Novi Sad, April 2nd, 2024

Dr. Marija Perović
Jaroslav Černi Water Institute,
Jaroslava Černog 80, Belgrade,
11 226 Pinosava,

Subject: Official Letter for Invited lecture at an international conference

Dear Dr. Marija Perović,

On behalf of the Scientific committee of the **SECOND INTERNATIONAL EUROSA CONFERENCE**, I am delighted to officially invite you to deliver a lecture at the **SECOND INTERNATIONAL EUROSA CONFERENCE**, which will be fully printed. The conference will take place from May 15 to 18, 2024, at the VRNJAČKE TERME Hotel in Vrnjačka Banja, Serbia. For further details, click eurosa 2024 - Eurosa.

Your expertise and contributions to environmental engineering, especially in the field of preserving the quality of water resources, have been widely recognized, making you an ideal candidate to enrich our conference program. We believe that your insights would greatly benefit our participants and contribute to the overall success of the event. Your session for Invited lecture at this international conference is scheduled for 15-May-24 at 17.30h. We kindly ask that you provide us with the speech of your Invited lecture by the 1st of May 2024.

Thank you for considering our invitation. We are eager to welcome you to Vrnjačka Banja and look forward to your valuable contribution to the SECOND INTERNATIONAL EUROSA CONFERENCE. Should you have any questions or require further information, please do not hesitate to contact me at +381642004875, or zorancepic@uns.ac.rs.

Kind regards,

President of Scientific Committee

Z Čepić

Assoc. Prof. Dr. Zoran Čepić
Faculty of Technical Sciences
University of Novi Sad

EUROSA 2024

2nd EUROSA Conference

May 15th - 18th, 2024
Vrnjačka Banja

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ФАКУЛТЕТ ТЕХНИЧКИХ НАУКА НОВИ САД

ФМГ КРАЉЕВО
УНИВЕРЗИТЕТ У КРАГУЈЕВЦУ

AGENDA May 15, 2024.

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16.00 - 16.15	Welcoming speech by Miodrag Meseldžija, President of the European Association of Occupational Safety
16.15 - 16.30	Welcoming speech by Zoran Čepić, President of the Scientific Committee of the EUROSA 2024 Conference
16.30 - 16.50	Maja Ilić, Labor Inspectorate
16.50 - 17.10	Representative from the Directorate for Occupational Safety and Health
17.10 - 17.30	Dr. Jovan Protić, International Labour Organization
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9.15 – 9.30	ANALYZING THE ENVIRONMENTAL EFFECTS OF MUNICIPAL WASTE MANAGEMENT IN NOVI SAD Jovanovic S, Ubavin D, Kosec B, Agarski B.
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11.15 – 11.30	REDUCTION OF CO AND NOX EMISSIONS IN THE DISTRICT HEATING SYSTEM OF THE CITZ OF KRALJEVO BY FUEL SWITCHING Marašević M, Stojić N, Vičovac A, Cvetkov G, Bošković G.
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	CONFERENCE CLOSURE
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Institut za preventivu - Novi Sad

Moderator: Dr Ivan Krstić

Fakultet zaštite na radu - Niš

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Lonza - Švajcarska

Panelist: Jovan Protić

Međunarodna organizacija rada

Moderator: Miodrag Meseldžija

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Vojnomedicinska akademija - Beograd

Panelist: Dr Tatjana Mihailović

Dom zdravlja Novi Beograd

Panelist: Dr Maja Popović

Univerzitetski klinički centar Srbije

Moderator: Dr Maja Petrović

Fakultet tehničkih nauka – Novi Sad

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La Farge - Beočin

Panelist: Dragan Jocić

Michelin - Pirot

Moderator: Srđan Novokmet

Yanfeng - Kragujevac

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- INTERHERMES
- HABERKORN

REGISTRATION FEE INFORMATION

- The participation fee for the conference is 12,000 Serbian dinars for in-person attendees and 6,000 Serbian dinars for online participants. This fee covers participation in the conference sessions, conference proceedings, and accompanying materials.

- The registration fee should be paid via bank transfer to the following account: European Association for Occupational Safety and Health, Raiffeisen Bank, Account Number: 265201031001046785

CONFERENCE TOPICS

- Engineering and Occupational Safety Management
- Environmental Engineering and Management
- Fire Protection Engineering and Management
- Engineering and Management of Disaster and Emergency Protection
- Good use of practice in protection

- The official language of the conference is English.
- Each participant can submit a maximum of 2 papers.
- All accepted papers will be published in the conference proceedings, in electronic format, with an ISBN number.

- Submit papers to euraznr@gmail.com by May 1, 2024.

HOTEL INFORMATION

- Accommodation is provided at the hotel on a half-board basis, with reservations exclusively through email at prodaja@vrnjacketerme.rs, with a mandatory note indicating that you are a conference participant. For additional information, please contact Jelena Jovanović and Bojana Benović at the following telephone numbers: +381 36 51 50 300 and +381 62 80 91 437.
- The price of hotel accommodation in double rooms is 26,430.00 Serbian dinars per person, with a surcharge of 8,960.00 Serbian dinars for single occupancy. The total amount for accommodation in the selected room includes 3 half-board meals, a welcome cocktail, and a gala dinner with live music.
- Staying at the hotel includes complimentary use of the Aqua zone (jacuzzi, five indoor and three outdoor recreational pools). All pools are filled with thermal mineral water ranging from 32° to 36°C. Guests also have access to the Wellness center (sauna, salt room, tepidarium, steam bath), sports facilities, Wi-Fi, and free outdoor parking.

EUROSA 2024

SECOND INTERNATIONAL EUROSA CONFERENCE

PROCEEDINGS

May 15-18, 2024

Hotel Vrnjačke terme, Vrnjačka Banja, Serbia

Hybrid event
May 15 – 18, 2024
Hotel Vrnjačke terme, Vrnjačka Banja, Serbia

<u>Organizers:</u>

Department of Environmental Engineering and Occupational Safety and Health, Faculty of Technical Sciences, University of Novi Sad, Serbia

Faculty of Mechanical and Civil Engineering in Kraljevo, University of Kragujevac

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Dr Bojana Zoraja

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PREFACE

On behalf of the Scientific and Organizational Committee, it is my honor and great pleasure to present the Proceedings of the 2nd EUROSA International Conference, held on 15-18 May 2024 in Vrnjačka Banja, Serbia.

The papers contained in this Proceedings represent current scientific and professional informations in the field of sustainable management of occupational health and safety, environmental protection, fire protection and emergency situations and represent a mix of scientific research and professional opinion, shared with us by participants from academia and industry professionals.

We sincerely thank all the conference participants for their contribution, ensuring the success of the conference. Special thanks to all the participants of the round tables and panel discussions, keynote speakers, chairmen of the sessions and of course the reviewers for their invaluable contribution.

Last but not least, I would like to express my sincere gratitude to all members of the Scientific and Organizing Committee, whose efforts and work led to the successful realization of the EUROSA 2024 conference.

Vrnjačka Banja, May 2024

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INTEGRATED ANALYSIS OF WATER POLLUTION: CASE STUDIES ON NITROGEN TRACING IN ANOXIC AND OXIC GROUNDWATER ENVIRONMENTS

Perović M.[1]

[1] *Jaroslav Černi Water Institute, 11 226 Pinosava, Belgrade, Serbia*
(marija.perovic@jcerni.rs)

Abstract: Presented research explores the critical role of water in sustainable development and the challenges it faces, particularly in managing groundwater pollution, with a focus on nitrogen sources. Groundwater, vital for human survival, undergoes increasing strain due to various factors, including agricultural and industrial activities. With less than 10% of wastewater in Serbia undergoing treatment and nitrogen being wastewaters' primary constituent, the research underscores the importance of utilizing diverse scientific methods to effectively trace nitrogen origin and transformations. Given that over 50% of Serbia's water supply is sourced from alluvial aquifers, three case studies demonstrate the integration of hydrogeochemistry, isotopic analysis, and microbiological tests as a comprehensive approach to understanding nitrogen transformations in shallow alluvial groundwaters. Based on physico-chemical data, the groundwater potential for nitrogen conservation or removal can be determined. To evaluate the ability of machine learning models to predict the ammonium concentration in groundwater, four machine learning models were applied: a three-layer neural network (NN), a deep neural network (DNN), and two variants of support vector regression (SVR) models: with linear and with Gaussian radial basis function kernel. Despite the complexities involved, this multidisciplinary approach offers valuable insights for groundwater management, emphasizing the importance of prevention over treatment.

Keywords: **N***itrogen; Nitrate; Groundwater; Isotopes.*

INTRODUCTION

Water is essential for sustainable development and human survival. It serves as the cornerstone of socio-economic progress, ecological health, and disease prevention, plays a crucial role in reducing the effects of climate change and protecting essential ecosystems (UN, 2024). As a finite and increasingly stressed resource, effective management is imperative to ensure its availability for future generations and to harness its potential as a catalyst for resilience in the face of evolving environmental challenges. Groundwater constitutes approximately 99% of the total global volume of fresh water (Shiklomanov and Rodda 2003). Globally, agriculture dominates freshwater withdrawals at approximately 70%, followed by an industry at just under 20%, and domestic (or municipal) use at about 12% (Ritchie and Roser, 2018; UN, 2024).

Higher-income nations utilize more water for industrial purposes, while lower-income countries allocate 90% or more of their water for agricultural irrigation (UN, 2024). In low-income countries, inadequate wastewater treatment largely contributes to poor water quality, while in more prosperous countries agricultural runoff emerges as the primary concern (UN, 2024). Roughly 75% of the EU population relies on groundwater for water supply.

The significance of groundwater in the Republic of Serbia as a water supply source is evidenced by the 75% share of groundwater used for water supply. Over 50% of groundwater utilized for water supply in Serbia originates from alluvial aquifers (SUVRS; Perović and Dimkić, 2021). Maintaining groundwater quality benefits from its slow flow (1 m/day to 1 m/year), enabling natural self-purification processes such as sorption, biodegradation, and redox reactions, along with dispersion, diffusion, and advection effects. Yet, this slow flow also burdens contamination removal, as pollutants persist in the groundwater for a long time, making purification economically challenging. Considering pressures on groundwater quality, the population connected to sewage systems and industrial facilities represents the most significant concentrated sources of water pollution. In the Republic of Serbia, approximately 54% of the total population is connected to public sewage systems. There is a significant disparity in the level of population connection to sewage compared to connections to the water supply network, especially in settlements with fewer than 50,000 inhabitants, posing a threat of groundwater quality, especially considering nitrates (SUVRS). Over the past few decades, over 50 urban wastewater treatment plants have been constructed in settlements with more than 2,000 inhabitants in the Republic of Serbia. Of the constructed plants, only 32 are operational, with only a few operating according to project criteria, while the rest operate with efficiency below projected levels (SUVRS). The overall treatment efficiency for organic load removal is less than 65%, for nitrogen components it's less than 35%, and for phosphorus components it's less than 25% (SUVRS). The Republic of Serbia ranks among moderately developed countries in terms of sewage infrastructure development, while lagging significantly in wastewater treatment capabilities.

Considering that less than 10% of wastewater in Serbia is treated and that nitrogen is wastewaters' primary component of concern, presented research demonstrates the possibilities and necessity of simultaneous application of different scientific methods from various scientific perspectives in order to determine the nitrogen origin in groundwater. The study presents a concise overview of three case studies in Serbia, showcasing the integration of hydrogeochemistry, isotopic analysis, and microbiological tests to trace nitrogen transformations and sources. The study aims to demonstrate that the only approach reducing ambiguity in drawing conclusions is highly complex, comprehensive, financially, and temporally demanding. Therefore, in the context of groundwater management, prevention outweighs treatment.

Environmental and health hazard of nitrogen compounds in water

Since the 1970s, elevated nitrate levels in groundwater have been recognized as a significant health and ecological risk, as highlighted by various reports from European Nations on nitrate contamination (Dimkić et al., 2008; EEA, 2018). Recharging surface waters with nitrate-laden groundwater and agricultural runoff nutrients can trigger eutrophication in aquatic ecosystems. This can harm biodiversity and populations of mammals, birds, and fish, leading to toxin production and decreased levels of dissolved oxygen (Pastén-Zapata et al., 2014). Eutrophication occurs naturally in aquatic ecosystems in response to elevated levels of nitrate and phosphate salts, leading to heightened primary production, specifically the growth of aquatic plants. Increased production of aquatic plants results in increased organic matter content, which is bacterially decomposed, producing unpleasant odors, consuming available oxygen, and affecting the development of other aquatic organisms. It has been found that even relatively low concentrations of N around 4.4-8.8 $mgNO_3/l$ (1-2 mgN/l) can trigger eutrophication in oligotrophic surface waters (Rivett et al., 2008). In nutrient-rich environments, phosphorus concentrations represent the limiting factor for eutrophication.

Increased nitrogen levels in aquifers indirectly contribute to nitrous oxide (N_2O) emissions, a potent greenhouse gas generated either as a necessary intermediate in denitrification reactions or as a by-product of nitrification. About 60% of the total N_2O present in the atmosphere is of natural origin. Natural N_2O emissions occur within the nitrogen cycle, which involves the circulation of nitrogen forms between the atmosphere, plants, animals, and microorganisms (in soil and water), in which nitrogen changes oxidation states from -3 to +5, including the state +1 (nitrous oxide). The main anthropogenic sources of N_2O are agriculture (application of mineral fertilizers and manure), burning of plant residues, fuel combustion, wastewater management, and industrial processes. N_2O molecules persist in the atmosphere for an average of 114 years, and this gas is 300 times more potent as a greenhouse gas than carbon dioxide (USEPA, 2018). Nitrous oxide (N_2O) is mitigated in the atmosphere through bacterial absorption, chemical reactions, or exposure to ultraviolet radiation.

Health problems due to constant intake of food rich in nitrogen compounds and water with elevated nitrogen concentrations have long been considered and well-documented (Canter, 1997; Camargo and Alonso, 2006; IARC, 2010; PSEP, 2018). The International Agency for Research on Cancer (IARC) categorizes nitrates and nitrites in Group 2A (vol. 94) as "probably carcinogenic to humans," potentially resulting in the formation of established carcinogens like N-nitroso compounds under specific circumstances (IARC, 2010). The most discussed disease caused by elevated nitrate concentrations in drinking water is a hematological disorder that occurs when erythrocytes contain more than 1% of methemoglobin - methemoglobinemia. Symptoms include cyanosis, bluish mucous membranes, digestive and respiratory problems, headache, drowsiness, fatigue, tachycardia, convulsions (methemoglobin concentrations 1-10%), brain damage, and fatal outcome (methemoglobin concentrations 50-70%) (PSEP, 2018). Nitrate in drinking water is considered a health threat in the general population when it is present at concentrations of 100-200 mgNO3-N/l, but individual negative effects depend on the amount of nitrates and nitrites ingested (PSEP, 2018). Studies have shown that long-term intake of drinking water containing nitrate levels nearing the maximum allowable limit (10 mg NO_3-N/l) can trigger the production of endogenous nitrosamines (Camargo and Alonso, 2006). There is scientific evidence that ingestion can have mutagenic, teratogenic effects, may contribute to the risk of lymphoma, as well as the occurrence of bladder, thyroid, ovarian cancers (Camargo and Alonso, 2006; IACR, 2010; PSEP, 2018). The presence of nitrates in groundwater can indicate the potential presence of bacteria, viruses, and protozoa, particularly if the nitrates originate from animal waste or septic system discharge (Almasri and Kaluarachchi, 2004). The European Union and the World Health Organization (WHO) have established a standard for nitrate concentration in drinking water at 11.3 mgN/l (50 mgNO_3/l). This same limit for drinking water applies in the Republic of Serbia. United States Environmental Protection Agency (USEPA) has set a standard for drinking water at 10 mg-N/l (45 mgNO_3/l) (Drinking Water Directive 98/83/EC; WHO, 2004). The Nitrate Directive (91/676/EEC) requires the protection of all natural freshwater bodies and sets a limit of 50 mgNO_3/l, which applies to all groundwater regardless of its purpose.

Characteristics and Parameters Relevant to Nitrogen Fate in Groundwater

The role of Hydrogeological characteristics

Hydrogeological characteristics encompass the physical properties of groundwater environment, such as aquifer structures, water table depth, porosity, permeability, and hydraulic conductivity, crucial for understanding groundwater origin, flow, and quality.

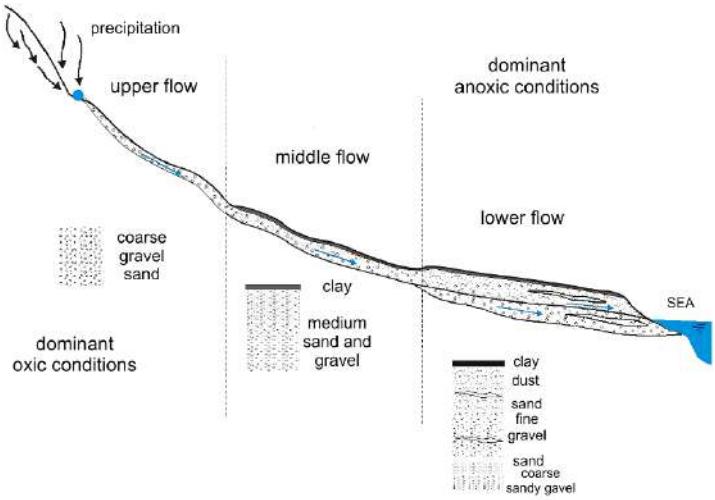

Figure 1. Schematic representation of alluvial groundwater aquifers (Dimkić, 2012)

For a thorough understanding of the vulnerability and protection of water resources, particularly alluvial aquifers, relevant knowledge of the following is essential: geological and hydrogeological composition, hydrological and hydrodynamic relationships within aquifers, knowledge of vegetation cover and plants' water and nutrient requirements, hydrochemical properties of water and soil in lithological layers (Perović and Dimkić, 2021). In the upstream section of the river basins, where water energy is higher, the deposition of fine particles is less pronounced. This results in coarse-grained layers that maintain oxic conditions, reducing the possibility of denitrification processes in the upstream parts of a river basin. As a result, the likelihood of elevated nitrate concentrations becomes more apparent (Figure 1).

Redox processes

The redox potential (R (mV)) is considered a dominant determining factor for pollutant transport, alongside oxygen concentration, and therefore a determinant of the fate of nitrogen species in groundwater. Depending on prevailing redox conditions, redox-sensitive compounds such as O_2, $NO_3^-/N_2/NH_4^+$, $SO_4^{2-}/HS^-/S^{2-}$, Mn^{4+}/Mn^{2+}, Fe^{3+}/Fe^{2+}, As^{5+}/As^{3+}, Se^{6+}/Se^{4+}, Cr^{6+}/Cr^{3+} will exhibit different mobility, degradation rates, solubility, bioavailability, and thus toxicity (Perovic, 2017). Once oxygen is consumed by aerobes, facultative anaerobes start utilizing nitrates as the next energetically favorable electron acceptors. Reduction reactions proceed with the consumption of Mn^{4+} and Fe^{3+}, followed by SO_4^{2-}, then H^+ and CO_2, ultimately leading to methane production. According to the literature review, the highest values of redox conditions where nitrate reduction can be expected are around 150 mV (<250 mV).

Oxygen content

Groundwater can be categorized as follows: oxic if the concentration of dissolved oxygen is greater than 30 μM (1 mg/l DO), suboxic if the oxygen concentration ranges from 1 μM < O2 < 30 μM (0.03 mg/l to 1 mg/l DO), anoxic if the oxygen content is less than 1 μM (0.032 mg/l DO), and anaerobic sulfidic or non-sulfidic (IJČ, 2010a). Feast et al. (1998) specifies the upper limit for denitrification occurrence as 0.2 mg/l, whereas Dimkić et al. (2008) states that the upper limit of dissolved oxygen concentration for denitrification processes is 0.5 mg/l. In aerobic aquifers, the aging of water abstraction facilities occurs significantly slower, and better self-purifying characteristics are expressed for most chemical compounds (Dimkić et al., 2008).

Environmental isotopes

In recent years, the ratio of stable nitrogen isotopes in nitrates has been widely used as a method to identify nitrate sources in water bodies (Nikolenko et al., 2018). Different sources of nitrogen compounds exhibit distinct isotopic signatures, which are used in identifying nitrogen sources

(Table 1). There are over 10 known isotopes of nitrogen, but only two, ^{14}N and ^{15}N, are stable. The atmospheric concentration of the heavier isotope ^{15}N remains relatively permanent, with a constant ratio of $^{15}N/^{14}N$ at 1/272 (0.36%) (Kendall and Aravena, 2000). As a result, the ratio of stable nitrogen isotopes ($\delta^{15}N$) is typically expressed in parts per thousand (‰) relative to atmospheric air according to equation (1):

$$\delta^{15}N_{(vs.air)}=[((^{15}N/^{14}N_{sample})/\ (^{15}N/^{14}N_{air}))-1]\cdot 1000 \quad (1)$$

The isotopic signature of nitrogen can be altered by various complex biogeochemical transformation mechanisms such as volatilization, nitrification, and denitrification, it cannot be exclusively relied upon for source identification, thus it is necessary to understand the influence of nitrogen compound transformation processes on isotopic fractionation (Pasten-Zapata et al., 2014; Perović et al., 2024). The isotope fractionation represents the enrichment of one isotope relative to another during a microbiological, chemical, or physical process, which is quantified by the enrichment factor ($^{15}\varepsilon$). This factor essentially signifies the ratio of enrichment/depletion of isotopes in the reaction product compared to the reactant (substrate). Isotopic fractionation is caused by the presence of stronger chemical bonds by the heavier isotope or due to mass-dependent processes such as diffusion (Sharp, 2007; Nikolenko et al., 2018). Isotopic fractionations are challenging to quantify because they are often metabolically driven, kinetically controlled, and non-equilibrium processes (Nikolenko et al., 2018). In Table 1 there is a review of characteristic isotopic signature results for different sources.

Table 1. The values of $\delta^{15}N\text{-}NO_3^-$ and $\delta^{15}N\text{-}NH_4^+$ for different sources in groundwater (based on data from Nikolenko et al., 2018; modified from Perović, 2019)

	Source	**Range $\delta^{15}N\text{-}NO_3^-$**
$\delta^{15}N\text{-}NO_3^-$	Soil nitrogen	+3 do +8 ‰
	Mineral fertilizers	-8 do +7 ‰
	Manure	+5 do +35 ‰
	Sewage water	+3 do +25 ‰
	Rain	-12 do +11 ‰
$\delta^{15}N\text{-}NH_4^+$	Organic matter	+2,4 do 4,1 ‰
	Rain	-13,4 do +2,3 ‰

	Mineral fertilizers	-7,4 do 5,1 ‰
	Municipal wastewater	+5 do +9 ‰
	Manure	+8 do +11 ‰

Biological activity reaction tests (BART tests)

Semiquantitative and semiqualitative biological activity reaction tests can be utilized to investigate specific bacterial groups involved in various processes in groundwater. The studied groups included bacteria capable of participating in nitrogen transformation processes, including denitrifying bacteria (DN-BART), sulfate-reducing bacteria (SRB BART), and iron-related bacteria (IRB BART). Specialized software, (BART-SOFTv6), was employed to calculate the correlation between the day, specific reaction type, and the quantity of active cells based on the BART test results. The application of BART tests in science is widely used for: assessing the environmental suitability for microbiological transformations, for indicating the potential for biofouling and biocorrosion of underground structures (well aging), as well as for predicting health risks.

MATERIALS AND METHODS

A comprehensive overview of three case studies was provided, covering research on the origin and fate of nitrogen in oxic alluvial groundwater, as well as anoxic alluvial groundwater. State-of-the-art methods were applied for predicting origin, form and nitrogen concentration, including isotopic signature analysis, microbiological activity reaction tests (BART) and machine learning techniques. First case study encompassed 20 groundwater sampling locations during the period 2010-2019, within oxic alluvial source named Ključ. This groundwater source is used for public water supply of the city of Požarevac (approximately 85,000 inhabitants). Within second case study 93 samples from three sampling sites were analysed within five days. Samples were collected from anoxic alluvial aquifer under the drainage system Kovin-Dubovac in Danube alluvium. In the third case study, data spanning a six-year monitoring period from 55 groundwater sampling sites, collected through the state monitoring network, which is part of the national monitoring network managed by the Environmental Protection Agency of Serbia, underwent statistical analysis.

RESULTS AND DISCUSSION

First case study

To solve the problem of increased nitrate concentrations in Ključ groundwater source, in the Velika Morava alluvium, the infiltration-protective system was established in 2006 year. Determined groundwater levels indicated that groundwater recharge depends on Velika Morava water level and on the work regime of extraction wells. The physico-chemical analysis sampling strategy encompassed both - river proximity and the hinterland. The groundwater quality data have shown that examined water is oxic (avg content of DO 5.27 ± 0.37 mg/l), with high redox potential (400.38 ± 17.01 mV). Nitrate concentration was in range from 0.61 mgN/l to 74.27 mgN/l. Statistical data analysis revealed significant correlation between human impact tracers (Na, Cl, NO_3, SO_4, B, pH and el. conductivity), between B and TOC, between pH, NH_4, DO and B. BART tests, along with the isotopic signature results ($\delta^{15}N$ and $\delta^{18}O$ in NO_3^-) were determined. The biological activity reaction tests (BART tests) revealed that nitrification detected in the hinterland alternates with denitrification in the coastal zone. Isotopic signature results showed that the most enriched nitrates with heavier isotope (^{15}N) are determined in the hinterland, while in the area near river the urea hydrolysis signature was observed. Details of conducted research are available in Perović et al., 2024. Based on a comprehensive, simultaneous analysis, zones of different influences were delineated, as depicted in Figure 2 (Perović et al., 2024).

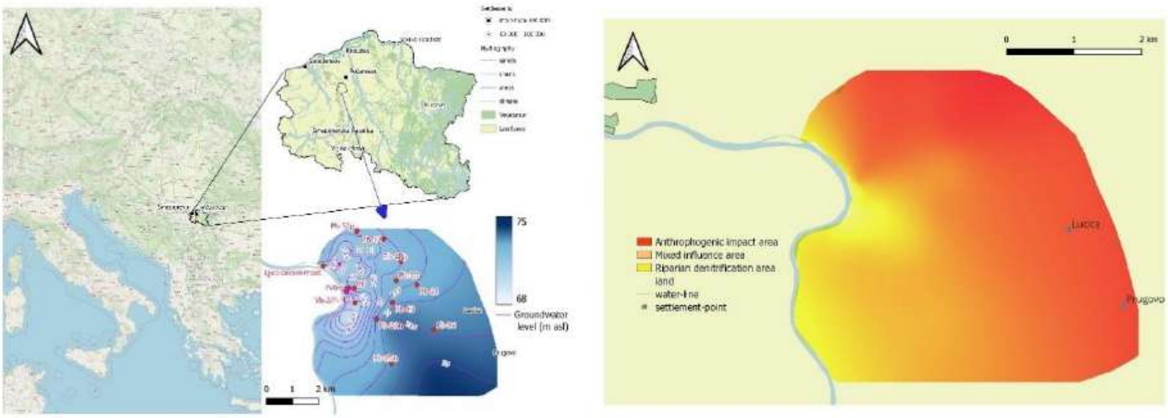

Figure 2. The position of examined area and delineated zone of nitrate origin and fate based on a comprehensive analysis (Perović et al., 2024)

Second case study

The research area holds significance as Kovin-Dubovac represents a potential future regional water supply source, while the area is characterized by the coexistence of intensive agricultural production and an open coal mine. The examined groundwater exhibited an increased concentration of ammonium ions, ranging from 0.02 mgN/l to 4.70 mgN/l and low oxygen concentration, ranging from below the quantification limit to 1.20 mgN/l. The research was conducted in order to determine the fate of nitrates in anoxic groundwater. The nitrate injection experiment ($NaNO_3$) followed tracer experiment (NaCl) and lasted for five days. Groundwater levels, physico-chemistry and BART tests were assessed. Based on conducted research the dominant process in anoxic groundwater environment was conversion of nitrates to ammonium ion by dissimilatory nitrate reduction. The combination of high DNRA (Dissimilatory Nitrate Reduction to Ammonium) and low respiratory denitrification, along with the results of the BART test, indicated that sulfate reducers with DNRA capability as a secondary metabolic pathway were predominant in the process (Perović et al., 2017) (Figure 3).

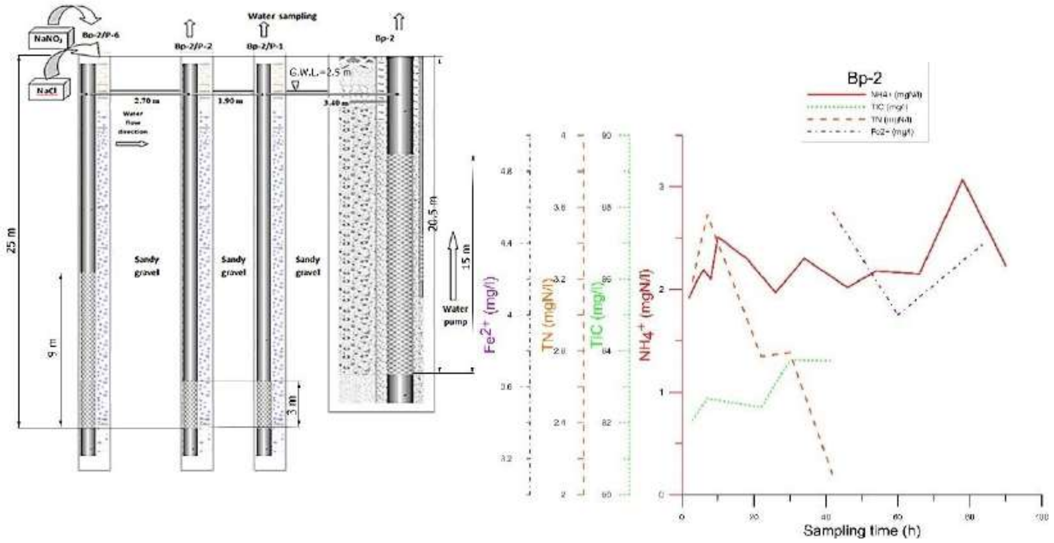

Figure 3. Nitrate and tracer experiment observational objects and observed concentration changes of selected parameters in the well Bp-2 (Perović et al., 2017)

Third case study

The aims of the study were – examination of the correlations between ammonium concentration influential parameters in shallow alluvial aquifers throughout the Serbia; followed by vulnerability map development; and the examination of the possibilities of machine learning models for predicting the ammonium concentration in groundwater in response to predictor variables representing physicochemical conditions in groundwater which are proved to be involved in N transformations (Perović et al., 2021). The map of overall groundwater potential for nitrogen conservation or loss was developed based on threshold values for certain N transformation processes. The figure 4 presents the status of groundwater quality considering ammonium concentrations and marked areas suitable for N conservation - DNRA - low oxygen content (DO) (below 1 mg/L) and C/N ratio over 3.5; denitrification process - places characterised by DO below 2 mg/L and total organic carbon above 3 mg/L and areas with DO content above 3.5 mg/L, which are designated as vulnerable for nitrate inflow. Four supervised learning regression models were applied: support vector regression (SVR) in two variants: with linear and Gaussian radial basis function, kernel, and artificial neural networks in two variants: a three-layer neural network (NN) and a deep neural network (DNN). Based on conducted results the nitrate vulnerability map was developed (Perović et l., 2021). The machine learning models were successfully applied for predicting the ammonium concentration with high determination coefficients (R^2) in tests: 0.84 for DNN and 0.64 for NN, while the SVR did not prove to be adequate with the best R^2 of 0.24 (Perović et al., 2021).

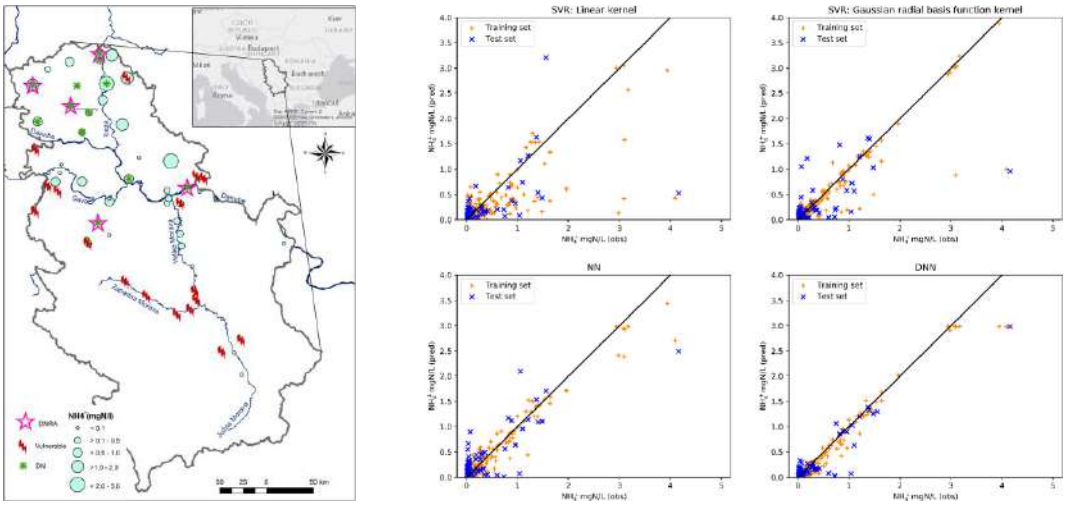

Figure 4. Mapped groundwater potential for nitrogen removal/conservation and Original measured values (NH_4^+ mgN/L (obs)) vs predicted values (NH_4^+ mgN/L (pred)) for ammonium concentration in groundwater compared with the identity line a) SVR model with linear kernel, b) SVR model with Gaussian radial basis function kernel, c) NN model with only one hidden layer, d) DNN model with four hidden layers (Perović et al., 2021).

CONCLUSION

Nitrogen is a unique element due to its abundance and ubiquity, as well as the multitude of oxidation states in which it can be found (-3 to +5) (Perović et al., 2017; Rivett et al., 2008). It provides a compelling example of the challenges in identifying the source of a pollutant that undergoes changes in forms, oxidation states, leading to changes in isotopic signatures, either partially or entirely through microbial metabolism. Methods to identify nitrate sources include isotopic analysis and comprehensive physicochemical, hydrogeological, and microbiological analyses. During the forensic analysis of nitrogen origin in groundwater, it is necessary to - determine groundwater flow and recharge; conduct a physicochemical analysis of groundwater from carefully selected sampling sites; perform microbiological tests to analyse the presence and abundance of bacteria involved in nitrogen transformation; and to extract conclusions with high reliability using appropriate data processing tools. The presented case studies demonstrate the theoretical and practical feasibility of detailed nitrogen source determination and transport and transformation mechanism. First case study showed the complementary analysis for nitrate origin determination in oxic alluvial groundwater. Statistical data analysis revealed significant correlation between anthropogenic impact parameters in the hinterland, where microbiological and isotopic data supplemented drawn conclusions. Second case study revealed that ammonium in examined anoxic groundwater originated from dissimilatory nitrate reduction, conducted by *Desufovibrio*. High determination coefficients of deep neural network and three layer neural network, calculated within third case study, showed that machine learning models could be applied with acceptable accuracy for predicting ammonium concentration in shallow alluvial aquifers.

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