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# 2nd International Conference on Chemo and Bioinformatics
# ICCBIKG_2023

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International Conference on Chemo and BioInformatics

# BOOK OF PROCEEDINGS

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# Biocorrosion, biofouling and health risk: biological activity reaction tests of selected brackish groundwater occurrences in Serbia

**Vladimir Šaraba[1*], Jasmina Nikodinovic-Runic[1], Vesna Obradović[2], Ivica Dimkić[3], Tamara Janakiev[3], Veselin Dragišić[4], Milica Ciric[1]**

[1]University of Belgrade, Institute of Molecular Genetics and Genetic Engineering, Laboratory for Molecular Genetics and Ecology of Microorganisms, Belgrade, Serbia; e-mail: vsaraba@imgge.bg.ac.rs , jasmina.nikodinovic@imgge.bg.ac.rs , milica.ciric@imgge.bg.ac.rs
[2]Jaroslav Černi Water Institute, Laboratory Jaroslav Černi, Belgrade, Serbia; e-mail: vesna.obradovic@jcerni.rs
[3]University of Belgrade, Faculty of Biology, Chair of Biochemistry and Molecular Biology, Belgrade, Serbia; e-mail: ivicad@bio.bg.ac.rs , tamara.janakiev@bio.bg.ac.rs
[4]University of Belgrade, Faculty of Mining and Geology, Department of Hydrogeology, Belgrade, Serbia, e-mail: veselin.dragisic@rgf.bg.ac.rs

** Corresponding author*

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**Abstract**: Targeted physiological groups of bacteria were cultivated and identified in the brackish groundwaters of Obrenovačka Banja (OB), Lomnički Kiseljak (LK) and Velika Vrbnica (VV) using biological activity reaction tests (BARTs) to assess the biocorrosion, biofouling and health risks. The highest density of iron-related, sulfate-reducing, slime-forming, facultatively anaerobic heterotrophic, denitrifying bacteria and representatives of *Pseudomonas* spp. was recorded in the OB sample, while the lowest density of the same physiological groups of bacteria was recorded in the LK sample. Facultatively anaerobic heterotrophic bacteria were the most abundant in the OB and LK samples, while, in contrast, heterotrophic aerobic bacteria were the most abundant in the VV sample. All tested samples were characterized by a high degree of biochemical activity associated with iron-related, sulfate-reducing, slime-forming, heterotrophic aerobic and facultatively anaerobic bacteria. Also, high biochemical activity of denitrifying bacteria was recorded in the OB sample, and the same activity of *Pseudomonas* species was recorded in the OB and VV samples. For OB and LK groundwaters, the highest degree of risk was estimated for biocorrosion process, while for the OB and VV occurrences, the highest degree of risk was estimated for biofouling process. The health risk was present for all examined groundwaters. Caution is warranted in further use of all investigated occurrences due to the established public health risk and an immediate revitalization of the OB, LK and VV wells is necessary.

**Keywords**: brackish groundwaters, biological activity reaction tests, Serbia.

## 1. Introduction

Wells, like any other part of the plumbing system, require preventive maintenance treatments and appropriate periodic cleaning [1], taking into account that bacteria are ubiquitous in wells [2]. In order for groundwater to have a satisfactory qualitative status, it is of the highest importance to prevent the occurrence of biofouling of wells. The development of biofouling contributes to the reduction of well capacity [1] and it is reported in North America as the major, most costly factor in the deterioration of groundwater system characteristics [3, 4]. Also, biofouling can represent a

source of pathogenic bacteria that can cause water-borne infections [5]. With its further development, a microenvironment contributing to the progress of biocorrosive processes is formed [6]. About 20% of all corrosive processes in various branches of industry, including the water industry, are the result of bacteriological activity, which amounts every year to billions of dollars in the US [7] and to 30-50 billion dollars worldwide [8]. Three occurrences, which are used for health-and-recreation and drinking purposes, which belong to the group of brackish groundwaters [9] and are captured by wells with a depth of 6.5-442.5 m [10, 11], were selected for cultivation and identification of targeted physiological groups of bacteria that can contribute to the development of biocorrosion and biofouling processes and may pose a health risk for groundwater users. Although bacteriological contamination of LK groundwaters was previously confirmed [12], continuous monitoring of its bacterial diversity and prediction of the risk of aging for LK well is warranted.

## 2. Methods

Sampling of studied groundwaters and transport of the three samples, each collected from corresponding wells was carried out in accordance with SRPS EN ISO - 19458:2009 standards during January 2020. The presence of targeted physiological groups of bacteria was determined using Iron-Related Bacteria (IRB), Sulfate-Reducing Bacteria (SRB), Slime-Forming Bacteria (SLYM), Heterotrophic Aerobic Bacteria (HAB), Denitrifying Bacteria (DN) and Fluorescent *Pseudomonas* (FLOR) Biological Activity Reaction Tests (BARTs). Portions of well-mixed, undiluted groundwater samples (7.5 mL) were carefully transferred *via* flotation ball to the inner test vial of the different BART biodetectors under sterile conditions and incubated at 22°C for a period of time standard for each type of BART test (5, 10 or up to 15 days). Protocols available from the manufacturer (DBI, Saskatchewan, Canada) were used. During the sample incubation period signature reactions of bacterial activity and their metabolic products were monitored daily (lag period) and photo-documented. Based on the time of occurrence of all recognized signature reactions in the applied BART system, the probable risks of biocorrosion, biofouling and public health risk were calculated using the BART-Soft v6 software and results were expressed as high, medium, and low aggressivity (HA, MA, and LA). For all examined occurrences, an assesment for each type of risk (biocorrosion, biofouling and health risk) was given numerically and it ranged from 0 (no risk) to 9 (maximum risk) [13].

## 3. Results and discussion

BART analysis recorded the highest bacterial density in the OB sample and the lowest in the LK sample. Facultatively anaerobic heterotrophic bacteria were the most abundant in the OB and LK samples, while heterotrophic aerobic bacteria were the most abundant in the VV sample. All examined occurrences exhibited a high degree of biochemical activity associated with iron-related, sulfate-reducing, slime-forming, heterotrophic aerobic and facultatively anaerobic bacteria, as well as the same degree of biochemical activity of denitrifying bacteria was recorded in the OB sample. It is important to note that high biochemical activity of fluorescent *Pseudomonas* species was recorded in the VV sample and the non-fluorescent pseudomonads, with the same degree of

biochemical activity, were detected in the OB sample. The calculated biocorrosion risk was highest for the OB and LK groundwaters, while the calculated biofouling risk was highest for the OB and VV occurrences. For all examined occurrences, health risk was present (**Table 1**).

**Table 1.** Density of populations of target physiological groups of bacteria in the studied groundwater occurrences.

| Investigated groundwaters | Population estimate (CFU/mL) | | | | | | | Biocorrosion risk<br>Biofouling risk<br>Health risk |
|---|---|---|---|---|---|---|---|---|
| | IRB | SRB | SLYM | HAB | DN | FLOR | Total | |
| | Aggressivity | | | | | | | |
| Obrenovačka Banja | 35.300 | 731.000 | 70.000 | 6.890.000 | 17.200 | 4.000** | 7.747.500 | 8.1<br>6.3<br>2.4 |
| | HA | HA | HA | HA | HA | HA | | |
| Lomnički Kiseljak | 8.820 | 731.000 | 70.000 | 454.000 | 2.140 | 400* | 1.266.360 | 8.1<br>4.5<br>5.4 |
| | HA | HA | HA | HA | MA | MA | | |
| Velika Vrbnica | 35.300 | 910 | 632.000 | 6.890.000 | 417 | 4.000* | 7.562.627 | 5.4<br>7.0<br>5.4 |
| | HA | HA | HA | HA | LA | HA | | |

**P. aeruginosa* confirmed as a result of detection of signature reaction PB (Pale blue); ***Non-fluorescent pseudomonads confirmed as a result of detection of signature reaction CL (Cloudy).

Based on the detected level of biochemical activity of iron-related, sulfate-reducing, slime-forming and heterotrophic aerobic bacteria, and taking into account the manufacturer's recommendations, revitalization of the OB, LK and VV wells is necessary in the shortest possible period. Due to the complexity of bacterial communities in groundwater and their tendency to grow in the form of biofilms, revitalization may include all or a combination of recommended treatments, such as the implementation of biocides, physical and chemical treatments [13]. According to the Rulebook on the quality and other requirements for natural mineral, spring, and table waters of Serbia [14], the presence of *P. aeruginosa* is not allowed. In contrast, the World Health Organization [15] and the United States Environmental Protection Agency [16] set no criteria for the acceptable level of *P. aeruginosa* in drinking water. *P. aeruginosa* does not pose a risk for gastrointestinal infections in the general human population using drinking water containing this bacteria. However, in vulnerable populations, e.g., patients with large wounds or burns, very young or elderly people, patients with acquired immunodeficiency syndrome (AIDS), or those undergoing immunosuppressive therapy, the presence of these bacteria in drinking or bathing groundwater may pose a health risk and may be associated with various infections of the ear, nose, throat, skin and the eye [15].

## 4. Conclusions

BARTs have a valuable place in informing water quality recommendations. Due to the established public health risk, caution is warranted in further use of the OB, LK and VV groundwaters. We recommend urgent revitalization of these wells, which will enable the

unhindered use of groundwater resources without negative indications on the service life of the wells and the health and safety of their users.

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and contact as:

+381 34 6100195

iccbikg@uni.kg.ac.rs

*Institute for Information Technologies Kragujevac*, University of Kragujevac, Jovana Cvijića bb, 34000 Kragujevac, Serbia