Correlation Analysis Among Selected Parameters of N Transformation in Kovin-Dubovac

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Abstract

Knowledge of the complex cycle of nitrogen transformation in soil and groundwater is of great importance, especially in aquifers that can potentially be used as sources of drinking water. Predicting the fate and therefore the final form of produced nitrogen compounds in specific groundwater environments provides an assessment of the impact of certain nitrogen sources on groundwater quality. The obtained results emphasize the importance of knowing concentration trends and relations of influential compounds in predicting the fate of applied fertilizers in anoxic groundwater in agricultural areas. In this paper we have identified the mutual interdependence and correlation strength between selected factors which are considered as dominant in the transformation of N compounds in anoxic groundwater. Applied Principal Component Analysis (PCA) and Cluster Analysis (CA) for 9 physico-chemical and microbiological parameters in 14 facilities (wells and connected piezometers) for anoxic alluvial groundwater from Kovin-Dubovac revealed 4 main factors, which explain 84,18% of overall variance. Statistical analysis demonstrated the existence of a strong correlation between redox potential (Eh), sulfide concentration (H_2S) and the number of iron related bacteria (IRB); between ammonium concentration ($NH4^+$), sulfate reducing (SRB) and denitrificating bacteria (DN); and between ferrous iron (Fe^{2+}) and total organic carbon (TOC) concentration.

Keywords: ammonium ion, anoxic groundwater, nitrogen, correlation.

Introduction

As a byproduct of poor agricultural practices (application of inorganic and organic fertilizers/manures), anthropogenic activity (septic tanks, sewage effluents) industry, fuel combustion, nitrates present one of the most widespread groundwater contaminants worldwide (Miljević et al. 2012). Excessive use of mineral fertilizers and pesticides poses a direct threat to existing ecosystems due to agricultural activities. The mobility of nitrates in groundwater is well studied and documented and is often defined as a tracer movement, due to its stability in the oxic environment, while the mechanism of transformation and transport of nitrates in anoxic aquatic environments is poorly documented (Perović et al., 2017). N compounds that have reached groundwater, participate in dynamic geochemical and various microbiologically mediated transformation nitrate reduction to ammonium (DNRA), nitrification, anammox (anaerobic ammonium oxidation), nitrifier denitrification, sorption and mineralization of organic matter (Nikolenko et al., 2018). Redox conditions, dissolved oxygen concentration and the availability of electron acceptors in groundwater environments predominantly determine the solubility, degradability and transformation pathway of various organic compound pollutants. The dissolved oxygen concentration <2.00 mg / l and Eh value <250 mV, and available organic matter are considered as necessary conditions for the denitrification process (Korom, 1992; Thayalakumaran et al., 2007). After oxygen is depleted different ionic and molecular species can be used in further microbiologically mediated transformations of organic matter and according to the decreasing values of the amount of energy released, suitable electron acceptors are: nitrates (NO_3^-), manganese (Mn^{4+}-

When prevailing environmental conditions are considered as suitable for nitrate reduction, there are three main nitrate removal processes: respiratory denitrification (Eqs. 1, 2a, 2b, 3), Anammox (eq 4) and Dissimilatory nitrate reduction (DNR), usually associated with ammonium production (DNRA eq 5) (Perović et al., 2017). Example stoichiometry for the listed reactions is presented in the following equations:

Heterotrophic denitrification eq. 1:

$$5\ CH_2O + 4\ NO_3^- \rightarrow 2\ N_2 + 4\ HCO_3^- + CO_2 + 3\ H_2O \quad \text{(Jorgensen et al., 2004)} \qquad (1)$$

Autotrophic denitrification using reduced iron, eq. 2a and 2b (Ottley et al., 1997):

$$10\ Fe^{2+} + 2\ NO_3^- + 14\ H_2O \rightarrow 10\ FeOOH + N_2 + 18\ H^+ \qquad (2a)$$

$$15\ Fe^{2+} + NO_3^- + 13\ H_2O \rightarrow 5\ Fe_3O_4 + N_2 + 28\ H^+ \qquad (2b)$$

Autotrophic denitrification using reduced sulfur (Korom, 1992):

$$5\ FeS_2 + 14\ NO_3^- + 4\ H^+ \rightarrow 7\ N_2 + 10\ SO_4^{2-} + 5\ Fe^{2+} + 2\ H_2O \qquad (3)$$

Anammox, as N loss pathway through reaction of ammonium and nitrite ion is presented through eq. 4 (Burgin and Hamilton, 2007):

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \qquad (4)$$

N conservation process in the form of ammonium ion occurs according to eq. 5 by a process known as Dissimilatory Nitrate Reduction to Ammonium (Rivett et al., 2008):

$$2\ H^+ + NO_3^- + 2\ CH_2O \rightarrow NH_4^+ + 2\ CO_2 + H_2O \qquad (5)$$

It is believed that nitrate nitrogen concentrations in groundwater exceeding 1 or 3 mgNO_3-N / l (Dimkić et al., 2008; Kaown et al., 2009) and ammonium ions above 0.2 mgN / l (WHO 2003) are an indicator of anthropogenic impact. NH_4^+ in groundwater can be of natural origin and bound to an aquifer matrix (different lithological and geological origins) and *allochthonous*, originating from anaerobic degradation of organic matter and as a result of contamination due to the disposal of organic waste (manure, sewage water, septic tanks). Extremely elevated concentrations of NH_4^+ 14-140 mg / L were observed in aquifers contaminated by landfill leachate waters or by discharge of wastewater (Böhlke et al., 2006). Agricultural practices and the existence of septic tanks may also result in elevated concentrations of NH_4^+ (Böhlke et al., 2006). Naturally elevated NH_4^+ concentrations (up to 3 mg / l) were observed in strata rich in humic materials, iron or in aquifers under forested land (Lindebaum, 2012). How much nitrogen will be available from leaching depends on: agricultural practices, the form and manner of the applied fertilizer formulations, the time of application, weather, the type of soil, the type of culture that is being fertilized. The area's characteristics, such as climate, geology, morphology and hydrology, have a significant impact on the fate of nitrogenous compounds in groundwater. Climatic factors influence the fate of nitrogenous compounds, with elevated temperatures accelerating denitrification and volatilization, elevated temperature and humidity mineralization,and increased precipitation accelerating transport of chemicals through an unsaturated zone to groundwater.

Contemporary research in this area is focused on defining the correlation between hydrogeological, physico-chemical and microbiological parameters of soil and water in order to define the dominant influence on the final transformation process of nitrogen compounds. According to the literature data, nitrogen transformation would mainly depend on oxygen concentration and redox potential, pH value, quantity and quality of available organic carbon, C: N ratio (DOC: NO_3) and concentrations and availability of other electron donors (mostly ferro-iron and sulfides) (Burgin and Hamilton 2007, Dimkić et al 2008, Rivett et al., 2008, Nikolenko 2018). The occurrence of elevated concentrations of NH_4^+ and NO_{3-} in water may indicate the potential presence of pathogens, if the quality deterioration is caused by manure applications or the inflow of unregulated sewage systems, and pesticides.

Site Area

The Kovin-Dubovac area occupies the southern part of the Banat depression and includes the alluvial plain of the Danube between the villages of Kovin and Dubovac (Dimkić and Pušić, 2014). Younger Quaternary-Upper Pleistocene and Holocene gravels and sandy gravels, with some cobbles even over 120 mm in diameter compose the deeper layers of the alluvial plain (Dimkić and Pušić, 2014). At the base, over the Pliocene strata are medium-grained sands, finely-grained silty sands, coal, alevrites and clay layers. The upper layer is composed of semi-permeable sediments: silty sands, alevrites, silty clays and clay. The north and northeast part of the alluvion are characterized with expressed heterogeneity and highly inconsistent

under the Kovin-Dubovac plain range from 1.5 to 35 m, with an average value of some 20 m (Dimkić and Pušić, 2014). The average width of the alluvial plain is 8 km. This area is characterized as an intensive agricultural area with shallow groundwater (2-4 m below the terrain surface), and elevated ammonium concentrations. The redox pair ratio as well as the redox potential indicates that this area can be categorized as a mixed post-oxy-anoxic environment, with elevated concentrations of organic matter, ammonium ions and ferrous-iron, with low oxygen concentrations and with an expressed potential for microbiologically mediated reduction of iron and manganese, nitrate and sulfate. The results of the conducted microbiological BART tests categorized this aquifer as reductive-anoxic, with a dominant anaerobic degradation of organic matter. Fermentation processes were noted as prevailing, and iron oxidation, denitrification and sulfate reduction varied in intensity, time and space.

Material and Methods

The applied statistical method, PCA analysis, explains the connection of interrelated variables, combining them into smaller groups of independent variables. PCA analysis is a technique often used to reduce a large number of data without losing information of importance. In the paper PCA and cluster analysis were applied in order to define the relations and extract significant factors, among the investigated physico-chemical and microbiological parameters: standard redox potential (Eh), NH_4^+, SO_4^{2-}, H_2S, Fe^{2+}, TOC concentration and the results of SRB, DN and IRB BART tests. For quantification of ammonium and ferrous iron, the spectrophotometric method was used, whereas a volumetric method was used for sulfide. Sulfate was analyzed using the turbidimetric method by WTW turbidimeter TURB 430IR (Germany). TOC (total organic carbon) was quantified using Hiper TOC analyzer Thermo Scientific (United Kingdom). Applied analytical methods are described in detail in the APHA, 2005. Measurements in the well and in piezometers were performed by lowering the probe to the screen level. The performed BART tests (Hach Company, United States) have enabled detection of the present environmental groups of bacteria through average values of duplicated tests and they have been interpreted according to the relevant literature (Cullimore R., 2010). The cluster analysis grouped the examined objects according to the similarity in the concentrations of the observed parameters.

Results and Discussion

Statistical methods can be considered a powerful tool for identifying the influence of each factor on the physico-chemical composition of groundwater and therefore many researchers have adopted cluster analysis (CA) and principal component analysis (PCA) as part of a multivariate statistical analysis for the assessment of groundwater quality (Kim et al., 2005). In general, CA indicates groupings of samples by linking intersample similarities and illustrates the overall similarity of variables in the data set, while PCA is used for assessing the associations between variables because it can indicate the participation of individual chemicals among several factors of influence (Kim et al., 2005).

The research results are grouped with the following objectives:

1. The selection and classification of N transformation parameters into groups using PCA analysis
2. The identification and description of the main factors influencing N transformation
3. The investigated objects are grouped into clusters according to the similarity of the observed concentrations

We applied PCA and CA analysis for the mentioned selected parameters (Eh, NH_4^+, SO_4^{2-}, H_2S, Fe^{2+}, TOC, SRB, DN and IRB BART tests). The parameters of interest were selected based on the: availability of data, listed chemical stoichiometry reactions and based on the reviewed literature considering N transformation conditions (Korom 1992; Burgin and Hamilton 2007; Rivet et al., 2008; Nikolenko et al., 2018).

Tabe 1: The correlation matrix between the selected parameters.

	Eh	NH_4	SO_4	H_2S	Fe^{2+}	TOC	IRB	SRB	DN
Eh	1,000	-0,264	0,183	-0,684	-0,310	-0,265	0,567	-0,136	0,215
NH_4	-0,264	1,000	0,360	0,089	0,319	0,601	0,015	0,310	-0,555
SO_4	0,183	0,360	1,000	-0,223	-0,112	0,324	0,038	-0,158	0,053
H_2S	-0,684	0,089	-0,223	1,000	-0,226	-0,272	-0,607	0,419	-0,388
Fe^{2+}	-0,310	0,319	-0,112	-0,226	1,000	0,707	-0,106	-0,132	0,082
TOC	-0,265	0,601	0,324	-0,272	0,707	1,000	-0,176	0,141	-0,178
IRB	0,567	0,015	0,038	-0,607	-0,106	-0,176	1,000	-0,156	0,182
SRB	-0,136	0,310	-0,158	0,419	-0,132	0,141	-0,156	1,000	-0,432
DN	0,215	-0,555	0,053	-0,388	-0,082	-0,178	0,182	-0,432	1,000

Table 1 shows the correlation matrix of the examined parameters in the groundwater of the selected wells and piezometers. In order to quantify the degree of correlation between physico-chemical and microbiological parameters, MSA values (measuring of sampling adequacy) greater than 0.5 (> 0.7 very high,> 0.6 high and> 0.5 mean correlation) are considered significant. Based on the inherent values, greater than 1, four main factors that represent hydrogeochemical processes of importance for the N transformation in groundwater are extracted.

The applied PCA analysis showed four main factors, which reveal 84.18% of the total variance (Table 2). Very high correlation (> 0.7), 31.93% was observed between: Eh and IRB, which are in inverse correlation with the H_2S concentration. This factor shows that a redox increase is followed by an increase in the number and activity of iron related bacteria, where no significant sulfide concentration in groundwater is expected. Fe^{2+} oxidation has been shown to be associated with denitrification or the DNRA process (Oshiki et al., 2013), while free sulfide is shown to inhibit denitrification and promote DNRA (Burgin and Hamilton, 2007). The second factor shows a significant relationship, a very high correlation (24.75%) between TOC and Fe^{2+} concentration. This relationship may indicate the common origin of bivalent iron and total organic carbon. It also indicates that conditions of elevated concentrations and availability of electron donor, organic carbon, may induce feri iron reduction. The third factor shows the relationship (15.86%) between the NH_4^+ concentration and the results of SRB BART tests, which are in inverted relation with DN BART test results. This factor indicates the potential mechanism of NH_4^+ production in the examined aquifer by DNRA process mediated by sulphate-reducing bacteria, which has already been observed and ascertained in previous research for the well from the first drainage line in Kovin Dubovac (Perović et al., 2017). The inverse relationship between sulfate reducing and denitrificating bacteria can be explained by competition for available electron acceptors and the inhibitory effects of low concentrations of the produced sulfides on respiratory denitrification. The fourth factor shows that the concentration of SO_4^{2-} is separated and that it is not in correlation with the selected examined physico-chemical and microbiological parameters. The concentration of sulfate is generally around the detection limit (2.5 mg / l) with the exception of 2 objects (Bp-16, B-64). The absence of SO_4^{2-} can be explained by organic matter oxidation by sulfate reducing bacteria and simultaneous use of sulfates and ferri iron as electron acceptors, with the precipitation of formed iron sulfide.

Tabe 2: Principal component loadings of these variables with the variances.

	Component					Rotation Sum of Squared Loadings		
	1	2	3	4		Total	% of Variance	Cumulative %
Eh	**.840**	-.300	-.131	.116	1	2.874	24.294	24.294
NH_4	-.022	.487	**.645**	.442	2	2.111	23.451	47.745
SO_4	.087	.031	-.067	**.976**	3	1.942	21.583	69.328
H_2S	**-.812**	-.333	.391	-.135	4	1.336	14.849	84.177
Fe	-.069	**.942**	-.072	-.179	5			
TOC	-.054	**.872**	.167	.325	6			
IRB	**.878**	-.054	.003	-.056	7			
SRB	-.111	-.102	**.800**	-.126	8			
DN	.149	-.109	**-.824**	-.012	9			

In Figure 1, the applied cluster analysis grouped the examined objects according to the concentration of NH_4^+, the results of SRB BART tests and DN tests. The objects are grouped into two main subclasses. The first sub cluster consists of the two groups. The first grouped objects are: B-19 / P-1, Bp-9, Bp-20, Bp-19, Bp-17, in which the lowest values of the concentrations of NH_4^+ ions were recorded and the results of SRB BART tests (NH_4^+ = 0.02 - 0.29 mgN / IL 0.9 - 2.3 log pac / ml). In the second group there are objects: Bp-12, Bp-2, Bp-25, Bp-24, Bp-16 / P-1, where observed concentrations are higher and are in the range of NH_4^+ = 0.62 - 1 , 7 mg N/L and 0.9-3.08 log p.a.c/ml. In the second subcluster objects: B-19/P-2, Bp-16, Bp-13 i Bp-5 whose NH_4^+ concentrations and SRB BART test results are the highest are gathered: NH_4^+ = 1,29-1,7 mgN/l i SRB BART 2,69-4,67 log p.a.c/ml. In Fig. 2 the NH_4^+ concentration gradient for the selected objects is shown.

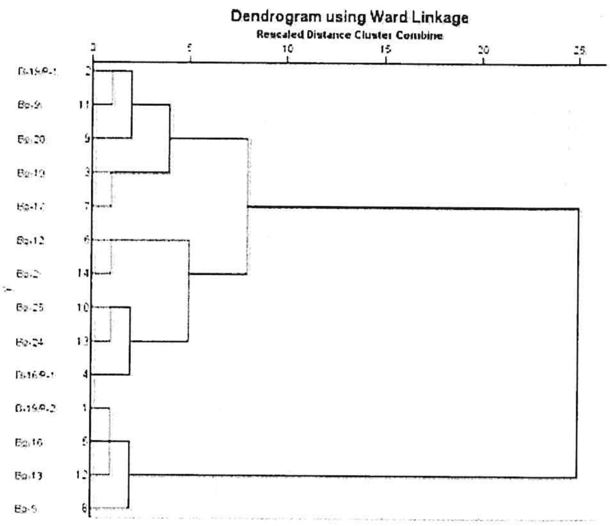

Figure 1: Dendrogram for factor 3.

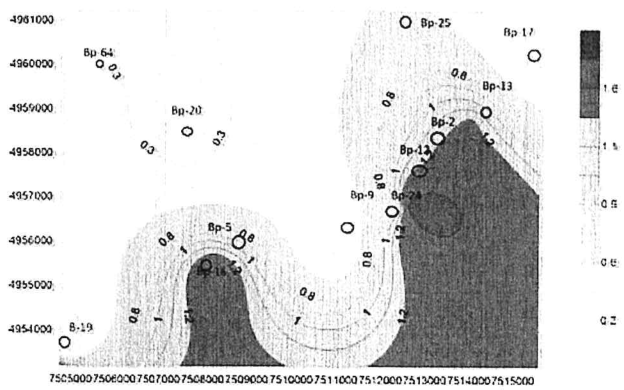

Figure 2: NH_4^+ concentration change for examined objects.

Conclusion

Application of PCA of 9 physico-chemical and microbiological parameters, which are considered significant for nitrogen transformation, on 14 selected wells and piezometers on Kovin-Dubovac, 4 main factors are revealed. Based on Initial Eigen Values the four main factors explained 84,18% of total variance. Factors showed the significant correlation between: Eh, H2S and IRB BART test results, Fe^{2+} and TOC concentration, NH_4^+ , SRB and DN BART, and the last factor singled out SO_4^{2-} concentration. Revealed correlations indicate that in an environment with expressed elevated Fe^{2+} and TOC concentrations, with no significant O_2, NO_{3-}, SO_4^{2-} concentrations, dissimilatory nitrate reduction to ammonium-ion (DNRA) is a favored process and probably mediated by:

1) the activity of sulfate-reducing bacteria that can use both nitrates and ferri ions as electron acceptors in their metabolism, depending on their bioavailability and prevailing fluctuating redox conditions and
2) nitrate-dependent oxidation of iron sulfide (Rivett et al., 2008; Oshiki et al., 2013).

The value of conducted research reflects a comprehensive, complex and simultaneous observation of the physico-chemical and microbiological parameters of an anoxic groundwater environment. The results of PCA analysis indicate a pronounced relationship between nitrogen compound transformations with sulfur and iron cycles in an anoxic groundwater environment, like Kovin-Dubovac. Further research would involve a more detailed analysis of the nitrogen transformation beneath agricultural areas, based on the determined flow directions and isotopic composition of N in formed ammonium ion, in order to confirm the origin of elevated NH_4^+ concentrations. The obtained results and the conclusions from scientific research could be used for the planning of future sustainable groundwater sources.

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