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Geochemical Fractionation and Risk Assessment of Potentially Toxic Elements in Sediments from Kupa River, Croatia

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Abstract: This study investigated the quality of Kupa River sediment using sequential extraction, ecological risk, and contamination indexes (Risk assessment code, Index of geoaccumulation, Enrichment factor, Ecological risk factor, Ecological risk index), determination of magnetic susceptibility of sediments, and statistical methods. The BCR sequential extraction technique was used for evaluating various element-binding forms. Most of the elements were considered to be immobile due to the high availability in the residual fraction. Lead was present mainly in the reducible fraction, while more easily mobile and bioavailable forms were predominant for cadmium and barium. Sediment samples from the river catchment exhibited low ecological risk. The most toxic element, Cd, is the main contributor to the total potentially ecological risk. Increased values of contamination factors have been observed for Zn, Cr, and Ba in some localities. Results of the comparison of element contents in sediments in a 15-year period (2018 vs. 2003) indicated that the situation with toxic element content in sediments along Kupa River improved formost of its course. Unfortunately, on the lower course of the river, the situation has worsened. Using the example of Kupa River sediments, it was shown that the magnetic susceptibility method is excellent indetecting increased values of Cr.

Keywords: potentially toxic elements; Kupa River; Croatia; sediment; chemical fractions

1. Introduction

Rivers perform a suite of ecological functions such as water transport, aquaculture, agricultural irrigation, domestic water, and tourism. Fluvial systems are the most important dynamic systems, wherein their interaction with the continental crust causes constant recycling of the materials of the Earth's crust. With this, rivers are the prime carrier of sedimentary materials from continents to the ocean, which results from continental denudation, i.e., the synergic action of rock weathering and erosion [1].

In river systems, sediments are a sink for potentially toxic elements (PTEs) and other contaminants [2]. Monitoring of sediment contaminants and assessment of sediment quality are usually carried out with the objectives of determining the extent to which the sediments are either a source or a sink for contaminants. Contaminated sediments are in direct contact with the biota, especially the benthic organisms that they provide with habitat and food, leading to toxic effects and allowing the

bioaccumulation of contaminant species, determining their potential transfer through food chains, and eventually causing harm to human health [3].

Sediment can be considered as a heterogeneous mixture of dissimilar particles [4]. In sediments, PTEs can be bound to various compartments in different ways: occluded in amorphous materials; adsorbed on clay surfaces or iron/manganese oxyhydroxides; present in the lattice of secondary minerals like carbonates, sulphates, or oxides; and complexed with organic matter or lattice of primary minerals such as silicates [5]. PTEs in the unpolluted soils and sediments are generally immobile and bonded to the silicate and primary minerals. However, in the polluted soils, the metals are generally more mobile and bound to the soil and sediment fraction [6].

The need and importance for determining chemical forms, bioavailability, contamination, and ecological risks of toxic metals in the rivers is listed as significant in Cao et al. [7]. The sequential extraction procedure (SEP) is a continual multistep analysis which is used for evaluating various element-binding forms [8] and to characterize the element fractions in soils and sediments and predict their mobility and bioavailability [9]. The community Bureau of Reference of the Commission of the European Union initiated a major effort to harmonize different extraction procedures. Their works produced the definition of an extraction protocol (the BCR protocol) and a purely operational definition of sequential fractionation [5]. According to the BCR method, the acid-soluble, reducible, and oxidizable fractions show a tendency of decreasing bioavailability, while the residual fraction is not available [10]. The summation of the mobile and exchangeable fractions can be used to assess the environmentally available components.

In addition to assessing the mobility of toxic elements using the BCR method, certain indices are often used in evaluating soil and sediment contamination. Calculation of pollution indices (contamination factor, enrichment factor, index of geoaccumulation, ecological risk factor, potential ecological risk index, pollution load index, combined pollution index, modified degree of contamination, and the toxic units) focuses on estimation of the anthropogenic input of toxic elements, quantifying the degree of metal enrichment in sediments, assessment of the pollution status of the area, and estimation of the potential acute toxicity of studied contaminants.

The Kupa River, with its length of almost 300 km, is the longest and largest river and is situated with its whole length inside Croatia or at its border. Its drainage basin is situated in the central part of Croatia, joining karstic areas of the Adriatic carbonate platform with lowlands of Pannonian basin. Also, it is an international drainage basin, of which its parts besides Croatia belong to neighboring countries Slovenia and Bosnia and Herzegovina. This drainage basin is very important due to its water resources, which are often bound to vulnerable karstic aquifers. Also, parts of the Kupa River drainage basin are protected areas of nature, e.g., Risnjak and Plitvice Lakes National parks. All these reasons lead to the awareness that it is important to investigate geochemistry and ecological status of this unique river, which until the beginning of the current century, was very poorly investigated in terms of its geochemistry and mineralogy. First, systematic research of this area started in 2003 with the Ph.D. Thesis of Frančišković-Bilinski [11], who investigated in detail the geochemistry and mineralogy of stream sediments in the Kupa River drainage basin. This research revealed several important problems in this drainage basin, which attracted further detailed research. The most important of those findings was the discovery of an extremely large barium anomaly in Kupa River [12]. Kupa River can therefore be treated as an excellent "natural laboratory" to study sediment transport processes, the behavior of many elements including toxic elements and other geochemical processes, and interactions between water, sediment, and biota.

A detailed study on fluvial geomorphology of the Kupa River drainage basin was carried out by Frančišković-Bilinski et al. [13]. Besides fluvial geomorphology itself, this study also investigated the ecological state of Kupa River, which was found to be poor in some locations. During the summer months, Kupa River is getting locally moribund and arrested within its course due to the fall of the river level and extremely uneven bed level configuration along both the transverse and longitudinal profiles of the river. An influx of pollutants and their restricted circulation in many

places have led to severe eutrophication with macroalgal blooms and generation of marsh vegetation and these processes are occurring mostly in its lower course. Human interference by construction of weirs for hydroelectrical power plants, construction of embankments for bank protections, and other activities like dredging and mining of river bed in some places has further caused modifications to the morphology, morphodynamics, and grain size characteristics of the river channel. Contamination from barite mine tailings, agriculture, industries, and sewage waters has resulted in an alarming state on some stretches and therefore Frančišković-Bilinski et al. [13] have finally suggested the need for systematic monitoring of the Kupa River and its drainage basin.

For the assessment of contamination in the Kupa River sediment, which is the aim of the current work, a combination of different methods was implemented. In addition to determining the content of elements in sediment samples and their chemical forms, various formulas and indexes for the assessment of environmental and human health risks were applied. For determining chemical forms and bioavailability, the optimized BCR procedure was used. To evaluate element pollution in sediments, in this research, we used assessment methods: RAC (risk assessment code), Igeo (index of geoaccumulation), EF (inrichment factor), Eri (ecological risk factor), and RI (ecological risk index). Also, magnetic susceptibility of sediments and statistical methods were applied in this research.

2. Materials and Methods

2.1. Study Area

The Kupa River basin occupies the west-central part of Croatia and is shared by two neighboring countries (Slovenia and Bosnia and Herzegovina). This drainage basin and its characteristics are described in detail in [13] and the following details were taken from there. The Kupa itself is a tributary to the Sava River and meets the latter at Sisak after traversing a distance of 294 km. The Sava River belongs to the Danube River watershed and enters the Danube River at Belgrade (Serbia). The Kupa River drainage basin is situated at the very south of the Danube drainage basin. The map of Croatia with (a) a rectangle indicating the position of the studied area of the Kupa River drainage basin and (b) the course of the Kupa River and its catchment area showing sediment sampling locations are presented in Figure 1. The total area, 10,605 km^2, of the Kupa River drainage basin is divisible into several sub-basins as per its countrywide distributions: 79.32% belongs to Croatia, 18.32% to Slovenia, and 2.36% to Bosnia and Herzegovina. The river basin is one of the most significant water resources in Croatia. Although shared by other adjoining countries, about 85% of the river water, being chiefly derived from carbonate karst springs, river springs, precipitation, and run off, discharges on the Croatian side [13]. The karst aquifers of Dinarides are highly vulnerable because of rapid water exchange with the groundwater through numerous shallow holes. The availability of about 3.5 m^3/s of very good quality spring water has given strategic importance to the area based on which the whole Adriatic coast and numerous settlements in the continental area have come into existence [14].

In addition, the upper part of the Kupa River basin offers a high heritage value as it covers the Dinaric karst region of the Risnjak National Park and some other areas of national heritage. The Kupa spring is a vauclusian spring (called "the turquoise karst lake" by local inhabitants) in the Risnjak national Park in Gorski Kotar region. In the middle and lower part, however, the Kupa flows through flat, low-lying, alluvial terrains where contributions from a large number of tributaries play a vital role in modifying the bed load capacity, transportation process, discharge, granulometry, geomorphology, mineralogy, and geochemical properties of river sediments. The landscape associated with the Kupa drainage basin and its catchments owes its origin to its setting in a seismo-tectonically active karstic region where there is a complex integration of the effects of vertical and horizontal motions of the crustal rocks and surficial erosion-deposition processes. The geomorphic features of Kupa are the resultant product of seismic and inter-seismic deformations of country rocks, differential subsidence and tilting in the karstic terrain, erosion-deposition behavior of the drainage basins, climatic forcing, and hydrodynamic conditions [13].

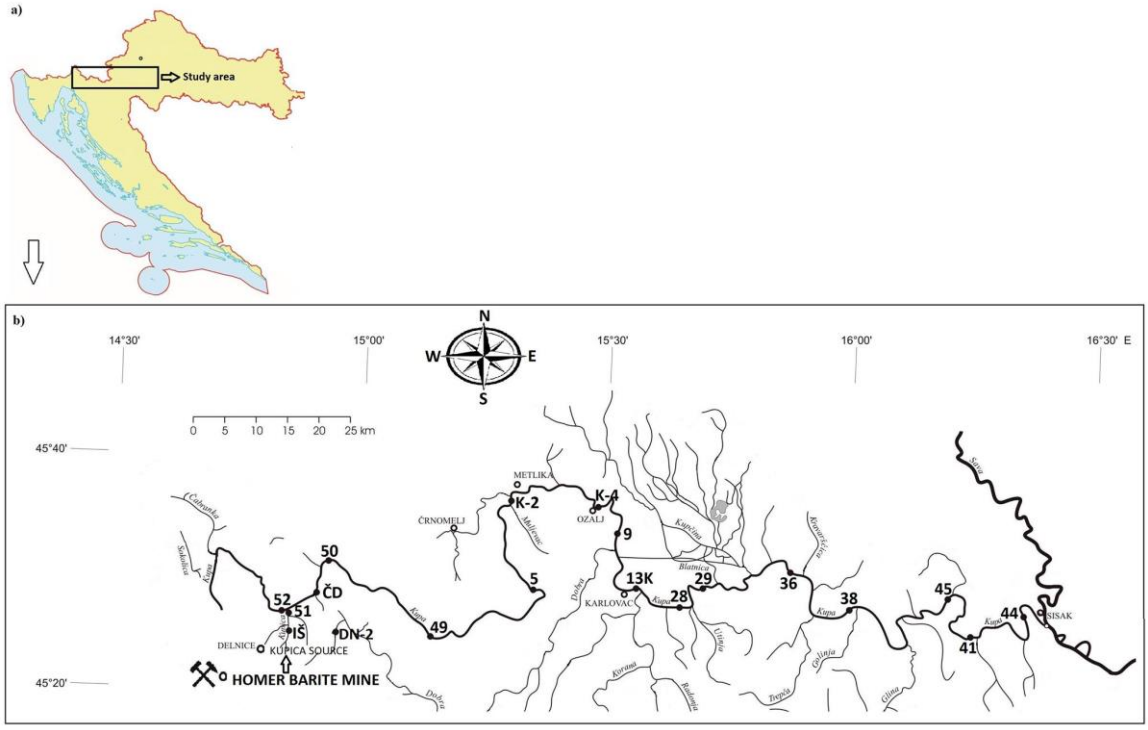

Figure 1. (**a**) Location of the study area; (**b**) Position of barite mine and sampling stations along the Kupa River.

2.2. Sampling and Sample Preparation

Sampling was performed on several most important locations along the Kupa River course in May 2018, exactly 15 years since the barium anomaly discovery [12], with the aim to see which processes happened during that time frame. During the 2018 sampling campaign, one sediment sample (DN-2) was taken in the uppermost part of Dobra River, one of the most important Kupa River tributaries, for comparison.

Positions of sampling locations taken along the Kupa River course are presented in Figure 1. Two locations (IŠ and 51) are on Kupica River, one location (52) is on Kupa River, upstream of the Kupica inflow, while all the other locations are in Kupa River, downstream from the Kupica River inflow. Locations where fine grained sediment accumulates along the river bank were chosen. On each sampling site, at least three grab samples of active fine-grained surface sediment (0–5 cm deep) were collected from different places in an area of 5 m^2. From this material, a composite sample was taken which weighed up to 1.5 kg. This procedure decreased the possible bias caused by local variability.

After sampling, the sediments were dried in air at room temperature and then sieved through 2000 µm and 63 µm sieves (Fritsch, Germany) to obtain two sediment fractions: fine fraction containing clay and silt (63 µm) and coarser fraction containing sand (63–2000 µm). Obtained sediment fraction < 63 µm were used for further analysis in this research.

2.3. Analytical Methods

2.3.1. Chemical Analysis

Sediment samples were analyzed by the optimized BCR three step sequential extraction procedure [15–18]. Sequential extractions were applied to 1 g of soil samples in 50 mL capacity centrifuge tubes. A detailed description of this method is shown in Supplementary Materials.

2.3.2. Determination of Element Concentrations and Quality Control

In this manuscript, the content of studied elements (Al, As, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, S, Si, Sr, Ti, V, and Zn) in the extracts obtained at each step of sequential extraction were determined by ICP/OES (iCAP-6500 Duo, Thermo Scientific, UK).

Quality control, accuracy, and precision of the measurement and concentration values were performed using certified reference material BCR 701. Acceptable accuracy (80–120%) and precision (≤20%) of elements was achieved for all steps of sequential extraction. The recovery rates for studied elements are shown in Table S1.

2.3.3. Determination of Magnetic Susceptibility

Magnetic susceptibility was measured using SM30, a small magnetic susceptibility meter, which thanks to its high sensitivity, can measure sediments and rocks with extremely low levels of magnetic susceptibility and in addition, can distinctly measure diamagnetic materials such as limestone, quartz, and also water. Sensitivity of SM30 is 1×10^{-7} SI units, which is about 10 times better than the sensitivity of most of the competitive instruments. Operating frequency is 8 kHz, measurement time is less than 5 s, and operating temperature is −20 °C to 50 °C. The SM30 has an 8 kHz LC oscillator with a large size pick-up coil as a sensor. The oscillation frequency is measured when the coil is put to the surface of the measured sample and when the coil is removed tens of centimeters away. Each sample was measured three times and the mean value was taken as the final result of measurement to assure as precise data as possible.

2.4. Assessment Methods

2.4.1. Risk Assessment Code (RAC)

The risk assessment code (RAC) was first introduced by Perin et al. [19] for evaluating the mobility and bioavailability of PTEs in sediments based on the exchangeable fraction (F1) content. The RAC can be calculated by the formula: RAC = $(C_{F1}/C_{tot}) \times 100\%$, where C_{F1} is the measured content of F1 and C_{tot} is the total content of the PTEs. In this manuscript, the total amounts of elements are defined as the sum of the five binding fractions. The degrees of RAC classification are presented in Table S2.

2.4.2. Index of Geoaccumulation (Igeo)

Index of geoaccumulation (Igeo) was applied to determine the metal pollution in the sediment samples. Index of geoaccumulation (Igeo) was calculated as Igeo = $\log_2$ [Cmetal/1.5 Cmetal (control)], where Cmetal is the concentration of the heavy metal in the sample and Cmetal (control) is the concentration of the metal in the unpolluted sample or control (background). The factor 1.5 is attributed to lithogenic a variation in the sediment [20]. This determination equation was introduced by Müller [21]. The geo-accumulation index (Igeo) scale consists of seven grades (0–6), ranging from unpolluted to highly polluted (as shown in Table S3).

2.4.3. Enrichment Factor (EF)

Enrichment Factor (EF) has been employed for the assessment of contamination in various environmental media by several researchers. The EF was calculated by using the following equation: $EF = (M/Y)_{sample}/(M/Y)_{background}$, where M is the concentration of the potentially enriched element and Y is the concentration of the proxy element. As mentioned by Sakan et al. [22], depleted elements have an EF < 1, which may reflect remobilization and loss of this element, whereas any enrichment due to either natural causes or anthropogenic influence is expressed by an EF > 1.

As background values for Igeo and EF, calculations in this manuscript used values for elements contents in sampling site K-4. These values were chosen as the background values because there are no significant anthropogenic sources of toxic elements at this locality and the sediment samples are similar

to the other investigated river sediments in geochemical characteristics and composition. Values of EF and pollution level are shown in Table S4.

2.4.4. Ecological Risk Factor (Eri) and Risk Index (RI)

Ecological risk index (RI) can measure the sensitivity of biological community to the overall heavy metal contamination at one site [23]. The RI was calculated using the following equation: $RI = \sum (Tri \times Ci/C_0)$, where Ti is the toxic-response factor for a given substance and indicates the toxicity level of heavy metal i and the sensitivity of organisms to heavy metal i. The Tri values for Zn, Pb, Cu, As, Ni, Cd, and Cr are 1, 5, 5, 10, 5, 30, and 2, respectively. Ci represents the metal content in the sediment and C_0 is the regional background value of heavy metals. Ecological risk factor (Eri) was calculated using the following equation: $Eri = Tri \times Ci/C_0$. Category of Eri and RI are shown in Table S5.

2.5. Statistical Analysis

Descriptive data analyses (mean, median, standard deviation, maximum and minimum concentrations) were performed in this research. The statistical analyses done in this study also include Pearson correlation coefficients, principal component analysis (PCA) and Hierachical cluster analysis (HCA). The statistical analyses were performed using IBM SPSS Statistics-Statistical Package for the Social Sciences (SPSS) (version 21.0).

3. Results and Discussion

3.1. Comparison of Element Contents in Sediments of Kupa River in 15-Year Period (2018 vs. 2003)

In the current part, element concentrations changes in the 15-year period (2018 vs. 2003) will be discussed going in the downstream direction (Table 1). Data from 2018 were obtained by the current study, while data from 2003 were taken from Frančišković-Bilinski [24]. The total amounts of elements in this manuscript (data from 2018) are defined as the sum of extracted elements in the four binding fractions.

Table 1. Concentrations of selected toxic elements (mg kg^{-1}) in fine sediment fraction (<63 μm) of the Kupa River [1].

Location/Year	As	Ba	Cd	Cr	Cu	Ni	Pb	Zn
IŠ/2003	-	-	-	-	-	-	-	-
IŠ/2018	8.3	1347	0.4	19.6	21.2	23.8	24.5	61.1
51/2003	9.5	5790	<0.7	29.1	25.0	30.7	24.5	71.7
51/2018	9.3	794	0.4	30.5	23.1	31.5	31.8	73.8
52/2003	8.0	75.2	0.3	22.6	23.1	31.3	20.6	70.9
52/2018	7.0	58.7	0.5	18.0	20.8	21.7	24.1	65.9
ČD/2003 [2]	8.6	1070	0.2	20.9	21.4	29.5	17.6	73.0
ČD/2018	6.2	429	0.4	19.1	21.3	25.6	26.3	61.6
K-2/2003	4.8	386	0.3	20.0	14.7	25.2	33.5	58.6
K-2/2018	4.1	398	0.4	14.1	13.0	15.3	16.4	47.0
K-4/2003	4.0 *	341	0.3	26.4	21.4	38.7	20.1	65.3
K-4/2018	2.6	241	0.3	12.4	10.2	13.4	10.6	37.5
38/2003	1.8	78.4	0.2	17.0	7.49	16.3	8.43	33.3
38/2018	4.7	209	0.6	65.5	18.1	23.2	25.9	71.0
44/2003	13.0	65	0.4	29.5	14.6	36.6	17.6	57.6
44/2018	5.3	63.8	0.5	22.5	15.2	26.3	20.1	50.3

[1] Element concentrations in 2003 for all elements (aqua-regia extraction and ICP-MS) are taken from Frančišković-Bilinski et al. [24]. [2] Data from 2003 are from location Žaga in Slovenia (about 5 km downstream from ČD, in sampling map 50), which is currently not available for sampling. Therefore, ČD was selected as the closest accessible point, which could be compared with Žaga location.

Location Iševnica (IŠ) at Kupica River does not have available data from 2003, therefore its data cannot be compared during the 15-year period. According to the fact that this location is closest to Kupica River spring, from which the Ba-anomaly described by Frančišković-Bilinski [12] entered Kupica and Kupa rivers, it could be assumed that Ba concentrations should be highest in this location. When they are compared with Ba-concentrations in 2003 on several km downstream locations in Kupica River at Brod na Kupi (51), just before its confluence with Kupa River, one can notice that Ba-concentration at IŠ in 2018 is significantly lower than it was in 2003 at location 51. When recent Ba-concentrations from 2018 are compared on both locations, one can see that concentrations drop going downstream. As a result of closing barite mine in Homer-Lokve, Ba-contamination stopped more than 20 years ago and karstic underground through which it penetrated to Kupica River source and also sediments of Kupica and Kupa rivers are slowly washing out of this contamination and Ba-concentrations are dropping significantly. At location 51, concentrations of all other observed elements besides Ba remained almost unchanged in the time frame of 15 years. Only Pb concentration slightly increased.

Location 52 is also in Brod na Kupi, very close to location 51, but it is located on Kupa River about 100 m upstream of Kupica River inflow. At this location, the concentration of most of the elements slightly decreased, or in the case of Pb, slightly increased, but are very similar to concentrations measured 15 years earlier.

Location Čedanj (ČD) is about 4 km downstream from the confluence of Kupica and Kupa rivers. The compared data from 2003 are from location Žaga in Slovenia (about 5 km downstream from ČD, in sampling map 50), which is currently not available for sampling due to current conditions at the Croatia–Slovenia border. Therefore, ČD was selected as the closest accessible point that could be compared. In this location, during 15 observed years, Ba-concentration significantly decreased due to the closing of barite mine in Homer. The concentration of the majority of studied elements remained similar or decreased a bit, except Cd, of which its concentration doubled and Pb, of which its concentration increased a bit.

Location K-2 is located at Jurovo beach on the Croatian bank of the Kupa River, just several hundred meters before Lahinja River inflows from the Slovenian side. In this location, concentrations of all elements in 2018 remained similar to those 15 years earlier or decreased significantly, like those of Cr, Ni, Pb, and Zn.

The next location going downstream is K-4, located just downstream of Ozalj hydropower plant dam. At these locations, concentrations of all observed elements significantly decreased during the 15-year period. This could indicate a decrease of pollution from upstream industry in the region of Bela Krajina in Slovenia, probably as a result of improving waste water purification or the closing of some factories.

Location 38, Pokupsko, is situated in the lower flow of Kupa River. This is the only location along the whole Kupa River flow where a significant increase of all studied elements was observed. Some of the toxic elements increased up to three times in the observed 15-year period, which is worrisome. In their study, Frančišković-Bilinski et al. [13] noticed the very poor and alarming ecological state of the river in this location during a dry summer period and concluded that parts of the river are moribund, with macroalgal bloom and marsh vegetation, and that intensive eutrophication is visible. Also, during the last few years, there have been many reports from local inhabitants, fishermen, and bathers stating that on this stretch of the river, conditions are poor and that many species of fish, shells, and other animals almost disappeared or have become rare. Therefore, our findings about such a significant increase of different polluting elements during the period of 15 years supports calls to perform an extensive study of this stretch of the river and to find causes of pollution. At this moment, we can only speculate about causes, but this pollution is most likely from industrial and municipal wastewaters of Karlovac or from industry in Jamnička Kiselica, upstream of Pokupsko.

The last studied location on Kupa River is 44, Zibel beach in Sisak, about 5 km before the end of Kupa River and its confluence with Sava River. The state of this location is much better than in upstream

Pokupsko and concentrations of all elements except as are lower than in Pokupsko. Concentrations of all elements except Cu and Pb decreased during the 15-year period, while Cu and Pb only slightly increased. At this location in 2003, As concentration was highest on the whole of Kupa River, but until 2018, it significantly decreased and now is not high anymore.

Finally, we can conclude that during the studied 15 years period, the situation with toxic element concentration along Kupa River improved on most of its course. But, unfortunately on the lower course of the river downstream of Karlovac, especially around location Pokupsko, the situation has worsened and reached an alarming state. Therefore, we call for conducting extensive research about pollution and the state of biota on this stretch of the river and for future monitoring of water, sediment, and biota.

3.2. Chemical Fractions and Risk Assessment Code of Potentially Toxic Elements in Kupa River Sediments

Descriptive statistics for total element content are presented in Table 2. The distributions of element contents by fractions for As, Cd, Cr, Cu, Ni, Pb, and Zn are given in Figure 2. These elements were selected because of their high degree of toxicity. An extreme barium anomaly in sediments of Kupica and Kupa rivers was discovered in 2003. Because of that, chemical fractionation of Ba is also shown in this manuscript.

Table 2. Descriptive statistics for total element content (mg kg^{-1}).

	Mean	SD [1]	Median	Min	Max
Al	9724	1796	10,552	6252	11,865
As	6.48	2.61	6.25	2.64	10.7
B	1.6	0.92	0.99	0.76	2.99
Ba	404	425	241	59.3	1347
Be	0.89	0.17	0.93	0.56	1.11
Ca	39,347	23,660	38,375	12,026	78,479
Cd	0.44	0.11	0.44	0.26	0.59
Co	9.44	2.39	10.0	5.52	12.7
Cr	24.8	16.1	19.6	12.4	65.5
Cu	18.8	5.13	20.8	10.2	26.2
Fe	28,115	6806	26,409	20,314	39,145
K	732	163	799	482	930
Li	24.9	3.25	24.9	18.0	29.4
Mg	9694	2705	9578	4949	14,670
Mn	442	249	318	164	881
Mo	0.41	0.17	0.42	0.24	0.81
Na	108	31.0	106	64.0	159
Ni	23.1	5.71	23.8	13.4	31.5
P	5842	859	6042	4414	6999
Pb	22.9	6.31	24.5	10.6	31.8
S	357	128	330	158	600
Si	1630	325	1567	1111	2115
Sr	24.2	7.67	26.2	10.1	37.2
Ti	74.2	15.4	76.9	49.3	104
V	19.3	3.7	19.5	11.9	25.0
Zn	61.5	14.7	61.6	37.5	85.3

[1] SD: Standard deviation.

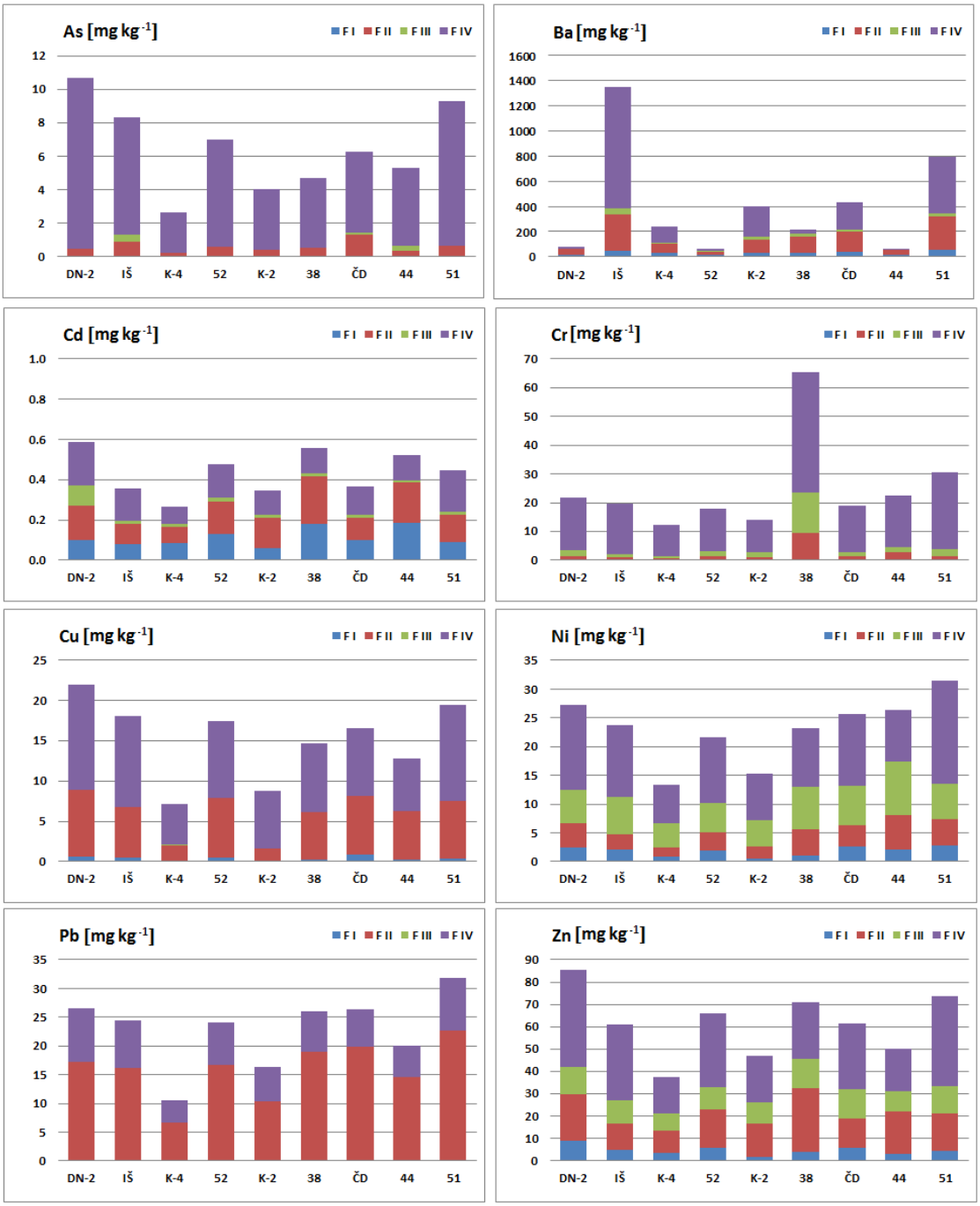

Figure 2. Distribution of elements by fractions. Explanation: Fraction F1/soluble in acid-exchangeable elements or associated with carbonates; Fraction F2/Reducible-elements associated with oxides of Fe and Mn; Fraction F3/oxidizable fraction-elements associated with organic matter and sulfides, and Fraction F4/residual fraction.

Distribution of elements in the fractions of the sequential extraction (F1-fraction soluble in acid; F2-reducible fraction; F3-oxidizable fraction; F4-residual fraction) is:

As: F4 > F2 > F3 > F1; Ba: F4 > F2 > F3 > F1; Cd: F4 = F2 > F1> F3; Cr: F4>>F3> F2> F1; Cu: F4 > F2 > F3 > F1; Ni: F4 > F3 > F2 > F1; Pb: F2 > F4 > F3 = F1; and Zn: F4 > F2 > F3 > F1.

Fractionation of As, Cd, Cr, Cu, Ni, Ba, and Zn showed that the major portion of these elements was extracted in the residual fraction (F4). Elements bound to residual fraction are "unreactive" and in the case of Kupa River, these elements are dominantly lithogenous in origin. Contents of these elements

are mainly controlled by continental weathering. Bilinski [25] found that Kupa River sediments are closest to average sandstone composition. X-ray diffraction from the same study of the <63 µm fraction suggested that chlorite, vermiculite, and mica are present in sediments. Depletion of some elements in sediments suggests their loss from sediments due to the dissolution of minerals, which leads to their release into water.

The least mobile elements are Cr and Ni since the significant content of these elements in addition to the F4 is bounded in the F3 (slightly lower percentage). In addition to being mostly bound in F4, As, Cd, Cr, Cu, Ni, Ba, and Zn are bound in F2 in some localities, indicating the importance of Fe and Mn oxides as well as the carbonates for the binding of these elements.

The most mobile element of the above is lead, since about 70% of this element is bound in F2, while some of the lead is bound in the organic/sulfide fraction (F4). In Mondal et al. [26], they show that organic matter and Fe-Mn oxides seemed to play an important role in controlling the adsorption of this element to the sediment surface. Also, in Mondal et al. [26], it is shown that lead species are strongly sorbed to Fe-Mn oxides which were reported to be more important than any association with clays and organic matter and any change in the anoxic conditions of sediment will influence the release or retention of this element in the reducible phase.

The results of the risk assessment codes with values given as percentages of the F1 fraction are shown in Figure 3. The sediments do not pose a high risk to the environment. The obtained results show that 3.31–23.76% of Ba, 0.16 to 0.64% of Cr, 3.36 to 10.22% of Ni, 3.56 to 10.56% of Zn, 17.23 to 35.32% of Cd, 0.67 to 4.22% of Cu, and 0% of As and Pb were present in exchangeable fractions. Ba showed light-medium risk; Cr, no risk; Ni, light-medium risk; Zn, light-medium risk; Cd, medium-high risk; Cu, no-light risk; and As and Pb, no risk to the local environment. The more easily mobile and bioavailable forms were predominant for cadmium and barium. A potentially dangerous situation in this area can derive mainly from Cd due to its toxicity and from Ba due to its very high concentrations.

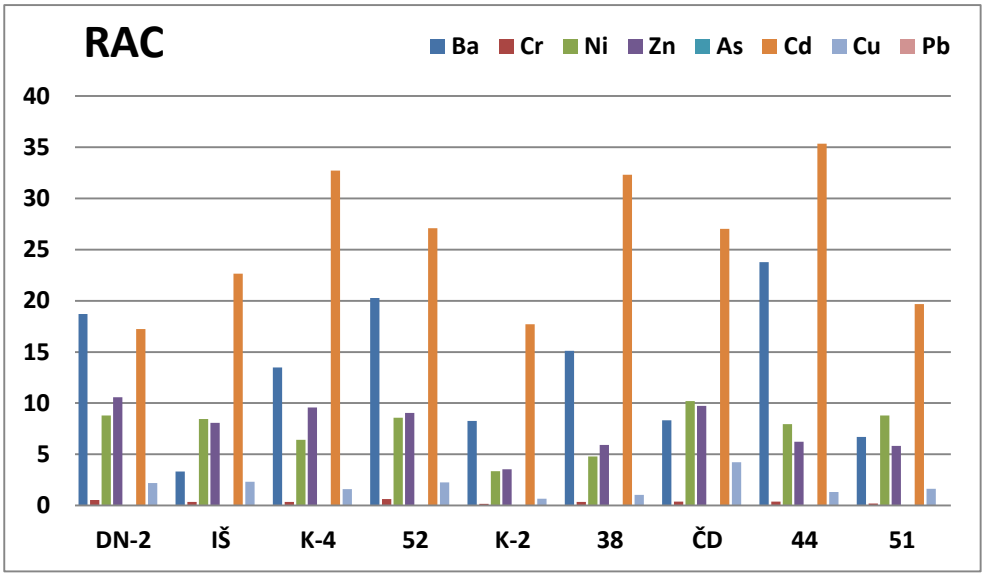

Figure 3. The risk assessment code (RAC) results for studied elements in the studied sediment.

3.3. Geo-Accumulation Index (Igeo)

The greatest number of elements belongs to Class 0 and Class 1 (Figure 4), i.e., uncontaminated and uncontaminated to moderately contaminated sediments (Ba, Ni, Zn, As, Cd, Cu, Pb, and Cr), and Class 2—moderately contaminated sediments (Zn, Ba, and Cr in some samples).

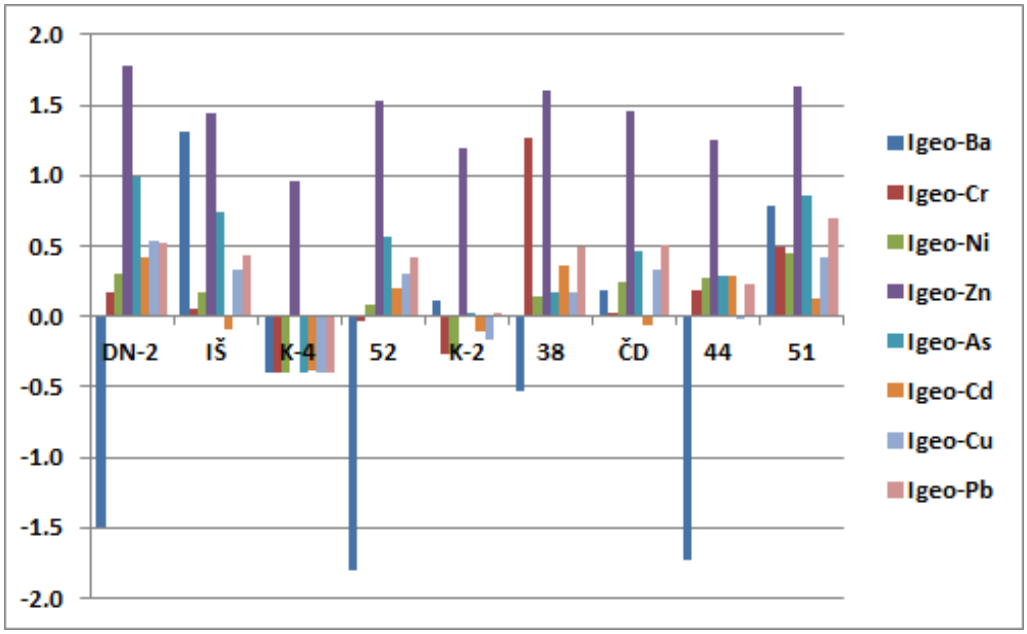

Figure 4. The values of the Igeo index.

3.4. Enrichment Factor (EF)

For calculation of EF, Al was used as the element for normalization. The EF values were calculated separately for all the sampling sites and results are shown in Figure 5. In general, in most localities and for most elements, the EF factor value is less than three, indicating no to minor enrichment. The highest values of EF factors were observed for Zn and have values between 3 and 5, indicating moderate enrichment with this element at all of the localities. At the locality marked with IŠ, the EF value for Ba is also slightly higher than 3, but only at that locality. The locality at which the increased barium content was observed is the Kupica River sediment, at a distance of 1.5 km upstream from its mouth in the Kupa River. As shown in Frančišković-Bilinski [12], the source of this Ba-contamination was an abundant barite mine in the Homer-Lokve area. Also, a slightly higher value of the EF factor for Cr was observed at locality 38 (Kupa, Sunny Beach near Pokupsko) rather than other localities, but the value was less than 3, indicating minor enrichment. These results are consistent with Igeo values.

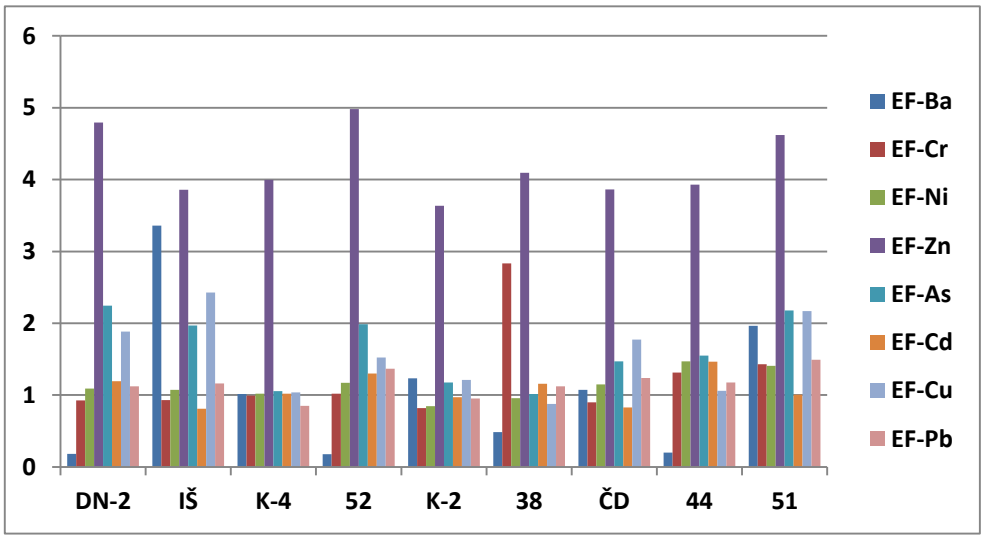

Figure 5. Distribution of studied elements enrichment based on enrichment Factor (EF).

3.5. Ecological Risk Factor (Eri) and Risk Index (RI)

The ecological risk assessment results are shown in Table 3. The Eri values of Pb, Cr, Ni, Zn, Cu, and As (except for arsenic at DN-2 (Dobra river)) were lower than 40, indicating a slight potential ecological risk of these elements in the Kupa river and its tributary. The Eri values of Cd were between 40 and 80, indicating moderate ecological risk. Amongst the studied elements, Cd presents a higher ecological risk because of their higher toxicity coefficients.

Table 3. Ecological risk factor (Eri) and risk index (RI).

	Pb_{Er}	Cr_{Er}	Ni_{Er}	Zn_{Er}	As_{Er}	Cd_{Er}	Cu_{Er}	RI
DN-2	12.53	3.54	10.15	8.92	40.36	67.92	5.95	149
IŠ	11.56	3.17	8.88	6.38	31.44	41.04	4.13	107
51	15.00	4.91	11.74	7.71	35.14	51.31	7.17	133
52	11.39	2.90	8.09	6.89	26.53	55.12	6.03	117
ČD	12.41	3.08	9.56	6.44	23.67	42.29	5.50	103
K-2	7.73	2.28	5.70	4.91	15.34	40.16	5.97	82.1
K-4	5.00	2.00	4.99	3.92	9.99	30.57	6.11	62.6
38	12.24	10.57	8.66	7.42	17.76	64.17	38.19	159
44	9.49	3.62	9.82	5.26	20.06	60.03	10.8	119

The RI values were generally lower than 150, which suggests that sediment samples from the river catchment exhibited low ecological risk. However, the RI value for sediment from locality 38 (Kupa, Sunny Beach near Pokupsko) was higher than 150, indicating moderate ecological risk of these elements. The contribution percent of the individual element to the overall potential ecological risk revealed that the most toxic element, Cd, is the main contributor to the total potentially ecological risk.

3.6. Magnetic Susceptibility of Sediments

Results of magnetic susceptibility measurements of fine sediment fraction (<63 μm) are presented in Table S6. As one can see, measured MS values are rather low and only in one sample (38, location Sunny Beach near Pokupsko in the lower flow of Kupa River), they are about three times higher than the average value. At this location, Cr was found to be present in an elevated concentration. So, on our example of Kupa River, it was the confirmed finding of [27] that concluded that two powerful applications of susceptibility measurements of soils (and sediments in our case) exist: the identification of polluted areas and the detailed mapping of these areas to reveal the extent of pollution. The other study [28] conducted in a designated Natura area of Giouchtas Mountain, Crete, Greece showed that studying the magnetic properties of surface soils, along with existing information, allowed the authors to characterize the natural and anthropogenic impacts.

Correlation analysis was performed to reveal statistical correlations between magnetic susceptibility and 26 elements analyzed by ICP-OES. Further, 10 elements of them showed a negative correlation (As, B, Ba, Fe, K, Li, Mg, Na, P, S), while other elements showed a positive correlation. Only elements with a positive correlation will be discussed. Chromium was the element with the highest correlation to MS (0.91). All other elements showed much weaker correlations with MS. The element with the strongest correlation to MS after Cr was vanadium (0.62), followed by Mn (0.52), Al (0.52), and Cd (0.50). All other elements were rather weakly correlated with MS, among which the highest were those of Sr (0.45), Zn (0.35), Be (0.28), Co (0.27), Pb (0.27), and Ti (0.26). The rest of the elements showed very low correlations. It is interesting to mention that MS does not have any positive correlation with Ba, which means that MS cannot serve as a method of choice for its detection. Kupa River sediments, especially in its upper and middle flow, are known to contain an extremely large Ba-anomaly, but it did not influence MS values at all. The obtained results, using the example of Kupa River sediments, showed that the MS method is excellent indetecting increased values of Cr and is rather good at detecting increased values of some other elements like V, Mn, Al, and Cd. In their study

of soils from Tallin, Estonia [29] found a strong positive correlation of magnetic susceptibility with Cr, Pb, Zn, and Cu in a central part of the city and concluded that they were conditioned by industrial contamination, mainly by metal-working factories and traffic.

3.7. Comparison of Total Extracted Element Contents with Threshold Values

Total concentrations of selected toxic elements (Cd, Pb, Ni, Cu, Cr, Zn, As, and Ba) in sediments were compared with proposed threshold values for freshwater sediments in Croatia [30]. The majority of those elements showed values below threshold values. The most interesting results are those of Cr in the lower flow and of Pb in its upper flow. The concentration of Cr is above the proposed threshold value (57 ppm for Cr) at Pokupsko (sample 6, location 38). Since Cr is predominantly bound to the residual fraction which is "unreactive", the increased content of this element does not pose at present a significant environmental problem. The sequential extraction procedure shows that chromium at Pokupsko in the lower course is distributed in fractions 2, 3, and 4 among which fraction 4 (residual fraction) predominates. Lead is distributed in fractions 2 and 4, among which fraction 2 (reducible fraction) predominates. When compared with proposed threshold values for freshwater sediments in Croatia (31 ppm for Pb), the concentration of Pb is slightly above this value in locations 51 (Kupica tributary, sample 9) and 52 (Kupa, sample 4) at Brod na Kupi in the area of vulnerable karstic aquifers [31]. This statement may indicate a problem for the environment since elements bound to reducible Mn and amorphous/crystalline Fe oxides can eventually become available under possible changes in redox conditions. Considering the increased lead and chromium content at the mentioned sites, more detailed monitoring of pollution including redox potential measurements along Kupa River is recommended.

3.8. Statistical Analysis

3.8.1. PCA

A PCA with Varimax normalised rotation was performed in this research. The results of PCA analysis (R mode) are given in Table S7 and shown in Figure S1. According to Kaiser's criterion (eigenvalues > 1), there were two main components (PC) identified:

PC1 explains 54.90% of the total variance and includes Cu, Pb, Zn, Ni, and As. These are elements mostly presented in the "residual fraction", which according to many authors, suggests a primarily geogenic rather than anthropogenic origin.

PC2 explains 33.63% of the total variance. Cd, Cr, and RI were clustered in component 2, indicating that of the investigated elements, Cd and Cr were likely responsible for the values of the risk ecological index.

3.8.2. HCA

Hierarchical cluster analysis (HCA) was performed on the dataset on both variables (R mode) and samples (Q mode) to identify clusters of elements and sites.

Figure S2 presents CA dendrogram (R mode), which produced three clusters. The first cluster was divided into two subclusters: (a) As, Cu, and Zn and (b) contains Pb and Ni. The second cluster contains Cd and the third Cr.

The results of a Cluster Analysis of the sampling stations (Q mode) are illustrated as a dendrogram in Figure S3. Two groups were identified as Cluster I and II. The sites in each cluster exhibited similar characteristics and possibly similar contributing sources. Cluster I could be separated into two subclusters; subcluster Ia represents the samples not significantly affected by pollution (location IŠ, ČD, 52, K-2, DN, and K-4) and subcluster Ib represents samples 44 and 51, at which little pollution was observed. Cluster II represents a contaminated site (location 38), at which increased content of chromium was observed.

4. Conclusions

In this manuscript, geochemical fractionation and risk assessment of potentially toxic elements in sediments from Kupa River (Croatia) were evaluated using different methods and indices. The chemical fractionation of sediment samples showed that studied elements are mainly derived from the source material, instead of anthropogenic contamination. Fe-Mn oxides seemed to play an important role in controlling the adsorption of lead to the sediment surface. Values for both EF and Igeo factors showed that As, Cu, Ni, and Pb were mostly derived from natural crustal contributions, whereas the values for Zn, Cr, and Ba indicated anthropogenic input in some localities. The results of the risk assessment codes indicate that more easily mobile and bioavailable forms were predominant for cadmium and barium. The statistical analysis shows that Cd and Cr were likely responsible for the values of the risk ecological index. Summarizing the results of the applied methods in this manuscript, it is possible to conclude that a significant part of Cd and Cr are dominantly of anthropogenic origin. Potential sources of increased content of these elements are probably from industrial and municipal wastewaters of the city Karlovac.

During the period of 15 years, the situation with toxic element concentration in sediments along Kupa River improved on most of its course, except on the lower course of the river downstream of Karlovac, especially around location Pokupsko, where the situation has worsened and reached an alarming state, with some parts of the river getting moribund and with obvious eutrophication. Therefore, detailed monitoring of pollution in the lower course of Kupa River is recommended.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4441/12/7/2024/s1. Details of BCR sequential extraction procedure; Figure S1: Loading plot of studied elements and RI, Figure S2: Dendrogram showing cluster of elements, Figure S3: Dendrogram showing cluster of studied sites, Table S1: Certified values, analytical values and recovery of the BCR Reference Material (BCR 701), Table S2: Category of risk assessment code, Table S3: Values of Igeo and the pollution level, Table S4: Values of EF and pollution level, Table S5: Category of potential ecological risk factor.

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