
University of Belgrade
Technical Faculty in Bor

31st International conference

# Ecological Truth & Environmental Research

Editor
Prof. Dr Snežana Šerbula

## PROCEEDINGS

Hotel Sunce, Sokobanja, Serbia
18–21 June 2024

# PROCEEDINGS
# 31st INTERNATIONAL CONFERENCE
# ECOLOGICAL TRUTH & ENVIRONMENTAL RESEARCH – EcoTER'24


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## *PREFACE*

*The 31st international conference Ecological Truth & Environmental Research – EcoTER'24 focuses on showing the latest research findings and innovations in the field of ecology, environmental protection and sustainable development. The conference will be held in Sokobanja (Serbia) in hotel Sunce in the period of 18–21 June 2024.*

*The aim of the conference is to connect the experts in various fields in order to transform attitudes and behaviors in everyday practices, as well as in the industry and economy sector which is essential for achieving the desired changes that our society must undergo.*

*The 31st international conference Ecological Truth & Environmental Research – EcoTER'24 is organized by the University of Belgrade, Technical Faculty in Bor, and co-organized by the University of Banja Luka, Faculty of Technology; the University of Montenegro, Faculty of Metallurgy and Technology – Podgorica; the University of Zagreb, Faculty of Metallurgy – Sisak; the University of Pristina, Faculty of Technical Sciences – Kosovska Mitrovica and the Society of Young Researchers – Bor.*

*These Proceedings encompass 119 papers from the authors coming from the universities, research institutes and industries in 15 countries: Brazil, Norway, USA, Spain, Austria, Libya, Italy, Israel, Slovenia, Croatia, Romania, Bulgaria, Montenegro, Bosnia and Herzegovina, North Macedonia, and Serbia. It is a great honor and pleasure to cordially wish a warm welcome to all the participants of the conference.*

*As a part of this year's conference, the 6th Student Section – EcoTERS'24 will be held. We appreciate the contribution of the students and their mentors who have also participated in the conference and hope that students will continue to explore and to be curious, since education is a never-ending process, and knowledge is continuously growing.*

*The organization of the EcoTER'24 conference has been financially supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia.*

*The support of the Donors and their willingness and ability to cooperate has been of great importance for the success of the EcoTER'24 conference. The organizing committee would like to extend their appreciation and gratitude to the Platinum donors of the conference – Serbia ZiJin Copper doo Bor and HBIS SERBIA, to the Gold donor of the conference – Elixir Group, as well as to the Silver donor of the conference – Serbian Chamber of Engineers.*

*We would like to express our sincere appreciation to all the authors who have contributed to the Proceedings. We would also like to express our gratitude to the members of the scientific, organizing and honorary committees, reviewers, speakers, chairpersons and all the conference participants for their support of the EcoTER'24. Sincere thanks go to all the people who have contributed to the successful organization of the EcoTER'24.*

*Prof. Snežana Šerbula,*

*President of the scientific and organizing committee*

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# PHYSICO-CHEMICAL AND MICROBIAL ANALYSIS IN SELECTED GROUNDWATER IN SERBIA

**Vesna Obradović[1], Marija Perović[1*], Predrag Pajić[1]**

[1]Jaroslav Černi Water Institute, Belgrade, Jaroslava Černog 80, 11226 Belgrade, SERBIA

[*]*marija.perovic@jcerni.rs*

## Abstract

*The conducted research aims to explore the intricate relationship between the physicochemical properties of groundwater and its microbiological composition within the selected wells of the Danube alluvial zone. The study aimed to determine levels of physicochemical and microbiological composition, as well as their involvement in various transformation processes, allowing insights into the groundwater potential for specific transformations. Although groundwater is commonly viewed as a reliable water source, its slow flow in certain conditions allows pollutants to persist once they infiltrate, posing potential risks to its safety for consumption. Hence, conducting a comprehensive and meticulous analysis becomes crucial to evaluate its suitability for diverse purposes and to establish the necessary construction standards for sustainable utilization. The obtained results have shown that the Danube alluvium exhibits significant biochemical diversity, which, along with changes in the concentration of redox-sensitive species, suggests dynamic hydrochemical and microbiological oxidation-reduction processes that can affect the primary quality of infiltrated Danube water in various ways.*

**Keywords**: groundwater, BART tests, quality, wells.

## INTRODUCTION

Microorganisms play a crucial role in groundwater ecosystems by facilitating key biogeochemical processes such as carbon and nitrogen cycling, as well as the natural breakdown of contaminants [1]. Rather than acting independently, various functional groups of microorganisms interact synergistically to orchestrate these processes. Aquifers provide extensive and complex environments that host a wide array of microbial communities [2]. In this study, the current microbial biodiversity of analysed groundwater with known compositions in the Danube alluvium is evaluated. The potential of modern techniques to deepen our comprehension of microbial biodiversity patterns and their correlation with environmental conditions was also explored. In 2021, a sampling campaign was carried out for seven wells located within the Danube alluvium, specifically within the zones of hydro-technical facilities B-1, B-2, B-3, B-4 near settlement Vinci, and B-10, B-13, and B-15, near the settlement of Veliko Gradište in Serbia. Physico-chemical and microbiological analyses of the water quality were conducted using standard procedures and methods.

## MATERIALS AND METHODS

Sampling was performed in accordance with SRPS EN ISO 19458:2009, Water Quality - Sampling for Microbiological Analysis, and according to instructions on sampling methods and laboratory analysis based on the Standard Methods for The Examination of Water and Wastewater, 21st Edition (2005). Microbiological samples were collected in sterile 500 mL bottles using a sterile metal sampler equipped with a cord. They were transported in handheld refrigerators maintaining a controlled temperature of <10°C and processed within the standard prescribed timeframe. Determination of the microbiological status and quality of groundwater was carried out by simultaneous application of five types of biological activity reaction tests (BART tests). The purpose of these investigations was to determine the presence and biochemical diversity, aggressiveness, and population size, primarily of indigenous bacterial groups [1,5]. The following BART tests were applied: IRB BART tests, for the detection of iron-associated bacteria ("iron bacteria") and some enteric species that can deposit iron; SLYME BART bio-tests, for detecting a wide range of bacteria that produce extracellular polymeric substances and are mostly biofilm-forming, including enteric and opportunistic pathogenic fluorescent *Pseudomonas* species; SRB BART bio-tests, for the detection of sulfate-reducing bacteria that generate biogenic $H_2S$ and cause localized corrosion; HAB BART bio-tests, for detecting a wide range of heterotrophic aerobic and facultatively anaerobic bacteria that are integral parts of biofilms and contribute to biocorrosion and biofouling processes, whose excessive abundance can compromise water quality through turbidity, changes in the organoleptic properties of raw water, and may pose a risk to public health. DN BART bio-tests, for the detection of denitrifying bacteria and the aquifer's potential for denitrification (nitrate removal). The results of BART analyses were processed using BART-SOFT V.6 software [1]. Based on the type and timing of appearance of signature reactions it enabled a determination of consortia and estimation of the abundance and aggressiveness of detected bacterial groups.

## RESULTS AND DISCUSSION

### The results of physicochemical analyses

The gained results are presented in Table 1. The dissolved oxygen (DO) was determined by *in situ* measurements and ranged from 2.03 mg/l to a 3.91 mg/l. The dissolved oxygen levels suggest that the examined groundwater was oxidized at the time of measurement, indicating the potential for aerobic oxidation of organic matter and other reduced chemical species. This process can be mediated through both chemical and microbiological means. The values of the redox potential, ranging from 363 to a maximum of 459 mV, suggest that oxidative processes likely dominate in the zone of the investigated objects. Electrolytic conductivity varied within a slightly broader range of oligosalinity, from a minimum of 362 µS/cm in B-4 to a maximum of 583 µS/cm in B-3. pH values were measured within a range of slightly basic values from 7.16 to 8.49. The observed concentrations of ammonium ions were increased, up to 1.36 mgN/L. The presence of nitrites and nitrates can indicate unfolding of nitrification processes and oxidation of ammonium ions. The organic carbon levels were slightly elevated, suggesting potential impact from organic pollution. This may be attributed to the presence of high numbers of aerobic heterotrophic bacteria, which utilize organic carbon as an electron donor. Consequently, there is a potential health risk due to the possible presence of pathogenic bacterial species. Additionally, determined certain content of divalent iron and oxygen at the same time of measurement suggests imbalance and disequilibrium of redox-sensitive species (redox states) due to artificially introduced oxygen, possibly because of lowering the static level or depression cone. A wide range of sulfate concentration values from a minimum of 3.33 mg/l in B-1 to a maximum of 30.03 mg/l, 33.45 mg/l and 34.30 mg/l in B-10, B-13 and B-15, respectively, suggests intensive microbial processes of sulfate reduction as well as oxidation of ferrous sulfide with nitrate reduction. Lack of sulfides may be attributed to rapid reaction with dissolved divalent iron (insoluble ferrous sulfides - black precipitates) and consequently precipitation from the water phase. The B-10 is characterised by the highest groundwater level and consequently highest redox potential.

***Table 1*** *Examined groundwater quality – selected parameters*

| Sampling site / Parameter | Unit | B-1 | B-2 | B-3 | B-4 | B-10 | B-13 | B-15 |
|---|---|---|---|---|---|---|---|---|
| pH | | 7.16 | 7.23 | 7.62 | 7.53 | 8.08 | 8.49 | 8.2 |
| Ec | µS/cm | 499 | 488 | 583 | 362 | 437 | 402 | 476 |
| Eh | mV | 371.5 | 394.2 | 363 | 389.9 | 458.7 | 414.3 | 406.6 |
| DO | $mgO_2/l$ | 2.5 | 3.59 | 3.75 | 2.96 | 2.03 | 3.91 | 2.49 |
| The consumption of $KMnO_4$ | mg/l | 8.89 | 9.05 | 8.51 | 16.84 | 6.71 | 4.52 | 5.93 |
| $NH_4$ | mgN/l | 1.36 | 0.72 | 1.02 | 0.14 | 0.97 | 0.06 | 0.14 |
| $NO_2$ | mgN/l | 0.006 | 0.054 | 0.038 | 0.028 | 0.01 | <0.005 | 0.011 |
| $NO_3$ | mgN/l | 0.15 | 0.81 | 0.62 | 2.83 | <0.05 | 0.63 | <0.05 |
| Cl | mg/l | 17.6 | 17.44 | 17.49 | 11.07 | 16.85 | 16.48 | 16.69 |
| $SO_4$ | mg/l | 3.33 | 15.5 | 18.35 | 25.47 | 30.03 | 33.45 | 34.3 |
| OP | mgP/l | 0.218 | 0.34 | 0.08 | 0.262 | 0.068 | 0.019 | 0.009 |
| $H_2S$ | mg/l | <0.04 | <0.04 | <0.04 | <0.04 | 0.12 | <0.04 | <0.04 |
| TDS | mg/l | 322 | 302 | 360 | 243 | 233 | 213 | 241 |
| Total hardness | mg $CaCO_3/l$ | 222.4 | 233.4 | 275.4 | 151.3 | 218.8 | 202.9 | 189.1 |
| TOC | mg/l | 2.48 | 1.98 | 3.8 | 3.89 | 1.96 | 1.16 | 1.2 |
| Iron (II) | mg/l | 0.72 | 0.4 | 0.21 | 0.26 | 1.35 | 0.32 | 0.12 |
| Iron | mg/l | 2.77 | 5.42 | 0.72 | 0.3 | 30.44 | 7.26 | 0.46 |

Compared to the prescribed values set by the Regulation on the Hygienic Potability of Drinking Water (“Official Gazette of the FRY”, No. 42/98 and 44/99, and “Official Gazette of the Republic of Serbia”, No. 28/2019) [4], the concentrations of ammonium, nitrites, iron, and manganese exceeded the permissible limits. Figure 1 and Figure 2 present the values of selected parameters.

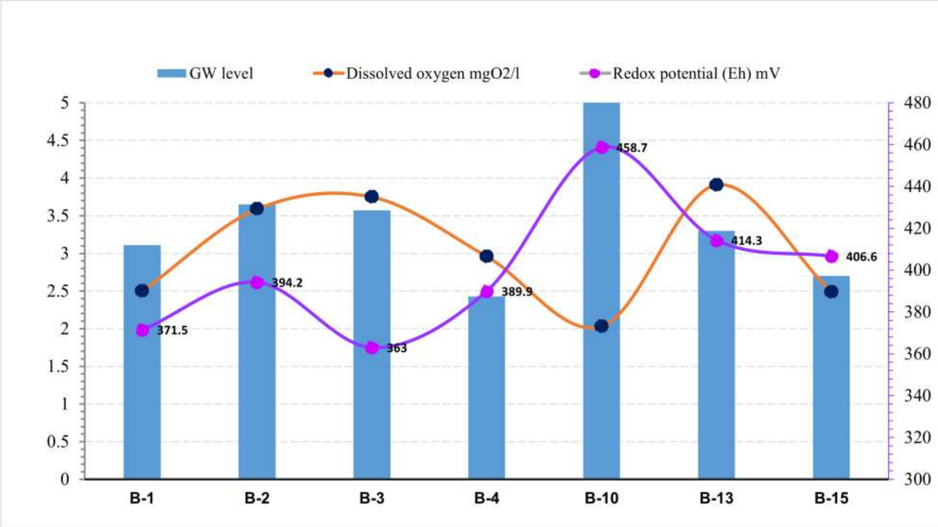


***Figure 1*** *The measurement of oxygen levels and redox potential in groundwater*

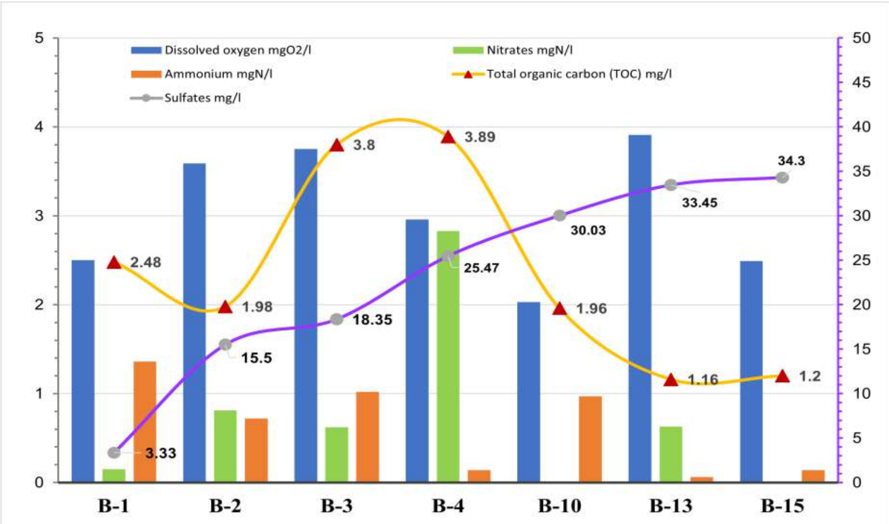


***Figure 2*** *The content of redox-sensitive chemical species and total organic carbon in groundwater*

**The results of the BART (Biological Activity Reaction Test) testing of groundwater**

The community structure is depicted in Figure 3 as relative abundance, represented in percentages, with highlighted color-coded tested groups. Based on the obtained results, it can be concluded that the groundwater within examined objects exhibits similar biochemical diversity and very high abundance of aerobic and facultatively anaerobic, heterotrophic bacteria and bacteria producing extracellular polymers, i.e., biofilm-forming groups. In all samples, a uniform population of sulfate-reducing bacteria was observed, with their aggressiveness (biochemical activity) assessed as high as 6000 cfu/ml. Due to the detection of denitrifying bacteria in four examined objects, it is recommended to continuously monitor faecal indicator bacteria groups, particularly the presence of Pseudomonas species (*Pseudomonas aeruginosa*), at these sites.

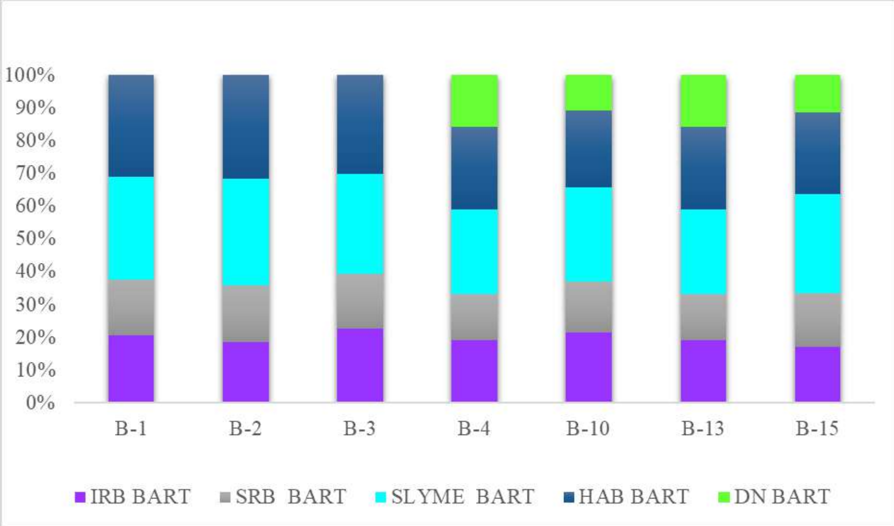


***Figure 3*** *The structure of the bacterial community in examined groundwater*

The determined biochemical diversity of the examined prokaryotic community in the groundwater alluvium of the Danube, besides the potential risk for the development of biofouling and biocorrosion processes on hydraulic elements, indicates a high potential for the removal of various pollutants. These pollutants are successfully degraded by aerobic and facultative anaerobic heterotrophic bacteria depending on prevailing conditions, as well as conditions of sulfate reduction, denitrification, and iron reduction.

For example, during the biodegradation of petroleum hydrocarbons, soil and groundwater microorganisms utilize oxygen ($O_2$), nitrate ($NO_3^-$), iron oxides [e.g., $Fe(OH)_3$], and sulfate ($SO_4^{2-}$) as electron acceptors to degrade petroleum hydrocarbons to $CO_2$ and $H_2O$ through aerobic and anaerobic degradation [denitrification, iron (III) reduction, sulfate reduction, methanogenesis]. When these transformations occur, relevant geochemical indicators (e.g., $NO_3^-$, $SO_4^{2-}$, $CH_4$) will change accordingly, which can demonstrate the respective biodegradation mechanisms.

## CONCLUSION

Groundwater abstracted through hydraulic structures installed parallel to rivers, tapping adjacent aquifers for water production purposes, is widely utilized in a significant proportion in most European countries and certain regions of North America. The principle of bank filtration involves various physical, chemical, and biochemical processes and is particularly known for effectively reducing and/or removing suspended solids, organic pollutants, pathogenic microorganisms, heavy metals, nitrogen, toxic algae, as well as trace organic compounds (e.g., pharmaceuticals), compounds causing salinity, taste, and odor. Depending on hydrogeological conditions, redox status, and microbiome composition with their metabolic capabilities, the potential for the existence or biodegradation of various pollutants can be inferred based on biochemical indicators and chemical redox-sensitive species to protect and preserve the quality of groundwater in the vicinity of large rivers.

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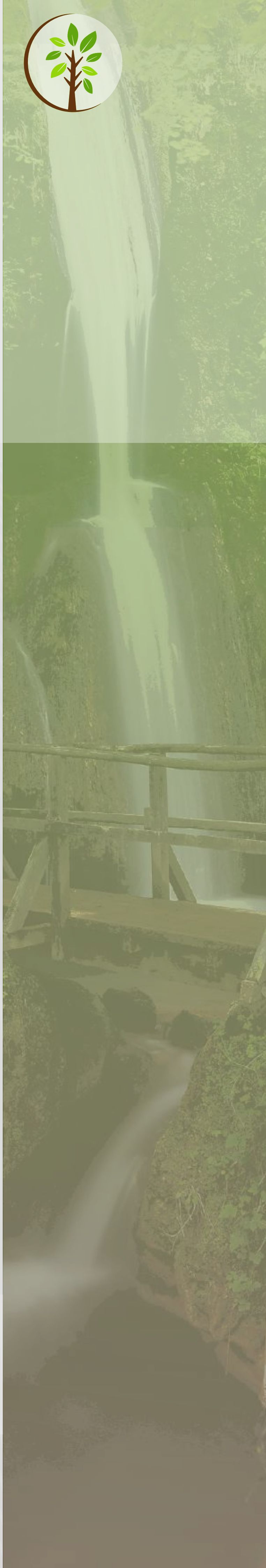

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