 1. Total Coliform bacteria in 5 levels of Gjakova Watershed Lake "Radoniqi"

In **chart 2** is presented the presence of faecal coliform bacteria of the samples that have resulted positive in 5 levels of the Gjakova watershed – Lake "Radoniqi". The high percentage of contamination was recorded in first, second and the fifth level of the lake with greatest number 212 CFU/100ml for level H1 and 201 CFU/100 ml in level H2 and the lowest number of FC 114 CFU/100 ml in level H1 and 112 CFU/100 ml in level H2.
The highest number of FC bacteria in level 5 was 182 CFU/100 ml and the lowest number in this level was 96 CFU/100 ml.
This percentage of Coliform bacteria was found during the months August, September, February and April as a result of environmental factors increase.

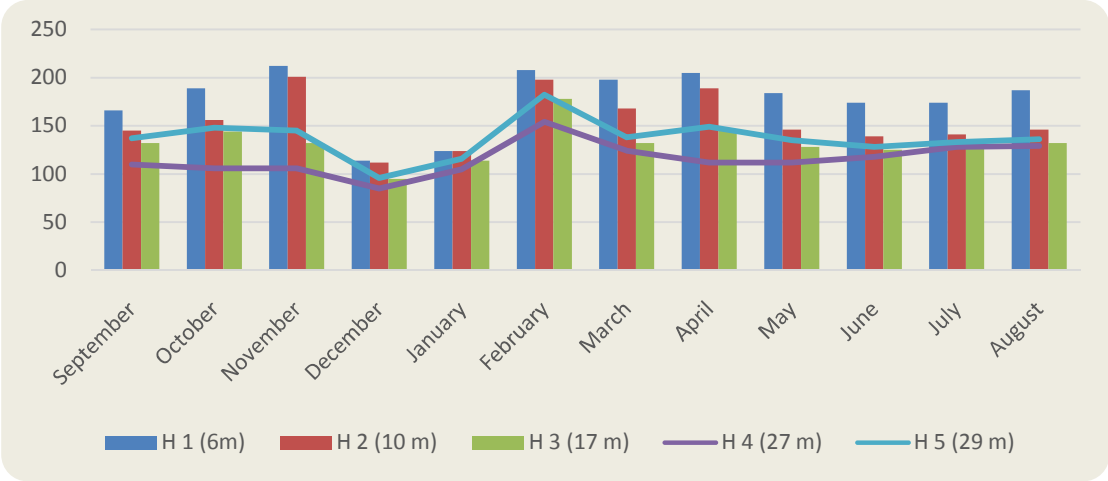


Chart 2. Faecal Coliform bacteria in 5 levels of Gjakova Watershed Lake "Radoniqi"

During the examination of *Steptococcus faecalis* as fecal indicator we notice that number of colonies in first level of watershed-lake "Radoniqi" was higher during the months September, April and June due to high temperatures and the effect of rainfall-**Chart 3**.In level H1maximal number was 14 CFU/ml in April 2016 and the lowest number 4 CFU/ml in January 2016. The number of bacteria has gone decreasing in other levels, with the exception of some periods when there was an increase in the number of bacteria as a result of higher temperatures.

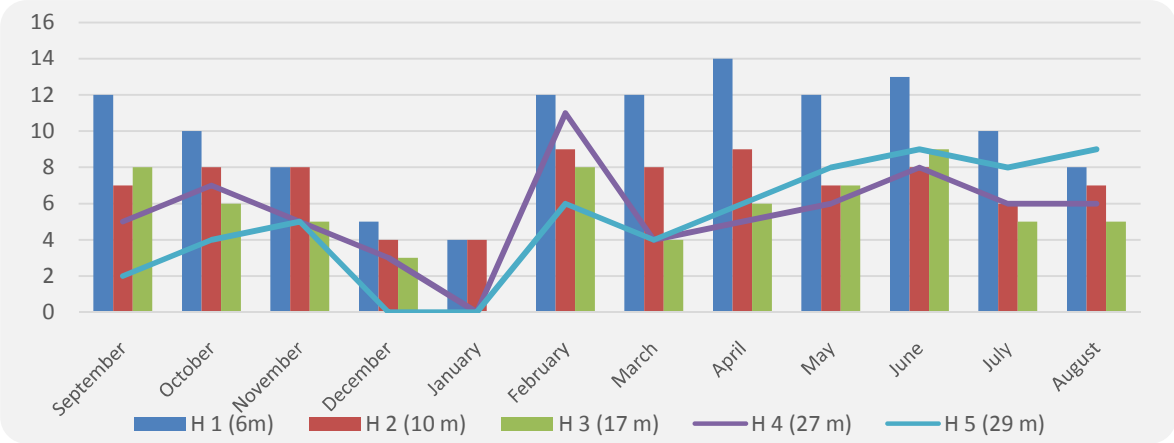


Chart 3. Streptococcus faecalis in 5 levels of Gjakova Watershed Lake "Radoniqi"

The number of Aerobic mesophilic bacteria as an indicator of water quality for drinking water was also determined during the period September 2015-August 2016 in 5 levels of Gjakova watershed-Lake "Radoniqi".The highest number of aerobic mesophilic bacteria is found in level H1, H2, H3, H4 and H5 with maximal number 44 CFU/100 ml in February 2016 and the minimal number is found in December 2015 with 4 CFU/ml. This results are presented in **Chart 4**.

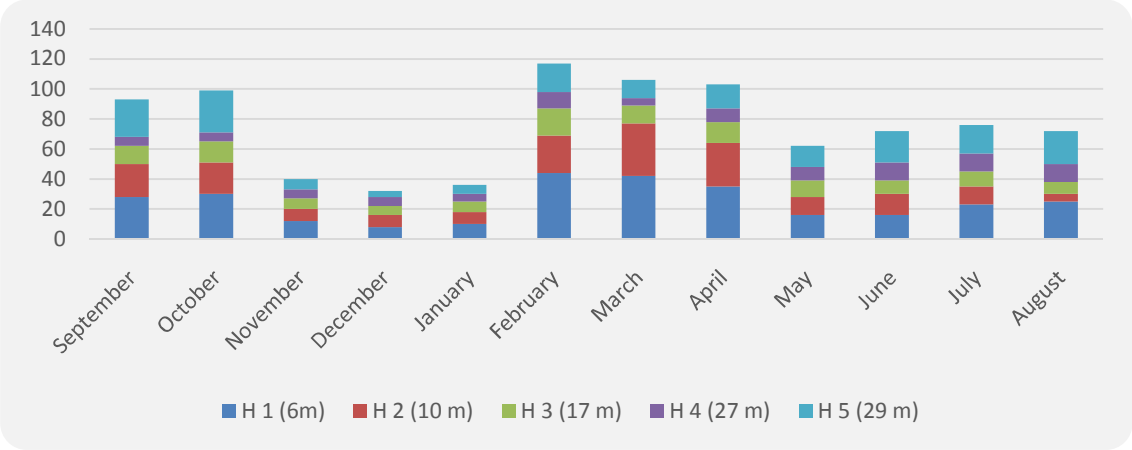


Chart 4. Aerobic mesophilic bacteria in 5 levels of Gjakova Watershed Lake "Radoniqi"

In Table. 1 We can see that average of Total Coliforms bacteria during the period September 2015-August 2016 was higher with 211.08 CFU/100 ml in Level 1 and lower in level 4 with 132 CFU/100ml. Level 5 had the higher value of bacteria than level 4 due to effect of rainfalls and presence of sediment in the bottom of the lake.
Faecal coliforms had higher average in level H1, H2 with maximum of 177 CFU/100 ml and in level H5 with 136 CFU/100ml.
Streptococcus faecalis average was higher in level H1 with 21 CFU/ml and in level H5 14 CFU/100 ml.
Average of Aerobic mesophilic bacteria was higher in level H1 with 10 CFU/100 ml and lower in level H5 with 5 CFU/100 ml.

Table 1. Average of bacteria in five levels of Gjakova Watershed-Lake "Radoniqi" during the period February 2015-February 2016

Sampling levels	Total coliforms Average	Fecal coliforms Average	Streptococcus faecalis/Average	Aerobic mesophylic bacteria/Average
Level H1	**211.08**	**177.9**	**21.75**	**10**
Level H2	**196.08**	**155.4**	**16.5**	**7.08**
Level H3	**153.4**	**132.2**	**10.6**	**5.5**
Level H4	**132**	**115.7**	**8.66**	**5.5**
Level H5	**154.6**	**136.9**	**14.75**	**5.08**

What we observe in our data is that the number of total coliform bacteria, fecal coliform bacteria, streptococcus faecalis and aerobic mesophilic bacteria was higher during the months February, March, April, June, September, October and November of the year. The higher number of bacteria was detected in Levels H1, H2 and in level H5. This situation is presented as a result of rainfall which had impact in presence of higher number of bacteria especially in level H5.

Foregoing studies have associated the occurrence of coliform bacteria, faecal coliform bacteria, streptococcus faecalis and aerobic mesophilic bacteria in drinking water system with rainfall events.[3,17]. According to these authors, rainfall is a complex variable and may have many different impacts on drinking water quality: rainfall can be a mechanism that introduces coliform bacteria into the system through leaks and cross-connections and rainfall can wash dissolved nutrients into the watershed and increase organic carbon levels.

4. CONCLUSION

1) This work indicated that seasonal precipitation directly increased the total coliform and faecal coliform levels in the investigated level of the lake. Statistical analysis demonstrated that TC and FC distribution were closely related to environment factors temperature, seasonal changes-especially rainfalls.

2) Further study is needed to determine the factors responsible for the presence of coliforms in the levels of watershed, so that effective intervention can be initiated. As far as possible water sources must be protected from contamination by human and animal waste.

5. REFERENCES

1. World Health Organization. Guidelines for drinking-water quality [electronic resource]. 1st addendum to
   third edition; Vol. 1, Recommendations. – 3rd ed. 2006
2. KAVKA, G. G. & POETSCH, E. (2002): Microbiology. - In: Technical Report of the International Commission for the Protection of the Danbue River. Eds. Péter Literáthy, Veronika Koller Kreimel, Igor Liska. Eigenverlag ICPDR. 138-150.
3. LeChavallier M W, Welch NJ, Smith DB. Full-scale studies of factors related to coliform regrowth in drinking water. *Appl Environ Microbial* 1996;62:2201-21. [Links]
4. APHA (American Public Health Association), 1998. StandardMethods for the Examination of Water and Wastewater(20th ed.). American Public Health Association, Washington DC.
5. GAUDY, F. A. Microbiology for environmental scientists and engineers. London: McGraw Hill International Book Company, 1998.
6. Houston, A.C. (1900) On the value of examination of water for Streptococci and Staphylococci with a view to detection of its recent contamination with animal organic matter. In Sup. 29th Ann. Report of the Local Government Board containing the Report of the Medical Officer for 1899–1900, p. 548, London City Council, London

7. (Glesson, C and Grey, N 1997) The Coliform index and waterborn desease.E and FN Spon, London. Pp 194.
8. *Guidelines for Drinking-Water Quality*, 3rd ed; World Health Organization: Geneva, Switzerland, 2008; Volume 1, Recommendations.
9. World Health Organization, Ainsworth, R, Ed.; *Safe Piped Water: Managing Microbial Water Quality in Piped Distribution Systems*; IWA Publishing: London, UK, 2004.
10. World Health Organization, Assessing Microbial Safety of Drinking Water. In *Improving Approaches and Methods*; IWA Publishing: London, UK, 2003.
11. Mohsin &Sfdar (2013) Assesment of Drinking water Quakity and its Impact on Residents Health in Bahawalpur City; International Journal of Hummanities and Social Science Vol.3 No.15;118
12. Scott et a. (2002). Microbial source tracking: Current methodology and future directions. Appl. Environ.Microbiol.68:5796-5803
13. World Health Organization (2004).Guidelines for Drinking water Quality, World Healt Organization, Geneva. World Health Organization (2000).
14. http/www.who.int/water-sanitarin-health/dwq/gdwq3rev/en/guidelines for drinking water quality/pdf
15. de.Kok,et.al (2001) Basic Environmental Health. New York:World Health Organization.
16. Sobsey M.D.(2002). In water, Sanitatob and Health Department of Protection of the Human Environment WHO, Geneva
17. Stukel A, Greenberg ER, Dain BJ, Reed FC, Jacobs NJ. A longitudinal study of rainfall and coliform contamination in small community drinking water supplies. *Environ Sci Technol* 1990;24:571-5[Links]