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Evaluation of biocorrosion, biofouling, and health risks in the two study locations in danube alluvium

ABSTRACT

Within conducted research the results of microbiological investigations on specific metabolic (phenotypic) groups of bacteria that play crucial roles in the biogeochemical cycling of iron, manganese, nitrogen, sulfur, and carbon are presented. These bacteria are also involved in the development of biocorrosion and biofouling processes, with some posing risks to public health. Utilizing results from applied biological activity reaction tests (BART tests), processed using specialized software, potential risks for the development of microbiologically mediated corrosion, biofouling, and health risks were calculated for seven wells within two oxic sites in the Danube alluvium – Vinci and Veliko Gradište, Serbia. Moderate to high corrosion risk was determined for all seven wells at both sites (CR=5.4). Microbiological fouling risk was very high in three out of the seven investigated wells (PR=8.10). Among the seven sites studied, one site stood out based on the calculated high value of health risk coefficient (HR=8.10). The research results provide new insights into the microbiological role in aging wells in oxic groundwater of the Danube alluvium. It is demonstrated that the physicochemical composition and chemical species such as minerals, organic matter, and the specific composition of microbial communities in the studied groundwater have the potential to stimulate biocorrosion and the formation of deposits and biofilms within well structures. In addition to biochemical analyses, hydrogeological characteristics of the analyzed area are presented to define the geological stratigraphy, for which specific microbiological transformations would be expected based on the obtained results.

Key words: *biocorrosion, biofouling, groundwater, health risk, Serbia*

1. INTRODUCTION

Well corrosion and biological clogging affect water quality, the operational lifespan of pumps, pipelines, and the quality of hydraulic structure operation in general. The occurrence of 'aging' wells and reduced yields, attributed to heightened resistance in the pre-filter zone, primarily stems from “unfavorable” groundwater chemistry that promotes chemical and biological incrustation (clogging) of the pre-filtering zone and the well filter structures. Specific water composition and increase of hydraulic resistance in the pre-filtering zone intensify these processes. The impact of well 'aging' is evident in wells operational limitations, particularly in their inability to maintain the required (adopted) protection criteria and achieve the permissible groundwater level relative to the terrain surface. Due to the expenses related to labor, equipment, and production caused by biofouling, this issue poses a notable economic concern [1]. Biofouling is the term used to describe the unwanted accumulation of microbial organisms and their extracellular substances [1, 2]. The micro-organisms (bacteria, archaea, and fungi) naturally present in groundwater can significantly impact the surface processes and expedite corrosion in environments where corrosion would not typically occur favorably [3, 4]. The biofilm forming community is diverse and contains species that have often been linked with corrosion, such as sulphate reducing bacteria (SRB) and methanogenic archaea (MA) [3]. Alongside the conventional assessment of well aging processes primarily centered around calculated risks (Langelier Saturation Index, Riznar stability index) associated with chemical corrosion and incrustation - chemical fouling [5], contemporary research is

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placing a growing emphasis on investigating the impact of microorganisms on well aging and the formation of biofouling [6]. The type of clogging and biofouling associated with the growth and activity of specific bacterial groups can vary and depends on the chemistry of groundwater, particularly on the availability of electron donors (mostly available reactive organic carbon), electron acceptors (oxygen, trivalent iron, manganese, nitrates, sulfates, carbon dioxide), and parameters such as temperature and pH of groundwater.

Bacterial activity oxidizing reduced iron can result in various consequences for water chemistry. These include scaling due to high carbonates, encrustation caused by elevated iron levels, nodules forming cores rich in iron and organic matter, and the production of slime. These alterations can impact the color, odor, and taste of groundwater, as well as induce turbidity. According to [7] bacteria most commonly associated with microbiologically influenced clogging (MIC) are a) Iron-reducing bacteria (IRB), which tend to dominate where iron accumulates (nodules, encrustations), and heterotrophic aerobic bacteria (HAB), particularly dominant when biomass resembles slime. Microbially mediated biofouling can occur through activity of aerobic and facultative anaerobic corrosive bacteria from groundwater reaching pipelines that have organic and inorganic film on the surface [7]. Heterotrophic bacteria producing extracellular polymeric saccharides initially attach to the walls of the pipeline/metal surfaces, creating a favorable environment for the adhesion of other microorganisms. The action of microaerophilic bacteria oxidizing iron in the biofilm results in the accumulation of iron oxide hydroxide deposits, leading to pipe volume reduction (clogging); generation of sulfuric acid released by sulfur-oxidizing bacteria, which accelerates environmental acidification, lowering pH [7]. Conditions with low oxygen concentration and organic acids released by acid-producing bacteria (e.g., fermentation) favor the attachment and development of sulfate-reducing bacteria, producing hydrogen sulfide (H_2S), accelerating the corrosion process and lowering pH, causing localized corrosion. In groundwater a certain chemical equilibrium is established under natural conditions. It can be disrupted by groundwater exploitation, resulting in the appearance or acceleration of sedimentation processes affecting the clogging of well filters and the pre-filter zone, as well as processes leading to corrosion of filter structures.

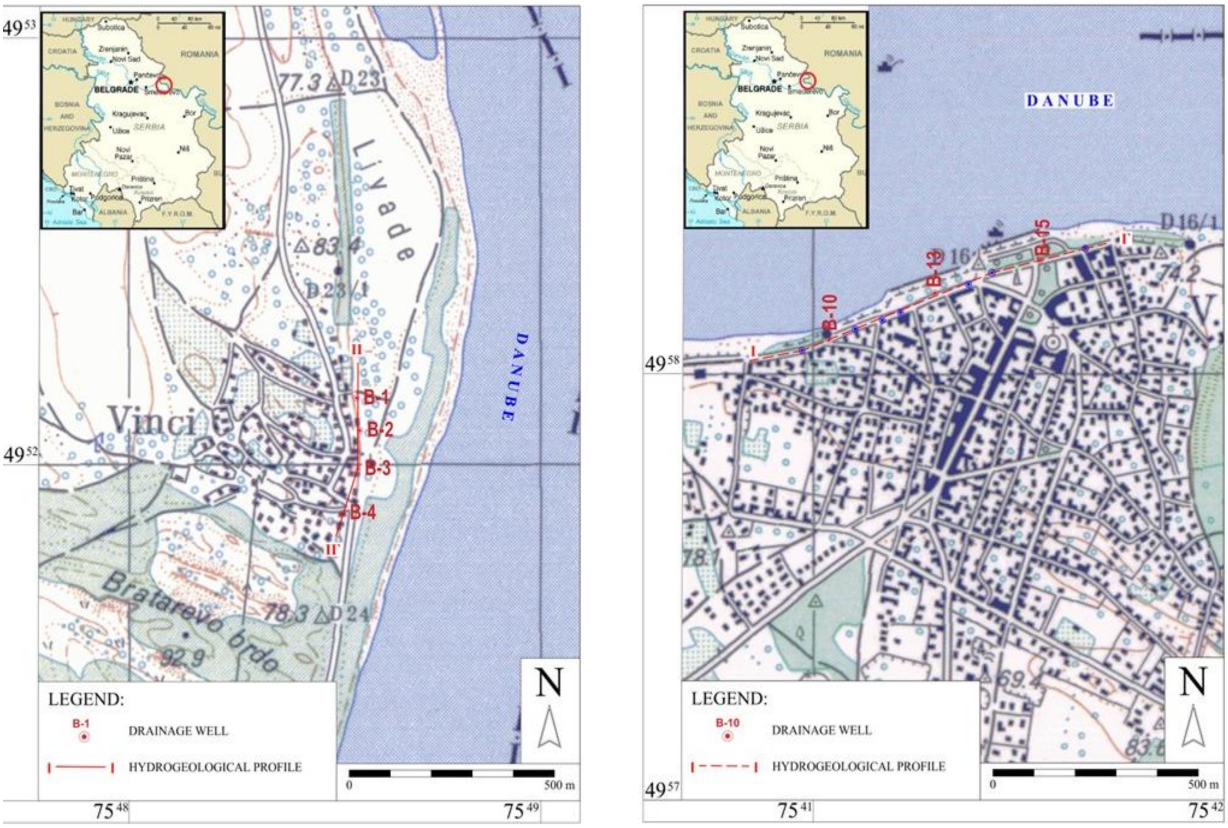

Figure 1. The position of examined areas with marked well's location (left Vinci, right Veliko Gradište)

This paper seeks to clarify the roles of sulfate-reducing, iron-related, heterotrophic-aerobic bacteria, denitrification and slyme forming microorganisms (SRB, IRB, HAB, DN, and SLYME, respectively) in the investigated biofouling and related health risk, that could substantially influence the maintenance of groundwater extraction facilities and associated costs. By employing a system of five types of Biological Activity Reaction Tests (BART tests), the risks for the development of biological processes leading to the formation of various types of deposits, encrustations, and clogging (biofouling), as well as the development of biocorrosion processes, primarily by indigenous bacterial groups, were assessed. The ubiquitous nature of biofouling and the substantial challenges associated with material protection for its prevention across various applications are well-documented in numerous studies [8-13].

Investigations were carried out at two groundwater sampling sites near the settlements of Vinci and Veliko Gradište, Serbia, where a yield decrease of installed drainage wells was observed. At the Vinci site, samples were collected from four drainage wells (B-1, B-2, B-3, B-4), whereas at the Veliko Gradište location, three drainage wells were analyzed (B-10, B-13, and B-15) (Figure 1). The observed decrease in yield led to an increase in the groundwater level, posing a threat to both the settlement and existing structures because of the shallow depth of the groundwater.

2. MATERIALS AND METHODS

IRB BART tests were utilized to identify iron-related bacteria and specific enteric species with the ability to precipitate iron. SLYME BART biotests are employed to identify various bacteria capable of generating extracellular polymeric substances, particularly those that form biofilms, which encompass enteric and opportunistic pathogenic fluorescent *Pseudomonas* species. SRB BART biotests are utilized to detect sulfate-reducing bacteria responsible for producing biogenic H_2S and inducing pitting corrosion. HAB BART biotests are used to identify a diverse array of heterotrophic aerobic and facultatively anaerobic bacteria crucial for biofilm development, playing a role in biocorrosion and biofouling processes. Applied DN BART biotests, besides the detection of denitrifying bacteria can indirectly indicate organic pollution as well as potential presence of pesticides and pathogens. Based on the timing of occurrence and type of reliably identified signature reactions in each applied BART biotest, entered into the dedicated software in the order they are observed, risks were calculated, ranging from 0 to 9, depending on community structure and estimated bacterial population size. Lower numerical values suggest lower potential risks for the development of biofouling processes, biocorrosion, as well as potential risks to public health.

The examined area - Vinci

The settlement of Vinci is located on the right bank of the Danube River (Figure 1). A section of the settlement, located at lower elevations ranging from 71 to 72 meters asl, is situated next to the Danube River. This area extends partially inland to the north and northwest, reaching towards the lower terrains of the Vinci-Požežena reclamation system, and towards the west, close to the "Vinci" water supply source for the Golubac municipality. Increase of groundwater levels, observed in the coastal part of the settlement, has posed a threat to existing structures. These levels have been recorded at depths of less than 2.0 meters from the ground surface. The necessary protection was obtained by constructed drainage system consisting of 4 wells (B-1, B-2, B-3, and B-4) with their own submerged pumping units and separate discharge outlets into the Danube. Within the hydrogeological investigations in the wider area of the Vinci settlement, aquifer is formed within the aquiferous sandy-gravelly complex of Quaternary age (Figure 2). Surficial aquifer layer, consisting of transposed fine-grained sands, mainly 2-3 meters thick, while it is absent in the coastal part of the settlement. Aquitard layer, poorly permeable, composed of clayey-silty sediments, mainly 1-3 meters thick. Semi-permeable layer in the upper part of the aquifer complex, composed of fine-grained sands, silts, mainly 1-4 meters thick. Main aquifer layer consists of medium to coarse-grained, highly gravelly sands, and in the lower part, gravelly sandy, even gravelly. The thickness of these sediments in the settlement area is up to 6 meters [14].

The examined area – Veliko Gradište

The settlement of Veliko Gradište is located on the right bank of the Danube upstream from the confluence of the Pek River, at station km 1059 (Figure 1). The lowest parts of the settlement are situated to the north along the Danube and in the central-eastern part of the town towards the lowlands of the reclaimed area of the Pek - Veliko Gradište. The southern and southwestern parts of the settlement are built on a high river terrace. Veliko Gradište settlement protection was obtained by constructed drainage system consisting of wells (B-9 to B-16) with their own submerged pumping units. The rise in groundwater levels, indicating the development of aging processes in the wells was observed. The alluvial complex in Veliko Gradište settlement consists of surface humus and sandy sediments overlaying a basic aquifer gravelly-sandy layer. The humus layer is located at the surface of the terrain and ranges in thickness from

0.5 to 2 meters. Beneath the humus, there are yellow clays, silts, and sandy loams, generally with a thickness of 3 to 5 meters, increasing slightly towards the hinterland. The aquifer complex consists of very gravelly sandy gravel and sand, with layers of medium-grained sand. The thickness of this aquifer complex varies but is typically within the range of 15 to 18 meters. Hydrogeological profile of examined area Veliko Gradište is presented in Figure 3.

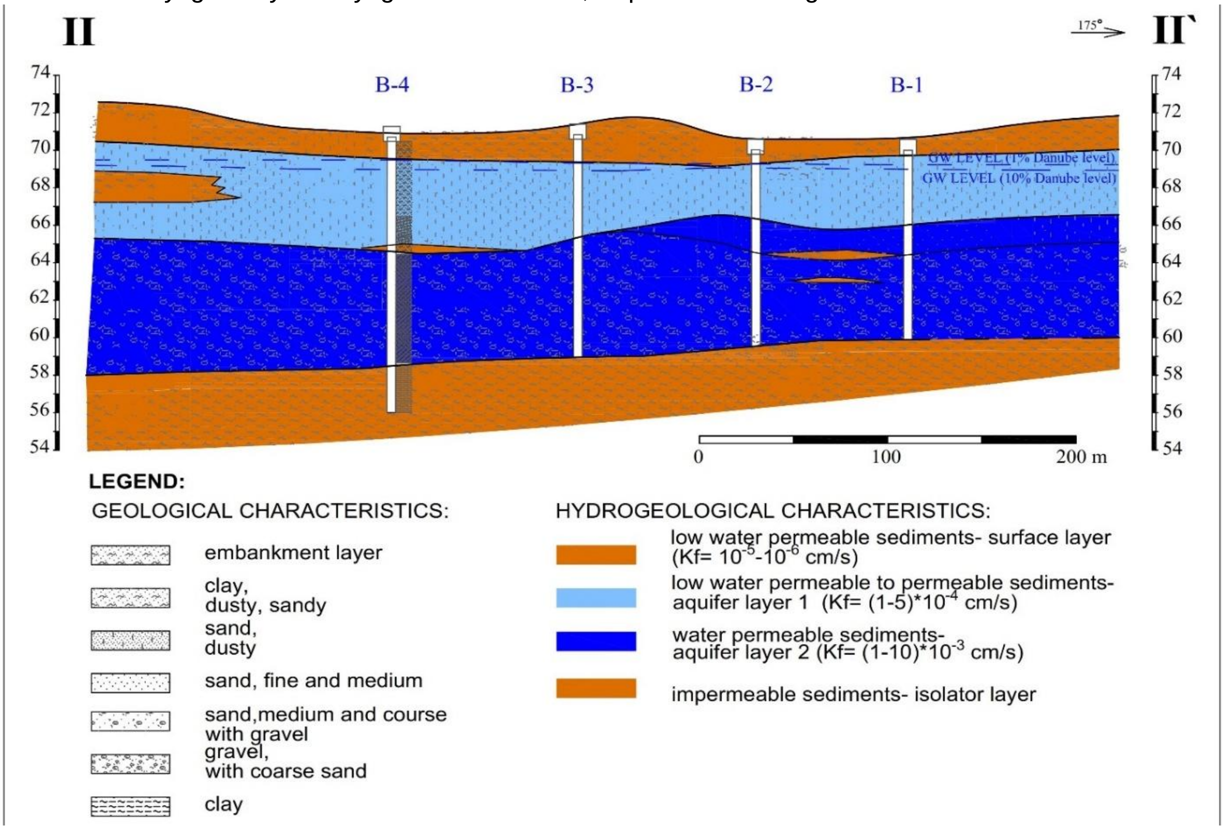

Figure 2. The characteristic hydrogeological profile of examined area - Vinci

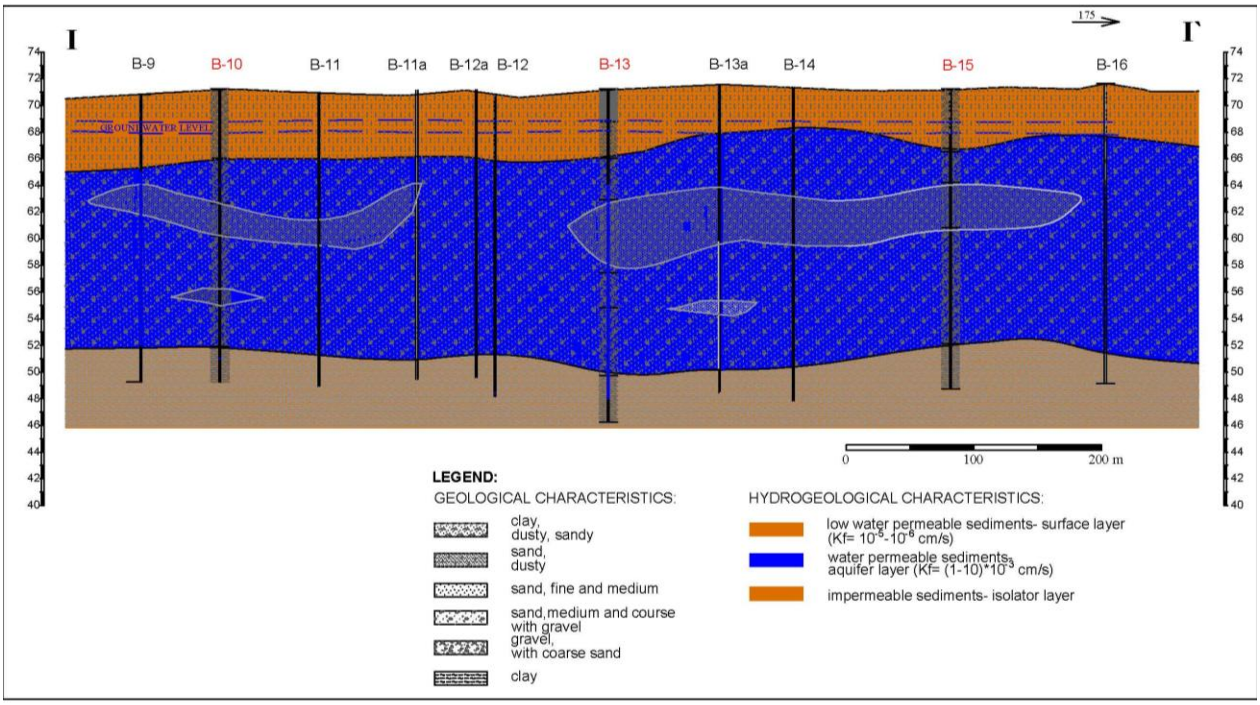

Figure 3. The characteristic hydrogeological profile of examined area - Veliko Gradište

Observation data of groundwater levels over time, from both locations, have indicated a weakening of the effects of well operation during the previous exploitation period, potentially due to the occurrence of "aging" of the wells because of long-term operation [14,15]. This has led to an increase in groundwater levels in the coastal area, with levels recorded in the lowest parts of the terrain behind the well array during the spring period at depths of less than 2.0 meters.

In 2021, the groundwater quality from the wells B-1, B-2, B-3, and B-4 in the Vinci area and B-10, B-13, and B-15 from Veliko Gradište area was analyzed in a one-time campaign (Figure 4 and Figure 5). The sampling was carried out in accordance with *SRPS EN ISO 19458:2009, Water Quality - Sampling for Microbiological Analysis standard. Quality control and quality assurance were guaranteed by ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories*. Details of applied physico-chemical analysis are stated in Standard Methods for the Examination of Water and Wastewater, 21st Edition (2005) [16]. The BART test results were processed using specialized software known as BART-SOFT V.6 [17, 18].

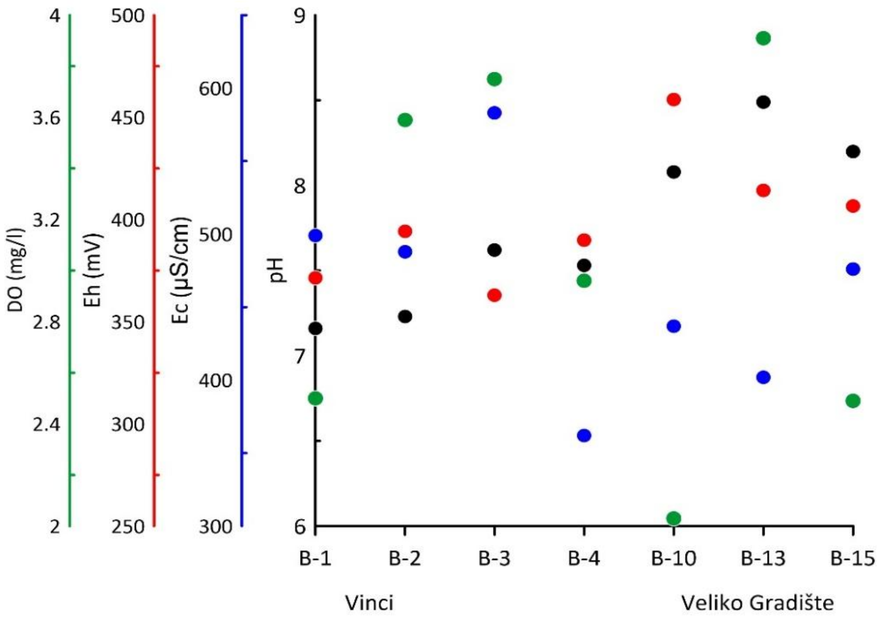

Figure 4. The values of groundwater state parameters in examined wells at two locations

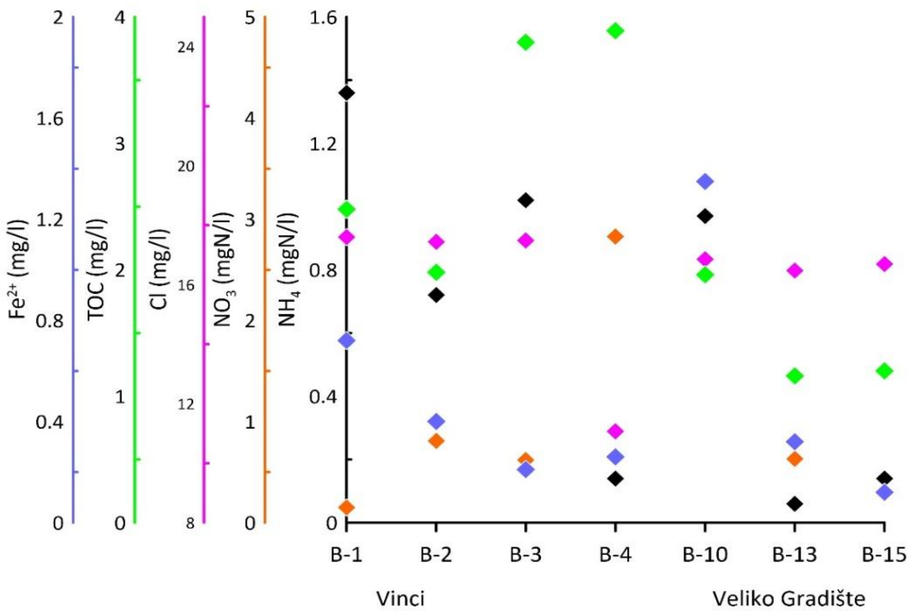

Figure 5. The values of groundwater quality parameters in examined wells at two locations

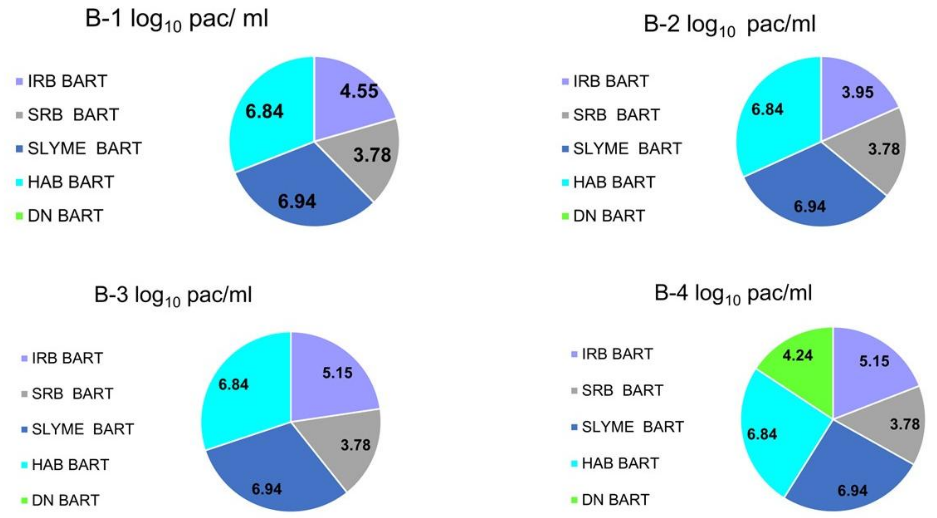

Figure 6. Abundance and Biochemical Diversity of Tested Samples - Vinci

Based on the type and time of signature reaction appearance the description of consortia and estimation of the abundance and aggressiveness of detected bacterial groups and the corrosion risk, clogging risk, and potential health risks were calculated. The corrosion risk (CR) and biofouling risk (BF) scale is represented by a numerical value ranging from 0 (indicating no risk) to 9 (denoting maximum risk) for the progression of these phenomena. During the Lag period (10-15 days) of sample incubation, the appearance of reactions in biodetectors was observed, photographed, and archived daily.

3. RESULTS AND DISCUSSION

Analysis of Groundwater Quality - Vinci

The oxygen content in groundwater in the Vinci wells B-1, B-2, B-3, and B-4 ranged from 2.5 mg/l to 3.75 mg/l. The standard redox potential ranged from 363 mV to 394 mV, confirming that oxidation processes of reduced chemical species likely dominate in investigated area. Electrolytic conductivity in the range of 362 μS/cm to 583 μS/cm proved oligosalinity. The pH value was within a narrow range from 7.16 to 7.62. The concentrations of ammonium ions were from 0.14 mgN/l to 1.36 mgN/L. The concentration of nitrite and nitrate were not significant, while iron and especially manganese were increased. The moderately increased concentrations of organic carbon in B-4, B-3, and B-1 (3.9 mg/l, 3.8 mg/l, 2.5 mg/l, respectively), could indicate the presence of organic pollution, thus possible high numbers of aerobic heterotrophic bacteria and a health risk due to possible presence of pathogens. The determined content of divalent iron and oxygen at the same time of measurement indicates imbalance and disequilibrium of redox-sensitive species (redox state) due to artificially intruded oxygen, likely because of lowering the static level and depression cone due to excessive pumping or infiltration through the unsaturated zone [6,14]. The mixing of different hydrochemical zones, reductive and slightly oxidative, due to significant content of reduced iron, suggests potential for chemical and microbiological oxidation and the formation of iron oxides-hydroxides precipitates, resulting in biofouling in the pre-filter zone and on well screens, as well as corrosion on hydro-technical elements and well construction. A wide range of sulfate concentrations from 3.33 mg/l to 25.47 mg/l suggests intensive microbiological processes of sulfate reduction, oxidation of ferrous sulfide with nitrate reduction (B-4). Dissolved sulfides were not detected in any groundwater sample, but their value may be underestimated due to precipitation by rapid reaction with dissolved divalent iron (insoluble ferrous sulfides-black precipitates) [19]. Groundwater in the B1, B2, B3 exhibits similar biochemical diversity and a very high number of aerobic heterotrophic bacteria and bacteria producing extracellular polymers, i.e., biofilm-forming groups, with values ranging from very high values of 6.84-6.95 log pac/ml (Figure 6). The population of iron-manganese oxidizing bacteria was present in the range of 3.95-5.15 log pac/ml. A uniform population of sulfate-reducing bacteria, with their aggressiveness assessed as high (3.78 log pac/ml), was observed in all four samples in Vinci. Groundwater in the B-4 stood out from the examined group due to the positive finding of fluorescent Pseudomonas species (*Pseudomonas aeruginosa*), biofilm-forming, motile, opportunistic pathogenic species. The population counts of the tested bacterial groups are given in the form of a logarithm for ease of representation due to the population size, with the tested groups indicated by

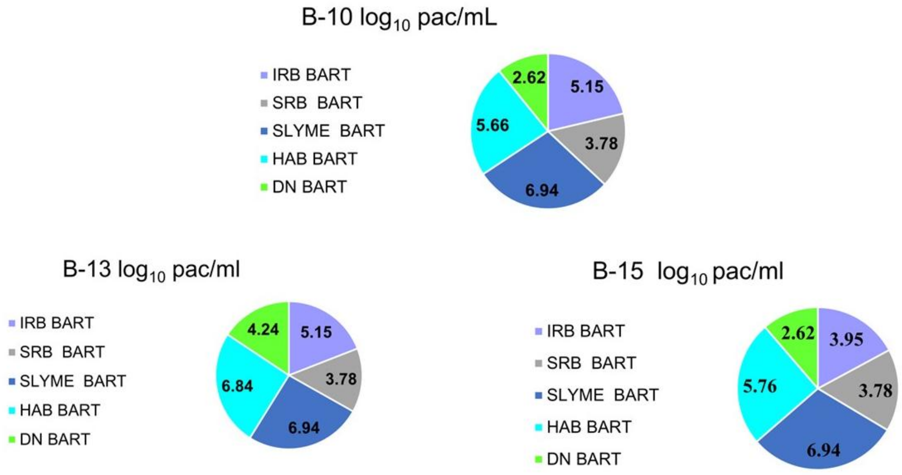

Figure 7. Abundance and Biochemical Diversity of Tested Samples – Veliko Gradište

color (Figure 6 and Figure 7).

Analysis of Groundwater Quality - Veliko Gradište

In the groundwater sampled from selected, tested drainage wells B-10, B-13, B-15, i*n situ* measurements indicated oxic environment, with the dissolved oxygen (DO) concentration in the range of 2.0 mg/l - 3.9 mg/l. The values of the standard redox potential in groundwater varied in the range of 406 mV - 459 mV. The electrolytic conductivity ranged from 402 to 476 µS/cm. The pH was within a narrow range of basic values of 8.1-8.5. Concentrations of iron and manganese in all wells were increased. Dissolved sulfides were detected in one groundwater sample (B-10). Sodium, chloride, and sulfate concentrations were not significant. Groundwater from B-10, B-13, B-15 exhibits similar biochemical diversity and very high number of aerobic heterotrophic bacteria and bacteria producing extracellular polymers, (i.e., biofilm-forming groups, ranging from 6.84 to 6.94 log pac/ml) (Figure 7). The population of iron-manganese oxidizing bacteria ranged from 3.95 log pac/ml in B-15 to 5.15 log pac/ml in B-10 and B-13. Sulfate reducing bacteria, whose aggressiveness (biochemical activity) was assessed as high (3.78 log pac/ml), were detected in the groundwater of all three examined wells.

Calculated risks for bio-corrosion, bio-clogging and health risk

Based on the timing of all recognized reactions in the system of five types of BART, the structure of consortia, and the estimated numbers and aggressiveness of detected bacterial groups, software-predicted risks for the development of biocorrosion and biofouling processes, as well as risks to public health, are calculated. The calculated risk for the development of corrosion processes beneath deposits and biologically generated pitting corrosion in the groundwater was uniform within moderately high-risk ranges (Table 1). Very high risks for the development of biologically induced clogging were determined for groundwater in B-4 (Vinci) B-10 (Veliko Gradište) and B-13 (Veliko Gradište). In B-4, denitrification activity has been detected, indicating a sanitary risk as well. Within the same well the positive finding of fluorescent pseudomonas species (*Pseudomonas aeruginosa*), which is biofilm-forming, motile, opportunistic pathogenic species was observed. Additionally, identified signature reactions within the IRB BART testing indicated that in the groundwater within zones B-1, B-3, and B-4, some enteric species were likely present as well. High risks to public health and the probable presence of pathogens have been identified for groundwater in B-13. In B-13 and B-15, the presence of biofilm-forming, motile, opportunely pathogenic species (Pseudomonas aeruginosa) was indicated. Also, signature reactions within IRB BART testing indicated that some enteric species were present in groundwater in B-10. Denitrifying bacteria were also detected in groundwater B-13 with significant numbers.

4. Conclusion

The potential impact of the analyzed chemical and microbiological parameters on the aging process of the wells was thoroughly analyzed on two alluvial groundwater sites. The results of the conducted physico-chemical analyses of groundwater in the drainage well zone indicated an oxic environment (elevated values of oxygen and redox potential) along with the increased

Table 1. Software-predicted risks for the development of biocorrosion, biofouling, and health risks in groundwater in the drainage wells zone (CR – biocorrosion risk; PR – biofouling risk; HR – health risk)

Software-predicted risks	B-1	B-2	B-3	B-4	B-10	B-13	B-15
	Vinci	Vinci	Vinci	Vinci	Veliko Gradište	Veliko Gradište	Veliko Gradište
CR	5.40	5.40	5.40	5.40	5.40	5.40	5.40
PR	7.20	7.00	7.20	8.10	8.10	8.10	6.30
HR	5.40	2.10	4.50	5.40	6.30	8.10	1.80

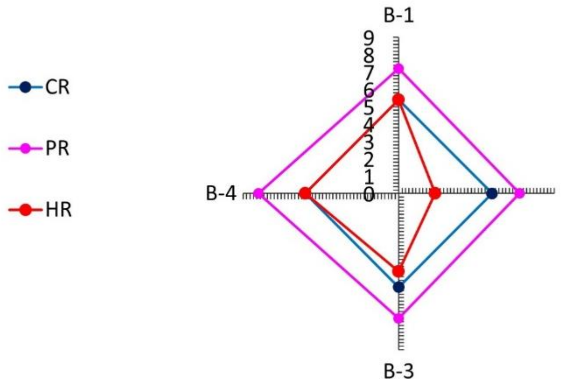

Figure 8. Graphical presentation of software-predicted risks for biocorrosion, biofouling, and health risk in groundwater – Vinci

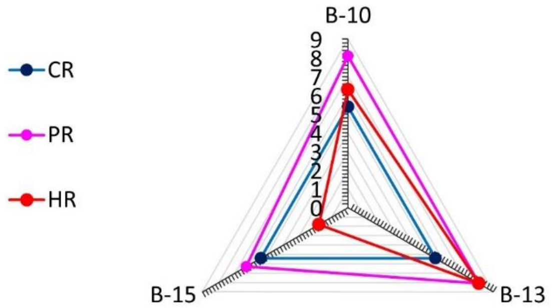

Figure 9. Graphical presentation of software-predicted risks for biocorrosion, biofouling, and health risk in groundwater – Veliko Gradište

concentrations of iron and manganese; thus, a potential for aerobic oxidation of reduced chemical species, which could be mediated both chemically and microbiologically. The rapid "aging" and decline in specific yield of examined wells are influenced by a combination of chemical instability (oxidation) of the environment conducive to this process and suboptimal well operation (over-pumping by high-capacity pumps, low engagement, with short intervals of operation). A moderately high risk was observed for corrosion of all examined wells, while a very high risk was calculated for biofouling for three of total seven wells. In one out of seven examined wells, the high risk for human health was determined. Special attention needs to be paid to the design of newly planned drainage wells to optimize their operation, ensuring a functional dependency on the installed capacities of the units, their engagement, and maintenance of required groundwater levels. This study highlights the significance of comprehensive understanding of physico-chemical and microbiological composition of water, as well as their interconnections and mutual dependencies. This interconnection not only signals potential health risks but also explicitly defines the probability of bio-induced corrosion and biofouling occurrences through coefficient calculations. Based on these results the engineers and technicians can implement preventive measures and treatments to mitigate clogging issues, ensuring the sustained functionality and efficiency of the well system.

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IZVOD

PROCENA BIOKOROZIJE, BIOOBRASTALJIVANJA I ZDRAVSTVENIH RIZIKA NA DVE ISTRAŽIVANE LOKACIJE U DUNAVSKIM ALUVIJUMIMA

U radu su prezentovani rezultati mikrobioloških istraživanja posebnih metaboličkih (fenotipskih) grupa bakterija koje igraju važnu ekološku ulogu u biogeohemijskom kruženju gvožđa , mangana , azota, sumpora i ugljenika, a koje učestvuju i u razvoji procesa biokorozije, biofulinga a neke od njih predstavljaju i rizik za javno zdravlje. Na osnovu dobijenih rezultata primenjenih reakcionih testova biološke aktivnosti (BART testovi), koji su obrađeni u namenskom softveru, izračunati su potencijalni rizici za razvoj mikrobiološki posredovanih procesa korozije, mikrobiološkog kolmiranja i zdravstveni rizici za podzemne vode u zoni sedam hidrotehničkih objekata dva oksična lokaliteta u aluvijonu Dunava - Vinci i Veliko Gradište, u Srbiji. Umereno visok rizik za biokoroziju određen je za svih sedam bunara na oba lokaliteta (CR=5.4). Rizik od mikrobiološkog kolmiranja bio je vrlo visok u tri od sedam ispitivanih bunara (PR=8.10). Među sedam ispitivanih lokaliteta, jedan se posebno izdvojio na osnovu izračunate visoke vrednosti koeficijenta zdravstvenog rizika (HR=8.10). Rezultati istraživanja su pružili nove uvide u mikrobiološku ulogu u starenju bunara u oksičnim podzemnim vodama dunavskog aluvijona. Pokazano je da fizičko-hemijski sastav i hemijske vrste poput minerala, organske materije i specifičan sastav zajednica mikroorganizama u ispitivanim podzemnim vodama imaju potencijal da podstaknu biokoroziju I formiranje naslaga i biofilmova unutar strukture bunara. Osim biohemijskih analiza, prikazane su i hidrogeološke karakteristike analiziranog područja, kako bi se definisala geološka stratigrafija za koju bi, na osnovu dobijenih rezultata, bilo očekivano odvijanje specifičnih mikrobioloških transformacija.
Ključne reči: *biokorozija, biozačepljavanje, podzemna voda, zdravstveni rizik, Srbija*

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