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# Spatial and temporal variability of dissolved metal(loid)s in water of the karst ecosystem: consequences of long-term exposure to wastewaters

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ABSTRACT

The metal(loid) variability in the sensitive karst ecosystem was addressed as possible indicator of short- and long-term exposure to the wastewaters, recognized as the main metal contamination sources. Industrial and municipal wastewaters are released without proper purification only 2 km upstream from the Krka National Park (KNP) in Croatia. The variability of dissolved metal(loid)s was studied in four seasons and eight locations: Krka River source (reference site, KRS), municipal wastewaters from the Town of Knin (KRK), industrial wastewaters from the screw factory (IWW), Brljan Lake in the KNP (KBL), and the tributaries Krčić (TKR), Kosovčica (TKO), Orašnica (TOR) and Butišnica (TBU). Water taken directly from IWW had several times higher concentrations of all elements than other locations and indicated industrial wastewater as the primary Mn, Zn, Co, Cs, and Fe source. Tributary Orašnica, flowing by IWW, was the most affected site, although higher metal concentrations were also found at other locations compared to KRS. Overall, spatial metal contamination followed the order: TOR>KRK>TKO>TBU>KBL>KRS. Seasonality was not pronounced, although the highest levels for most metals were observed in summer, dry season when the self-purification processes are reduced. Almost all elements had low tendency to bind with particles, therefore showing high presence in dissolved fraction and confirming their bioavailability and potential toxicity. Although metal concentrations increased over time, they were still low compared to metal-contaminated rivers. However, observed metal exposure and inter-site differences present a warning and indicate the need for the targeted continuous monitoring of potential hotspots to protect this karst ecosystem.


## 1. Introduction

Karst aquifers are considered extremely sensitive ecosystems, susceptible to contamination due to their unique hydrological (rapid flow of contaminants through fractures), morphological (presence of numerous cavities) and geological (fractured carbonates) characteristics (Brinkmann and Parise, 2012; Campanale et al., 2022; Kovačič and Ravbar, 2003; Padilla and Vesper, 2018; Selak et al., 2022). Contaminants are easily dispersed and transported over long distances in

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karst aquifers, whether through sinking streams, caves, sinkholes or open fractures and shafts in carbonate rocks, which are characterized by conduit porosity and highly permeable zones (Padilla and Vesper, 2018; Kalhor et al., 2019). In addition, the porosity and high sediment content of karst aquifers allow significant storage capacity for various types of contaminants, which can then be released into the water column slowly and over a long time (Padilla et al., 2011; Padilla and Vesper, 2018; Vadillo and Ojeda, 2022).

Among contaminants, metal(loid)s always pose a major threat due to their enhanced releases from natural and anthropogenic sources, persistence, toxicity and possible accumulation. As seasonal variations in precipitation regime, temperature, interflow or groundwater flow have a significant impact on river discharge and consequently on the concentration of metals in river water (Vega et al., 1998), the assessment of spatial, as well as temporal variability of metal(loid) concentrations is important for the ecological characterization of aquatic ecosystems and estimation of water quality.

Although groundwater from karst aquifers is one of the most important drinking water sources and reservoirs (Bakalowicz, 2005), not many studies have focused on the water quality assessment of the karst rivers in Europe, even worldwide, as confirmed by Šariri et al. (submitted for publication) using CiteSpace. Namely, combination of the keywords "karst", "water quality" and "river" resulted in only 237 published papers around the world, mostly in China and USA. Further, only small portion of these papers deals with water pollution and anthropogenically-induced degradation, which was indicated as major environmental problem investigated in the modern world. Since many karst terrains in the world have also been declared protected areas due to their biotic and abiotic values together with high sensitivity and vulnerability (Telbisz and Mari, 2020), special awareness and establishment of permanent monitoring and protection measures in such areas are necessary.

The Krka River is a typical karst river in the Dinaric region of Croatia, the largest part of which has been declared a national park, with the aim of protecting and preserving this region of exceptional natural value characterized by tufa-barriers, waterfalls and great plant and animal biodiversity. Although most of the Krka River watercourse is relatively sparsely populated and seemingly pristine, the upper part is exposed to anthropogenic influences including industrial and municipal wastewaters, agricultural runoff, fertilizers, tourism and gypsum factory (Cukrov et al., 2008; Filipović Marijić et al., 2018). In recent years, the limited number of studies in the upper course of the Krka River investigated the potential threat to the national park and confirmed ecological disturbances and negative impact of wastewaters by variety of physico-chemical and microbiological parameters and metal(loid) contamination at the sites downstream of the wastewater's discharges in the period 2004–2021 (Cukrov et al., 2008, 2012; Filipović Marijić et al., 2018; Sertić Perić et al., 2018; Šariri et al., submitted for publication). As for the temporal changes of metal(loid)s in water, previous studies in the upper part of the Krka River covered only some seasons in a limited spatial area (usually two characteristic locations, pristine and contaminated) or presented only few elements (Cukrov et al., 2012; Filipović Marijić et al., 2018; Sertić Perić et al., 2018), while a comprehensive study covering temporal changes in all four seasons at a number of target locations in the wider area was never conducted. Therefore, we presented for the first time long-term trends in variability of 20 metal(loid) concentrations in the karst river and estimated the use of their current and long-term levels as chemical indicators of pollution impacts.

As a continuation of the mentioned research in this important area, we aimed to conduct a comprehensive study of the metal exposure assessment of the upper part of the Krka River by: (a) characterizing for the first time seasonal dynamics of metal(loid) concentrations at locations of variable pollution impact; (b) comparing the results with previously reported data to assess long-term trends and changes in metal concentrations; (c) estimating and comparing the bioavailability of 20 elements at unpolluted and polluted sites by calculating ratios of dissolved to total metal concentrations; (d) characterizing the main pollution sources and their direct and long-term impact on the Krka River watercourse; (e) identifying the influence and consequences of long-term exposure to industrial and municipal wastewaters on this sensitive ecosystem.

## 2. Materials and methods

### 2.1. Study area

Presented research was conducted in the Dinaric karst Krka River in Dalmatian part of Croatia. Although its lower part is protected as a national park, the upper course of the river is nowadays under influence of anthropogenic activities, which mostly include technological wastewaters from the screw factory, municipal wastewaters from the Town of Knin and agricultural runoff (Filipović Marijić et al., 2018; Sertić Perić et al., 2018). Wastewater outlets represent serious threat for the living world of the Krka River, as well as for the KNP, considering that wastewater is discharged into the river without adequate treatment only about 2 km upstream of the KNP border (Fig. S1). Previous research in this area has pointed to ecological disturbances and evidently higher metal concentrations and negative impact downstream of the existing wastewater outlets (Filipović Marijić et al., 2018). Conductivity, chemical oxygen demand, levels of ammonium, total nitrogen and phosphorus, nitrates and bacteria counts were below good water quality status even near the border of the KNP (Filipović Marijić et al., 2018). Further, 2–400 times higher levels of Al, Co, Fe, Li, Mn, Ni, Sr, Ti, and Zn were previously recorded in the technological/municipal wastewaters and the locations under the anthropogenic influence downstream of the Town of Knin (Filipović Marijić et al., 2018; Sertić Perić et al., 2018) compared to the Krka River source.

Therefore, to make comprehensive spatial and temporal assessment of environmental conditions in the upper part of the Krka River, 8 locations were selected: Krka River source without known anthropogenic impact as the reference site (KRS), downstream of the municipal wastewaters of the Town of Knin (KRK), industrial wastewaters from the screw factory (IWW), Brljan Lake situated in the Krka National Park (KBL), tributary Krčić flowing near the Krka River source (TKR), tributary Kosovčica influenced by gypsum factory (TKO), tributary Orašnica which flows along IWW (TOR) and tributary Butišnica, affected by agricultural activities (TBU) (Fig. S1).

### 2.2. Water sampling

The river water samples for the analysis of inorganic elements, except total mercury (THg), were collected in pre-cleaned polyethylene bottles in triplicates in all four seasons during 2021. Exception was TKR, where water was not collected in summer and autumn since this tributary had dried up. In all samplings, appropriate aliquots from each bottle were transferred into pre-cleaned 20-mL polyethylene bottles for determination of total metal concentrations. Other aliquots from each bottle were filtered using a 0.45-μm pore diameter cellulose acetate filter (Sartorius, Germany) mounted on syringes for the measurements of dissolved metal(loid) concentrations. All samples were acidified with concentrated nitric acid (Rotipuran Supra 69%, Carl Roth, Germany) and stored at 4 °C before analysis in the laboratory.

Water samples for THg analysis were collected concurrently with the collection of samples for other elements according to the method described by Bravo et al. (2018). Briefly, unfiltered and filtered (0.45-μm CA filters; Sartorius, Germany) water samples for the analysis of total and dissolved THg fraction and corresponding locational blanks were collected in pre-cleaned 100-mL amber borosilicate glass bottles, acidified with hydrochloric acid (HCl, 30%, Suprapur, Merck, Darmstadt, Germany) and stored at 4 °C in plastic zip-sealed bags for further THg analyses.

### 2.3. Measurements of trace and macroelement concentrations in water samples

Concentrations of trace and macroelements in collected and acidified water samples were measured directly, without prior dilution using inductively coupled plasma mass spectrometry (ICP-MS) on an Agilent 7500cx instrument (Agilent Technologies, Tokyo, Japan) under working conditions presented in Table S1. Helium and hydrogen collision gases were used to remove interference and an internal standard solution containing 3 $\mu g\ L^{-1}$ Ge, Rh, Tb, Lu and Ir (SCP Science, Quebec, Canada) was used to correct for instrumental drifts and plasma fluctuations. Standard solutions used for external calibration were prepared from individual PlasmaCAL Single-Element Standard solutions (SCP Science, Quebec, Canada). The preparation and analysis of samples were carried out in a laboratory with a HVAC system (Heating, Ventilating and Air Conditioning) combined with HEPA filters. The limits of detection (LODs) of the analyzed elements ranged from 0.0005 $\mu g\ L^{-1}$ for Cs to 30 $\mu g\ L^{-1}$ for K (individual LOD values are given in Table S2). Three standard certified reference materials (NIST SRM 1643e, NIST SRM 1643f, NRCC SLRS-5) were analyzed as part of quality control. The accuracy for the analyzed elements in the referent water samples was within ±10% of the certified values, with recoveries ranging from 92% (Ni) to 109% (Ba). Detailed data on all elements in reference materials is given in Table S2.

### 2.4. Measurements of mercury in water samples

Concentrations of THg in filtered and un-filtered water samples were analyzed directly, using cold-vapor atomic absorption spectrometry (CV-AAS) on AMA 254 Mercury Analyzer (LECO, Korea). Briefly, an aliquot of the water sample was added to the sample boat and gradually heated to 750 °C. After thermal decomposition and catalytic removal of impurities, mercury vapors were concentrated on a gold trap. Mercury was then released from the gold trap by heating to 900 °C and measured on the detector. Standard solutions used for external calibration were prepared from standard solution of inorganic mercury (10 $mg\ L^{-1}$; Inorganic Ventures, Christiansburg, USA). The LOD of the method was 0.03 $\mu g\ L^{-1}$. Standard certified reference material NIST SRM 1641e was analyzed with each batch of samples as part of quality control, with recoveries ranging from 95% to 101% of the certified value.

### 2.5. Data processing and statistics

Statistical analyses and creation of images was performed in SigmaPlot 11.0 for Windows. We presented data on metal concentrations as mean ± standard deviation (S.D.). Variability of metal concentrations in water samples between the sites and seasons was tested using two-way ANOVA and Holm-Sidak test. In all cases level of significance was set at $p < 0.05$. As TKR was dried up in two seasons, it was not analyzed and presented in graphs. Statistically significant differences between sites within each season are always indicated with different numbers in the graphs: 0 Significant difference from all other locations; 1–6 Significant difference from the location indicated by a specific number as follows: 1—KRS; 2—KRK; 3—KBL; 4—TOR; 5—TBU; 6—TKO. Differences between seasons for each location are indicated by different letters or asterisk: * Significant difference between all seasons; a-d Significant difference from season indicated by a specific letter as follows: a—winter; b—spring; c—summer; d—autumn.

## 3. Results and discussion

### 3.1. Concentration ranges of dissolved metal(loid)s in IWW, point source of pollution

Data related to the inadequately purified wastewaters from the screw factory were characterized and presented separately from other riverine locations, as an artificial pool presenting point source of pollution and the most serious threat for the whole karst ecosystem of the Krka River. The concentrations of trace elements in IWW were in the following ranges, depending on the season ($\mu$g $L^{-1}$): 50-3850 (Mo<Ba<Mn≤Fe≤Sr<Zn); 0.5–10 (As<Cu<Cr<Ni≤Co); 0.001–0.5 (Tl<Cd<Cs≤Se<Sb) (Table S3). Macroelements were found in the following concentration ranges (mg $L^{-1}$): Ca (424–1560) > Na (102–246) ≥ K (85.7–318) > Mg (16.4–27.1). We have also measured THg in waters, but all the values, even at IWW, were close to the limit of detection (0.03 $\mu$g $L^{-1}$), indicating there was no significant pollution with total mercury in this area. Contrary to expectations, given that the values of certain physico-chemical parameters and nutrients (chemical oxygen demand (COD), ammonium, nitrites, total nitrogen, phosphorus) at this location exceeded the regulatory emission limits (Šariri et al. submitted for publication), obtained concentrations of investigated elements in IWW in all seasons were below the legally defined threshold values for wastewater emissions (GRC, 2020). This particularly applies to As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, Se and Zn concentrations, which were many times lower than prescribed by the regulation.

Concentrations of most elements at IWW were significantly higher compared to all riverine locations, and especially compared to the river source water (KRS) with up to 46689 times higher values for Mn, 566 for Zn, 405 for Co, 263 for Cs, 114 for Fe and 80 for Ni, depending on the season (Table S3). That could be expected considering that these are all elements often used in the industry of iron and steel alloys, as present in this area (Filipović Marijić et al., 2018; Sertić Perić et al., 2018). Besides KRS, significant differences in metal(loid) levels were also found between IWW and other locations, even TOR, as presumably the most contaminated site on the Krka River, which is directly influenced by IWW due to possible spillover of wastewaters during rains and/or floods, and karst porosity.

Comparison of our data with the previous research of these technological wastewaters in autumn 2015 (Filipović Marijić et al., 2018) indicated a significant increase in the concentrations of many elements (including As, Ba, Cr, Cu, Mo, Sr, Ca, K, Mg), but also a decrease in the concentrations of Cd, Fe, Mn, Ni, Se and Zn over time at the IWW. Since concentrations of most of the "industrial" elements (Fe, Mn, Ni, Zn) at the IWW were lower in 2021 than in 2015, this may reflect positive improvements in the treatment of the wastewater and construction of the dam between the waste basins and TOR to prevent spillover of wastewater. As a follow up to 2015 research, the study of the of organic and inorganic pollution in sludge and waters in lagoons in the immediate vicinity of the screw factory in 2019 showed extreme contamination with different types of hydrocarbons, while sludge, as the only medium in which inorganic pollutants were measured, was highly contaminated with Zn, Cr, Ni, Mn, Al, Mg, Ca and K (Kisić et al., 2019), many of which were also highly increased in this research. Therefore, enhanced metal releases are still evident and are a cause for concern due to the karst nature of the terrain, which allows industrial wastewaters to reach the groundwater and enter the Krka River through the numerous underground fracture networks.

### 3.2. Concentration ranges of dissolved metal(loid)s in the Krka River catchment

Generally, element concentrations in the Krka River watercourse were in the following ranges ($\mu$g $L^{-1}$): ≤ 0.5 (Tl≤Cs<Cd<Sb≤Se≤As; Fig. 1); 0.5–3.0 (Co≤Cu<Mo≤Cr; Fig. 2); 3.0–2800 (Zn≤Ba≤Ni≤Mn≤Fe≤Sr; Fig. 3). As expected, the highest metal concentrations in water were observed for macroelements (0.2–300 mg $L^{-1}$, K<Na≤Mg<Ca; Fig. 4). Since THg concentrations of more than 50% of samples at all locations and seasons were below the LOD of the used method and the other values were only slightly higher than the LOD, THg was not further presented and discussed.

Among investigated elements, only Cd, Hg and Ni are regulated by Croatian and European legislations (EPCEU, 2013; GRC, 2019) and Cd and Hg are additionally considered as priority hazardous substances by the Water Framework Directive (EPCEU, 2013). According to these legislations, tolerable annual average concentrations of dissolved forms ($\mu$g $L^{-1}$) are 0.15 for Cd and 4 for Ni, while maximum allowable concentrations (MAC; $\mu$g $L^{-1}$) are 0.9 for Cd, 34 for Ni, and 0.07 for THg, respectively. Even the highest concentrations of Cd measured in our study were around 7 times lower than the recommended values, while THg values, being below 0.03 $\mu$g $L^{-1}$, were also few times lower than the recommended MAC, indicating the good condition of the upper reaches of the Krka River with regard to Cd and THg concentrations. Several times lower values compared to the recommended annual average were also observed for Ni, except for the values at KRK and TOR in winter (~29 $\mu$g $L^{-1}$ and ~14 $\mu$g $L^{-1}$, respectively) (Fig. 3), which were close to the MAC.

Maximum concentrations of most elements were obtained at TOR and KRK, directly impacted by industrial and municipal wastewaters, respectively. As exception, Cd and Tl had the highest concentrations at the reference site KRS (Fig. 1), probably reflecting the geological background of the catchment area. This applies especially to Cd considering that Jurassic dolomites, as the main feature of the geological background of the Krka River basin, may contain naturally high Cd concentrations (Cukrov et al., 2008).

Comparison of the obtained values with already available data on metal(loid) concentrations in the Krka River in period from 2004 to 2015 (Cukrov et al., 2008, 2012; Filipović Marijić et al., 2018) revealed mostly higher values in our research, indicating an increase of concentrations of most elements over time (Table S4). That was especially evident for Cr, Cu,

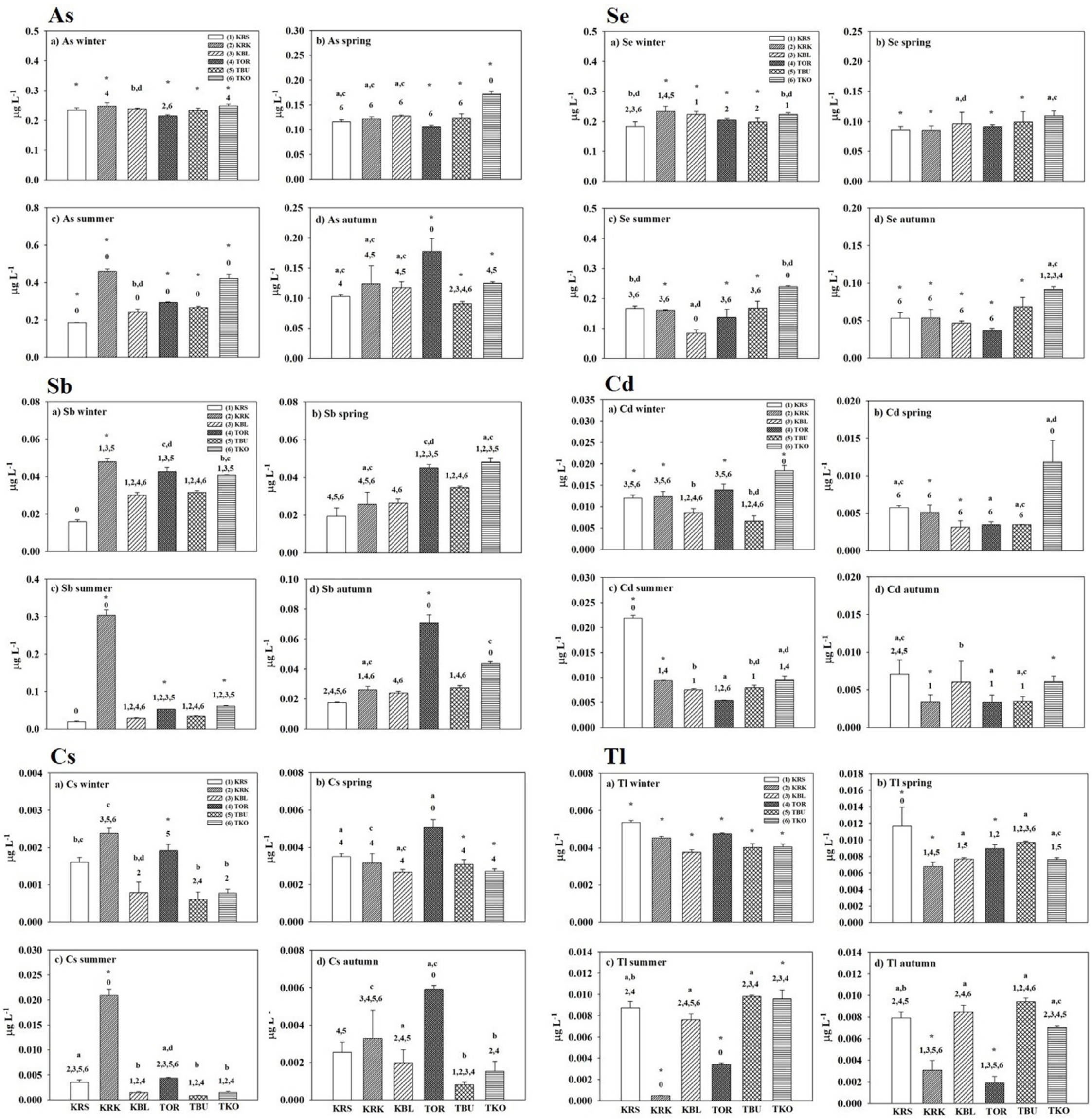


**Fig. 1.** Dissolved concentrations of metal(loid)s with maximum average concentrations $\leq 0.5$ $\mu g$ $L^{-1}$ in water from six sites of the Krka River sampled in four seasons. The description of the labels (numbers and letters) are given in Section 2.5 "Data processing and statistics".

Fe, Mn and Ni, which are all often used in industrial and agricultural activities. The only elements whose concentrations were mostly comparable in all research during the past 18 years are Mo, Sb, Se, Tl and Zn (Table S4). Comparison with another research conducted only in the Kosovčica River (TKO) in 2011 also indicated an increase in concentrations of Cd, Cr, Sr, Zn, and especially Fe and Mn during 11 years (Ternjej et al., 2014). Although **"**industrial" elements measured directly at IWW showed certain decrease over the years, the same trend could not be seen in the Krka River watercourse probably as a consequence of long-term contamination, whereby sediments serve as the potential sink and, in the case of resuspension, important source of metals over longer period (Filipović Marijić et al., 2018).

Comparison of our results with the data for typical Croatian karst ecosystems with low level of anthropogenic pollution, such as Plitvice Lakes and Una River, showed lower concentrations of all elements except Cd, and Mg in the Plitvice Lakes, and Ba, Ca, and Mn in the Una River (Dautović, 2006; Dautović et al., 2014). Since Ca, Cd and Mg are elements characteristic for the karst ecosystems, these differences are due to differences in natural characteristics of the areas of origin and dependence on the weathering of the dolomite carbonates (Hartmann et al., 2014). Comparison with the karst Mrežnica River showed comparable concentrations of most elements at the reference sites of both rivers. However, higher concentrations of Ba, Cr, Cu, Fe, Mn, Ni, Sr, Zn, Ca, K, and Na were observed in anthropogenically affected sites of the Krka River (Dragun et al., 2022). Although concentrations of most of the elements mentioned above, except Ba, Na and Sr, are

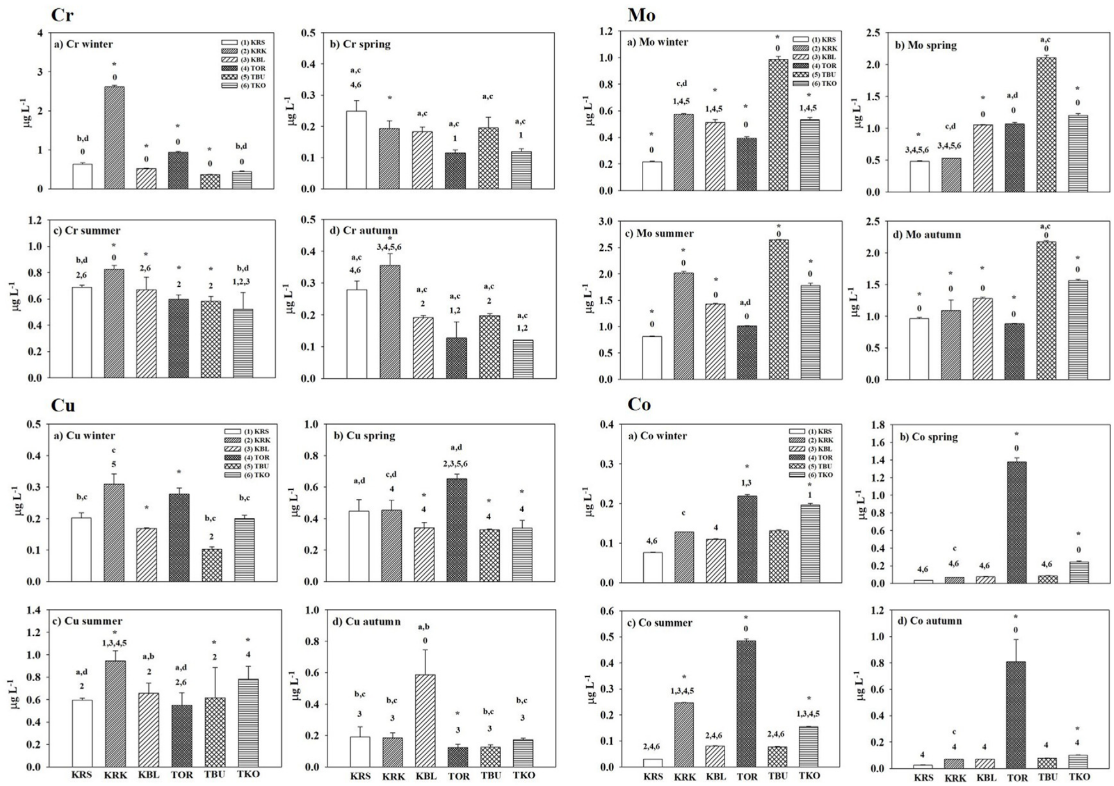


**Fig. 2.** Dissolved concentrations of metal(loid)s with maximum average concentrations 0.5–3 μg $L^{-1}$ in water from six sites of the Krka River sampled in four seasons. The description of the labels (numbers and letters) are given in Section 2.5 "Data processing and statistics".

naturally higher in the soils of coastal Croatia (where Krka River is located) than in central Croatia (location of Mrežnica River) due to differences in geologic and lithogenic composition in these two regions (Halamić and Miko, 2009; Halamić et al., 2012), Krka River water samples probably also reflect a more significant impact of the active screw factory on the Krka River compared to the influence of the former textile industry (closed in 2015) which was located on the Mrežnica River.

However, although the Krka River is more contaminated than those pristine Croatian karst ecosystems mentioned above, comparison with other Croatian rivers of different degrees of contamination (Sava, Ilova and Sutla rivers) pointed to lower concentrations of most elements in the Krka River than in those moderately to heavily contaminated rivers (Table S4). The main exceptions were Co, Cr, Cu, Mn and Ni which sometimes showed even higher concentrations at some sites of the Krka River than at the polluted sites of the Sava, Sutla and Ilova rivers (Dragun et al., 2009, 2011; Filipović Marijić et al., 2016; Mijošek et al., 2020).

Based on the above, the water quality of the Krka River is still mostly good considering metal contamination and the impact of wastewaters and agriculture can be considered as moderate. Nevertheless, we were still able to recognize possible threats for the whole karst ecosystem, especially national park (Figs. 1–4).

### *3.3. Spatial and temporal variability of dissolved metal(loid) concentrations in the upper part of the Krka River catchment*

Metal(loid) concentrations varied considerably between sites and seasons (Figs. 1–4), although elements accumulation was shown to be more site than season dependent. Therefore, temporal (4 seasons) and spatial trends (8 locations, including tributaries) of metal(loid) concentrations were presented for the first time for the upper part of the Krka River watercourse showing some specific but also long-term trends.

#### *3.3.1. Spatial variability of dissolved metal(loid) concentrations in the upper course of the Krka River basin*

Concentrations of dissolved metal(loid)s in water mostly pointed to significant element increases at anthropogenically affected sites compared to the reference location, but differences between all sites were often observed (Figs. 1–4). Concentrations of elements at TKR, sampled only in two seasons, were in similar ranges as KRS in winter and spring, so it can be considered as an additional reference site. Spatial differences were only sometimes comparable, but generally, concentrations of Co, Fe, K, Mn and Zn were the highest at TOR, of Cr and Cu at KRK, of Ca and Se at TKO, of Mg, Mo,

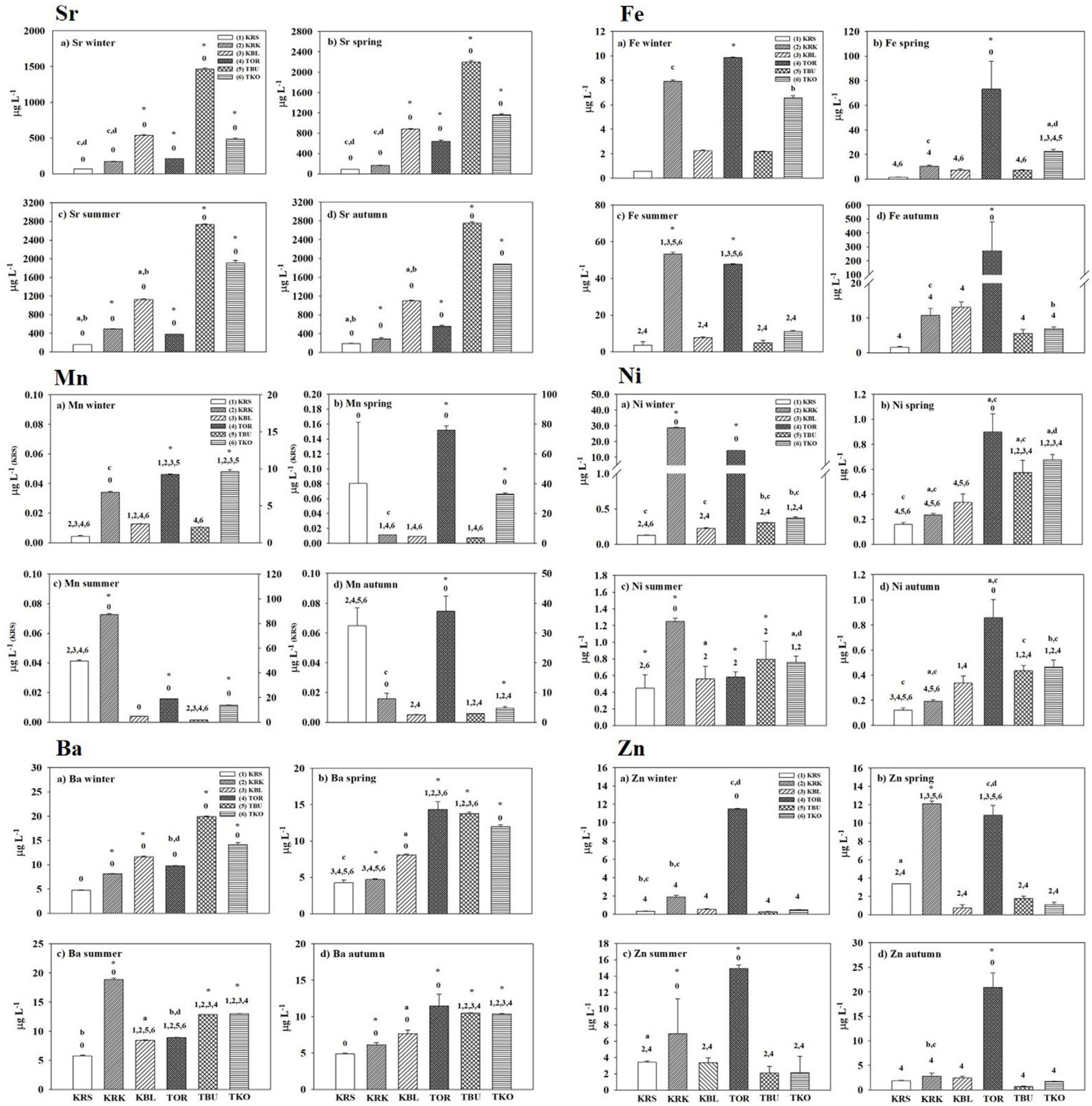


**Fig. 3.** Dissolved concentrations of metal(loid)s with maximum average concentrations ≥3 μg L$^{-1}$ in water from six sites of the Krka River sampled in four seasons. The description of the labels (numbers and letters) are given in Section 2.5 "Data processing and statistics".

and Sr at TBU, and of Cd and Tl at KRS, while other elements showed considerable variations depending on the season (Figs. 1–4). Overall, metal contamination followed the order: TOR>KRK>TKO>TBU>KBL>KRS.

Altogether, the most pronounced spatial differences existed between KRS and the two wastewater impacted sites, TOR and KRK (Figs. 1–4). That was especially evident for Ba, Co, Fe, K, Na, Ni, Sb and Zn, as consistent with the previous research in this area (Cukrov et al., 2008; Filipović Marijić et al., 2018). Many of these elements, including Co, Fe, Ni and Zn are regularly used in different kinds of industrial activities (Wang et al., 2005; Sertić Perić et al., 2018; Gemeda et al., 2021). If KRK and TOR are compared, Mg, K, Ca, Na, Mn, Zn, Ba and Sr were mostly significantly higher at TOR, influenced by industrial wastewaters, while As, Cr, and Mo had higher concentrations at KRK, influenced by municipal wastewaters. Therefore, TOR, a potential recipient of waters from IWW, represents a direct connection and the most significant source of metal(loid)s contamination directly in the Krka River watercourse.

Nevertheless, many significant differences were also observed between other two tributaries (TBU and TKO) and KRS (Figs. 1–4). If TBU and TKO are compared, more elements had higher concentrations in TKO, except Ba, Cr, Mg, Mo, Sr, and Tl, which were higher in TBU, probably as a result of more extensive agricultural activity in this area and nearby field Kninsko polje. Tributary Kosovčica is also influenced by agriculture from the nearby Kosovo polje, but also by gypsum factory, whose wastewaters, resulting from mining activities and gypsum production, may be contaminated with variety of

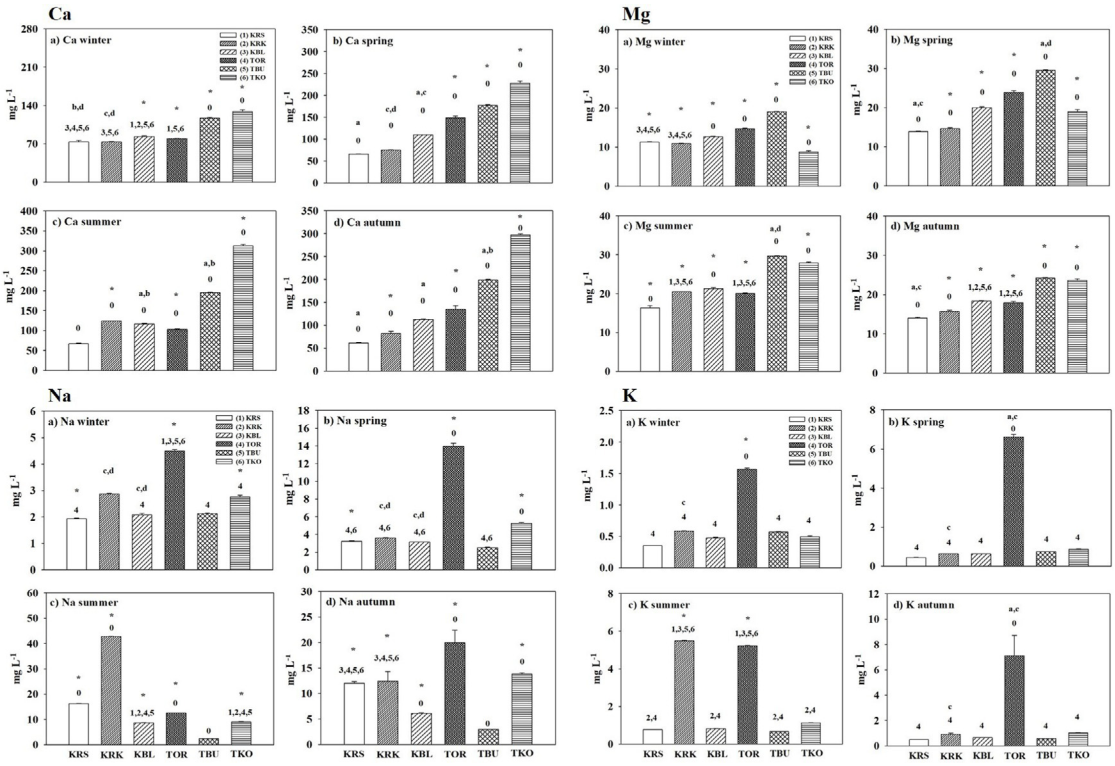


**Fig. 4.** Dissolved concentrations of macroelements in water from six sites of the Krka River sampled in four seasons. The description of the labels (numbers and letters) are given in Section 2.5 "Data processing and statistics".

metals (Carbonell-Barrachina et al., 2002; Mihara et al., 2008; Ternjej et al., 2014). In the previous research of Ternjej et al. (2014), investigating the impact of gypsum mine water on organisms in Kosovčica River, concentrations of most elements were mostly lower than in our research, indicating an increase of the pollution impact over time. However, concentrations at TBU and TKO are still mostly lower than at KRK and TOR, with the exceptions of Ca, Cd, Mg, Mo, Sb, Se, and Sr in some seasons (Figs. 1–4), which are elements that can often be found in fertilizers and/or phosphogypsum (Carbonell-Barrachina et al., 2002; Thomas et al., 2012; Mijošek et al., 2020). Concentrations of Cd and Tl tended to be higher in the reference site than in the downstream, anthropogenically affected sites of the Krka River. Martinez et al. (2002) assumed that elevated Cd concentrations in river sources are often associated with leaching of soil enriched with organic matter and sulphur. Another explanation is that limestones and dolomites, predominant in Dinaric area, are enriched with Cd compounds in karst areas (Cukrov et al., 2008).

Comparison of concentrations of metal(loid)s at KBL, chosen site located in the protected KNP, with river source water (KRS) showed higher concentrations of Ba, Ca, Cu, Fe, Mg, Mo, and Sr, and lower of Cd, Cs, Mn, and Na at KBL (Figs. 1–4), while concentrations of other elements were comparable. In general, metal concentrations were higher in the anthropogenically affected sites than in KBL, while the higher levels in the national park were found for Mo and Tl than in TOR, for Sr than in KRK and TOR, and for Cs than in TBU (Figs. 1–3). Such trends confirm that the impact of wastewaters is still not significant in the area of KNP. However, enhanced accumulation of some elements and higher concentrations of many elements compared to the river source, represent a possible danger to preserving this sensitive ecosystem in the future. This is still not cause for concern since numerous small cascade lakes are formed by tufa-barriers in the central course of the Krka River and they serve as traps for trace elements, resulting in self-purification of the water and prevention of downstream metal(loid)s increase (Cukrov et al., 2008). This process is enabled by the strong and intensive sedimentation, which starts in KBL, so even lower metal concentrations can probably be expected in the area of the KNP in the downstream direction (Cukrov et al., 2008). This is also supported by a comparison of physico-chemical parameters, which over the years did not show an evident increase in pollution impact in the area of KNP (Filipović Marijić et al., 2018; Šariri et al., submitted for publication).

Observed differences in metal concentrations between sites were generally supported by the spatial distribution of physico-chemical parameters (Šariri et al., submitted for publication). Namely, waters at TOR, KRK and TKO were classified as waters below good quality (GRC, 2019) in all seasons due to COD and content of nutrients, which, together with metal accumulation, confirmed long-term impact of municipal and industrial wastewaters (Mihaljević et al., 2011; Ternjej et al., 2014; Filipović Marijić et al., 2018; Sertić Perić et al., 2018; Šariri et al., submitted for publication). On the other hand, the best ecological conditions considering physico-chemical parameters were confirmed in KRS and TKR, which were classified as waters of very good quality, while the water parameters at TBU were either within the range for very good or good ecological status (GRC, 2019), all supporting the patterns of metal accumulation in our research (Šariri et al., submitted for publication), but also previous findings of Filipović Marijić et al. (2018).

### 3.3.2. Temporal variability of dissolved metal(loid) concentrations in the Krka River basin

Temporal variability of metal(loid) concentrations may depend on different factors such as water level and flow, rain intensity, soil erosion, pH values, water hardness, agricultural activities or wastewater discharge. In our research, higher water levels representing wet seasons were recorded in winter and spring (219 cm in January; 117 cm in April) and lower in summer and autumn as dry seasons (77 cm in July; 80 cm in October), according to Croatian Meteorological and Hydrological Service.

Dissolved concentrations of many metal(loid)s often differed considerably between seasons in many locations (Figs. 1–4). However, the uniform seasonal variations pattern of most elements was not obvious, except for significantly higher values observed in summer at most locations, probably due to the lowest water level (77 cm), which additionally reduces the dilution effects and the self-purification ability of the Krka River. The high summer concentrations pattern was evident for Cs, Cu, Mg, Mo, Na, Sb, Sr, and mostly for As, Ba, and Ca (Figs. 1–4). On the contrary, the lowest concentrations of Ca, Co, Cs, Cu, Fe, K, Mg, Mn, Mo, Na, Sr, Tl, and Zn were observed in winter (Figs. 1–4), the season specified by the highest precipitation, when high water level and water velocity contribute to the dilution and more effective purification processes which decrease metal concentrations. However, some elements showed the opposite trend and the highest concentrations in winter were mostly observed for Cd, Cr, Ni and Se, as well as for As and Ba in some locations (Figs. 1–3), probably due to considerable rainfalls which wash down the waste and can cause soil erosion and metal desorption from sediments to the water column (Dural et al., 2007; Gunes, 2022). Some of these elements, like As, Cd, Cr, and Ni, are often found in fertilizers (Thomas et al., 2012) and additional rains or floods can contribute to the wash-up of agricultural soils to the river water (Mijošek et al., 2020).

Most elements showed differences among seasons in almost all locations, except Fe, K, Mn, Sb and Zn, which showed poor temporal changes compared to other elements (Figs. 1–4). Namely, significant differences between seasons were observed for Fe and K only at KRK and TOR, for Mn and Sb at KRK, TOR and TKO, and for Zn in KRS, KRK and TOR, showing that temporal variability of many elements at the wastewater impacted sites probably often depends on their (ir)regular wastewaters discharges.

Therefore, seasonal changes in the dissolved metal(loid)s concentrations are partially influenced by wastewaters inputs and partially by the rainfalls and water level, which were at a maximum in winter and a minimum in summer.

Previous research by Filipović Marijić et al. (2018) in this area, which covered only two seasons (spring and autumn), pointed to significantly higher levels of majority of elements in dry season. That was not the case in our study, but such inter-annual differences are normal for water samples, depending on the changes in flood season duration, rain intensity or the level of anthropogenic pressure (Guo et al., 2022). This highlights the need of long-term monitoring, to get the most reliable results and accurate estimation of water quality of the ecosystem.

## 3.4. Ratios of dissolved and total metal(loid) concentrations in the upper course of the Krka River

Dissolved elements are easily transported over longer distances and are much more available, and, as such, are potentially more toxic to aquatic organisms than the elements which are mostly attached to particles (Janssen et al., 2003; de Paiva Magalhães et al., 2015; Adams et al., 2020). Therefore, it is important to know the distribution of elements between dissolved and particulate phase since average ratios of dissolved and total concentrations of elements might give us basic information on the metal(loid)s behavior in the Krka River and their tendency to bind with particulate matter (Table 1). It is commonly expected that the dissolved concentrations of trace metals in waters, which are available to aquatic organisms, are lower than the total concentrations of those elements (Cleven et al., 2005).

All macroelements were almost completely ($\geq$95%) present in the dissolved fraction in all locations of the Krka River, regardless of the season (Table 1). Many trace elements (Co, Sb, Ba, Se, Sr and Mo) also showed high presence ($>$90%) in dissolved fraction. Average ratios of dissolved to total concentrations of Tl, Ni, As, Mn, Cr, Cd and Cu ranged between 70 and 90% pointing to their still relatively low affinity of binding to particles in this ecosystem and high bioavailability for organisms. Average ratio of Zn was 68.6%, while the only elements with average presence in dissolved form below 50% were Cs and Fe (Table 1), pointing to their predominant association with particles and/or suspended matter and consequently lower risk of manifestations of their toxicity.

Regarding the spatial distribution of the dissolved/total ratios, the presence of macroelements in dissolved form at the IWW location did not differ significantly from the locations in the Krka River watercourse. However, majority of trace elements, including Co, Ba, Se, Mo, As, Cr, Cu, Zn and Fe, showed lower ratios of dissolved to total concentrations at IWW than other locations, indicating higher portion of trace metals associated to particulate matter of the industrial source (Table 1). Smaller spatial differences were also evident between other locations in the Krka River, showing lower ratios of many elements (Se, Sb, Co, Tl, Ni, As, Mn, Cr, Cd, Cu, Fe) in some of the anthropogenically affected locations (e.g. KRK, TOR, TBU, TKO), which have more particulate and suspended matter from an anthropogenic source. This is also supported by the highest values of TDS, TOC and DOC recorded previously in IWW and anthropogenically influenced sites of the

**Table 1**
Average ratios of dissolved and total metal(loid) concentrations in the investigated locations of the Krka River in four seasons.

| | Mg | Na | Mo | K | Sr | Se | Ca | Ba | Sb | Co | Tl | Ni | As | Mn | Cr | Cd | Cu | Zn | Cs | Fe |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | % | % | % | % | % | % | % | % | % | % | % | % | % | % | % | % | % | % | % | % |
| **IWW** | | | | | | | | | | | | | | | | | | | | |
| **average** | 100.3 | 102.1 | 90.5 | 98.6 | 96.8 | 85.9 | 99.1 | 87.0 | 84.1 | 74.9 | 75.5 | 79.1 | 60.1 | 89.8 | 14.8 | 91.6 | 32.6 | 58.1 | 96.1 | 4.0 |
| **SD** | 4.4 | 5.6 | 13.8 | 2.8 | 3.6 | 10.1 | 5.7 | 17.2 | 15.0 | 39.3 | 3.4 | 31.6 | 26.1 | 11.8 | 11.1 | 21.1 | 20.0 | 44.4 | 4.9 | 2.2 |
| **KRS** | | | | | | | | | | | | | | | | | | | | |
| **average** | 100.8 | 101.2 | 98.1 | 98.8 | 98.1 | 97.8 | 95.6 | 95.6 | 86.0 | 95.5 | 98.2 | 88.2 | 92.9 | 76.6 | 86.9 | 92.4 | 74.8 | 68.0 | 64.9 | 33.3 |
| **SD** | 4.5 | 8.1 | 2.8 | 3.8 | 5.0 | 11.4 | 11.3 | 7.7 | 7.4 | 3.7 | 5.2 | 5.8 | 4.8 | 7.4 | 12.4 | 10.7 | 47.9 | 57.5 | 22.3 | 26.5 |
| **KRK** | | | | | | | | | | | | | | | | | | | | |
| **average** | 103.1 | 102.1 | 96.8 | 99.8 | 98.7 | 93.8 | 99.8 | 93.8 | 98.1 | 90.3 | 79.5 | 92.0 | 89.9 | 91.9 | 91.3 | 65.8 | 65.8 | 78.4 | 54.6 | 24.1 |
| **SD** | 3.3 | 5.4 | 7.5 | 3.2 | 3.0 | 22.1 | 4.3 | 2.2 | 17.8 | 6.1 | 33.8 | 14.5 | 11.8 | 10.0 | 15.1 | 33.0 | 35.5 | 6.5 | 12.5 | 8.5 |
| **KBL** | | | | | | | | | | | | | | | | | | | | |
| **average** | 102.8 | 102.7 | 100.2 | 98.8 | 99.6 | 103.2 | 96.4 | 98.2 | 94.3 | 96.3 | 97.4 | 84.0 | 92.1 | 79.8 | 89.4 | 72.6 | 73.0 | 61.1 | 42.6 | 35.4 |
| **SD** | 4.4 | 4.7 | 3.5 | 4.6 | 2.8 | 6.0 | 6.0 | 2.8 | 3.7 | 6.8 | 14.7 | 12.4 | 5.3 | 14.9 | 6.2 | 6.2 | 17.9 | 13.4 | 27.8 | 36.1 |
| **TOR** | | | | | | | | | | | | | | | | | | | | |
| **average** | 101.4 | 102.2 | 100.7 | 98.4 | 98.3 | 98.0 | 97.4 | 95.4 | 95.2 | 88.4 | 76.8 | 82.2 | 74.6 | 89.3 | 57.6 | 37.2 | 72.1 | 62.8 | 50.4 | 25.6 |
| **SD** | 4.1 | 4.8 | 2.9 | 2.7 | 2.1 | 15.8 | 4.7 | 3.7 | 5.7 | 11.3 | 14.0 | 24.5 | 6.6 | 9.4 | 14.0 | 16.7 | 17.8 | 21.0 | 28.5 | 27.4 |
| **TBU** | | | | | | | | | | | | | | | | | | | | |
| **average** | 102.5 | 100.5 | 100.7 | 99.1 | 98.6 | 98.4 | 94.6 | 96.3 | 86.0 | 86.1 | 88.3 | 89.6 | 87.6 | 63.2 | 72.1 | 81.8 | 82.4 | 74.6 | 32.2 | 19.5 |
| **SD** | 3.9 | 5.8 | 3.9 | 4.2 | 2.3 | 13.3 | 1.4 | 2.3 | 9.5 | 19.1 | 11.8 | 9.9 | 10.9 | 24.0 | 3.3 | 15.6 | 29.2 | 33.1 | 29.2 | 17.1 |
| **TKO** | | | | | | | | | | | | | | | | | | | | |
| **average** | 103.6 | 102.4 | 101.7 | 100.3 | 100.3 | 97.0 | 97.9 | 98.0 | 97.7 | 86.0 | 84.2 | 86.8 | 83.3 | 78.0 | 69.8 | 76.8 | 55.4 | 66.7 | 22.9 | 10.8 |
| **SD** | 3.5 | 5.4 | 2.4 | 3.2 | 2.3 | 17.1 | 4.1 | 1.8 | 5.8 | 14.1 | 8.4 | 7.8 | 7.6 | 25.8 | 20.0 | 5.7 | 13.9 | 28.0 | 12.8 | 2.3 |
| **Average*** | 102.4 | 101.9 | 99.7 | 99.2 | 98.9 | 98.0 | 97.0 | 96.2 | 92.9 | 90.4 | 87.4 | 87.1 | 86.7 | 79.8 | 77.9 | 71.1 | 70.6 | 68.6 | 44.6 | 24.8 |
| **SD** | 1.1 | 0.8 | 1.9 | 0.7 | 0.8 | 3.0 | 1.8 | 1.7 | 5.5 | 4.5 | 9.0 | 3.6 | 6.9 | 10.3 | 13.5 | 18.9 | 9.2 | 6.7 | 15.3 | 9.1 |
| **RSD** | 1.0 | 0.8 | 1.9 | 0.7 | 0.9 | 3.1 | 1.9 | 1.7 | 5.9 | 5.0 | 10.3 | 4.1 | 7.9 | 12.9 | 17.3 | 26.5 | 13.0 | 9.8 | 34.3 | 36.6 |

*Final average value corresponds to the locations in the Krka River watercourse, without IWW which were separated as in the whole paper.

Krka River (Šariri et al., submitted for publication) which are all well known to complex trace elements (Buffle, 1988). Seasonal patterns were not always uniform and were probably caused by both natural and anthropogenic environmental conditions. Average ratios of dissolved and total metal(loid) concentrations in locations of the Krka River followed the order: Mg>Na>Mo>K>Sr>Se>Ca>Ba>Sb>Co>Tl>Ni>As>Mn>Cr>Cd>Cu>Zn>Cs>Fe (Table 1).

Altogether, high variability in average ratios of dissolved and total metal(loid) concentrations between locations, supported by higher RSD values (>15%), were confirmed only for Cr, Cd, Cs and Fe (Table 1), possibly as a consequence of irregular discharges of these elements from industry, agriculture and municipal activities. Although more particulate matter at wastewater impacted locations can bind higher amount of some metals and decrease their dissolved concentrations, generally much higher concentrations at these sites, despite binding capacity, still point to significant risk of negative effects on the Krka River and possible toxic effects for biota.

## 4. Conclusions

Conducted research showed spatial and temporal variations in metal(loid) concentrations in the Krka River, reflecting direct and long-term consequences of wastewater discharges and physico-chemical water properties in this sensitive karst area. The highest element concentrations were mostly observed in summer, as the dry season characterized by the lowest water level and poor purification processes in the river and its tributaries. Dissolved metal concentrations indicated clean, pristine conditions at the river source, while industrial wastewaters contained the highest concentrations of all elements, particularly Mn, Zn, Co, Cs, and Fe, than other locations. The most significant impact of IWW was observed at its closest site TOR, which turned out to be the most contaminated site in the upper reaches of the Krka River. Although IWW was found to be the most important source of many elements, municipal effluents of the Town of Knin, agricultural practice, and gypsum factory were the main route of contamination with As, Ca, Cr, Mg, Mo, and Sr, showing the highest concentrations in KRK, TBU or TKO. Brljan Lake (KBL), as the location in the KNP, had higher concentrations of Ba, Ca, Cu, Fe, Mg, Mo, and Sr than KRS. However, most of the levels were still much lower than at other anthropogenically affected locations, confirming the moderate impact of the contamination sources on the KNP and self-purification processes specific to karst flow systems.

Most elements, except for Zn, Cs and Fe, were present in dissolved fraction with >70%, showing a low tendency to bind with particulate matter and suggesting possible high risk of toxicity during time. The long-term comparisons showed increased concentrations of most elements over time in the Krka River watercourse, especially for Cr, Cu, Fe, Mn, and Ni, as elements often used in industrial activities. Altogether, the influence of industrial and municipal activities over time still seems moderate in the Krka River but observed differences and metal increases emphasize possible accumulation over time. Therefore, continuous monitoring and control of contamination sources and adequate wastewater treatment are required to protect this karst ecosystem.

Our study contributes to a better understanding of the dynamics of dissolved and total metal(loid) concentrations in sensitive karst river systems under anthropogenic impact, represented by the Krka River, a typical karst basin of the Dinaric region, which is one of the largest karst areas in Europe. In this way, both the vulnerability of the karst ecosystem in general and the potential threat to the Krka National Park were assessed.

## CRediT authorship contribution statement

**Tatjana Mijošek:** Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Zorana Kljaković-Gašpić:** Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Tomislav Kralj:** Investigation. **Damir Valić:** Investigation. **Zuzana Redžović:** Investigation. **Sara Šariri:** Formal analysis, Investigation. **Ivana Karamatić:** Investigation. **Vlatka Filipović Marijić:** Conceptualization, Investigation, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

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## References

Adams, W., Blust, R., Dwyer, R., Mount, D., Nordheim, E., Rodriguez, P.H., Spry, D., 2020. Bioavailability assessment of metals in freshwater environments: A historical review. Environ. Toxicol. Chem. 39 (1), 48–59.

Bakalowicz, M., 2005. Karst groundwater: a challenge for new resources. Hydrogeol. J. 13 (1), 148–160. http://dx.doi.org/10.1007/s10040-004-0402.

Bravo, A.G., Kothawala, D.N., Attermeyer, K., Tessier, E., Bodmer, P., Amouroux, D., 2018. Cleaning and sampling protocol for analysis of mercury and dissolved organic matter in freshwater systems. MethodsX 5, 1017–1026. http://dx.doi.org/10.1016/j.mex.2018.08.002.

Brinkmann, R., Parise, M., 2012. Karst environments: problems, management, human impacts, and sustainability. J. Caves Karst Stud. 74 (2), 135–136. http://dx.doi.org/10.4311/2011JCKS0253.

Buffle, J., 1988. Complexation reactions in aquatic systems: an analytical approach. Ellis - Horwood Ltd, Chichester, England.

Campanale, C., Losacco, D., Triozzi, M., Massarelli, C., Uricchio, V.F., 2022. An overall perspective for the study of emerging contaminants in Karst aquifers. Resources 11 (105), http://dx.doi.org/10.3390/resources11110105.

Carbonell-Barrachina, A., DeLaune, R.D., Jugsujinda, A., 2002. Phosphogypsum chemistry under highly anoxic conditions. Waste Mgmt 22, 657–665.

Cleven, R., Nur, Y., Krystek, P., van den Berg, G., 2005. Monitoring metal speciation in the rivers meuse and rhine using DGT. Water Air Soil Pollut. 165, 249–263.

Cukrov, N., Cmuk, P., Mlakar, M., Omanović, D., 2008. Spatial distribution of trace metals in the Krka River, Croatia. An example of the selfpurification. Chemosphere 72 (10), 1559–1566. http://dx.doi.org/10.1016/j.chemosphere.2008.04.038.

Cukrov, N., Tepić, N., Omanović, D., Lojen, S., Bura-Nakić, E., Vojvodić, V., Pižeta, I., 2012. Qualitative interpretation of physico-chemical and isotopic parameters in the Krka River (Croatia) assessed by multivariate statistical analysis. Int. J. Environ. An. Ch. 92 (10), 1187–1199. http://dx.doi.org/10.1080/03067319.2010.550003.

Dautović, J., 2006. Determination of Metals in Natural Waters using High Resolution Inductively Coupled Plasma Mass Spectrometry Bachelor of Science thesis. University of Zagreb (in Croatian).

Dautović, J., Fiket, Ž., Barešić, J., Ahel, M., Mikac, N., 2014. Sources, distribution and behavior of major and trace elements in a complex karst lake system. Aquat. Geochem. 20 (1), 19–38. http://dx.doi.org/10.1007/s10498-013-9204-9.

de Paiva Magalhães, D., da Costa Marques, M.R., Baptista, D.F., Buss, D.F., 2015. Metal bioavailability and toxicity in freshwaters. Environ. Chem. Lett. 13, 69–87.

Dragun, Z., Kapetanović, D., Raspor, B., Teskeredžić, E., 2011. Water quality of medium size watercourse under baseflow conditions: the case study of river Sutla in Croatia. Ambio 40 (4), 391–407. http://dx.doi.org/10.1007/s13280-010-0119-z.

Dragun, Z., Roje, V., Mikac, N., Raspor, B., 2009. Preliminary assessment of total dissolved trace metal concentrations in Sava river water. Environ. Monit. Assess. 159, 99–110.

Dragun, Z., Stipaničev, D., Fiket, Ž., Lučić, M., Udiković Kolić, N., Puljko, A., Repec, S., Šoštarić Vulić, Z., Ivanković, D., Barac, F., Kiralj, Z., Kralj, T., Valić, D., 2022. Yesterday's contamination–A problem of today? The case study of discontinued historical contamination of the Mrežnica River (Croatia). Sci. Total Environ. 848, 157775.

Dural, M., Goksu, M.Z.L., Ozak, A.A., 2007. Investigation of heavy metal levels in economically important fish species captured from the Tuzla Lagoon. Food Chem 102, 415–421. http://dx.doi.org/10.1016/j.foodchem.2006.03.001.

European Parliament and the Council of the European Union (EPCEU), 2013. Directive 2013/ 39/EU of the European parliament and of the council of 12 2013 amending directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. O. J. L. 226. p. 1.

Filipović Marijić, V., Kapetanović, D., Dragun, Z., Valić, D., Krasnići, N., Redžović, Z., Grgić, I., Žunić, J., Kružlicová, D., Nemeček, P., Ivanković, D., Vardić Smrzlić, I., Erk, M., 2018. Influence of technological and municipal wastewaters on vulnerable karst riverine system, Krka River in Croatia. Environ. Sci. Pollut. Res. 25, 4715–4727. http://dx.doi.org/10.1007/s11356-017-0789-1.

Filipović Marijić, V., Sertić Perić, M., Matoničkin Kepčija, R., Dragun, Z., Kovarik, I., Gulin, V., Erk, M., 2016. Assessment of metal exposure, ecological status and required water quality monitoring strategies in small- to medium-size temperate rivers. J. Environ. Sci. Health A 51 (4), 309–317.

Gemeda, F.T., Guta, D.D., Wakjira, F.S., Gebresenbet, G., 2021. Occurrence of heavy metal in water, soil, and plants in fields irrigated with industrial wastewater in Sabata town, Ethiopia. Environ. Sci. Pollut. Res. 28, 12382–12396. http://dx.doi.org/10.1007/s11356-020-10621-6.

Government of the Republic of Croatia (GRC), 2019. Directive on water quality status. Official gazette of the republic of Croatia no. 96 (NN 96/19).

Government of the Republic of Croatia (GRC), 2020. Regulation on limit values for waste water emissions. Official gazette of the Republic of Croatia no. 26 (NN 26/20).

Gunes, G., 2022. The change of metal pollution in the water and sediment of the Bartın River in rainy and dry seasons. Environ. Eng. Res. 27, 200701.

Guo, W., Zou, J., Liu, S., Chen, X., Kong, X., Zhang, H., Xu, T., 2022. Seasonal and spatial variation in dissolved heavy metals in Liaodong Bay, China. Int. J. Environ. Res. Public Health 19 (608), http://dx.doi.org/10.3390/ijerph19010608.

Halamić, J., Miko, S. (Eds.), 2009. Geochemical Atlas of the Republic of Croatia. Croatian Geological Survey, Zagreb.

Halamić, J., Peh, Z., Miko, S., Galović, L., Šorša, A., 2012. Geochemical atlas of Croatia: Environmental implications and geodynamical thread. J. Geochem. Explor. 115, 36–46. http://dx.doi.org/10.1016/j.gexplo.2012.02.006.

Hartmann, A., Goldscheider, N., Wagener, T., Lange, J., Weiler, M., 2014. Karst water resources in a changing world: Review of hydrological modeling approaches. Rev. Geophys. 52, 218–242.

Janssen, C.R., Heijerick, D.G., De Schamphelaere, K.A.C., Allen, H.E., 2003. Environmental risk assessment of metals: tools for incorporating bioavailability. Environ. Int. 28, 793–800.

Kalhor, K., Ghasemizadeh, R., Rajić, L., Alshawabkeh, A., 2019. Assessment of groundwater quality and remediation in karst aquifers: A review. Groundw. Sustain. Dev. 8, 104–121. http://dx.doi.org/10.1016/j.gsd.2018.10.004.

Kisić, I., Zgorelec, Ž., Galić, M., Delač, D., 2019. Analysis of Mud and Water in Lagoons Polluted By Waste Materials in Knin. Zagreb, https://knin.hr/wp-content/uploads/2019/07/Zavrsno-izvijesce-Analiza-mulja-DIV-ove-lagune.pdf.

Kovačič, G., Ravbar, N., 2003. Karst aquifers vulnerability or sensitivity? Acta Carsologica 32, 307–314.

Martinez, C.E., McBride, M.B., Kandianis, M.T., Duxbury, J.M., Yoon, S., Bleam, W.F., 2002. Zinc sulfur and cadmium-sulfur association in metalliferous peats: evidence from spectroscopy, distribution coefficients, and phytoavailability. Environ. Sci. Technol. 36, 3683–3689.

Mihaljević, Z., Ternjej, I., Stanković, I., Ivković, M., Želježić, D., Mladinić, M., Kopjar, N., 2011. Assessment of genotoxic potency of sulfate-rich surface waters on medicinal leech and human leukocytes using different versions of the Comet assay. Ecotoxicol. Environ. Safety 74, 1416–1426.

Mihara, N., Soya, K., Kuchar, D., Fukuta, T., Matsuda, H., 2008. Utilization of calcium sulfide derived from waste gypsum board for metal-containing wastewater treatment. Glob. NEST J. 10, 101–107.

Mijošek, T., Filipović Marijić, V., Dragun, Z., Ivanković, D., Krasnići, N., Redžović, Z., Sertić Perić, M., Vdović, N., Bačić, N., Dautović, J., Erk, M., 2020. The assessment of metal contamination in water and sediments of the lowland Ilova river (Croatia) impacted by anthropogenic activities. Environ. Sci. Pollut. Res. 27, 25374–25389.

Padilla, I.Y., Irizarry, C., Steele, K., 2011. Historical contamination of groundwater resources in the north coast karst aquifer of Puerto Rico. Dimension 25 (3), 7–12.

Padilla, I.Y., Vesper, D.J., 2018. Fate, transport, and exposure of emerging and legacy contaminants in karst systems: State of knowledge and uncertainty. In: White, W., Herman, J., Herman, E., Rutigliano, M. (Eds.), Karst Groundwater Contamination and Public Health. In: Advances in Karst Science, Springer, Cham, http://dx.doi.org/10.1007/978-3-319-51070-5_5.

Šariri, S., Valić, D., Kralj, T., Cvetković, Ž., Mijošek, T., Redžović, Z., Karamatić, I., Filipović Marijić, V., n.d. Long-term and seasonal trends of water parameters in the karst riverine catchment and general literature overview based on CiteSpace. Appl. Water Sci. (submitted for publication).

Selak, A., Reberski, J.L., Klobučar, G., Grčić, I., 2022. Ecotoxicological aspects related to the occurrence of emerging contaminants in the Dinaric karst aquifer of Jadro and Žrnovnica springs. Sci. Total Environ. 825, 153827.

Sertić Perić, M., Matoničkin Kepčija, R., Miliša, M., Gottstein, S., Lajtner, J., Dragun, Z., Filipović Marijić, V., Krasnići, N., Ivanković, D., Erk, M., 2018. Benthos-drift relationships as proxies for the detection of the most suitable bioindicator taxa in flowing waters – a pilot-study within a Mediterranean karst river. Ecotoxicol. Environ. Safety 163, 125–135. http://dx.doi.org/10.1016/j.ecoenv.2018.07.068.

Telbisz, T., Mari, L., 2020. The significance of karst areas in European national parks and geoparks. Open Geosci. 12, 117–132.

Ternjej, I., Mihaljević, Z., Ivković, M., Previšić, A., Stanković, I., Maldini, K., Želježić, D., Kopjar, N., 2014. The impact of gypsum mine water: a case study on morphology and DNA integrity in the freshwater invertebrate, Gammarus balcanicus. Environ. Pollut. 189, 229–238. http://dx.doi.org/10.1016/j.envpol.2014.03.009.

Thomas, E.Y., Omueti, J.A.I., Ogundayomi, O., 2012. The effect of phosphate fertilizer on heavy metal in soils and *Amaranthus caudatus*. Agric. Biol. J. N. Am. 3, 145–149.

Vadillo, I., Ojeda, L., 2022. Carbonate aquifers threatened by contamination of hazardous anthropic activities: Challenges. Curr. Opin. Environ. Sci. Health 26, 100336. http://dx.doi.org/10.1016/j.coesh.2022.100336.

Vega, M., Pardo, R., Barrado, E., Deban, L., 1998. Assessment of seasonal and polluting effects on the quality of river water by exploratory data analysis. Water Res. 32 (12), 3581–3592.

Wang, X.S., Qin, Y., Sang, S.X., 2005. Accumulation and sources of heavy metals in urban topsoils: a case study from the city of Xuzhou, China. Environ. Geol 48, 101–107. http://dx.doi.org/10.1007/s00254-005-1270-x.