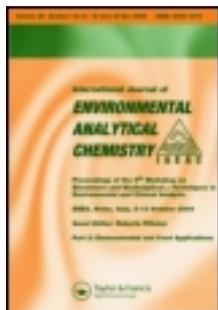


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Qualitative interpretation of physico-chemical and isotopic parameters in the Krka River (Croatia) assessed by multivariate statistical analysis

Neven Cukrov^{a*}, Nataša Tepić^b, Dario Omanović^a, Sonja Lojen^c,
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Multivariate statistical analyses were applied on the measured physico-chemical (Cd, Pb, Cu, Zn, Mg, Ca, O₂, alkalinity, temperature, pH, SAS, DOC and DIC) and isotopic parameters ($\delta^{13}\text{C}$ and $\delta^{18}\text{O}$) to estimate and distinguish anthropogenic from natural influences to the water system of the Krka River. Analyses were conducted on the data collected during six years from twelve sampling sites. On the basis of orientation, positioning and grouping of parameters arranged by biplots, four main hypotheses were defined and finally statistically confirmed. Thereof, two main and distinct processes occurring in the Krka River could be highlighted: (i) upstream pollution, caused by the inflow of untreated waste-waters of city of Knin and (ii) downstream self-purification, caused by the sedimentation and/or co-precipitation of pollutants coupled by the inflow of clean subterranean water (groundwater recharge). Grouping of (i) hydrological and carbon cycle connected parameters, and (ii) anthropogenically influenced correlated parameters were proposed as a result of statistical analysis. Regarding the pH, it is shown that a stream section influenced by the subterranean inflow of Zrmanja River is statistically significantly different for all sampling campaigns during six years, being lower for about 0.5 pH unit.

Keywords: Krka River; self-purification; trace metals; stable isotopes; organic matter; biplot; MANOVA

1. Introduction

Krka is a typical groundwater-fed karstic river in Dalmatian karst that enters in the Adriatic sea, receiving a considerable fraction of its surface water flow through subsurface groundwater discharge. Most of it derives from the Zrmanja river watershed through submerged or ephemeral springs, as well as from a diffuse recharge which is not yet localized. The hydrogeological drainage area of the Krka is around 2 427 km² [1,2]. The average annual river discharge at Skradinski Buk Waterfalls (end of freshwater part) in last 50 years has varied between 40 and 60 m³ s⁻¹, with a minimum flow of 5 m³ s⁻¹ and maximum of 565 m³ s⁻¹ [1,2]. The length of the fresh-water section is 49 km, while the estuary is extended for additional 23.5 km.

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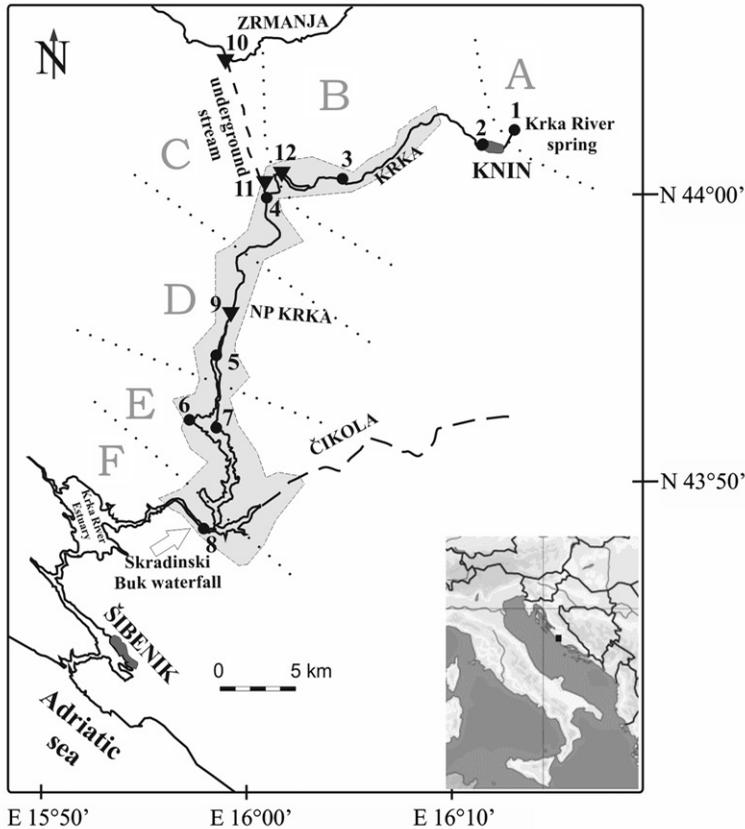


Figure 1. Map of the sampling area with marked area of National Park Krka. Black dots represent primary sampling stations and black triangles additional sampling stations. Capital letters represent regions.

The canyon of the Krka River is located at the Dinaric carbonate platform, consisting of carbonate rocks, mostly limestones and dolomites. Sub-recent and recent carbonate deposits in the river consist of calcareous cascade and barrage tufa. Tufa depositing is a constant, dynamic process, involving physico-chemical factors and organisms living in the water [3,4]. Owing to the picturesque scenery of tufa cascades stretching over tens of km, as well as its biological and geological characteristics, the lower part of the river region is protected as a National park Krka.

Anthropogenic pollution of the river by trace metals and radionuclides has been identified downstream of city of Knin that is located a few kilometers downstream of the Krka River spring (Figure 1) [5,6]. In the 2001 census, the population of Knin was 11,128 in the city and 15 190 in the municipality inhabitants, respectively. Up to now, all waste waters from the city have entered the Krka River without any pretreatment. This considerably influences the water quality before entrance into National park Krka. However, a significant self-purification process takes place through several small cascades lakes (Manojlovacki Waterfalls) formed behind tufa barriers in the middle part of the

river flow, where intensive scavenging through sedimentation and precipitation results in significant decrease of trace metal concentrations [5]. At the moment, a waste water treatment system for the city of Knin and its municipality is under construction.

Motivated by the interesting findings regarding basic water chemistry presented by Cukrov et al. [5], a new set of parameters (stable isotope composition of oxygen and carbon, dissolved organic carbon (DOC) and surface active substances (SAS)) was added in the succeeding field studies of the Krka River. The presented results are from two three-year projects with different sampling strategies, supplemented by all available results from previous sporadic test studies. Such a complex data structure prompted the application of multivariate techniques. Four of the most common multivariate techniques are multiple regression analysis, factor analysis, path analysis and multiple analysis of variance or MANOVA. In this work we used multiple analysis of variance and multidimensional preference (MDPREF) analysis [7]. Multidimensional preference analysis [7] is a descriptive statistical graphical method which is developed typically for marketing research to investigate relationships among products and individual differences in preferences for those products. The end result is a two-dimensional scatter plot – biplot [8] that shows the most salient information in the data matrix. According to the versatile study design, the number of variables, stations and samplings, the biplot was used for visualization and for the analysis of variance for exploring differences between subgroups of data. Usage of the analysis of variance on the different subdatasets should confirm the existence and persistence of processes in different parts of the Krka River. Such statistic analysis was found to be very useful in interpretations of environmental quantitative data parameters [9]. Also, multivariate statistical analysis should help ease understanding of complex processes, which are characteristic for the environment and in extracting pieces of information derived from observations of the river system that are worthy of synthesis and interpretation.

A multivariate statistical analysis applied to obtained data provided useful parameters which were used in order to confirm known, and possibly find some additional (dominant) influences or regularities of the Krka River water chemistry.

2. Experimental

2.1 Sampling strategy

Sampling campaigns were performed between 2001 and 2007 at 12 stations (Figure 1). Over the course of six year there were 14 sampling expeditions: February 2001, May 2001, July 2001, November 2001, April 2002, June 2002, August 2002, November 2004, January 2005, May 2005, July 2005, July 2006, February 2007 and July 2007. Corresponding average monthly flows ($\text{m}^3 \text{s}^{-1}$) at Skradinski buk (sampling site 8) were 83, 41, 15, 78, 4730, 39, 37, 61, 46, 14, 13, 61 and 20, respectively. Measurements of physico-chemical parameters (water temperature, pH, dissolved oxygen) were performed in situ. pH was measured with a portable instrument Mettler MP 12, dissolved oxygen with Oxymeter Mettler MO 128, and water temperature with a mercury thermometer submerged under water for 5 min or with a thermometer built in a Mettler Oxymeter probe. The oxymeter and pH probe were checked prior to each sampling event and daily recalibrated.

For alkalinity measurements, water samples were taken into glass Pyrex[®] bottles (250 mL) and determined by titration with 0.1 M hydrochloric acid solution using a Bromocresol Green-Methyl Red indicator.

Samples for trace metal analyses (zinc, cadmium, lead and copper) were taken using the principle of a 'clean sampling technique' [5], into high-density polyethylene (HDPE) bottles (1 L), previously cleaned with nitric acid and thoroughly rinsed with Milli-Q water. Surface water samples were taken 0.5–1 m away from the river banks at 20 cm depth.

Samples for the analysis of stable isotope composition of oxygen ($\delta^{18}\text{O}$) were taken in 60 mL HDPE bottles, whereas the samples for the analysis of stable isotope composition of dissolved inorganic carbon ($\delta^{13}\text{C}$ of DIC) were filtered through 0.20 μm membrane filters, stored in 12 mL septum vials and preserved with saturated HgCl_2 (1 mL L^{-1}) to suppress bacterial growth and related C isotope fractionation.

Samples for determination of dissolved organic carbon (DOC) and surface active substances (SAS) were collected with Pyrex[®] bottles (1 L) pre-washed with chromic-sulphuric acid and rinsed with MilliQ water. Filtration of the samples was performed on precombusted glass fibre filters (Whatman, GF/F, pore size 0.7 μm) in all-glass Millipore filter holder. Subsamples for DOC analysis were collected in precombusted 20 mL glass tubes with Teflon[®]-lined screw caps, poisoned with mercury chloride (10 mg L^{-1}) and stored for further analysis. Surface active substances (SAS) were analyzed in nonfiltered (SAS_{NF}) and filtered (SAS_F) samples within 24 h from sampling.

2.2 Equipment and experimental procedure

Analysis of both, total (unfiltered) and dissolved (filtered) trace metals (Cd, Pb, Cu, Zn) was carried out. Dissolved fractions were determined after filtration under nitrogen pressure, through 0.45 μm cellulose nitrate membrane filters (Sartorius, Gottingen, Germany). Water samples were digested by acidification with conc. HNO_3 (suprapur, Merck, Darmstadt, Germany) to $\text{pH} < 2$ (2 mL of acid to 1 L of sample) and additionally irradiated by UV-light (mercury lamp 150 W, Hanau, Germany) for at least 24 h. Typical blank values for all measured metals were below the detection limit, or were insignificant in comparison with concentrations measured in analysed samples.

Trace metal concentrations were measured by differential pulse anodic stripping voltammetry (DPASV) using AUTOLAB PGSTAT12 instrument (Eco-Chemie, Utrecht, Netherlands) equipped with three electrode system. Hanging mercury drop was used as a working electrode (VA stand 663, Metrohm, Herissau, Swiss).

The stable isotope composition of oxygen of water ($\delta^{18}\text{O}$) was determined in triplicate by the CO_2 equilibration technique (12 h at 25°C , [10,11]). The analyses were performed using a Varian MAT 250 mass spectrometer. Working standards (water) were calibrated versus IAEA-VSMOW and IAEA-GISP reference materials. The internal precision of $\delta^{18}\text{O}$ measurement was better than 0.05%, the standard uncertainty of the entire procedure was 0.1%.

The stable isotope composition of dissolved inorganic carbon (DIC) was determined in duplicate in 5 mL samples injected into He-flushed septum vials containing 100% H_3PO_4 . The CO_2 released was analysed using a Europa 20-20 Stable Isotope Analyser with an ANCA TG trace gas separation module. The working standard (natural CO_2) was calibrated versus CO_2 derived from NBS18 and NBS19 reference materials at 25°C . The internal precision of $\delta^{13}\text{C}$ measurement was $\pm 0.1\%$, while the standard uncertainty of the $\delta^{13}\text{C}$ -DIC analysis was 0.25%.

Stable isotopic compositions of C and O were determined as $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$ ratios, respectively, and reported in % as relative $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values:

$$\delta = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000 [\text{‰}]$$

where R is the $^{13}\text{C}/^{12}\text{C}$ or $^{18}\text{O}/^{16}\text{O}$ ratio of the sample and the standard. The results for carbon were reported relative to the V-PDB (Vienna – Pee Dee Belemnite), while those for oxygen were reported relative to the V-SMOW (Vienna – Standard Mean Ocean Water [12]).

The DOC concentrations were analysed in duplicate using the sensitive high-temperature catalytic oxidation (HTCO) technique. A model TOC-VCPH (Shimadzu) with platinum silica catalyst and nondispersive infrared (NDIR) detector for CO_2 measurements was used.

Samples acidified with hydrochloric acid ($\text{pH} \sim 2$) were purged with organic free air for 10 min prior to analysis to remove inorganic carbonate fraction. DOC concentrations were calculated by subtracting the measuring system and Milli-Q water blanks. Potassium hydrogen phthalate was used as a standard. The concentration was calculated as an average of three to five replicates. The average instrument and Milli-Q blank correspond to 0.03 mg L^{-1} ($n = 32$) and the reproducibility was high (1.6%).

Determination of surface active substances (SAS) was performed by phase sensitive alternating current (a.c.) polarography at the mercury electrode in the manner described elsewhere [13–16]. For quantitative determination of adsorption effects of an unknown mixture of SAS (mg L^{-1}) in the samples, the calibration curve prepared with an arbitrary model nonionic surface active substance, Triton-X-100 was used. The detection limit of SAS determination is 0.02 mg L^{-1} eqv. Triton-X-100. The relative standard deviation for multiple analyses of the same solution (0.1 mg L^{-1}) is $<5\%$.

Surface active substances were determined in the original (non-filtered) samples (SAS_{NF}) as well as in the filtered containing only dissolved surface active substances (SAS_{F}). Electrochemical measurements were performed with a BAS-100 B electrochemical analyser (Bioanalytical Systems, West Lafayette, IN), using a hanging mercury drop electrode, and Ag/AgCl electrode as the reference electrode.

Symbols used in the text represent: pH (negative decimal logarithm of the activity of dissolved hydrogen ions); t (water temperature in $^{\circ}\text{C}$); O_2 (dissolved oxygen in mg L^{-1}); ^{13}C ($\delta^{13}\text{C}$ in $\%$ V-PDB); ^{18}O ($\delta^{18}\text{O}$ in $\%$ V-SMOW); Alk (total alkalinity in mmol L^{-1}); Ca_{D} and Mg_{D} (concentration of dissolved Ca^{2+} and Mg^{2+} in mmol L^{-1}); trace metal concentrations are given in ng L^{-1} with their chemical symbols (Cd, Pb, Zn and Cu) and phase (dissolved or total); SAS_{F} and SAS_{NF} (filtered and nonfiltered surface, i.e. dissolved and total surface active substances in mg L^{-1}), DOC (dissolved organic carbon in mg L^{-1}) and DIC (dissolved inorganic carbon in mmol L^{-1}).

2.3 Statistical analysis

The data analysis in this paper was performed using SAS/STAT software, Version 9.1.3 of the SAS System for Windows. We used the PRINQUAL procedure to perform a nonmetric multidimensional preference (MDPREF) analysis [7] for generating biplots [8] on raw data.

In order to explore the spatial distribution patterns, univariate and multivariate analysis of variance was performed for the representative stations, regions and different sampling periods. We have used multivariate analysis of variance (GLM procedure with manova option) for testing the differences between the subgroup means of our data with 90% confidence level (Hotelling-Lawley trace, Pillai's trace, Wilks' criterion, and Roy's maximum root criterion tests). Logarithmic transformation ($\ln(x)$) was used to meet the

Table 1. Concentration range, median, 1st and 3rd quartile of all measured parameters.

Variable	Minimum	1st quartile	Median	3rd quartile	Maximum
pH	6.96	7.63	7.99	8.21	8.70
t (°C)	3.60	10.4	12.6	16.2	26.9
O ₂ (mg L ⁻¹)	6.60	8.30	10.2	11.1	14.3
¹³ C (‰)	-13.9	-11.8	-10.9	-9.83	-7.40
¹⁸ O (‰)	-9.03	-8.33	-7.91	-7.67	-6.82
Alk (mmol L ⁻¹)	3.29	4.33	4.69	5.00	5.92
Ca (mmol L ⁻¹)	0.91	1.87	2.12	2.33	3.21
Mg (mmol L ⁻¹)	0.17	0.33	0.41	0.51	0.70
Zn _D (ng L ⁻¹)	54.0	155	356	756	3890
Zn _T (ng L ⁻¹)	83.0	268	549	1245	64181
Cd _D (ng L ⁻¹)	0.50	1.00	3.10	4.30	7.10
Cd _T (ng L ⁻¹)	0.80	3.73	4.80	6.43	58.8
Pb _D (ng L ⁻¹)	1.00	6.23	10.5	16.9	180
Pb _T (ng L ⁻¹)	8.20	27.7	49.5	84.8	7772
Cu _D (ng L ⁻¹)	20.0	104	136	163	1300
Cu _T (ng L ⁻¹)	50.9	134	163	216	6053
SAS _{NF} (mg L ⁻¹)	0.02	0.04	0.07	0.10	1.02
SAS _F (mg L ⁻¹)	0.02	0.03	0.04	0.07	0.56
DOC (mg L ⁻¹)	0.26	0.44	0.53	0.68	1.75
DIC (mmol L ⁻¹)	3.17	4.27	4.75	5.33	6.70

normality and variance homogeneity (fundamental assumptions required by this model, but not for the MDPREF model). We tested only hypothesis of high interest owing to the limited degrees of freedom.

3. Results and discussion

Table 1 presents summary values of all 20 measured parameters.

The presented biplot display in Figure 2 shows a separation between the data groups according to different effects (station and sampling period). Each combination of numbers (sampling stations) and letters (sampling periods) includes values from all twenty measured variables and represents the multivariate observation based on the non-metric multidimensional preference model. The vectors represent measured variables and the vector positions are determined by the high-value observations of individual variables.

This biplot implies diverse patterns for individual samplings and stations along the Krka River. Vector positions, or in other words, their grouping is under the influence of different effects which variables are passing through this system. As a result, the biplot is composed of four groups. The first one is combined from seven variables (Pb_D, Cu_T, SAS_{NF}, SAS_F, Zn_T, Pb_T, Cd_T), which are dominated by high values from station 2. The Krka River water is polluted with untreated municipal waste waters rich in particles (station 2) which act as metal carriers [5], so all metal variables connected with the total phase are placed in this group, as expected. Surface active substance (SAS) concentrations are the highest at station 2 pointing out dominance of anthropogenic over natural origin in Krka water. In the case of SAS natural origin, its vector should be associated with the DOC vector, indicating connection with primary production; however, that is not the case

Table 2. Multivariate Hotelling-Lawley trace, Pillai's trace, Wilks; criterion, and Roy's maximum root criterion tests for contrasts (90% confidence level). *p*-values for all four tests were identical.

Contrast station vs. station	<i>p</i> -value
1 vs 8	0.0038
1 vs 2	0.0115
2 vs 3	0.0166
2 vs 8	0.0076
3 vs 4	0.0004

flow sections, waterfalls and cascades, increases the $\delta^{13}\text{C-DIC}$ values. The only metal in this group is Cu_D , while Zn_D has position on the boundary zone between the first and the second group.

The third group, placed opposite to the first one and linked with observations from May 2005 and February 2007, is composed of three variables (Mg_D , ^{18}O , Ca_D), while O_2 stays in border zone between the second and the third group. Vectors from this group are characterized by the smallest length. Grouping of dissolved Ca^{2+} and Mg^{2+} concentrations with $\delta^{18}\text{O}$ of water is related to the river recharging mode from relatively deep, thoroughly homogenized groundwater with retention times of at least 3–6 months [3]. This implies that the chemical and isotopic composition of the river water is subject to different processes (including equilibration with the host rock and dissolution/precipitation of carbonate) taking place within the aquifer, as well as those occurring in the surface flow of the river and mixing with surface runoff [1,3]. The relatively long retention time and homogenization of groundwater within the aquifer mask the influence of seasonal variability of $\delta^{18}\text{O}$ of precipitation, which is by far larger and positively correlated with ambient temperature [20], in opposite to the $\delta^{18}\text{O}$ of the river water.

The fourth group is composed of three variables (Cd_D , DIC, Alk) where DIC and Alk represent the influence of subterranean waters.

This biplot indicates possible spatial and temporal differences between various groups, but owing to non-sufficient seasonal measurement data, we only analyse variance according to sampling stations and regions. In accordance with our previous knowledge about this water system and information from biplot, the following research hypotheses were defined:

- (1) quantities of the measured chemical species are different at the spring and at the end of the fresh water part of the Krka River (1 vs 8),
- (2) the municipal waste waters of city of Knin pollute the Krka River (1 vs 2, 2 vs 3),
- (3) there is a self-purification process of the Krka River concerning trace metals (1 vs 8, 1 vs 2, 2 vs 3 and 2 vs 8) and
- (4) the entrance of Zrmanja River subterranean water between stations 3 and 4 influences the Krka River.

MANOVA showed that there were strong statistical evidences for the differences between examined groups (Table 2).

Table 3. Univariate estimations of differences between group means. Only statistically significant ($p < 0.10$) values are presented. Units are given in section 2.3.

Transformed variable	Estimation				
	st 1 vs st 8	st 1 vs st 2	st 2 vs st 3	st 2 vs st 8	st 3 vs st 4
ln Pb _T	–	-3.20 ± 0.64	1.18 ± 0.64	2.50 ± 0.64	1.19 ± 0.64
ln Zn _T	–	-4.16 ± 0.61	1.31 ± 0.64	3.16 ± 0.61	1.75 ± 0.61
ln SAS _F	-0.97 ± 0.39	-1.85 ± 0.39	1.23 ± 0.39	0.89 ± 0.39	–
ln SAS _{NF}	-1.01 ± 0.33	-1.27 ± 0.33	0.71 ± 0.33	–	–
ln Zn _D	-1.51 ± 0.45	-2.09 ± 0.45	–	–	1.57 ± 0.45
ln pH	-0.05 ± 0.01	-0.05 ± 0.01	-0.03 ± 0.01	–	0.10 ± 0.01
ln DOC	-0.46 ± 0.25	-0.48 ± 0.25	–	–	–
ln ¹³ C	–	–	–	–	0.84 ± 0.24
ln ¹⁸ O	-0.82 ± 0.10	-0.33 ± 0.10	–	-0.49 ± 0.10	-0.55 ± 0.10
ln Ca _D	-0.32 ± 0.09	-0.33 ± 0.09	–	–	–

According to all four tests (Multivariate Hotelling-Lawley trace, Pillai's trace, Wilks' criterion, and Roy's maximum root criterion test) there is very strong evidence against the null (conservative) hypotheses. In other words, there is a significant difference between means of stations at the spring and at the end of the fresh water part of the Krka River (Table 2, 1 vs 8). The same is valid for differences between the spring and the station located downstream of the city of Knin wastewater outflow (1 vs 2), Knin station vs. Bilušića buk waterfall located 10.5 km downstream (2 vs 3), Knin station vs the end of the fresh water part (2 vs 8) and for stations 3 and 4 in between the Zrmanja River subterranean waters discharge into the Krka River.

By analysing each individual variable we arrive to the variables which are potential reason for the significant difference found by MANOVA. Only transformed variables with significant differences between the means for chosen stations are listed in Table 3.

The difference between the spring and the end of the fresh water part of the Krka River (1 vs 8) is statistically significant for SAS_F, SAS_{NF}, Zn_D, pH, DOC, ¹⁸O and Ca_D showing higher values at the river end comparing with spring.

The relations between the spring and the city of Knin show that the municipal waste waters elevated (negative values) all of the variables referred to in Table 3 except ¹³C (which is unrelated to the anthropogenic activities, but reflects the predominant source of DIC from the soil CO₂). Comparing the differences between (1 vs 8) and (1 vs 2) it is notable that those between the spring and the end of the Krka river are lower compared with those between the spring and the city of Knin which are also the result of self-purifications.

The self-purification process of the Krka River concerning trace metals is justified by the fact that 1 vs 8 stations have no significant difference in Pb_T and Zn_T concentrations. It is evident that from the spring to the city of Knin there is a significant increase of those metal concentrations, which consequently decrease from the city of Knin to the next sampling point (3), and even more from the city of Knin to Skradinski Buk (end of fresh water part, sampling point 8).

The fourth hypothesis (the influence of Zrmanja River subterranean water between stations 3 and 4) is approved for Pb_T, Zn_T, Zn_D, pH, ¹³C and ¹⁸O. Different stable isotope composition of both O and DIC (¹⁸O and ¹³C) of Zrmanja River evidently generate

changes to isotope composition of Krka River (3 vs 4). However, until the end of the fresh water river flow the $\delta^{13}\text{C}$ -DIC values evolve according to the changes in the carbonate equilibrium owing to the degassing and carbonate precipitation. In contrast to $\delta^{13}\text{C}$, the original $\delta^{18}\text{O}$ signal (which has all characteristics of a conservative tracer for the origin, e.g. groundwater recharge area) of the Zrmanja River contribute to considerable fraction in the water of the Krka River and, remains retained until the end of the fresh water flow (1 vs 8, [3]).

The information extracted from the first biplot (Figure 2) directed towards more specific grouping and interpretations shown in Figures 3 and 4. According to the analysis of eight stations and 20 parameters, and including additional data taken on four other locations along the Krka River region (labelled 9 to 12, denoted as black triangles in Figure 1), the whole Krka River stream is divided into six regions named A to F (Figure 1). Parameters corresponding to carbon cycle (t, SAS_F , SAS_{NF} , DOC, DIC, ^{18}O , ^{13}C , pH, Alk, Ca, Mg, Cd_D , Cu_D) and anthropogenic influence (Zn_T , Cd_T , Pb_T , Cu_T , Zn_D and Pb_D) from these six regions were sorted out and statistically analyzed. Multivariate tests for effects and contrasts (90% confidence level) for carbon cycle as well as for anthropogenic influence showed statistically significant difference between regions A vs F, A vs B, A vs C, B vs C and C vs F.

In Figure 3(a) the grouping of the parameters t, DOC, pH, ^{13}C and Cu_D accentuated the effects of primary production, while grouping of DIC, Alk and ^{18}O refer to Zrmanja River influence. All variables in this biplot are grouped similarly to that in Figure 2. with different data set, affirming the same processes. In Figure 3(b) sampling expedition 11 (held in July 2005) is dominantly influenced by primary production (t, DOC, pH, ^{13}C and Cu_D). For sampling expeditions 9 and 10 (January and May of 2005) the high influence of Zrmanja River water is evident (DIC, Alk, ^{18}O grouped). Sampling expedition 14 (February 2007) is strongly influenced by the event of the city of Knin municipal water outflow.

The more pronounced anthropogenic influence was sorted out by extracting the information for Zn_T , Cd_T , Pb_T , Cu_T , Zn_D and Pb_D according to the six regions. In Figure 4 it is seen that the region B (2) (Knin) has an influence on dissolved metals and also the sampling expedition that caught directly the municipal waste waters is highly related to the total metal concentrations as well.

Analysis of only pH parameter allowed us to take into consideration for statistical analysis 16 distinct sampling expeditions during 6 years. The whole Krka River stream was grouped into four distinct regions I (A & B), II (C), III (D) and IV (E & F) in order to make the most of available data sets. That grouping was statistically approved because there was less variability of pH values between sampling expeditions (data are not showed in the current article owing to the limited space) than between regions. The region II (characterized by subterranean inflow of Zrmanja River) is statistically significantly different from other regions. The difference between region II and others was around -0.5 and differences between any other two regions were less than -0.1 (Table 4).

4. Conclusions

Despite the huge variability and relatively small data set, multivariate statistical analysis was successfully used to arrive at the conclusions that waste waters of the city of Knin statistically significantly pollute the Krka River with lead and zinc as well as with organic matter, while the self-purification process downstream of the city of Knin compensates

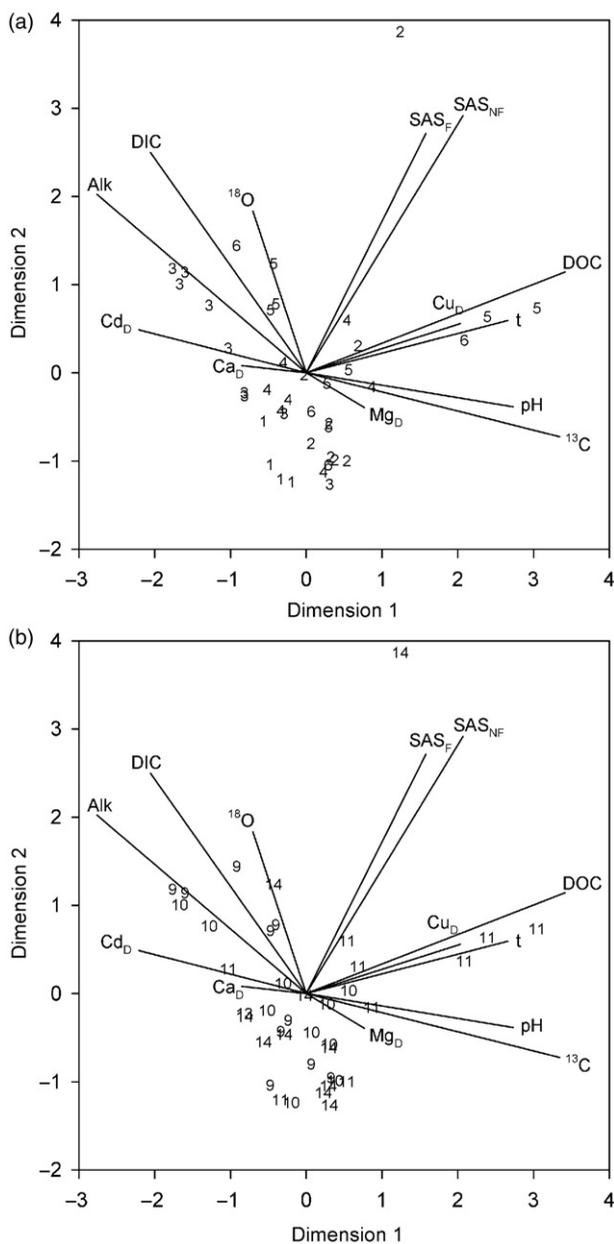


Figure 3. Biplot for carbon cycle corresponding parameters (40 cases). This display represents approximately 48% of the total data variance. (a) Coding for six regions, (b) coding for 5 from 14 sampling expeditions.

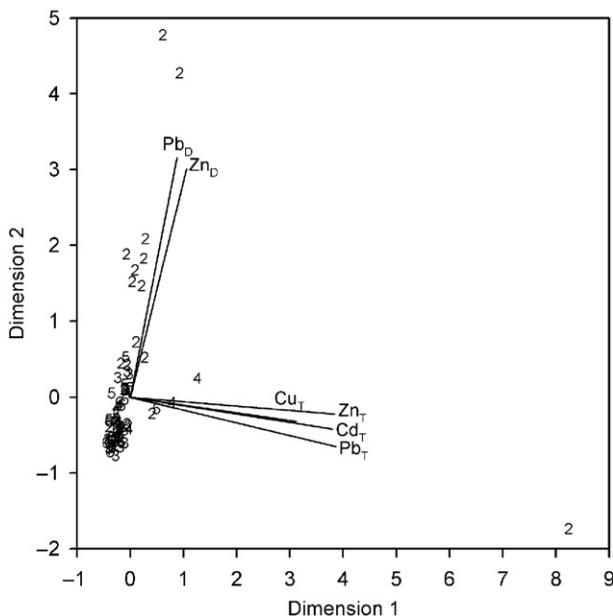


Figure 4. Biplot for six regions and anthropogenic influenced corresponding parameters (74 cases). This display represents approximately 79% of the total data variance.

Table 4. Contrasts and estimates for univariate analysis of variance for differences of regions means with their standard errors for the pH variable. Confidence level was 90%.

Contrast region vs region	<i>p</i> -value	Estimates Δ mean \pm st.err
I vs II	<0.0001	0.49 \pm 0.07
I vs III	0.6757	-0.03 \pm 0.08
I vs IV	0.1845	-0.09 \pm 0.06
II vs III	<0.0001	-0.52 \pm 0.08
II vs IV	<0.0001	-0.58 \pm 0.07
III vs IV	0.4839	-0.05 \pm 0.08

this pollution. Input of clean water upstream of station 4 helps the process of self-purification and changes the composition of stable isotopes C and O. There is also clear evidence that isotope composition of dissolved inorganic carbon changes seasonally, while the oxygen isotope composition of water remains reasonably stably retained.

Also, the statistical analysis enabled grouping of parameters connected with carbon cycle (t, DOC, pH, ^{13}C , Cu_D) and those connected to anthropogenic activities (total metal concentrations). The most exhaustive data set of pH parameter allowed a clear distinction of the region influenced by subterranean inflow of Zrmanja River, being statistically significantly different, consistently lower for about half of the pH unit from the others, regardless of the sampling expedition during the six years' measuring period.

Such analysis opens new perspectives for further investigations: enables focusing on parts of the Krka River flow where biggest changes of parameters occurred, in order better

to record and explain the event and the responsible processes. Also, for monitoring purposes, on the basis of the results of such analysis one could optimize the number of the parameters and the measuring stations according to the processes of interest.

Despite the waste water treatment system for the city of Knin that is under construction, respecting the results of this study, a monitoring programme is proposed to National Park Krka authority. Besides monitoring main physico-chemical parameters, program is intended to be focused on determination of trace metal and organic matter concentration and stable isotopes composition during dry and wet season.

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