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Groundwater quality assessment of protected aquatic eco-systems in cross-border areas of Serbia and Croatia

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Abstract: Research results define basis for specific monitoring programs of groundwater quality in wetland eco-systems in Serbia and Croatia. The main purpose of the research was to determine the impact of nonpoint diffuse source pollution on the groundwater quality, as well as seasonal variations on the concentration levels of selected physicochemical parameters. Statistical analyses, PCA, HCA, ANOVA and *t*-test, encompass 18 monitored parameters in groundwater. Statistical data indicated that protected area in Serbia has a significantly higher load of pollution from agricultural activities compared to Wetlands Tompojevci. The highest load in groundwater was detected from total nitrogen, ammonia and nitrogen anions, indicating contamination of groundwater by nitrogen-based fertilizers. The results obtained within the two-year seasonal monitoring program, from 2018 to 2020, are highly essential for achieving a comprehensive database that could be used as platform for high-quality groundwater management in selected protected areas with the aim of minimizing environmental pollution.

Keywords: sensitive water bodies; physicochemical parameters; monitoring; agricultural activities.

INTRODUCTION

Environmental impact assessment (EIA) of protected areas is an imperative, particularly in the terms of groundwater quality, conservation of biodiversity, environmental sustainability and finally climate changes. Long-term anthropogenic pollution, as well as the changes within groundwater quality could be improved with data obtained by long-term monitoring programmes. The information

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on the groundwater quality in protected areas is sporadic and scarce. The lack of comprehensive and reliable data and databases on the current state of the groundwater quality in protected areas require development of specific monitoring programmes for minimization of groundwater and surrounding areas contamination.

Lakes and wetland areas are sensitive and vulnerable ecosystems where all human activities promptly affect groundwater contamination and quality.[1,2] Anthropogenic activities, such as agriculture, industry, urbanization and waste disposal, disturb and impair the natural aquatic ecosystems.[3] Groundwater contamination is attributed to nonpoint sources of contamination (excessive use of pesticides, mineral and natural fertilizers), adaptation of old wells into permeable septic tanks, unsanitary landfills, uncontrolled waste disposal and discharge of untreated wastewater.[4–6]

Temporal and spatial distribution of physicochemical pollutants, mainly nitrogen compounds is essential for evaluation of groundwater quality.[7–9] The uncontrolled urbanization in the vicinity of protected areas affect groundwater quality causing the contamination with nutrients from agriculture, waste disposal and discharge of untreated mixed urban wastewater.

Within the Interreg IPA CBC Croatia–Serbia Project Active sensor monitoring network and environmental evaluation for protection and wiSe use of wetlands and other surface waters – SeNs Wetlands, the groundwater quality in two cross-border protected areas, Lake Zobnatica, Serbia and Wetlands Tompojevci, Croatia, was investigated.[10] The two selected sites are within hydrological systems of watercourses recognised as ecological corridors. The Wetlands Tompojevci in Croatia has a direct influence on the quality of the Natura 2000 site. The Natura 2000 is the largest coordinated network of protected areas in the world that has the aim to ensure the long-term survival of Europe's most valuable and threatened species and habitats.[11] The protected area of the national Nature Park of Lake Zobnatica, Serbia, is environmentally interconnected with the Tompojevci Wetlands, indirectly linked to the Natura 2000 Network. Recognized protected ecosystems have similar and comparative characteristic – urban settlements around the water body, agricultural land in the close proximity of water bodies with the same types of crops (wheat, corn and sunflower), connection to the Danube River and Sava River Basin, and unevolved management and protection of sensitive aquatic systems.[12] Throughout the two years monitoring period (2018–2020), physicochemical parameters that affect the quality of the groundwater in protected areas were monitored – pH, electrical conductivity (σ), dissolved oxygen (DO), chemical oxygen demand (COD), orthophosphate, nitrite, nitrate, ammonia, total nitrogen (N_{tot}), sulfate, chloride, fluoride, total chlorine (Cl_{tot}), chromium (VI), nickel, iron, zinc and copper ions.

The aim of this research paper is to identify the sources of groundwater contamination in cross-border protected areas.

To reduce the volume of collected data the multivariate statistical analysis was applied, extracting the data relevant for EIA. Seasonal variations of the parameter concentration levels in groundwater and long-term groundwater pollution were also assessed. The results of this study can provide successful design and application of protective measures on the specific environmental problems and weak points in protected areas and sensitive water bodies.

The operative groundwater protective measures considered in selected protected areas are development and maintenance of multifunctional vegetation belt (vegetation that have a potential of sorption of pollutants); optimization of chemicals used for fertilization and pest control (fertilizers, pesticides, herbicides, *etc.*); and recommendations for water control and management in the vicinity of the sensitive aquatic systems.

This type of research was initiated and conducted for the first time on sensitive cross-border protected areas in Western Balkan Region, as one of planed objectives within the Interreg IPA Project SeNs Wetlands.

EXPERIMENTAL

Details related to the sampling sites are given in the Supplementary material to this paper.

Samples were collected with mechanical bailer than transferred into 1 L bottles. After proper collection, the samples were stored in refrigerator at 4 °C, and transported to the laboratory for analyses. The sampling procedure was conducted according to the Standards SRPS EN ISO 5667-1:2008 (Guidelines for development of sampling programs), SRPS EN ISO 5667-3:2007 (Guidelines for protection and handling of the sample), SRPS EN ISO 5667-11:2005 (Guidance on sampling of groundwater).

Measurement of pH, dissolved oxygen and electrical conductivity was performed *in-situ via* a multiparameter device using standard EPA methods (EPA 150.1, EPA 360.1, EPA 120.1).

Orthophosphates were prepared and analysed by standard EPA method (EPA 365.3), while chemical oxygen demand, nitrite, nitrate, sulphate, chloride, fluoride, ammonium ions, total nitrogen and total chlorine were measured according HACH methods (LCI 500, HACH 8507, HACH 8192, HACH 8155, HACH 10208, HACH 8051, HACH 8113, HACH 8029 and HACH 8167, respectively). Metal ions chromium (VI), nickel, iron, zinc and copper were also analysed according to HACH methods (HACH 8023, Method 8150, Method 8146, Method 8009 and Method 8143, respectively). The concentrations of selected parameters were measured in the laboratory by UV–Vis spectrophotometer (DR 5000, HACH, Germany). The measurements were performed on maximum wavelengths suggested in EPA and HACH methods based on Standard Methods for the Examination of Water and Wastewater.[13] The recoveries were carried out by the addition of the standards of each element at different levels. Recoveries ranged from 89 to 97 %. Blanks were included in each batch of analysis. Calibration curves for the determination of selected parameters were processed with different dilutions of the standard stock solutions. The linear regression lines were acquired with correlation coefficient value of more than 0.9.

The IBM Statistical Package of Social Science (SPSS) software package version 25 was used for statistical data analyses. Descriptive statistic, principal component analyses (PCA), hierarchical cluster analyses (HCA), one-way ANOVA and independent sample *t*-test were performed for the purpose of data evaluation. A statistical analysis was applied for determining the basic aggregated functions (mean, minimum and maximum) of the observed physicochemical parameters. PCA was used to extract the relevant data and determine the most polluted locations of protected areas, Lake Zobnatica and Wetlands Tompojevci. One-way ANOVA was applied to detect the effect of seasonal variations on the concentration levels of physicochemical parameters in analysed samples of groundwater. Independent sample *t*-test was used to compare the cumulative groundwater pollution between the two observed cross-border protected areas.

RESULTS AND DISCUSSION

Within two-year monitoring programme (2018–2020), eighteen physicochemical parameters in the vicinity of Lake Zobnatica and Wetlands Tompojevci were investigated in groundwater. Statistical analysis was used to determine mean and the range for all physicochemical parameters (Table I).

TABLE I.Statistical results for analysed parameters in groundwater; COD: chemical oxygen demand; DO: dissolved oxygen; N_{tot}: total nitrogen; Cl_{tot}: total chloride; TV: threshold value from Croatian legislation; *SD*: standard deviation

Parameter	Zobnatica Lake			Wetlands Tompojevci			TV
	Mean ± *SD*	Min.	Max.	Mean ± *SD*	Min.	Max.	
pH	7.77±0.461	6.95	9.14	7.82±0.95	6.961	12.8	–
σ / µS cm^{-1}	1016±319.45	477	1728	858.71±253.3	248	1442	2500
c_{O_2} / mg L^{-1} (DO)	5.01±1.287	0.8	7.53	4.48±1.789	0.1	9	–
$c_{PO_4^{3-}}$ / mg L^{-1}	0.469±0.788	0.005	4.075	0.942±1.587	0.005	9.741	0.2
$c_{NO_2^-}$ / mg L^{-1}	0.076±0.149	0.001	0.628	0.028±0.052	0.001	0.291	0.5
$c_{NO_3^-}$ / mg L^{-1}	0.965±0.971	0.01	4.52	0.34±0.632	0.01	2.73	50
$c_{NH_4^+}$ / mg L^{-1}	0.119±0.285	0.005	1.51	0.837±1.152	0.005	3.88	0.5
$c_{SO_4^{2-}}$ / mg L^{-1}	46.17±28.4	1	85	24.13±27.57	1	98	250
c_{Cl^-} / mg L^{-1}	43.22±42.15	0.05	232.8	14.06±10.93	0.6	45.4	250
c_{F^-} / mg L^{-1}	0.25±0.367	0.01	2.56	0.58±0.872	0.01	4.32	–
$c_{Cl_{tot}}$ / mg L^{-1}	0.033±0.039	0.01	0.16	0.116±0.152	0.01	0.45	–
$c_{Cr^{6+}}$ / mg L^{-1}	0.0131±0.2	0.005	0.11	0.066±0.148	0.04	0.743	–
$c_{N_{tot}}$ / mg L^{-1}	12.06±14.577	0.5	76.23	7.08±10.3	0.5	46.99	–
c_{Ni} / mg L^{-1}	0.0054±0.014	0.003	0.112	0.009±0.013	0.003	0.062	–
c_{Fe} / mg L^{-1}	0.113±0,14	0.01	0.88	0.486±0.65	0.01	2.92	–
c_{Zn} / mg L^{-1}	0.455±0.355	0.005	1.53	0.466±0.31	0.01	1.31	–
c_{Cu} / mg L^{-1}	4.476±7.16	0.5	35	8.15±11.96	0.5	50	–
c_{O_2} / mg L^{-1} (COD)	17.843±29.859	0	155	30.61±35.15	0.137	170	–

According to the Groundwater Quality Standards (GWQS) of the respective national legislation (Official Gazette no. 50/2012 for Serbia and Official Gazette no. 96/19 for Croatia) and EU Groundwater Directive (GWD) 2006/118/EC, nitrates are set as definitive parameter for monitoring of groundwater pollution with threshold value of 50 mg L^{-1}. In Serbian legislation, nitrates are also determined as key parameter; however, the threshold value of 50 mg L^{-1} is determined as annual average concentration.

Furthermore, the other significant parameter is stated as active component of pesticides, their metabolites and products of degradation and chemical reaction, with threshold value of 0.1 μg L^{-1}, with total concentration of all detected and quantified pesticides no higher than 0.5 μg L^{-1}.

The EU GWD is stating that EU member states have to develop threshold values for arsenic, cadmium, lead, mercury, ammonia, chloride, sulphate, trichloroethylene, tetrachloroethylene and electrical conductivity, and if necessary to expend the list. The defined threshold values from Croatian By-Law are stated in the Table I. In Serbian By-Law, other pollutant are only listed (Lists I and II) without defined threshold values: organic compounds of halogens, phosphorus; oils and hydrocarbons; metals, metalloids and their compounds; cyanides; biocides and derivate; ammonia, fluorides, inorganic compounds of phosphorus and other.

Comparing the results in Table I and requirements of respective national legislation (Official Gazette of the RS 50/2012 for Serbia and Official Gazette no. 96/19 for Croatia), it was concluded that following parameters can be excluded from further analyses: pH, total chlorine and fluoride (as parameters that are not listed for assessment of groundwater quality status in legislation), cations of nickel, chromium and copper (the low concentrations were found to be insignificant for this research).

Correlation analysis was used to classify the strength of relationships between parameters (Table II).

TABLE II. Correlation coefficient values for the observed parameters; N_{tot} – total nitrogen; A – Serbia, Zobnatica lake; B – Croatia, Wetlands Tompojevci

Parameter		B											
		σ	c_{O2}[a]	c_{PO43-}	c_{NO2}	c_{NO3}	c_{NH4+}	c_{SO42}	c_{Cl-}	c_{Ntot}	c_{Fe}	c_{Zn}	c_{O2}[b]
		μS cm^{-1}	mg L^{-1}										
A	σ	1	–0.23	0.26	–0.47	–0.58[a]	0.11	–0.24	0.21	–0.13	0.39	0.18	–0.07
	c_{O2}[a]	–0.24	1	–0.16	0.24	0.07	–0.16	0.16	–0.14	–0.06	–0.28	–0.28	–0.33
	c_{PO43-}	–0.09	–0.16	1	–0.31	–0.09	0.13	–0.1	0.02	0.11	0.07	0.24	0.14
	c_{NO2-}	0.39	–0.25	0.01	1	0.79[a]	–0.09	0.48	–0.08	0.11	–0.17	–0.22	–0.14
	c_{NO3-}	0.08	0.29	–0.23	–0.01	1	–0.17	0.41	–0.24	0.11	–0.19	–0.13	–0.13
	c_{NH4+}	–0.1	–0.35	0.20	0.03	–0.15	1	–0.24	0.17	0.77[a]	0.54[a]	0.34	0.42
	c_{SO42-}	–0.01	0.34	–0.27	–0.14	0.59[a]	–0.26	1	–0.19	–0.22	–0.34	–0.17	–0.13
	c_{Cl-}	0.10	–0.01	–0.20	–0.16	0.30	0.10	0.27	1	–0.03	0.63[a]	0.13	0.37
	c_{Ntot}	–0.01	0.31	–0.20	–0.16	0.47	–0.16	0.35	0.20	1	0.27	0.19	0.32
	c_{Fe}	0.11	–0.42	0.37	0.13	–0.27	0.51[a]	–0.37	–0.13	–0.18	1	0.27	0.26
	c_{Zn}	–0.01	–0.06	0.08	–0.11	0.23	0.12	0.29	0.13	0.15	0.21	1	0.2
	c_{O2}[b]	0.22	–0.18	0.05	0.64[a]	–0.28	–0.09	–0.15	–0.26	–0.15	0.17	–0.33	1

[a]DO – dissolved oxygen; [b]COD – chemical oxygen demand

Based on the correlation coefficient, it was concluded that there is a high positive correlation between nitrate and sulphate ions ($r = 0.59$), nitrite and chemical oxygen demand ($r = 0.64$), as well as ammonia and iron ions ($r = 0.51$) in groundwater in Zobnatica, Serbia. Samples from Croatia revealed a significant positive correlation between nitrite and nitrate ($r = 0.79$), total nitrogen and ammonia ion ($r = 0.77$), ammonia and iron ions ($r = 0.54$), iron and chloride ions ($r = 0.63$); and negative correlation between electrical conductivity and nitrate ($r = –0.58$).

Elevated concentration levels of nitrates in groundwater can be explained by the widespread use of nitrogen-based fertilizers in agriculture and atmospheric deposition, whereas sulphate groundwater pollution can be explained by atmospheric deposition, geothermal processes, and mineral dissolution and precipitation processes.[14] The product of nitrogen-based fertilizers decomposition is ammonia, enabling infiltration and migration of ammonia into the groundwater through the permeable soil. Iron naturally occurs in groundwater as a result of mineral dissolution processes in the soil.[15]

Principal component analyses (PCA) was applied to minimize the volume and abundance of data by extracting the key physicochemical parameters for monitoring and evaluation of groundwater quality. Kaiser's coefficient for overall measure of sample adequacy is 0.61 (Zobnatica Lake) and 0.60 (Wetlands Tompojevci) and Bartlett's test of sphericity ($p < 0.05$) indicate that the data are appropriate for the PCA. The scree diagram, as graphical tool, was used to determine the number of significant factors in PCA. To interpret the scree plot it is necessary to identify the scree point, the point at which the curve sharply changes shape and direction (Fig. S-2 of the Supplementary material). The number of significant factors contributing the most to total variance is the number of points which are equal to or above the scree point. The scree plots (Fig. S-2) derived from PCA indicate that two factor score (Zobnatica Lake) and three factor score (Wetlands Tompojevci) are suitable for examined data. Significant factors were adopted to clarify groundwater quality status. Following the selection of significant factors, varimax rotation was used to redistributing the influence of factors from the main factor (Table III).

Measure of sampling adequacy (MSA) of 0.5 and greater was used to quantify the correlation of physicochemical parameters and for factor analysis confirmation (Table IV).

Two significant factors for Zobnatica Lake explained 42.786 % of total variance, while three significant factors for Wetlands Tompojevci explained 57.731 % of the total variance pointing groundwater pollution.

In Factor 1, nitrate, sulphate, iron and zinc ions were isolated as significant parameters for Zobnatica Lake, while electrical conductivity, nitrite, nitrate and sulphate were isolated for Wetlands Tompojevci. Nitrate and sulphate as the key parameters for assessment of groundwater pollution, overlap in cross-border areas for Factor 1. The main source of nitrate in groundwater is treatment of wheat, corn, and sunflower with fertilizer such as potassium nitrate (KNO_3 – 13 % nitrogen), urea-carbamide, uric acid ($CO(NH_2)_2$ – 46 % nitrogen) and ammonium nitrate (NH_4NO_3 – 33–34 % nitrogen), while main source of groundwater contamination with sulphate are natural phenomena such as atmospheric deposition, geothermal processes and mineral dissolution and precipitation processes. Based on the obtained results Factor 1 is interpreted as “contamination of groundwater by nitrate-based fertilizers”.

TABLE III. Factor loading of variables obtained by principal component analysis (PCA); DO: dissolved oxygen; N_{tot}: total nitrogen; COD: chemical oxygen demand; λ: eigenvalue; $Var(X)$: variance; $Var(X)_c$: cumulative variance; PC – principal component

Parameter	Component				
	Zobnatica Lake		Wetlands Tompojevci		
	PC1	PC2	PC1	PC2	PC3
σ / µS cm^{-1}	0.095	0.121	–0.653[a]	–0.197	0.204
c_{O_2} / mg L^{-1} (DO)	0.355	–0.613[a]	0.079	–0.005	–0.294
$c_{PO_4^{3-}}$ / mg L^{-1}	–0.087	0.444	–0.043	0.045	–0.107
$c_{NO_2^-}$ / mg L^{-1}	–0.051	0.053	0.890[a]	0.017	0.001
$c_{NO_3^-}$ / mg L^{-1}	0.791[a]	–0.141	0.901[a]	0.026	–0.106
$c_{NH_4^+}$ / mg L^{-1}	–0.194	0.733[a]	–0.129	0.864[a]	0.250
$c_{SO_4^{2-}}$ / mg L^{-1}	0.745[a]	–0.271	0.630[a]	–0.338	–0.121
c_{Cl^-} / mg L^{-1}	0.233	0.137	–0.110	–0.046	0.916[a]
c_{Ntot} / mg L^{-1}	–0.131	0.784[a]	0.097	0.934[a]	–0.008
c_{Fe} / mg L^{-1}	0.610[a]	0.509[a]	–0.229	0.308	0.769[a]
c_{Zn} / mg L^{-1}	0.648[a]	–0.186	–0.155	0.261	0.084
c_{O_2} / mg L^{-1} (COD)	–0.254	–0.128	0.024	0.398	0.457
λ	2.950	2.184	2.676	2.271	1.980
$Var(X)$ / %	24.586	18.200	22.299	18.929	16.503
$Var(X)_c$ / %	24.586	42.786	22.299	41.228	57.731

[a]MSA at the level ≥ 0.5

TABLE IV. Comparison of seasonal variations for Zobantica Lake; DO: dissolved oxygen; N_{tot}: total nitrogen; SD: standard deviation

Parameter	Spring	Summer	Autumn	Winter	p-value
	Mean ± SD				
c_{O_2} / mg L^{-1} (DO)	5.84±0.58	4.65±1.53	4.17±1.03	5.28±1.12	<0.0005[a]
$c_{NO_3^-}$ / mg L^{-1}	0.92±1.05	0.704±0.62	1.18±0.89	1.4±1.49	0.25
$c_{NH_4^+}$ / mg L^{-1}	0.09±0.135	0.068±0.091	0.264±0.52	0.033±0.026	0.124
c_{Ntot} / mg L^{-1}	34.17±24.32	6.21±7.94	7.73±4.98	13.09±9.46	0.178
$c_{SO_4^{2-}}$ / mg L^{-1}	52.62±22.47	36.65±31.41	47.31±28.81	58.37±24.79	<0.0005[a]
c_{Fe} / mg L^{-1}	0.065±0.052	0.14±0.085	0.132±0.225	0.111±0.16	0.391
c_{Zn} / mg L^{-1}	0.603±0.288	0.296±0.376	0.544±0.387	0.435±0.147	0.035[a]

[a]Statistical significance at the level of 0.05

In Factor 2, dissolved oxygen, ammonium ion, total nitrogen and iron ion were isolated as significant parameters for Zobnatica Lake, while ammonium ion and total nitrogen were isolated for Wetlands Tompojevci. Ammonium ion and total nitrogen overlap in Factor 2 which could lead to the conclusion that the main potential source is treatment of surrounding agricultural soil with ammonium phosphate ($(NH_4)_3PO_4$ – 11 % nitrogen and 52 % phosphorus), ammonium nitrate and nitrogen–phosphorus–potassium (NPP, 15 % N, 15 % P and 15 % Na). Based on the obtained results Factor 2 could be interpreted as "contamination of groundwater by ammonia-based fertilizers".

Factor 3 for Wetlands Tompojevci isolated iron and chloride ions as significant parameters and could be interpreted as "infiltration processes and dissolution of minerals and rocks" since iron and chloride ions reach groundwater naturally by infiltration from soil.[15,16]

Based on the conducted PCA, it can be concluded that the main sources of groundwater pollution are agricultural activities (use of nitrogen-based fertilizers as the most dominant factor, F1), which corresponds to the fact that wheat, corn, and sunflower are grown on the studied area.

The most polluted locations were investigated using hierarchical cluster analysis (HCA) in respect to observed selected parameters in groundwater. From Fig. S-3 of the Supplementary material it can be concluded that the piezometers B3, B7, B1, B4 and B8 were in the separate cluster due to the lowest concentration level of physicochemical parameters (Table S-II of the Supplementary material). Locations B2 and B5 were isolated in the second cluster, as the most polluted sites and differ from other piezometers in groundwater quality, as a consequence of the high concentrations of nitrates, sulphates, chloride ions and total nitrogen for the Lake Zobnatica (Table S-II). Locations P2 and P8 in Wetlands Tompojevci (Fig. S-3) were grouped in a specific cluster and correspond to the most polluted sampling areas. Concentrations of nitrite, nitrate and total nitrogen are highly elevated on these locations compared to other locations (Table S-III of the Supplementary material). Locations P4, P5, P3, P7, P1, P6 and P8 are in second cluster which correspond to low groundwater pollution.

Seasonal variations on concentration levels for selected parameters extracted by PCA were assessed within research activities. One-way ANOVA test was used to examine differences in concentration levels of physicochemical parameters in relation to seasonal variations. Four groups of parameters were compared, arranged by seasons. Statistically significant differences in seasonal concentration levels were determined for dissolved oxygen, sulfate and zinc ions (Zobnatica Lake) and chloride ion (Wetlands Tompojevci), Tables IV and V.

Dissolved oxygen is an important parameter to consider when assessing quality of every water type and it is a highly sensitive factor in terms of seasonal variations and other prevailing conditions. The predominant part of groundwater recharge process is in the winter-spring period and higher concentrations of dissolved oxygen in groundwater are observed in winter, as the solubility of oxygen is lower in water with higher temperature. The lower concentration of dissolved oxygen in groundwater can also be the consequence of the intensified oxidation and reduction processes of organic and inorganic pollutants.[17,18]

TABLE V. Comparison of seasonal variations for Wetlands Tompojevci; N_{tot}: total nitrogen; *SD*: standard deviation

Parameter	Spring	Summer	Autumn	Winter	*p*-value
	Mean ± *SD*				
σ / µS cm^{-1}	900.56±260.18	912.42±226.6	798.14±306.18	820.46±220.42	0.541
$c_{NO_2^-}$ / mg L^{-1}	0.032±0.027	0.02±0.035	0.033±0.075	0.03±0.053	0.923
$c_{NO_3^-}$ / mg L^{-1}	0.432±0.812	0.425±0.628	0.208±0.49	0.242±0.49	0.72
$c_{NH_4^+}$ / mg L^{-1}	0.571±1.01	0.991±1.24	0.8±1.16	0.9±1.26	0.878
c_{Ntot} / mg L^{-1}	6.31±16.43	6.55±5.69	7.24±7.07	7.96±11.96	0.403
$c_{SO_4^{2-}}$ / mg L^{-1}	30.12±26.78	30.07±33.65	13.85±19.74	25.75±28.25	0.99
c_{Fe} / mg L^{-1}	0.121±0.067	0.464±0.73	0.72±0.81	0.483±0.34	0.229
c_{Cl^-} / mg L^{-1}	10.15±4.28	5.4±4.38	21.62±12.73	19.9±7.71	<0.0005[a]

[a]Statistical significance at the level of 0.05

The concentration of sulphate anion is the highest in spring and late autumn, when agricultural land is treated with fertilizers and fungicides. In autumn, the concentration of sulphate anion increases in comparison to the summer due to higher precipitation, which increases soil erosion. Chloride anions occur naturally in groundwater by dissolution of minerals and their concentration depends on atmospheric deposition.[16] Chloride anions concentration is directly correlated to the atmospheric precipitation, when the precipitation is lowest, in summer months, the concentration levels of chlorides is lower and inversely.

Independent sample *t*-test was used to examine difference in concentration of parameters between two compared groups, Zobnatica Lake and Wetlands Tompojevci. There is a statistically significant difference between the groundwater quality of two observed protected areas for the concentration levels of nitrite, nitrate, ammonia, sulphate and iron ions (Table VI).

TABLE VI. Comparison of physicochemical parameters in groundwater by locations; N_{tot}: total nitrogen; *SD*: standard deviation

Parameter	Zobnatica Lake	Wetlands Tompojevci	*p*-value
	Mean ± *SD*		
$c_{NO_2^-}$ / mg L^{-1}	0.076±0.149	0.028±0.052	0.021[a]
$c_{NO_3^-}$ / mg L^{-1}	0.965±0.971	0.34±0.632	<0.0005[a]
$c_{NH_4^+}$ / mg L^{-1}	0.119±0.28	0.837±1.152	<0.0005[a]
c_{Ntot} / mg L^{-1}	12.06±14.57	7.08±10.3	0.085
$c_{SO_4^{2-}}$ / mg L^{-1}	46.17±28.4	24.13±27.57	<0.0005[a]
c_{Fe} / mg L^{-1}	0.115±0.139	0.486±0.65	0.001[a]

[a]Statistical significance at the level of 0.05

Based on the statistical comparison, it can be concluded that Zobnatica Lake has a higher groundwater contamination load (Table VI). The concentrations of nitrite and nitrate in groundwater are higher in Zobnatica Lake, which confirm the oxic conditions compared to the anoxic environment in Wetlands Tompojevci (higher concentration of ammonia).

Agricultural activities as the main source of contamination indicate that nitrate-based fertilizers are predominantly used in Zobnatica Lake, while ammonia-based fertilizers are predominantly used in Wetlands Tompojevci. Mineral dissolution as the main source of groundwater pollution with sulphate confirms that Zobnatica Lake is more exposed to sulphate contamination due to mineral composition compared to Wetlands Tompojevci. Iron cation concentrations were evaluated in anoxic environment which corresponds to higher concentrations of iron in Wetlands Tompojevci compared to Zobnatica Lake.

Data obtained within the scope of the IPA Project SeNs Wetlands enabled the proposition of the coastal multifunctional vegetation belt which contributes to a greater reduction of pollutants that enter the water from the surrounding areas.

The multifunctional vegetation belt is buffer system and physical barrier for the sensitive water bodies.[19] In order to ensure that the amount of pollution that reaches the water is halved, a green buffer strip 16 m wide is needed, made of grass and shrubs, but species-rich plant communities. Multi-layered green buffer system 20 m wide retains up to 75 % of pollutants, also in experiments with phosphorus.[20] Woody vegetation has the best results in restricting the pollutants wind transmission and is considerably more effective than medium and low height vegetation.

CONCLUSION

The conducted study research indicates the agricultural activities as the main source of the groundwater pollution in the cross border protected areas in Serbia and Croatia. The groundwater quality status assessment of selected protected areas, 18 physicochemical parameters were examined from 2018 to 2020. Six parameters (pH, total chlorine, fluoride, nickel, chromium and copper ions) have been excluded from discussion due to the results of statistical evaluation. The most significant correlations have been observed between chemical oxygen demand, electrical conductivity, nitrite, nitrate, sulfate, ammonia, iron and chlorine ions on both sites, Lake Zobnatica, Serbia and Wetlands Tompojevci, Croatia. The PCA analyses indicate that a two-factor score is appropriate for Zobnatica Lake data and three factor score for data from Wetlands Tompojevci. Based on the statistical assessment, it could be concluded that protected area in Serbia is more affected by agricultural activities compared to Wetlands Tompojevci due to the higher groundwater contamination load. Locations with the observed highest level of pollution were B2 and B5 in Zobnatica, Serbia and P2 and P6 in Tompojevci, Croatia. The nitrogen compounds have been identified as the most dominant pollutants, since the nitrite, nitrate and total nitrogen were isolated in statistical evaluation. The results of this research have shown that the most significant pathways of pollution are agricultural activities and use of nitrogen and phosphorus-based fertilizers. The findings of this study have been used for development and maintenance of vegetation belt in protected areas. The results obtained within the two-year monitoring programme are highly essential on achieving a comprehensive database that could be used as platform for high-quality groundwater management in selected protected areas with the aim of minimizing environmental pollution.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/11194, or from the corresponding author on request.

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ИЗВОД

ПРОЦЕНА КВАЛИТЕТА ПОДЗЕМНИХ ВОДА ЗАШТИЋЕНИХ АКВАТИЧНИХ МОЧВАРНИХ ЕКОСИСТЕМА У ПРЕКОГРАНИЧНИМ ПОДРУЧЈИМА СРБИЈЕ И ХРВАТСКЕ

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Резултати истраживања дефинишу основу за специфичне програме праћења квалитета подземних вода у мочварним екосистемима у Србији и Хрватској. Основни циљ истраживања био је утврђивање утицаја загађења из дифузних извора на квалитет подземних вода, као и сезонских варијација концентрационих нивоа селектованих физичко-хемијских параметара. Статистичке анализе, PCA, HCA, ANOVA и *t*-тест, обухватају 18 параметара анализираних у подземним водама. Статистички подаци су показали да заштићено подручје у Србији има значајно веће оптерећење загађивачима из пољопривредних активности у поређењу са мочварним подручјем Томпојевци. Највеће оптерећење подземних вода забележано је од укупног азота, амонијака и азотних анјона, што указује на загађење подземних вода ђубривима на бази азота. Резултати добијени у оквиру двогодишњег мониторинг програма, од 2018. до 2020. године, од изузетног су значаја за формирање свеобухватне базе података која би се могла користити као

платформа за добро управљање подземним водама у изабраним заштићеним подручјима са циљем смањења загађења животне средине.

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