



University of Belgrade  
Technical Faculty in Bor

EcoTEK

31<sup>st</sup> International conference

# Ecological Truth & Environmental Research

Editor

Prof. Dr Snežana Šerbula

## PROCEEDINGS

Hotel Sunce, Sokobanja, Serbia  
18–21 June 2024

## PROCEEDINGS

### 31<sup>st</sup> INTERNATIONAL CONFERENCE

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## **PREFACE**

*The 31<sup>st</sup> 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 31<sup>st</sup> 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 6<sup>th</sup> 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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## EVALUATING CORROSION AND BIOFOULING POTENTIAL BASED ON GROUNDWATER MICROBIOLOGICAL COMPOSITION

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### Abstract

*Microbiologically influenced corrosion was recognized in the mid-20<sup>th</sup> century, but the study of corrosion influenced by microorganisms (prokaryotes) is relatively new compared to other research. Microbiologically induced corrosion (MIC) can manifest in diverse environments, extending beyond aqueous conditions to thrive even in humid atmospheres. Biofouling pertains to the adhering of biological matter (including bacteria, algae, and various microorganisms) on surfaces submerged in water. In groundwater settings, biofouling manifests when these organisms attach to well screens, pipes, and other infrastructure components. This buildup can restrict water flow, reduce well efficiency, and deteriorate water quality, affecting overall groundwater utilization. By simultaneously applying a system of six types of bioactivity reaction tests (BART biotests), the biochemical diversity and estimated population size of ecological groups of bacteria participating in the transformation of organic matter and redox-sensitive species were determined. These bacteria predominantly form biofilms at the water-matrix interface, water-metal interfaces, and other surfaces. The goal was to comprehensively assess the microbiological status of groundwater and detect the biochemical activity of bacteria primarily posing risks for corrosion and biofouling processes on hydraulic structures, which could also pose risks to public health. Conducted research indicated the existence of potential for possible intensive and highly aggressive biofouling processes. The forecasted risk was in the range of high risks for the development of corrosion processes.*

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

### INTRODUCTION

Corrosion is an electrochemical process consisting of an anodic reaction involving the ionization (oxidation) of the metal (corrosion reaction) and cathodic reactions based on the reduction of chemical species. These reactions can be influenced by microbial activities, especially when organisms are in close contact with the metal surface and form a biofilm. The result of microbial biochemical activity is the degradation of metal known as Microbiologically Influenced Corrosion (MIC). Microorganisms involved in the biocorrosion of metals such as iron, copper, and aluminium, and their alloys, are physiologically diverse. Biocorrosion processes on metal surfaces are associated with the colonization of microorganisms and the products of their metabolic activities, including enzymes, exopolymers (slime), organic and inorganic acids, as well as volatile compounds such as ammonia or hydrogen sulfide. Bacteria, with their activity and metabolic by-products, can influence cathodic and/or anodic reactions, thus altering the electrochemistry at the biofilm/metal interface. Various mechanisms of biocorrosion have been identified and

recognized, reflecting the diversity of physiological activities performed by different types of microorganisms [1–6]. Many contemporary studies focus on the corrosion of copper and copper alloys under the influence of microbes, with particular attention given to sulfate-reducing bacteria and metal-oxidizing bacteria that deposit metals (especially iron and manganese) [2]. Based on numerous environmental and laboratory studies, the importance of microbial consortia (community-associations) and the role of extracellular polymeric substances (slime) in biocorrosion and biofouling have been emphasized [1–6]. It has been noted that microbiological growth in the form of biofilm promotes biofouling. The presence of biofilm can promote physicochemical reactions that are not typical for the given physicochemical conditions. In both natural and artificial environments, corrosion occurs when materials made of pure metals and/or their mixtures (alloys) undergo a chemical change from the neutral-basic state to an ionic form.

The metabolic activity of analysed bacterial groups can cause biofouling and corrosion, as well as turbidity, changes in taste, odor, and color, deteriorating the overall quality of raw water. Through their biochemical-physiological capabilities, these bacterial groups participate in the self-purification of groundwater and in biofouling processes on filters and in the filter zone. The examined bacterial groups can also be the cause of corrosion on hydraulic elements and structures beside leading to changes in overall water quality.

## MATERIALS AND METHODS

In a one sampling campaign conducted in 2023, microbiological quality testing was performed using a system comprising six types of bioactivity reaction tests (BART analysis) on three groundwater samples: Bn1, Bn4, Bn6, all obtained from the Bn well zone. Sampling of groundwater for microbiological analysis was carried out in accordance with the standard SRPS EN ISO 19458:2009, Water Quality - Sampling for Microbiological Analysis. Prior to sampling, the piezometers were flushed with at least three water column changes using a pump and an aggregate. Following the flushing of the piezometers and upon return to static level, in situ measurements of temperature, dissolved oxygen content, oxygen saturation, redox potential, specific electrolytic conductivity, and pH values were conducted using a HACH probe. Samples were collected in sterile 500 mL bottles using a sterile metal sampler with a cord.

By simultaneously applying a system of six types of BART biotests, the biochemical diversity and estimated population size of ecological groups of bacteria participating in the transformation of organic matter and redox-sensitive species were determined. These bacteria predominantly form biofilms at the water-matrix interface, water-metal interfaces, and other surfaces. The following BART tests were employed to detect the presence of: Iron-Related Bacteria (IRB BART) for detecting iron-associated bacteria and certain enteric species capable of iron deposition; Slime Forming Bacteria (SLYME BART) for detecting a wide range of bacteria producing extracellular polymeric substances, predominantly biofilm-forming, including enteric and opportunistic fluorescent *Pseudomonas* species; Sulfate Reducing Bacteria (SRB BART) for detecting sulfate-reducing bacteria that generate biogenic H<sub>2</sub>S and cause localized corrosion; Total Aerobic Bacteria (TAB) or Heterotrophic Aerobic Bacteria (HAB BART) for detecting a broad range of heterotrophic aerobic and facultatively

anaerobic bacteria, which contribute to biofilm formation and biofouling processes. An excessive abundance of these bacteria may compromise water quality by causing turbidity, changes in raw water organoleptic properties, and may pose risks to public health. Denitrifying Bacteria (DN BART) for detecting denitrifying bacteria and assessing the aquifer's potential for denitrification (nitrate removal), indicating organic pollution and potential presence of pesticides and pathogenic species.

Results of the BART analyses were processed using BART-SOFT V.6 software designed for this type of testing. The appearance and patterns of observed signature reactions and metabolism products (turbidity, color, gases, fluorescence, precipitates) in each individual biotest were entered into the software application. The size of the population for each tested bacterial group, aggressiveness, and calculated risks for corrosion, biofouling, and health risks were assessed. During the lag period of sample incubation, lasting ten to fifteen days, the analysis progress and reactions in biotests were observed daily, photographed, and archived as photo documentation. The scale of estimated risks of corrosion and biofouling is presented as numerical values ranging from 0 (no risk) to 9 (maximum risk).

## RESULTS AND DISCUSSION

According to the results of BART biotest, the biochemical diversity in groundwater in the Bn zone included metabolic activity of iron-oxidizing, sulfate-reducing, heterotrophic aerobic, and facultative anaerobic bacteria, fluorescent *Pseudomonas* species (*Pseudomonas aeruginosa*), and aerobic and facultative anaerobic bacteria capable of producing extracellular polymeric substances. Denitrification activity in groundwater in the Bn zone was not detected, likely due to the suppression of nitrate reductase activity by sulfate-reducing bacteria (Figure 2).

The highest abundance and biochemical activity (aggressiveness) of bacteria were observed in SLYM and HAB BART biotests (8810000 and 454000 pac/ml, equivalent to the number capable of forming colonies). Specifically, a very high abundance of aerobic and facultative anaerobic heterotrophic bacteria capable of synthesizing and secreting extracellular polymeric substances (EPS) was detected compared to other tested groups, indicating a high risk (potential) for biofilm development and somewhat lower than corrosion, both in the aquifer matrix and on all metal surfaces (Figure 2). These bacterial groups also perform biomineralization of organic matter and participate in its removal from groundwater, whereby resulting degradation products can alter the primary water quality (e.g., changes in water pH, CO<sub>2</sub> content, and HCO<sub>3</sub>). The abundance of heterotrophic bacteria and bacteria excreting extracellular polymers (mostly polysaccharides) is also important because opportunistic and pathogenic species, protected within these groups of biofilms from predation and within cooperative metabolism consortia of biofilm, persist longer in the environment.

The abundance of fermentation bacteria detected within the IRB BART system was also high, with an average of 9000 presumed active cells per 1 ml. Fermentation bacteria can participate in the transformation of iron (oxidation-reduction), and during incomplete degradation of organic matter, they generate acidic products (e.g., organic acids and alcohols), H<sup>+</sup>, and CO<sub>2</sub>, which can contribute to both iron deposition processes and accelerate corrosive

processes on well metal structures and pipelines. Due to signature reactions indicating possible presence of enteric and pathogenic species (e.g., appearance of blackening along the entire length of the IRB BART detector reaction tube), it is necessary to conduct an analysis of the sanitary-hygienic suitability of groundwater in the Bn zone.

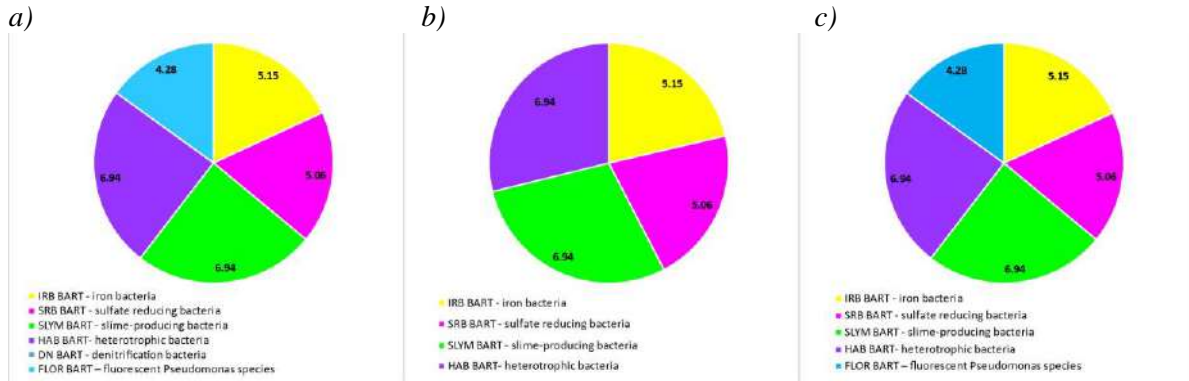


Figure 1 Abundance of tested bacterial groups, as log pac/ml a) Bn1; b) Bn4; c) Bn6

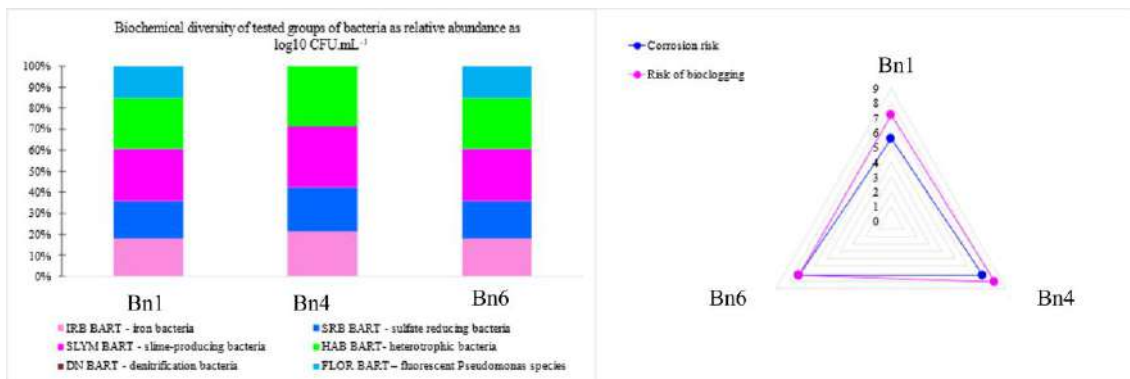


Figure 2 Relative abundance of tested bacterial groups (community structure based on abundance and manifestation of biochemical capabilities) and terminal reactions in bart biodetectors in the Bn zone

Sulfate-reducing bacteria were detected in all groundwater samples from observational piezometers with a constant count of 115,000 presumed active cells per ml, indicating a possible development of localized corrosion on well metal surfaces, equipment, and pipelines. Sulfate-reducing bacteria, strict anaerobes, utilize sulfates as primary electron acceptors in the degradation of organic matter, resulting in the production of H<sub>2</sub>S and thus contributing to the unpleasant odour of groundwater. The metabolic versatility of sulfate-reducing bacteria in the degradation of organic matter is important because they can perform fermentation and use other electron acceptors besides sulfates. In the case they use nitrate, the product of this transformation is ammonium ion. They can also use trivalent iron as an electron acceptor, thus contributing to the increase in reduced iron content in groundwater. The activity of hydrogenase enzymes in sulfate-reducing species *Desulfovibrio vulgaris* (Hildenl) is regulated by the availability of Fe<sup>2+</sup>, offering another mechanism under which corrosion could occur. Thus, the influence of dissolved iron on the development of corrosion processes under the influence of SRB is a complex phenomenon. SRB can lead to corrosion of zinc and zinc-

based alloys with sphalerite as a product, while the activity of SRB with lead carbonates leads to the formation of galena (PbS). Biogenically produced sulfides pose health and safety issues, are hazardous to the environment, and result in significant economic losses due to equipment corrosion. These organisms can coexist in natural biofilms, often forming synergistic communities (consortia) capable of influencing electrochemical processes through cooperative metabolism not observed in individual species.

During microbiological analyses, within the SRB BART biotesting, subsequent generation of substrate blackening along the tester was observed, indicating the activity of some proteolytic species in H<sub>2</sub>S generation. The primary appearance of reactions around the flotation ball in the SRB system, in groundwater samples in the Bn zone, indicates that sulfate-reducing bacteria are active in the community with primarily aerobic heterotrophs and producers of extracellular polymers and can cause sudden and dramatic drops in production of extraction wells, primarily caused by corrosion and pump failure.

Based on observed signature biochemical reactions and the time of their occurrence in each individual tester, using software BART Softv6, the population abundance of bacterial groups was estimated, and predicted risks for biofouling and corrosion were provided (Figure 2 and Table 1).

The software-calculated risk in the forecast of biofouling (biofilm formation) on a scale of 0–9 (from no risk to maximum risk) was >5 in all samples, suggesting possible intensive and highly aggressive biofouling processes. The forecasted risk for corrosive processes was >7, ranking in the range of high risks for the development of corrosion processes (Table 1).

**Table 1** Software-predicted risks for corrosion and biofouling for each sample from the Bn zone

<b>Piezometer</b>	<b>Bn1</b>	<b>Bn4</b>	<b>Bn6</b>
The risk of corrosion	5.6	7.2	7.2
The risk of fouling	7.2	8.1	7.2

Several signature reactions within all applied tests may indicate the potential presence of opportunistically pathogenic and enteric-type bacteria. Based on these identified reactions or the absence thereof, the predicted health risk and the potential presence of pathogenic species in the groundwater in the zone of the examined observational piezometers were moderately high to high, ranging from 6.3 to 8.1 (HRaver-6.9).

## CONCLUSION

The main groups of bacteria associated with the corrosion of cast iron and stainless-steel structures are sulfate-reducing bacteria (SRB), sulfur-oxidizing bacteria, iron-oxidizing/reducing bacteria, and manganese-oxidizing/reducing bacteria (IRB - iron and manganese associated bacteria), bacteria emitting organic acids and exopolymers or slime (EPS or slime-producing). It is known that some bacteria primarily adhere to corrosive products and can be present in large numbers, even when they do not play any role in primary corrosion processes, and some among them belong to the group of pathogenic bacteria.

The conducted analysis of fouling and corrosion risk revealed a significant risk of intensive and highly aggressive biofouling, with all samples indicating a risk level surpassing 5 on a scale of 0–9. Moreover, the forecasted risk for corrosive processes exceeded 7, indicating a substantial likelihood of corrosion development in the studied samples. These findings underscore the importance of proactive measures to mitigate both biofouling and corrosion in relevant systems.

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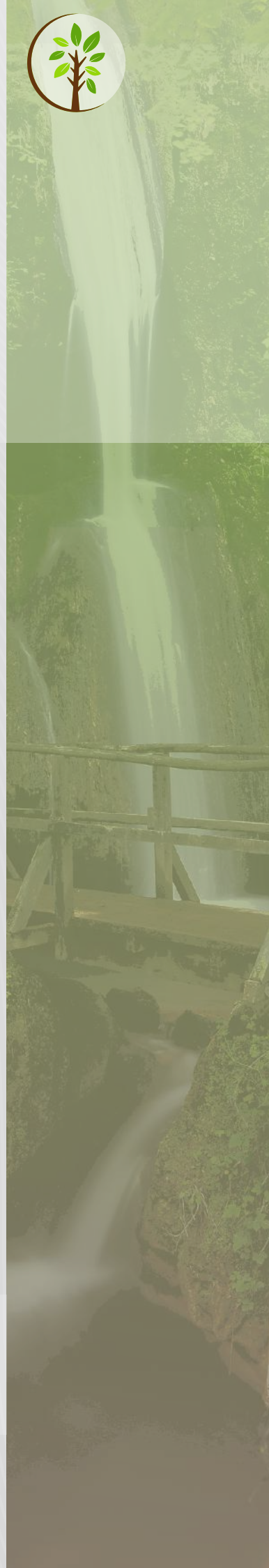
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