Arch Environ Contam Toxicol (2014) 66:514–528
DOI 10.1007/s00244-014-0012-z

# Surface Water Characterization of Three Rivers in the Lead/Zinc Mining Region of Northeastern Macedonia

**Sheriban Ramani · Zrinka Dragun · Damir Kapetanović · Vasil Kostov · Maja Jordanova · Marijana Erk · Zehra Hajrulai-Musliu**

Received: 9 December 2013 / Accepted: 11 February 2014 / Published online: 14 March 2014


**Abstract** Mine waste is recognized as being one of the most serious threats for freshwater ecosystems, and it still represents one of the greatest environmental concerns in Macedonia. The aim of our investigation was to obtain an in-depth understanding of mining influence on freshwater systems from water contamination to effects on aquatic organisms. In this study, we assessed the impact of active lead (Pb)/zinc (Zn) mines Zletovo and Toranica on the water quality of three rivers in northeastern Macedonia (Bregalnica, Zletovska, and Kriva rivers) based on data collected in spring and autumn of 2012. The Bregalnica River, near Shtip, was characterized mainly by weak contamination with arsenic, barium, iron, molybdenum, titanium, uranium, vanadium, nitrate, and phosphate, as well as critical faecal pollution, which alltogether could be connected to agricultural activities; however, an impact of the mines was not observed. Contrary, both the Zletovska and Kriva rivers showed a clear impact of Pb/Zn mines on water quality. In the Zletovska River, increased concentrations of cadmium (Cd), cobalt, cesium, copper, lithium, manganese (Mn), nickel, rubidium, tin, strontium, thallium, Zn, sulphates, and chlorides were found, especially in autumn (*e.g.*, Cd 2.0 μg $L^{-1}$; Mn 2.5 mg $L^{-1}$; Zn 1.5 mg $L^{-1}$). In the Kriva River, increased Cd (0.270 μg $L^{-1}$) and Pb (1.85 μg $L^{-1}$) concentrations were found only in spring, possibly due to sediment resuspension during greater water discharge. The selected sampling sites on the Bregalnica, Zletovska, and Kriva rivers were confirmed as being appropriate locations for further studies of mining waste's impact on freshwater ecosystems, the first one as a non-impacted site and the other two as possible areas of increased exposure of aquatic organisms to metals.

S. Ramani
Hydrology and Ecology Department, National Hydrometeorological Service, 1000 Skopje, Macedonia

Z. Dragun (✉) · M. Erk
Laboratory for Biological Effects of Metals, Division for Marine and Environmental Research, Ruđer Bošković Institute, 10002 Zagreb, Croatia
e-mail: zdragun@irb.hr

D. Kapetanović
Laboratory for Aquaculture and Pathology of Aquatic Organisms, Division for Marine and Environmental Research, Ruđer Bošković Institute, 10002 Zagreb, Croatia

V. Kostov
Institute of Animal Sciences, 1000 Skopje, Macedonia

M. Jordanova
Faculty of Natural Sciences and Mathematics, Ss. Cyril and Methodius University in Skopje, 1000 Skopje, Macedonia

Z. Hajrulai-Musliu
Faculty of Veterinary Medicine, Ss. Cyril and Methodius University in Skopje, 1000 Skopje, Macedonia

Metal mine discharges have resulted in severe degradation of many rivers across the globe (Cerqueira *et al*. 2011; Silva et al. 2011a, b, c; Byrne *et al*. 2012). Therefore, after diffuse agricultural pollution, metal mine drainage is recognized by environmental agencies as being the second most serious threat to water quality (Environment Agency 2006). Some of the reasons for its toxicity are acidity and high content of trace metals (Stuhlberger 2010). In rivers that receive mine waters with high levels of one or more ecotoxic metals, significant loss of biodiversity can be expected (Stuhlberger 2010). Thus, the composition and health of plant and animal communities can be severely impaired (as reviewed by Byrne *et al*. 2012). Increased concentrations of trace metals can be found around both abandoned and active mines as the result of discharging and dispersion of mine waste materials into nearby soils, food crops, and stream sediments (Dolenec *et al*. 2005).

In Macedonia, discharges of untreated wastewater from mining are among the most serious water-pollution concerns (Stuhlberger 2010). Macedonia's most significant mineral deposits are lead (Pb) and zinc (Zn) ores, the exploitation of which is performed in extraction, smelting, and metal-processing industrial plants located in the northeastern part of the country (Zletovo in Probishtip, Toranica in Kriva Palanka, and Sasa in Makedonska Kamenica) (Midžić & Silajdžić 2005). In the Probishtip concentration plant, where ore from the Zletovo mine is processed, the tailing-storage facility is known to be prone to failure (Stuhlberger 2010). For example, in 1975, the tailing dam at Zletovo failed, and the lagoon discharged, flooding villages and agricultural land downstream (Stuhlberger 2010). In addition, wastewater from the Zletovo mine, which is contaminated with metals, is pumped from the concentration plant into the Kiselica River, a tributary of the Zletovska River, without cleaning or neutralization. Consequently, there is not much life in the Kiselica River, and high levels of metals have been found in fish and other biological samples (Midžić & Silajdžić 2005).

Information obtained from existing monitoring programmes for rivers in northeastern Macedonia (Milevski *et al*. 2004), combined with common knowledge of the hazards for aquatic systems associated with mining, were the incentive for the start of a comprehensive investigation on the impacts of the currently active Pb/Zn mines Zletovo and Toranica on rivers receiving their waste. The overall aim of the investigation was to obtain an in-depth understanding of mining's influence on freshwater systems based on the assessment of (1) metal exposure by determining metal concentrations in surface water, (2) metal bioavailability and bioaccumulation by determining metal concentrations in fish tissues, and (3) finite effects of water contamination on fish by defining histopathological changes in fish tissues as well as fish health status. In this article, the impact of mining on different aspects of surface water quality of selected aquatic systems will be presented, including physicochemical parameters, nutrient concentrations, microbiological parameters, and concentrations of dissolved macroelements and trace elements. Two rivers were chosen for this study, the Zletovska River, which receives waste from the Zletovo mine, and the Kriva River, which receives waste from the Toranica mine; the Bregalnica River was chosen as a nonimpacted aquatic system. The specific aim of this study was to define the contamination level for each of these three rivers and to ascertain if they could serve as good systems for further evaluation of the impact of mining on aquatic life.

## Materials and Methods

### Study Area

The northeastern part of Macedonia encompasses several ore districts, among which are the Kratovo-Zletovo and Sasa-Toranica districts. We studied the influence of Pb/Zn mineralization and mining on river water quality near the Zletovo and Toranica mines. Three rivers were included in this study: Bregalnica, Zletovska, and Kriva rivers (Fig. 1, Table 1).

Based on information gathered during existing monitoring programmes (Milevski *et al*. 2004), Bregalnica was selected as nonimpacted river because it is less contaminated compared with the other two rivers. It is the longest left tributary of the Vardar River, the principal river in Macedonia. Bregalnica has a length of 225 km and a catchment area of 4307 $km^2$. Its water discharge in 2012 ranged from 1.24 to 66.30 $m^3$ $s^{-1}$ (Table 2) (these data were obtained courtesy of the Hydrometeorological Service of the Republic of Macedonia). To avoid the influence of contamination from the Zletovska River, we chose a sampling point at Bregalnica, situated approximately 35 km downstream from the mouth of the Zletovska River into the Bregalnica River. However, this site is located downstream from the city of Shtip, the largest town in the eastern part of Macedonia, and therefore is influenced by sewage and household water discharges as well as waste from industrial facilities and farms, which is partly released in the collection system and partly released directly into the river (Spasovski 2011; Rebok 2013).

The Zletovska River is one of the most polluted tributaries of the Bregalnica River (Dolenec *et al*. 2005). It is 56-km long with a catchment area of 460 $km^2$. Water discharge of the Zletovska River in 2012 ranged from 0.167 to 26.55 $m^3$ $s^{-1}$ (Table 2). This river drains the central part of the Kratovo-Zletovo volcanic complex, the abandoned old mining sites and bare tailings, as well as the effluents from the Pb/Zn mine Zletovo and its ore-processing facilities located near the town of Probishtip in northeastern Macedonia (Alderton *et al*. 2005; Dolenec *et al*. 2005). In the town of Probishtip, a battery factory is also a potential source of contamination (Spasovski & Dambov 2009). As the sampling point at the Zletovska River, we chose a site 5 to 6 km downstream from the tailings impoundment of the Zletovo mine. The main ore minerals in the Zletovo mine are galena (PbS) and sphalerite (ZnS), followed by pyrite ($FeS_2$) and chalcopyrite ($CuFeS_2$), as well as a number of other minerals that occur only sporadically (Alderton *et al*. 2005; Serafimovski *et al*. 2006). The mine started operating actively in the 1940 s, and its production still continues, although there have been several short-term interruptions. Ores from the Zletovo

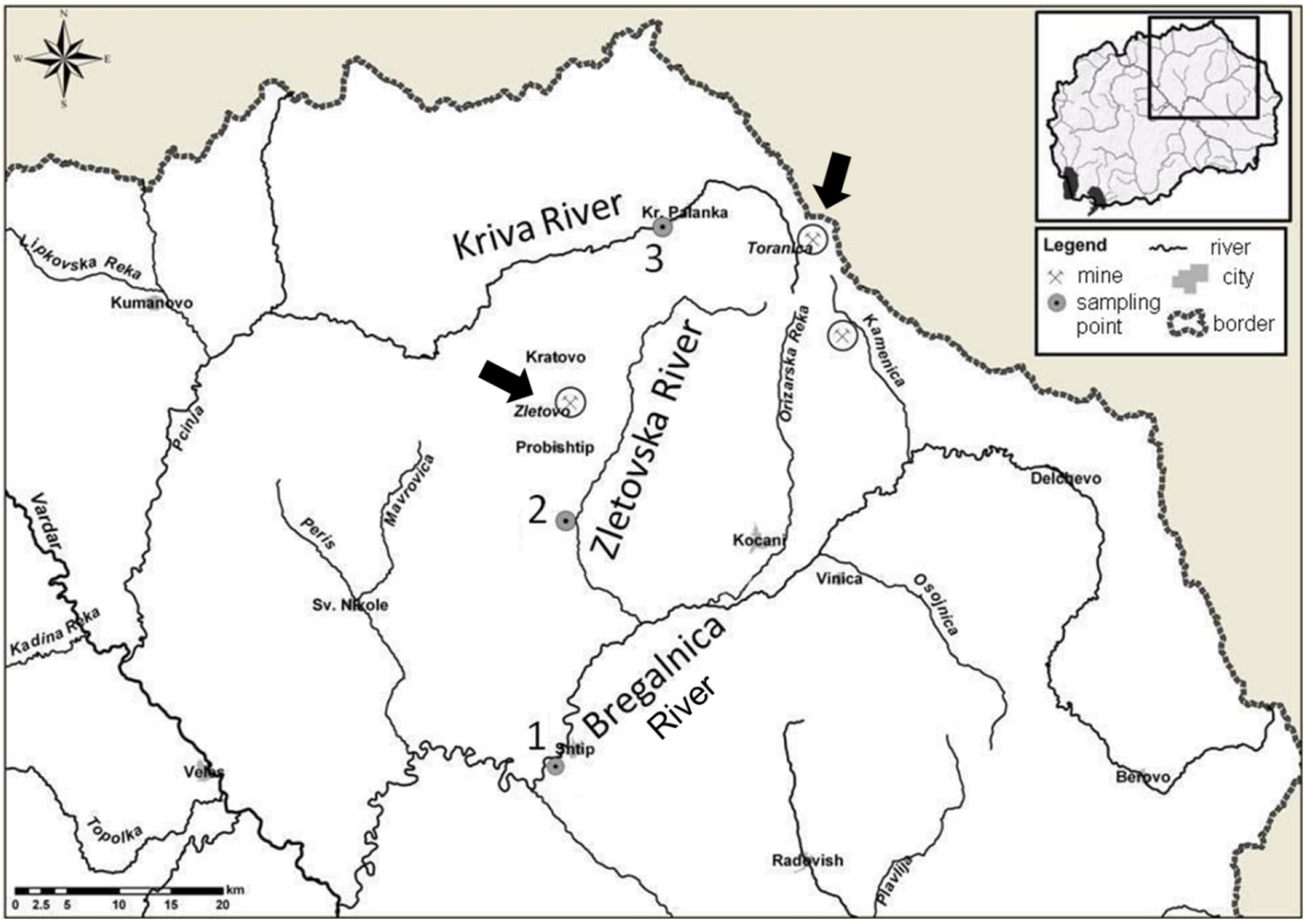


**Fig. 1** Map of the sampling area with marked sampling sites. *Black arrows* point to studied Zletovo and Toranica Pb/Zn mines

mine, with grades higher than 9 % Pb and 2 % Zn, and with a significant presence of silver (Ag), bismuth (Bi), cadmium (Cd), and copper (Cu) (Alderton *et al*. 2005), are concentrated by flotation at Probishtip, whereas tailings are disposed of in two impoundments situated in adjacent valleys. Two tributaries of the Zletovska River, namely the Kiselica and Koritnica rivers, drain the flotation plant at Probishtip and the main excavation points of the Zletovo mine (Alderton *et al*. 2005).

The Kriva River is the longest tributary of the Pčinja River, which is the left tributary of the Vardar River. The length of the Kriva River is 78.7 km, and the catchment area is 968 $km^2$. Water discharge of the Kriva River in 2012 ranged from 0.08 to 8.42 $m^3\ s^{-1}$ (Table 2). As the sampling point at the Kriva River, we chose a site 15 to 20 km downstream from the Toranica mine. The Toranica deposit is situated in northeastern Macedonia close to the Sasa deposit but in a separate watershed. Production of Pb and Zn from the Toranica mine has lasted from 1987 with a few year-long interruptions that occurred after 2000 (Alderton *et al*. 2005). The ore from Toranica mine consists predominantly of galena, sphalerite, chalcopyrite, and pyrite (Fidancev *et al*. 2011). Ore grades are approximately 6.5 % of Pb + Zn with additional increased concentrations of Cd, Cu, manganese (Mn), Ag and Bi (Serafimovski *et al*. 2007). Ore milling and flotation occur at the mine, and there is a tailing dam below the mine with a culvert directing the Toranica River, which is a tributary of the Kriva River, beneath the dam (Alderton *et al*. 2005). During the autumn sampling, water of the Kriva River was additionally collected at an upstream location closer to the mine near Zhidilovo (N 42°13,50’ E 22°22,18’).

## Water Sampling

River water was sampled for analyses of dissolved metals and physicochemical parameters in each river once in spring when there was greater water discharge and once in autumn when discharge was close to the annual minimum (Tables 1 and 2). On each occasion, river water samples were collected by grab sampling in polyethylene plastic bottles (three bottles of 0.25 L for metal analyses and one bottle of 1.0 L for physicochemical parameters), which were rinsed with acid (for metal

**Table 1** List of sampling sites with coordinates, known pollution sources, and sampling dates

| Sampling site | Coordinates | Pollution sources | Sampling dates |
|---|---|---|---|
| Bregalnica (1) | N 41°43,57’ | Municipal wastewater from the city of Shtip, agricultural waste | Spring: May 11 2012 |
| | E 22°10,27’ | | Autumn: October 17 2012 |
| Zletovska River (2) | N 40°58,54’ | Waste from Pb/Zn mine Zletovo and from Pb-battery factory | Spring: May 11 2012 |
| | E 21°39,45’ | | Autumn: October 16 2012 |
| Kriva River (3)[a] | N 42°11,39’ | Waste from Pb/Zn mine Toranica, municipal wastewater from Kriva Palanka | Spring: June 13 2012 |
| | E 22°18,34’ | | Autumn: October 18 2012 |

[a] In the autumn sampling, water was additionally collected at an upstream location closer to the mine near Zhidilovo (N 42°13,50’ E 22°22,18’)

analyses = v/v 10 % $HNO_3$, p.a., Merck, Germany; for physicochemical parameters = v/v 5 % HCl, p.a., Merck) and Milli-Q water before sampling. The samples were stored at 4 °C for at most 24 h before filtration through a cellulose nitrate filter (0.45-µm pore diameter; Sartorius, Germany). For metal analyses, the filtrates were acidified with nitric acid (acid concentration in the samples = 0.65 %; Suprapur, Merck) and stored at 4 °C.

For microbiological analyses, water samples were collected in each river only during autumn. Samples were collected in sterilized plastic bottles (0.5 L) from the subsurface layer of river water (0.5 m). Samples were immediately transported to the laboratory in refrigerated containers, and all bacterial counts (total coliform bacteria and enterococci) were performed in duplicate.

**Table 2** Hydrological information for Bregalnica, Zletovska, and Kriva rivers at the time of water sampling (water discharge [$m^3$ $s^{-1}$])

| Sampling period | Bregalnica River | Zletovska River | Kriva River |
|---|---|---|---|
| Spring sampling | 2.90 | 9.51 | 0.933 |
| Autumn sampling | 1.60 | 0.424 | 0.099 |
| Annual average | 6.36 | 2.65 | 0.705 |
| Annual minimum | 1.24 | 0.167 | 0.080 |
| Annual maximum | 66.30 | 26.55 | 8.42 |

### Physicochemical Parameters

Several physicochemical parameters (Table 3), such as temperature, pH, redox potential (pE), conductivity, and dissolved oxygen (DO), were measured on site with a portable meter (WTW Multi 340i/SET; Germany). The other physicochemical parameters were measured subsequently in river water samples in the laboratory by the methods listed in Tables 3 and 4. For all analyses, standardized ISO, American Public Health Association (APHA) (1998), and United States Environmental Protection Agency (USEPA) methods, as well as high-purity reagents (Merck and Fluka Analytical) and Milli-Q water (Model Milli-Q 5), were used.

### Dissolved Metals and Metalloids in River Water

Dissolved trace elements were measured directly in filtered river water samples, whereas macroelements (sodium [Na], potassium [K], calcium [Ca], and magnesium [Mg]) were measured in 10 × diluted filtered samples due to their higher concentrations. The measurements of both trace and macroelements were performed on a high-resolution inductively coupled plasma mass spectrometer (HR ICP-MS Element 2, Thermo Finnigan, Germany) equipped with an autosampler ASX 510 (CETAC Technologies, USA). Measurements of $^{7}$Li, $^{85}$Rb, $^{98}$Mo, $^{111}$Cd, $^{120}$Sn, $^{121}$Sb, $^{133}$Cs, $^{205}$Tl, $^{208}$Pb, and $^{238}$U were operated in low-resolution mode; $^{23}$Na, $^{24}$Mg, $^{42}$Ca, $^{47}$Ti, $^{51}$V, $^{55}$Mn, $^{56}$Fe, $^{59}$Co, $^{60}$Ni, $^{63}$Cu, $^{66}$Zn, $^{86}$Sr, and $^{138}$Ba were operated in medium-resolution mode; and $^{39}$K and $^{75}$As were measured in high-resolution mode. Indium (1 µg $L^{-1}$; indium atomic spectroscopy standard solution, Fluka) was added to the samples as internal standard (Dautović 2006). External calibration was performed using standard solutions prepared from multielement stock standard solution for trace elements (100 mg $L^{-1}$; Analitika, Czech Republic) in which single element standard solutions of Sb and tin (Sn) (1 g $L^{-1}$; Analytika) were added. Separate external calibration was performed for macroelements using standards (Ca 2.0 g $L^{-1}$; Mg 0.4 g $L^{-1}$; Na 1.0 g $L^{-1}$; K 2.0 g $L^{-1}$; Fluka, Germany) adequately diluted in 2 % $HNO_3$ (Suprapur; Merck, Germany). Measurements were also performed in filtration blanks (Milli-Q water filtered and acidified in the same way as river water samples). If necessary, blank corrections of measured trace element concentrations in river water were made. The accuracy of metal determination was controlled with 100 × diluted quality-control (QC) sample for trace metals (QC trace metals, catalog no. 8072, lot no. 146142-146143; UNEP GEMS, Burlington, Canada) and with 10 × diluted QC sample for macroelements (QC minerals, catalog no. 8052, lot no. 146138-146139; UNEP GEMS). Limits of detection for trace element measurement in the filtered river water

**Table 3** General physicochemical parameters determined in river water of Bregalnica, Zletovska, and Kriva rivers during spring and autumn 2012

| General physicochemical parameters | Bregalnica River | | Zletovska River | | Kriva River | | Measurement method |
|---|---|---|---|---|---|---|---|
| | Spring | Autumn | Spring | Autumn | Spring | Autumn | |
| Tempurature (°C) | 19.8 | 17.1 | 13.7 | 13.1 | 17.7 | 12.4 | Measured on site |
| pH | 8.12 | 8.11 | 6.88 | 6.52 | 7.90 | 8.02 | Measured on site |
| Alkalinity (mg $CaCO_3$ $L^{-1}$) | 140.1 | 255.2 | 365.3 | 410.4 | 94.2 | 144.0 | Titration with acid (HCl) (ISO 9963-1:1994) |
| pE (mV) | −68 | −63 | 5 | 28 | −54 | −57 | Measured on site |
| Turbidity (NTU) | 16 | 6 | 27 | 3 | 7 | 10 | Nephelometric method (APHA 2130B) |
| Conductivity (µS $cm^{-1}$) | 390 | 595 | 1,490 | 2,020 | 211 | 347 | Measured on site |
| TDS (mg $L^{-1}$) | 316 | 510 | 987 | 1,568 | 153 | 261 | Measured gravimetrically (APHA 2540C) |
| DO (mg $O_2$ $L^{-1}$) | 10.38 | 9.40 | 9.17 | 8.23 | 8.87 | 8.97 | Measured on site |
| $COD_{KMnO4}$ (mg $O_2$ $L^{-1}$) | 2.96 | 0.89 | 6.08 | 0.40 | 2.24 | 4.84 | Titrimetric method (ISO 8467:1993) |
| Total hardness ($^o$dH) | 10.43 | 14.27 | 37.60 | 70.00 | 5.92 | 8.62 | EDTA titrimetric method (APHA 2340 C) |

EDTA = ethylene diamine tetraacetic acid

For parameters measured on site, applied equipment is described in Physicochemical Parameters

were as follows (in µg $L^{-1}$): As (0.005), barium (Ba) (0.020), Cd (0.001), cobalt (Co) (0.001), cesium (Cs) (0.001), Cu (0.010), iron (Fe) (0.100), lithium (Li) (0.010), Mn (0.010), molybdenum (Mo) (0.020), nickel (Ni) (0.020), Pb (0.020), rubidium (Rb) (0.010), Sb (0.001), Sn (0.001), strontium (Sr) (0.020), titanium (Ti) (0.020), thallium (Tl) (0.002), uranium (U) (0.001), vanadium (V) (0.002), and Zn (0.100) (Dautović 2006; Roje 2008).

### Bacterial Counts in River Water

Microbiological analyses of river water were performed in the same way as previously described for marine water by Kapetanović *et al.* (2013). Total coliform bacteria were identified using Colilert (IDEXX Laboratories., Westbrook, USA), a defined substrate technology (Edberg *et al.* 1990). Enterococci were identified similarly using Enterolert-E (IDEXX Laboratories). Total coliforms and enterococci were enumerated using Quantitray 2000 (IDEXX Laboratories), which used a 97-test-well system and provided the most probable number (MPN) of bacteria/100 mL (Table 4).

### Data Processing and Statistical Analyses

Statistical program SigmaPlot 11.0 for Windows was applied for graph creation and statistical analyses. Due to the small number of data, several nonparametric statistical tests were applied. Kruskal–Wallis one way analysis of variance on ranks with *post hoc* Dunn's test was used for comparison of metal and metalloid concentrations measured in the three rivers, separately for each sampling. Mann–Whitney rank sum test was used for comparison of the results obtained during spring and autumn, separately for each site. Although some differences were observed between the spring and the autumn samples, these were not statistically significant ($p > 0.05$) due to small number of data per site during each season ($n = 3$).

## Results and Discussion

The assessment of river water quality presented in this article was a part of larger study that also encompassed evaluation of the impact of water contamination on the health of native fish. Therefore, one of the basic criteria for sampling-site selection was that some essential characteristics of the river water, such as pH and water oxygenation, should be compatible with possibility for fish survival. Accordingly, the sampling sites of two mining-impacted rivers were selected at locations being far enough from the mines to assure the presence of the aquatic life. As a result of the targeted selection of sampling locations, both pH and DO level in all three rivers in this study (Table 3) were categorized as the first class of surface waters (Table 5; GRM 1999). However, as expected, a number of measured parameters did not comply with the requirements for first-class water. For example, turbidity in all samples of studied rivers was high, and characteristic for fourth-class of water (Table 5). Increased turbidity in the vicinity of mines can be the result of Fe precipitates. It decreases the incidence of light in a water body, thus impeding photosynthesis and causing a breakdown of food chains, which finally results in decreased biodiversity in the affected areas (Stuhlberger 2010). Some of obtained results also indicated serious water pollution, especially in the Zletovska and Kriva rivers, which will be discussed later in the text, separately for each studied watercourse.

**Table 4** Concentrations of nutrients and other anions, as well as microbiological parameters, determined in river water of Bregalnica, Zletovska, and Kriva rivers during spring and autumn 2012

| | Bregalnica River | | Zletovska River | | Kriva River | | Measurement method |
|---|---|---|---|---|---|---|---|
| | Spring | Autumn | Spring | Autumn | Spring | Autumn | |
| Nutrients and anions | | | | | | | |
| $NO_3^-$ (mg N $L^{-1}$) | 0.639 | 1.131 | 0.364 | 0.033 | 0.596 | 0.744 | Spectrophotometric method (AQUANAL®-plus test) |
| $NO_2^-$ (mg N $L^{-1}$) | 0.014 | 0.037 | 0.0001 | 0.0013 | 0.008 | 0.097 | Spectrophotometric method (APHA 4500-$NO_2^-$ B) |
| $NH_4^+$ (mg N $L^{-1}$) | 0.053 | 0.096 | 0.019 | 0.123 | 0.360 | 2.18 | Spectrophotometric method (APHA 4500-$NH_3$ D; ISO 7150/1) |
| TN (mg N $L^{-1}$) | 0.90 | 1.90 | 1.50 | 0.50 | 0.95 | 5.10 | Spectrophotometric method (DIN EN ISO 11905-1) |
| $PO_4^{3-}$ (mg $PO_4^{3-}$ $L^{-1}$) | 0.230 | 0.188 | 0.028 | 0.044 | 0.175 | 0.916 | Ascorbic acid method (APHA 4500-P E) |
| TP (mg P $L^{-1}$)[a] | <0.5 | 0.089 | <0.5 | 0.032 | <0.5 | 0.325 | Ascorbic acid method (APHA 4500-P E) |
| $SO_4^{2-}$ (mg $L^{-1}$) | 53.78 | 44.47 | 453.2 | 883.9 | 29.75 | 25.02 | $Ba(ClO_4)_2$ titration method (APHA 4500- $SO_4^{2-}$) |
| $Cl^-$ (mg $L^{-1}$) | 10.18 | 18.72 | 20.62 | 43.83 | 4.89 | 12.13 | Ferricyanide colorimetric method (USEPA 325.2 Chloride) |
| Number of bacteria (MPN/100 mL)[b] | | | | | | | |
| Total coliforms | | 15,214 ± 543.2 ($n = 3$) | | 3,864 ± 254.6 ($n = 2$) | | 435,170 ($n = 1$) | |
| Enterococci | | 1,004.9 ± 174.6 ($n = 3$) | | 23.5 ± 11.8 ($n = 3$) | | 15,653 ± 7,837 ($n = 2$) | |

[a] For total phosphorus determination, Spectroquant phosphate cell test 114729 was used during the spring period, whereas Spectroquant phosphate test 114848 was used in the autum period; these two tests have different detection limits, which is the reason for the different manner of expressing the results for the two seasons

[b] Microbiological analyses were performed only in the autumn sampling; methods are described in Bacterial Counts in River Water

**Table 5** Permitted ranges and upper limits of several physicochemical parameters[a] and faecal indicator bacteria[b] defined for five classes of surface water quality as well as classification of three rivers in two sampling periods according to each specific parameter

| | | pH | Turbidity (NTU) | TDS (mg $L^{-1}$) | DO (mg $O_2$ $L^{-1}$) | Total coliforms (MPN/100 mL) | Enterococci (MPN/100 mL) |
|---|---|---|---|---|---|---|---|
| 1st class surface water | | 6.5–8.5 | <0.5 | 350 | >8.0 | ≤500 | ≤40 |
| 2nd class surface water | | 6.3–6.5 | 0.5–1.0 | 500 | 6.00–7.99 | >500–10,000 | >40–400 |
| 3rd class surface water | | 6.0–6.3 | 1.1–3.0 | 1,000 | 4.00–5.99 | >10,000–100,000 | >400–4,000 |
| 4th class surface water | | 5.3–6.0 | >3.0 | 1500 | 2.00–3.99 | >100,000–1,000,000 | >4,000–40,000 |
| 5th class surface water | | <5.3 | >3.0 | >1,500 | <2.00 | >1,000,000 | >40,000 |
| River classification | | | | | | | |
| Bregalnica | Spring | 1st | 4th–5th | 1st | 1st | – | – |
| | Autumn | 1st | 4th–5th | 3rd | 1st | 3rd | 3rd |
| Zletovska River | Spring | 1st | 4th–5th | 3rd | 1st | – | – |
| | Autumn | 1st | 4th–5th | 5th | 1st | 2nd | 1st |
| Kriva River | Spring | 1st | 4th–5th | 1st | 1st | – | – |
| | Autumn | 1st | 4th–5th | 1st | 1st | 4th | 4th |

[a] Government of the Republic of Macedonia (1999)

[b] Kavka *et al.* (2006)

## Bregalnica River

The Bregalnica River receives water from the Zletovska River, which is directly influenced by the active Zletovo mine (Fig. 1). However, a location was selected at Bregalnica, which is presumably far enough from the source of contamination to be considered a nonimpacted site. Downstream and further away from the mining regions, decrease in dissolved metal concentrations can be expected as a result of dilution in noncontaminated river water as well as a result of a removal from solution due to precipitation of the oxide, hydroxide, and sulphate phases and coprecipitation or adsorption of metals onto these phases in sediments (Hudson-Edwards *et al.* 1999; Alderton *et al.* 2005; Ribeiro *et al.* 2013a, b; Silva *et al.* 2013). Furthermore, the contamination also generally decreases in river sediments, not only in water, downstream from the contaminant source (Byrne *et al.* 2012) due to hydraulic sorting (Wolfenden & Lewin 1978), dilution by uncontaminated sediments (Marcus 1987), hydrogeochemical reactions (Hudson-Edwards *et al.* 1996), and biological uptake (Lewin & Macklin 1987).

Although within the limits for first-class water (Table 5), the pH of Bregalnica River water was slightly alkaline and comparable with pH values characteristic for sites with high-intensity agricultural activity, which were reported to be approximately 8 (Cooper & Fortin 2010). In addition, total dissolved solids (TDS) (Table 3) were rather low in the spring samples, but they somewhat increased in the period of low water level during autumn (Table 5). Levels of nitrates and phosphates were slightly increased compared with the Zletovska River (Table 4). Similarly, indicators of faecal pollution, i.e., levels of total coliforms and enterococci, which were determined only during the autumn sampling, were also higher compared with the Zletovska River (approximately 4 and 40 times, respectively) but were much lower than values obtained for the Kriva River (approximately 30 and 15 times, respectively) (Table 4). They exceeded 10,000 and 400 MPN/100 mL, respectively, which are the upper limits defined for good bathing water quality (Council of the European Communities (CEC) 1976; European Parliament and the Council of the European Union [EPCEU] 2006). Kavka *et al.* (2006) defined five classes of faecal pollution of surface water (Table 5) considering the values recommended by European directives (CEC 1976; EPCEU 2006). Based on that classification, Bregalnica could be classified as third class or river water with critical faecal pollution (Table 5; Kavka *et al.* 2006). Somewhat disrupted physical and organoleptic properties of the water, accompanied by an increased level of suspended substances and high microbiological contamination, have already been reported for the Bregalnica River under the town of Shtip as a result of drainage of sewage from households, the collection system, and industry (Spasovski 2011).

An interesting finding for the Bregalnica River was the several degrees higher water temperature compared with the other two rivers during both sampling periods (Table 3), which can have a profound influence on the aquatic ecology (Huet 1986), e.g., by affecting the solubility of gases (Cokgor *et al.* 2009). The cause of this increase could be found in the geothermal system Kezhovica-Ldzhi, which is situated nearby on the right bank of the Bregalnica River and approximately 2 km to the southwest from the center of Shtip. The temperature increase of river water was possibly the result of the mixing of river water with hot water from the deep springs of cracked

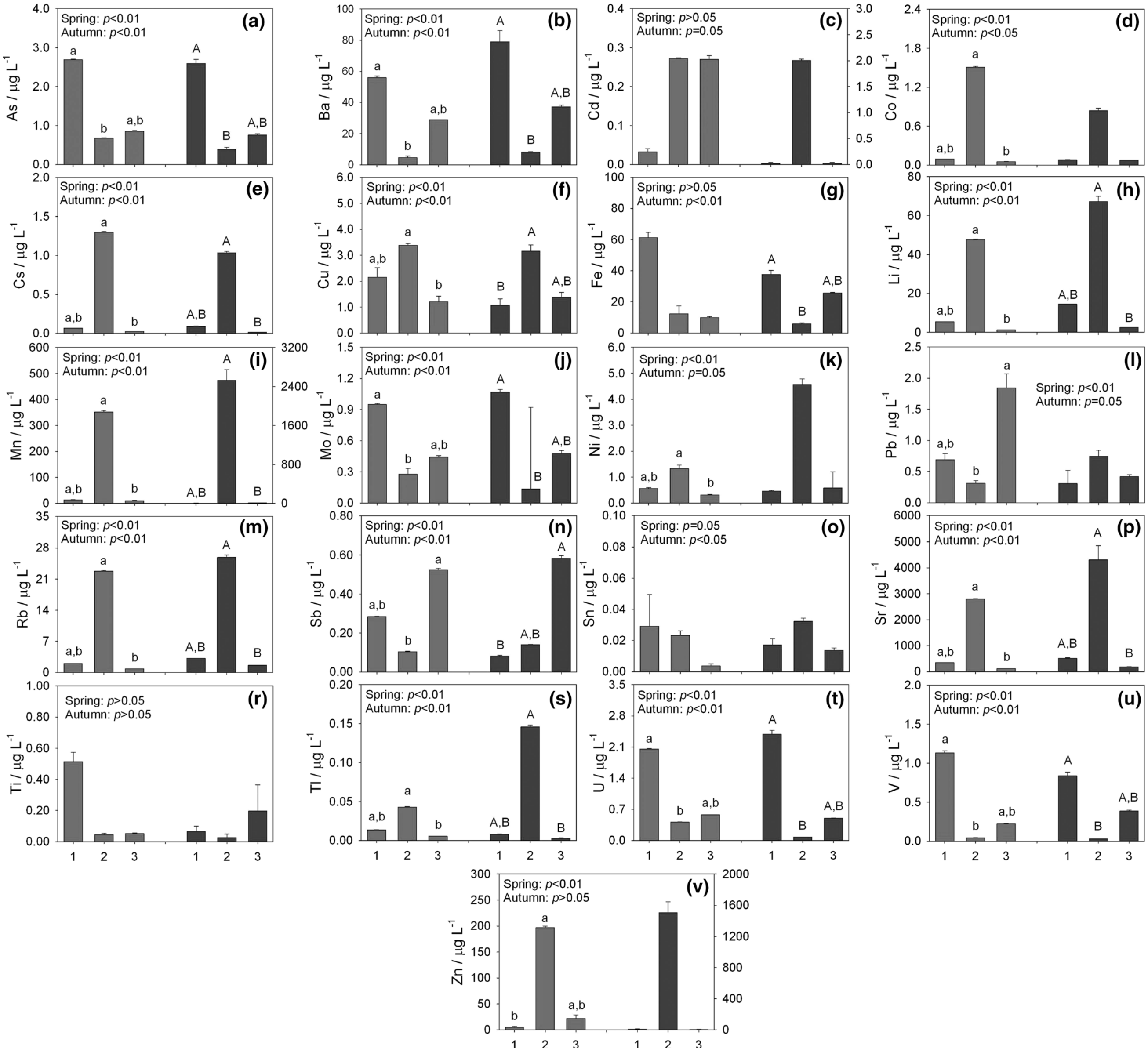


**Fig. 2** Concentrations (µg $L^{-1}$) of dissolved trace elements in river water of three rivers in northeastern Macedonia (1 = Bregalnica; 2 = Zletovska River; 3 = Kriva River): **a** As, **b** Ba, **c** Cd, **d** Co, **e** Cs, **f** Cu, **g** Fe, **h** Li, **i** Mn, **j** Mo, **k** Ni, **l** Pb, **m** Rb, **n** Sb, **o** Sn, **p** Sr, **r** Ti, **s** Tl, **t** U, **u** V, **v** Zn. Results are presented as mean and SD ($n = 3$). Statistically significant differences between sites according to Kruskal–Wallis one-way ANOVA on ranks (levels of significance are indicated in the figure) followed by *post hoc* Dunn's test ($p < 0.05$) are indicated by *different letters* (*lower-case letters* for spring and *upper-case letters* for autumn). *Light gray bars* spring; *dark gray bars* autumn

granites, which are the reservoirs of this geothermal system (Spasovski 2012).

In addition, the concentrations of several dissolved trace elements, namely As, Ba, Fe, Mo, Ti, U, and V, were moderately increased in surface water of this river (Fig. 2a, b, g, j, r, t, and u). This increase was mostly notable when a comparison was made with the Zletovska River with concentrations being approximately 4–30 times higher. However, the concentrations of metals defined as priority toxic substances by the European Water Framework Directive (EU WFD), such as Cd, Ni, and Pb (EPCEU 2008, were not increased in this river. Among seven listed elements, only Ba concentration surpassed the freshwater screening benchmark as defined by the USEPA (Fig. 2b, Table 6). The concentrations of other six elements did not exceed either USEPA benchmarks (http://www.epa.gov/reg3hscd/risk/eco/btag/sbv/fw/screenbench.htm) or the strict limits defined by the Canadian guidelines for the protection of aquatic life (Table 6), which are derived based on a goal of no long-term observable adverse effects on aquatic ecosystems (http://st-ts.ccme.ca/).

The specificity of these moderate changes of the river water quality observed at the Bregalnica River was that

**Table 6** EQS proposed by the EU WFD, the Canadian water-quality guidelines for the protection of aquatic life or protection of agriculture (for irrigation) for dissolved trace elements in surface waters, and freshwater-screening benchmarks defined by the USEPA for dissolved trace and macroelements

| | EQS[a] | Canadian guidelines[d] | USEPA[g] |
|---|---|---|---|
| Element (μg $L^{-1}$) | | | |
| As | – | 5 | – |
| Ba | – | – | 4 |
| Cd | 0.08–0.25[b] | 0.031–0.112[e] | 0.25[h] |
| Co | – | 50[f] | 23 |
| Cu | 8.2[c] | 2.25–4[e] | 9[h] |
| Fe | – | 300 | – |
| Li | – | 2,500[f] | 14 |
| Mn | – | 200[f] | 120 |
| Mo | – | 73 | – |
| Ni | 20.0 | 91.3–150[e] | 52[h] |
| Pb | 7.2 | 2.95–7.00[e] | 2.5[h] |
| Sb | – | – | 30 |
| Sn | – | – | 73 |
| Sr | – | – | 1500 |
| U | – | 15 | 2.6[h] |
| V | – | 100[f] | 20 |
| Zn | – | 30 | 120[h] |
| Element (mg $L^{-1}$) | | | |
| Na | – | – | 680 |
| K | – | – | 53 |
| Ca | – | – | 116 |
| Mg | – | – | 82 |

[a] European Parliament and the Council of the European Union (2008)

[b] EQS for Cd depends on the concentration of $CaCO_3$ in river water: Kriva River EQS for Cd = 0.150 μg $L^{-1}$, Zletovska and Bregalnica rivers EQS = 0.250 μg $L^{-1}$

[c] Crane *et al*. (2007)

[d] http://st-ts.ccme.ca/

[e] For some elements, Canadian guidelines for the protection of aquatic life are based on the concentration of $CaCO_3$ in water. The lower limit given in the table is based on the lowest $CaCO_3$ level measured in the Kriva River, whereas the upper limit is based on the highest $CaCO_3$ level measured in the Zletovska River

[f] For several elements, Canadian guidelines for the protection of aquatic life are not defined, and thus values presented in the table refer to the guidelines for the protection of agriculture

[g] http://www.epa.gov/reg3hscd/risk/eco/btag/sbv/fw/screenbench.htm

[h] Defined for hardness = 100

they could be usually seen in rivers flowing through agriculturally developed regions. In the Sutla River in Croatia, for example, a mild increase of the same elements as in the Bregalnica River (As, Ba, Fe, U, V, and Ti) was observed at the agriculturally impacted river section (Dragun *et al*. 2011). Both synthetic and natural fertilizers, herbicides, and insecticides, which were used in agriculture, were reported as sources of metals (e.g. As [Bednar *et al*. 2002], Ba [Senesi *et al*. 1983], Ti [Anke & Seifert 2004], and V [Vachirapatama *et al*. 2002]) that might contaminate nearby rivers (Nash *et al*. 2003; Bolan *et al*. 2004; Cooper & Fortin 2010). Increased concentrations of As, Cu, and Zn have been previously associated with the poultry litter (Jackson & Bertsch 2001; Jackson *et al*. 2003); therefore, an increase of As concentration could be also associated to the presence of a poultry farm in the town of Shtip (Spasovski 2011). In contrast, increased U concentrations at agriculturally impacted sites can occur due to its complexation with humic substances (Sachs *et al*. 2007) abundantly present in the river water because of their use as additives to fertilizers (Peña-Méndez *et al*. 2005). Finally, increased levels of phosphorus and nitrogen in aquatic ecosystems was also reported in association with agricultural activities as well as an increased number of enterococci in river water, which indicates water contamination with manure because enterococci are present in the faeces of warm-blooded animals (Carpenter *et al*. 1998; Lata *et al*. 2009; Dragun *et al*. 2011; http://www.oasisdesign.net/water/quality/coliform.htm).

The concentrations of several elements (Cu, Mn, Pb, and Ti [Fig. 2f, i, l, and r]), otherwise present in rather low concentrations, were somewhat increased in spring compared with autumn samples (by 2–8 times), which could be also connected to leaching from agriculturally used soils during rainy periods. Previous analyses of the sediments indicated that the effects of the Zletovo mine extended all the way to the Bregalnica River because even at the confluence between the Zletovska and Bregalnica rivers, sediment still contained Zn in a concentration of 990 mg/kg (Alderton *et al*. 2005). However, the effect of mining was not visible in river water downstream from the confluence of these two rivers even during the spring period of greater water discharge when sediment resuspension and a consequent increase in metal concentrations could be anticipated (Neal *et al*. 2000; Dragun *et al*. 2009).

### Zletovska River

The sampling location at the Zletovska River had slightly acidic water (Table 3), probably due to the influence of drainage waters from the tailings impoundment of the Zletovo mine. Furthermore, characteristic findings for the Zletovska River in both sampling periods were positive pE, the highest water hardness, and the highest conductivity and TDS (Table 3), classified as third- and fifth-class of water, respectively, during spring and autumn (Table 5). Total hardness, the concentration of $CaCO_3$ (Table 3), and the concentrations of macroelements (Na, K, Ca, and Mg [Fig. 3a–d]) were also the highest in that river with Ca concentration being even higher than USEPA benchmark (Table 6). Because conductivity is especially sensitive to sulphate ions (Jiménez *et al*. 2009), concentrations of $SO_4^{2-}$

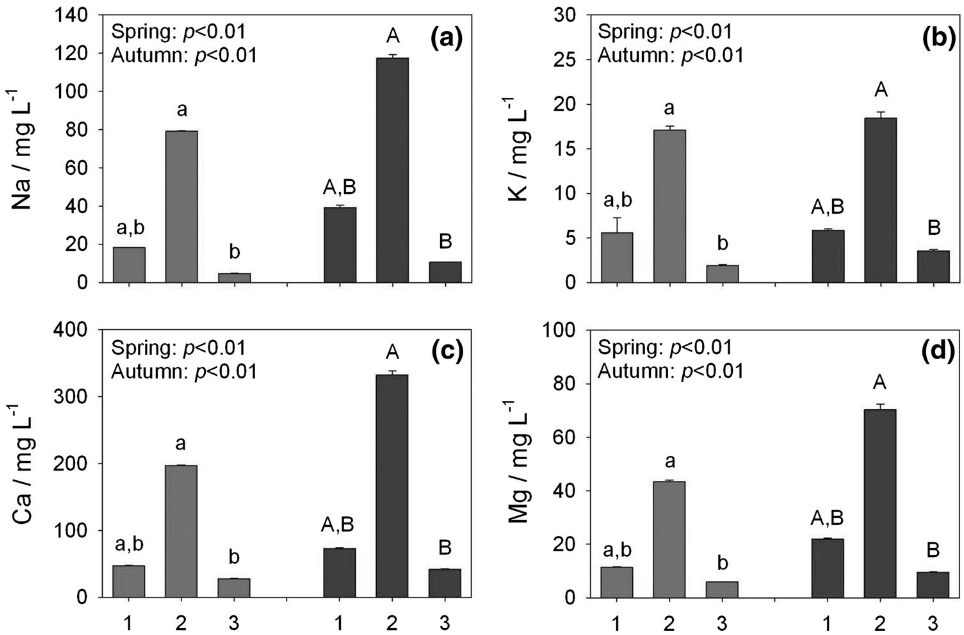


**Fig. 3** Concentrations (mg $L^{-1}$) of dissolved macroelements in river water of three rivers in northeastern Macedonia (1 = Bregalnica; 2 = Zletovska River; 3 = Kriva River): **a** Na. **b** K. **c** Ca. **d** Mg. Results are presented as described in the caption of Fig. 2

were accordingly higher in this river compared with the Bregalnica and Kriva rivers by 8–15 times in the spring and 20–35 times in the autumn (Table 4). An observed increase of $Cl^-$ concentrations in the Zletovska River was somewhat less pronounced (Table 4). Sulphate concentrations were approximately 2–4 times higher than the recommended limit for drinking water (250 mg $L^{-1}$ [Stuhlberger 2010]). Both sulphate and conductivity are useful indicators of acid mine drainage contamination because they remain increased even when pH approaches neutral values due to large dilutions (Jiménez *et al*. 2009) as observed at the sampling site at the Zletovska River. This occurs because sulphur is not easily adsorbed and thus can migrate further than heavy metals; its migration can represent the largest range of mine-tailing impact (Gray 1996).

Similar changes as in the Zletovska River were observed in the Kocacay River, near the Balya mine in Turkey, where metallurgic wastes were composed mainly of metal sulphides: pyrite >galena >sphalerite, which was similar to the ore composition in the Zletovo mine (Aykol *et al*. 2003). The majority of acid production by mine wastes occurs precisely due to the oxidation of iron sulphide minerals, such as pyrite and pyrrhotite (Stumm & Morgan 1981; Lapakko 2002), which is the reason why an increase in pyrite content, such as is characteristic for the Zletovo mine, commonly results in greater acidity (Alderton *et al*. 2005) as well as in the formation of soluble metal sulphates in waste dumps (Aykol *et al*. 2003). Consequently, the first indicator of sulphide mineral oxidation is the presence of sulphates as the dominant anions and $H^+$ in mine-drainage waters (Nordstrom & Alpers 1997; Lapakko 2002; Aykol *et al*. 2003). Next to sulphates and low pH, mine drainage is commonly characterized by high levels of dissolved toxic metals (Braungardt *et al*. 2003; Robb & Robinson 1995). Such acidic, metal-rich waters, which flow from abandoned or active mines, can contaminate streams and rivers far downstream from the drainage source and consequently can have toxic effects on biota (Aykol *et al*. 2003).

With increasing distance from the contamination source, the acidity of mine water is generally buffered to a greater or lower degree by dilution (Aykol *et al*. 2003). Relatively high pH of the river water (>6.1) favours the incorporation of metals into the particulate phase through the processes of sorption (Bird *et al*. 2010). However, even then river water can contain considerable amounts of metals, which can seriously endanger the aquatic ecosystem as seen in the Kocacay River, in which several elements (As, Cd, chromium, Cu, Fe, Mn, Pb, and Zn) were reported to be an important environmental concern (Aykol *et al*. 2003). Similarly, the analysis of trace elements in the Zletovska River, at pH of 6.52–6.88 (Table 3), indicated serious contamination of the river water with a number of metals (Cd, Co, Cs, Cu, Li, Mn, Ni, Rb, Sn, Sr, Tl, and Zn [Fig. 2c, d, e, f, h, i, k, m, o, p, s, and v]), among which Cd and Ni belong to priority toxic substances and Cd even exceeded the environmental quality standards (EQS) set by the EU WFD for inland surface waters (European Parliament and the Council of the European Union 2008; Table 6). Comparison with the Canadian guidelines and USEPA benchmarks (Table 6) indicated a possible troublesome increase of several elements above their recommended limits, namely Cd, Cu, Li, Mn, Sr and Zn. The increase of Zn concentrations as a result of the activities in the Pb/Zn mine Zletovo has already been reported in the water of the Zletovska River and its tributary, the Kalnistanska River, accompanied by increase of Cd concentrations because Cd geochemically follows Zn-containing minerals (Spasovski & Dambov 2009). The increase of Cu and Mn concentrations in the water of the Kalnistanska River was also observed where the Cu increase was a result of a chalcopyrite presence associated with Pb and Zn minerals (Spasovski & Dambov 2009). Concentrations of a few metals (Cd, Mn, Ni, Pb, Tl, and Zn) were especially high in October during low water discharge. Specifically, dissolved Cd was approximately 10 times higher than its EQS and 20 times higher than Canadian recommendations, whereas dissolved Mn and Zn were as much as 12 and 50 times higher than Canadian limits, respectively (Fig. 2c, i, and v, Table 6). From our results, it was obvious that contamination of the river water with Zn was much more pronounced than with Pb, even though ZnS and PbS are present in equal amounts in Pb/Zn mines (Barnes 1979) such as Zletovo mine. This could be explained by greater solubility of ZnS compared with PbS (Barnes 1979) as well as the fact that Pb is readily adsorbed by aluminum and Fe oxide phases in sediment (Lee *et al*. 2002), thus resulting in a much quicker decrease of dissolved Pb than Zn concentration (Zhang *et al*. 2004). Therefore, Pb is to a lesser degree present in water in the dissolved phase, which is why Zn is a better indicator of the effects of mining (Alderton *et al*. 2005).

Previously, Zn and Cd, bound to Fe and Mn oxides/hydroxides, as well as several other metals (Cs, Cu, and Tl) were also found in highly increased concentrations in paddy soil samples in the vicinity of the Zletovska River (Dolenec *et al*. 2005). This was undoubtedly the consequence of the discharge of untreated acid mine water and effluents from tailings, rich in Zn and Cd (39 and 176 mg $L^{-1}$, respectively), into river water that was used for the irrigation of the paddy fields on the western side of the Kočani field (Dolenec *et al*. 2005). However, not only acid mine drainage, but also a high regional geochemical background, must be considered as a source of high trace-element concentrations in Zletovska River water because previous studies have also reported high metal levels upstream of the mine (e.g., Zn 330 and Mn 900 μg $L^{-1}$ [Alderton *et al*. 2005]).

Contrary to the other two rivers, the Zletovska River does not seem to be affected by faecal pollution. Levels of total coliforms and enterococci were <10,000 and 400 MPN/100 mL (Table 4), respectively, indicating good microbiological water quality according to the European recommendations (Council of the European Communities 1976; European Parliament and the Council of the European Union 2006). If the classification by Kavka *et al*. (2006) was applied, this river would belong to the first and second class, which would indicated weak or moderate faecal water pollution (Table 5). The level of nutrients was also lower compared with the other two rivers (Table 4), and low inputs of inorganic nutrients and organic matter, next to high pyrite availability, could be an additional cause of the poor water quality regarding trace elements in the Zletovska River (Aykol *et al*. 2003).

### Kriva River

Kriva River water had slightly alkaline pH and negative redox potential similar to the Bregalnica River (Table 3). However, conductivity, TDS, and alkalinity were lower (Table 3), indicating lower concentrations of various salts in this river, which was also confirmed by low levels of macroelements (Fig. 3a–d). Accordingly, although both rivers had higher concentrations of the same trace elements compared with the Zletovska River, being characteristic for agricultural-type contamination, such as Ba, Fe, Mo, and V (Senesi *et al*. 1983; Vachirapatama *et al*. 2002; Dragun *et al*. 2011), their concentrations were much lower in Kriva River water (Fig. 2b, g, j, and u). Furthermore, it would be expected to find a similar type of contamination in the Kriva River as in the Zletovska River because the sampling location was situated downstream from the Toranica mine, which exploits similar Pb- and Zn-rich minerals as those at Zletovo and exhibits similar sediment contamination with Pb, Zn, Cd, and other ore-related metals (Alderton *et al*. 2005). However, the water at this locality was much less contaminated as has been shown in previous studies (Alderton *et al*. 2005). As explained by Alderton *et al*. (2005), this was probably caused by lack of pyrite in the ore compared with Zletovo as well as buffering of acid waters by carbonate host lithologies (limestone), which kept metal concentrations low. A similar finding was reported for parts of the English Peak District with predominating carbonate lithology (Carboniferous limestone), which were characterized by neutral to basic mine discharges and significantly lower concentrations of dissolved toxic metals (Smith *et al*. 2003). In the carbonate areas, acid formed in the oxidation of sulphides can be neutralized by carbonate rock, such as limestone and dolomite, thus resulting in slightly alkaline surface water and retarding the migration of heavy metals (Holmstrom *et al*. 1998; Zhang *et al*. 2004). This could explain the low dissolved metal concentrations in surface water of the Kriva River as well as the alkaline pH, which was even higher at an upstream location closer to the mine near Zhidilovo (autumn pH = 8.16).

Characteristic findings for the Kriva River, however, were severe temporary water contaminations with different contaminants. For example, high concentrations of Cd and Pb (Fig. 2c and l) were found in river water in the spring sampling, which could be associated with the impact of the nearby mine. The concentration of Cd in the spring was higher than its EQS, whereas Pb concentration, although increased, was still lower than its EQS by approximately four times (Table 6). In the autumn sampling, however, Cd and Pb concentrations decreased at the selected sampling site, but closer to the mine they were still rather high and comparable with spring values (at Zhidilovo: Cd 210 ng $L^{-1}$ and Pb 1.95 μg $L^{-1}$).

The contamination event observed in the autumn referred to an increased number of faecal bacteria accompanied by an increase of $NH_4^+$, $PO_4^{3-}$, total nitrogen, and total phosphorus (Table 4). The levels of total coliforms and enterococci were far greater than 10,000 and 400 MPN/100 mL, respectively, indicating unsatisfactory microbiological water quality (Council of the European Communities 1976; European Parliament and the Council of the European Union 2006). In the autumn sampling, the Kriva River was classified as fourth class (Table 5), which indicates strong faecal water pollution (Kavka *et al*. 2006). The concurrent presence of high levels of both nutrients and faecal bacteria could be explained by the fact that nutrients play an important role in faecal bacteria survival in natural systems (Korhonen & Martikainen 1991) by prolonging their persistence or instigating their growth (Findlay *et al.* 2002). The sampling site on the Kriva River is also surrounded by gardens and cultivated land, which could be associated with the presence of faecal bacteria and high level of nutrients in river water.

However, with exception of the continuous slight increase of several trace elements characteristic for agricultural use (Ba, Fe, Mo, and V [Fig. 2b, g, j, and u]), it seems that these occasional contamination events were not the consequence of continuous leaching either from mine tailings or from agricultural soil considering that they were rather extreme but of short duration. Therefore, it could be hypothesized that contamination of the Kriva River was caused by periodic waste input directly into the river water either from the mine or manure from the farms. In the spring sampling, it is even more probable that sediment resuspension, as a consequence of greater water discharge, resulted in the concentration increase of elements sequestered in sediment, such as Cd and Pb, as has often been reported for trace elements in river water (Neal *et al*. 2000; Dragun *et al*. 2009). The contamination of river sediments has been reported in most metal mining regions of the world with metal concentrations usually being several orders of magnitude higher than those in the water column (Macklin *et al*. 2006). sediments of the Toranica River, tributary of the Kriva River, were reported as being highly contaminated with Pb, Zn, and sulfur; although the metal concentrations decreased after the confluence with the Kriva River, they were still significantly increased (Alderton *et al*. 2005). Similarly, in the Twymyn River in the United Kingdom, in the vicinity of a former Pb/Zn mine, Pb concentrations in sediment were ≤100 times higher than levels reported to have deleterious impacts on aquatic ecology, and they were especially high in the acid-soluble phases (Byrne *et al*. 2010). It is characteristic for metals introduced to sediments through human activities, such as mining, that they often exist in weakly bound chemical forms (Jain 2004) and therefore potentially pose serious threats for the aquatic systems (Byrne *et al*. 2010). Because of that, sediments are not a permanent sink for metals, and sulphide-/organic-bound metals may be released into the water column whenever suitable conditions for dissolution occur, such as, for example, the disturbance and oxidation of sediments during flood flows (Byrne *et al*. 2010). Therefore, even when concentrations of toxic elements in water decrease due to dilution or precipitation and pH is not very low, river sediments still can be the source of high pollution level and high toxicity (Sarmiento *et al*. 2011).

## Conclusion

The results of this survey have confirmed the appropriateness of the sampling-site selection for the study of how mining impacts aquatic life in freshwater ecosystems of northeastern Macedonia. It was shown that the selected sampling site on the Bregalnica River could serve as a nonimpacted site in such studies because it does not have water contamination characteristic for mining-impacted rivers but only weak contamination by several trace elements and faecal bacteria, which is characteristic for agriculturally developed regions. In contrast, both the Zletovska and Kriva rivers exhibited clear signs of water contamination as a result of mining activities with high concentrations of several trace elements, among which Cd and Pb should be pointed out as priority toxic substances. An interesting finding was that the contamination of the Zletovska River was evident already in the surface water, whereas in the Kriva River it seemed that the contamination was probably associated with sediments based on a comparison with previously published data. Further study of these two rivers will thus enable investigation of the effect of mining on freshwater fish as a result of two different routes of exposure: exposure through water and exposure originating from sediment.

**Acknowledgments** This study was performed as a part of two bilateral projects between Macedonia and Croatia entitled "The Assessment of the Availability and Effects of Metals on Fish in the Rivers Under the Impact of Mining Activities" and "Bacterial and Parasitical Communities of Chub as Indicators of the Status of Environment Exposed to Mining Activities." The authors are grateful to project participants Vlatka Filipović Marijić, Damir Valić, Katerina Rebok, and Nesrete Krasnići, for valuable assistance in the field. Financial support by the Ministry of Science, Education and Sport of the Republic of Croatia (Projects No. 098-0982934-2721 and 098-0982934-2752) is acknowledged. The hydrological data were obtained courtesy of the Hydrometeorological Service of the Republic of Macedonia. The authors are especially thankful to Nevenka Mikac for the opportunity to use HR ICP-MS and to Željka Fiket for insightful contribution to the discussion.

## References

Alderton DHM, Serafimovski T, Mullen B, Fairall K, James S (2005) The chemistry of waters associated with metal mining in Macedonia. Mine Water Environ 24:139–149

American Public Health Association (1998) Standard methods for the examination of water and waste water (20th ed). APHA, Washington, DC

Anke MK, Seifert M (2004) Titanium. In: Merian E, Anke M, Ihnat M, Stoeppler M (eds) Elements and their compounds in the environment, vol 2., Metals and their compounds. Wiley, Weinheim, pp 1125–1140

Aykol A, Budakoglu M, Kumral M, Gultekin AH, Turhan M, Esenli V et al (2003) Heavy metal pollution and acid drainage from the abandoned Balya Pb-Zn sulfide mine, NW Anatolia, Turkey. Environ Geol 45:198–208

Barnes HL (1979) The solubilities of ore minerals. In: Barnes HL (ed) The geochemistry of hydrothermal ore deposits, 2nd edn. Wiley, New York, pp 404–460

Bednar AJ, Garbarino JR, Ranville JF, Wildeman TR (2002) Presence of organoarsenicals used in cotton production in agricultural water and soil of the southern United States. J Agric Food Chem 50:7340–7344

Bird G, Brewer PA, Macklin MG, Nikolova M, Kotsev T, Mollov M et al (2010) Dispersal of contaminant metals in the mining-affected Danube and Maritsa drainage basins, Bulgaria, Eastern Europe. Water Air Soil Pollut 206:105–127

Bolan NS, Adriano DC, Mahimairaja S (2004) Distribution and bioavailability of trace elements in livestock and poultry manure by-products. Crit Rev Environ Sci Technol 34:291–338

Braungardt CB, Achterberg EP, Elbaz-Poulichet F, Morley NH (2003) Metal geochemistry in a mine-polluted estuarine system in Spain. Appl Geochem 18:1757–1771

Byrne P, Reid I, Wood PJ (2010) Sediment geochemistry of streams draining abandoned lead/zinc mines in central Wales: The Afon Twymyn. J Soil Sed 10:683–697

Byrne P, Wood PJ, Reid I (2012) The impairment of river systems by metal mine contamination: A review including remediation options. Crit Rev Environ Sci Technol 42:2017–2077

Carpenter SR, Caraco NF, Correll DL, Howarth RW, Sharpley AN, Smith VH (1998) Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecol Appl 8:559–568

Cerqueira B, Vega FA, Serra C, Silva LFO, Andrade ML (2011) Time of flight secondary ion mass spectrometry and high-resolution transmission electron microscopy/energy dispersive spectroscopy: a preliminary study of the distribution of $Cu^{2+}$ and $Cu^{2+}$/$Pb^{2+}$ on a Bt horizon surfaces. J Hazard Mater 195:422–431

Cokgor EU, Oktay S, Tas DO, Zengin GE, Orhon D (2009) Influence of pH and temperature on soluble substrate generation with primary sludge fermentation. Bioresour Technol 100:380–386

Cooper S, Fortin C (2010) Metal and metallothionein content in bullfrogs: study of a whole watershed impacted by agricultural activities. Ecotoxicol Environ Safe 73:391–399

Council of the European Communities (1976) Council Directive of 8 December 1975 concerning the quality of bathing water (76/160/EEC). Official J L 31

Crane M, Kwok KWH, Wells C, Whitehouse P, Lui GCS (2007) Use of field data to support European water framework directive quality standards for dissolved metals. Environ Sci Technol 41:5014–5021

Dautović J (2006) Determination of metals in natural waters using high resolution inductively coupled plasma mass spectrometry. Bachelor's thesis, University of Zagreb, Zagreb

Dolenec T, Serafimovski T, Dobnikar M, Tasev G, Dolenec M (2005) Mineralogical and heavy metal signature of acid mine drainage impacted paddy soil from the western part of the Kočani field (Macedonia). RMZ Mater Geoenviron 52:397–402

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, 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 40:391–407

Edberg SC, Allen MJ, Smith DB, Kriz NJ (1990) Enumeration of total coliforms and *Escherichia coli* from source water by the defined substrate technology. Appl Environ Microbiol 56:366–369

Environment Agency (2006) Attenuation of mine pollutants in the hyporheic zone. Environment Agency, Bristol

European Parliament and the Council of the European Union (2006) Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC. Official J L 64/37

European Parliament and the Council of the European Union (2008) Directive 2008/105/EC of the European Parliament and of the Council on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC, and amending Directive 2000/60/EC of the European Parliament and of the Council. Official J L 348/84

Fidancev B, Ilievski D, Kostadinov L, Straseski A (2011) Substitution of the conventional method with a new method for building the future over height flotation tailing disposal from Toranica lead and zinc mine, Kr. Palanka, Macedonia. XIV Balkan Mineral Processing Congress, Tuzla

Findlay S, Tank J, Dye S, Valett HM, Mulholland PJ, McDowell WH et al (2002) Cross-system comparison of bacteria and fungal biomass in detritus pools of headwater streams. Microbial Ecol 43:55–66

Government of the Republic of Macedonia (1999) Regulation for classification of water. Official Gazette of the Republic of Macedonia No. 18/99

Gray NF (1996) Field assessment of acid mine drainage contamination in surface and groundwater. Environ Geol 27:358–361

Holmstrom H, Ljungberg J, Ohlander B (1998) Role of carbonate in mitigation of metal release from mining waste: Evidence from humidity cells tests. Environ Geol 37:267–280

Hudson-Edwards KA, Macklin MG, Curtis CD, Vaughn DJ (1996) Processes of formation and distribution of Pb, Zn, Cd and Cu bearing minerals in the Tyne Basin, northeast England: Implications for metal-contaminated river systems. Environ Sci Technol 30:72–80

Hudson-Edwards KA, Schell C, Macklin MG (1999) Mineralogy and geochemistry of alluvium contaminated by metal mining in the Rio Tinto area, southwest Spain. Appl Geochem 14:1015–1030

Huet M (1986) Text book of fish culture, 2nd edn. Fishing News Book Ltd., Farnham

Jackson BP, Bertsch PM (2001) Determination of arsenic speciation in poultry wastes by IC-ICP-MS. Environ Sci Technol 35:4868–4873

Jackson BP, Bertsch PM, Cabrera ML, Camberato JJ, Seaman JC, Wood CW (2003) Trace element speciation in poultry litter. J Environ Qual 32:535–540
Jain CK (2004) Metal fractionation study on bed sediments of the River Yamuna, India. Water Res 38:569–578
Jiménez A, Aroba J, de la Torre ML, Andujar JM, Grande JA (2009) Model of behaviour of conductivity versus pH in acid mine drainage water, based on fuzzy logic and data mining techniques. J Hydroinform 11:147–153
Kapetanović D, Dragun Z, Vardić Smrzlić I, Valić D, Teskeredžić E (2013) The microbial marine water quality associated with capture-based bluefin tuna aquaculture: a case study in Adriatic Sea (Croatia). Fres Environ Bull 22:2214–2220
Kavka GG, Kasimir GD, Farnleitner AH (2006) Microbiological water quality of the River Danube (km 2581–km 15): Longitudinal variation of pollution as determined by standard parameters. Proceedings of 36th International Conference of IAD. Austrian Committee Danube Research/IAD, Vienna, pp 415–421
Korhonen LK, Martikainen PJ (1991) Survival of *Escherichia coli* and *Campylobacter jejuni* in untreated and filtered lake water. J Appl Bacteriol 71:379–382
Lapakko K (2002) Metal mine rock and waste characterization tools: An overview, mining, minerals and sustainable development, No. 67. Available at: http://pubs.iied.org/pdfs/G00559.pdf. Accessed: April 2002
Lata P, Ram S, Agrawal M, Shanker R (2009) Enterococci in river Ganga surface waters: Propensity of species distribution, dissemination of antimicrobial-resistance and virulence-markers among species along landscape. BMC Microbiol 9:140–149
Lee G, Bigham JM, Faure G (2002) Removal of trace elements with Fe, Al and Mn from natural waters contaminated with acid mine drainage in the Ducktown mining district, Tennessee. Appl Geochem 17:569–581
Lewin J, Macklin MG (1987) Metal mining and floodplain sedimentation in Britain. In: Gardiner V (ed) International geomorphology 1986 Part I. Wiley, London, pp 1009–1027
Macklin MG, Brewer PA, Hudson-Edwards KA, Bird G, Coulthard TJ, Dennis IA (2006) A geomorphological approach to the management of rivers contaminated by metal mining. Geomorphology 79:423–447
Marcus WA (1987) Copper dispersion in ephemeral stream sediments. Earth Surf Proc Land 12:217–228
Midžić S, Silajdžić I (2005) Macedonia. In: Wolkersdorfer C, Bowell R (eds) Contemporary reviews of mine water studies in Europe Part 2. Mine Water Environ 24:2–37
Milevski J, Karamanolevski Z, Tognacca C (2004) RIMSYS—river monitoring system. BALWOIS, Ohrid, pp 1–10
Nash D, Halliwell D, Hannah M, Clemow L, Webb B (2003) Phosphorus and selected metals mobilized from two commercial fertilizers into overland flow during border irrigation. Nutr Cycl Agroecosyst 67:255–264
Neal C, Williams RJ, Neal M, Bhardwaj LC, Wickham H, Harrow M et al (2000) The water quality of the River Thames at a rural site downstream of Oxford. Sci Total Environ 251–252:441–457
Nordstrom DK, Alpers CN (1997) The environmental geochemistry of mineral deposits Part A. Processes, methods and health issues. In: Plumlee GS, Logsdon MJ (eds) Reviews in economic geology. vol. 6. Society of Economic Geologists, Littleton, CO.
Peña-Méndez EM, Havel J, Patočka J (2005) Humic substances. Compounds of still unknown structure: application in agriculture, industry, environment and biomedicine. J Appl Biomed 3:13–24
Rebok K (2013) A toxicopathology survey of the River Bregalnica using the barbel (*Barbus peloponnesius,* Valenciennes 1844) as bioindicator. Doctoral Thesis, University Ss. Cyril and Methodius, Skopje, Macedonia
Ribeiro J, DaBoit K, Flores D, Kronbauer MA, Silva LFO (2013a) Extensive FE-SEM/EDS, HR-TEM/EDS and ToF-SIMS studies of micron- to nano-particles in anthracite fly ash. Sci Total Environ 452–453:98–107
Ribeiro J, Taffarel SR, Sampaio CH, Flores D, Silva LFO (2013b) Mineral speciation and fate of some hazardous contaminants in coal waste pile from anthracite mining in Portugal. Int J Coal Geol 109–110:15–23
Robb GA, Robinson JDF (1995) Acid drainage from mines. Geogr J 161:47–54
Roje V (2008) Determination of metals in environmental samples by inductively coupled plasma-mass spectrometry. Doctoral thesis, University of Zagreb, Zagreb
Sachs S, Brendler V, Geipel G (2007) Uranium(VI) complexation by humic acid under neutral pH conditions studied by laser-induced fluorescence spectroscopy. Radiochim Acta 95:103–110
Sarmiento AM, DelValls A, Nieto JM, Salamanca MJ, Caraballo MA (2011) Toxicity and potential risk assessment of a river polluted by acid mine drainage in the Iberian Pyrite Belt (SW Spain). Sci Total Environ 409:4763–4771
Senesi N, Polemio M, Lorusso L (1983) Evaluation of barium, rubidium and strontium contents in commercial fertilizers. Fertil Res 4:135–144
Serafimovski T, Dolenec T, Tasev G (2006) New data concerning the major ore minerals and sulphosalts from Pb-Zn Zletovo Mine, Macedonia. RMZ Mater Geoenviron 52:535–548
Serafimovski T, Dolenec T, Tasev G, Dolenec M, Rogan N (2007) Acid mine drainage systems and metal pollution around the active polymetallic mines in the Eastern Macedonia. Geol Maced 21:69–73
Silva LFO, Izquierdo M, Querol X, Finkelman RB, Oliveira MLS, Wollenschlager M et al (2011a) Leaching of potential hazardous elements of coal cleaning rejects. Environ Monit Assess 175:109–126
Silva LFO, Querol X, da Boit KM, Fdez-Ortiz de Vallejuelo S, Madariaga JM (2011b) Brazilian coal mining residues and sulphide oxidation by Fenton's reaction: An accelerated weathering procedure to evaluate possible environmental impact. J Hazard Mater 186:516–525
Silva LFO, Wollenschlager M, Oliveira MLS (2011c) A preliminary study of coal mining drainage and environmental health in the Santa Catarina region, Brazil. Environ Geochem Health 33:55–65
Silva LFO, Fdez-Ortiz de Vallejuelo S, Martinez-Arkarazo I, Correa OK, Oliveira MLS et al (2013) Study of environmental pollution and mineralogical characterization of sediment rivers from Brazilian coal mining acid drainage. Sci Total Environ 447:169–178
Smith H, Wood PJ, Gunn J (2003) The influence of habitat structure and flow permanence on invertebrate communities in karst spring systems. Hydrobiologia 510:53–66
Spasovski O (2011) Heavy and toxic metals and nutrients in separate places in the river Bregalnica, (eastern Macedonia). Annual of the University of Mining and Geology St. Ivan Rilski. vol. 54. Part II: Mining and mineral processing. pp 118–120
Spasovski O (2012) Potential and geochemical characteristics of geothermal resources in Eastern Macedonia. In: Panagiotaras D (ed) Geochemistry—Earth's system processes. InTech, Rijeka, pp 291–322
Spasovski O, Dambov R (2009) Heavy metals in the water of the river Kalnistanska and the vicinity. Balkanmine 2009—3rd Balkan Mining Congress. Izmir, Turkey, pp 667–670
Stuhlberger C (ed) (2010) Mining and environment in the Western Balkans. Zoï Environment Network, Geneva
Stumm W, Morgan JJ (1981) Aquatic chemistry—An introduction emphasizing chemical equilibria in natural waters. Wiley, New York, p 470

Vachirapatama N, Macka M, Haddad PR (2002) Separation and determination of vanadium in fertiliser by capillary electrophoresis with a light-emitting diode detector. Anal Bioanal Chem 374:1082–1085

Wolfenden PJ, Lewin J (1978) Distribution of metal pollutants in active stream sediments. Catena 5:67–78

Zhang G, Liu C-Q, Yang Y, Wu P (2004) Characterization of heavy metals and sulphur isotope in water and sediments of a mine-tailing area rich in carbonate. Water Air Soil Pollut 155:51–62