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Surface water characterization of three rivers in the Pb/Zn mining region of north-eastern Macedonia

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Abstract

The mine waste was recognized as one of the most serious threats for freshwater ecosystems, and it still represents one of the biggest environmental concerns in Macedonia. The aim of our investigation was obtaining of in-depth understanding of the mining influence on freshwater systems, from water contamination to effects on aquatic organisms. In this paper we have assessed the impact of active Pb/Zn mines Zletovo and Toranica on water quality of three rivers in north-eastern Macedonia (Bregalnica, Zletovska and Kriva), based on data collected in spring and autumn of 2012. The Bregalnica River, near Shtip, was characterized mainly by weak contamination with As, Ba, Fe, Mo, Ti, U, V, nitrate and phosphate, and critical faecal pollution, which alltogether could be connected to agricultural activities, whereas the impact of the mines was not observed. Contrary, both Zletovska and Kriva rivers revealed a clear impact of Pb/Zn mines on water quality. In the Zletovska River increased concentrations of Cd, Co, Cs, Cu, Li, Mn, Ni, Rb, Sn, Sr, Tl, 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 higher water discharge. The selected sampling sites on Bregalnica, Zletovska and Kriva rivers were confirmed as the appropriate locations for further studies of mining waste impact on the freshwater ecosystems, the first one as non-impacted site, and the other two as possible areas of increased exposure of aquatic organisms to metals.

Key words: bacterial counts, metals, mining, rivers, surface water quality

1. Introduction

Metal mine discharges have resulted in the 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, the metal mine drainage is recognized by the environmental agencies as the second most serious threat to water quality (Environment Agency 2006). Some of the reasons for its toxicity are the acidity and high content of trace metals (Stuhlberger 2010). In the rivers which are receiving 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, the discharges of untreated wastewater from mining are among the most serious water pollution concerns (Stuhlberger 2010). Macedonian most significant mineral deposits are Pb and Zn ores, the exploitation of which is carried out in extraction, smelting, and metal-processing industrial plants located in the north-eastern part of the country (Zletovo in Probishtip, Toranica in Kriva Palanka, and Sasa in Makedonska Kamenica) (Midžić and Silajdžić 2005). In the Probishtip concentration plant, where the 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). Also, wastewater from the Zletovo mine, which is contaminated with metals, is pumped from the concentration plant into the Kiselica River, 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 the fish and other biological samples (Midžić and Silajdžić 2005).

Information obtained from the existing monitoring programmes for the rivers in the north-eastern Macedonia (Milevski et al. 2004), combined with common knowledge on the hazards for the aquatic systems associated with mining, were the incentive for the start of a comprehensive investigation on the impacts of currently active Pb/Zn mines Zletovo and Toranica in the rivers receiving their waste. Overall aim of that investigation was obtaining of in-depth understanding of the mining influence on freshwater systems, based on the assessment of: (1) metal exposure by determining metal concentrations in the surface water, (2) metal bioavailability and bioaccumulation by determining metal concentrations in fish tissues, and (3) the finite effects of water contamination on the fish by defining histopathological changes in fish tissues, as well as fish health status. In this paper, the impact of mining on different aspects of the surface water quality of selected aquatic systems will be presented, such as physico-chemical parameters, nutrient concentrations, microbiological parameters, and the concentrations of dissolved macro and trace elements. Two rivers were chosen for this study, the Zletovska River, which received the waste from the mine Zletovo, and the Kriva River, which received the waste from the mine Toranica, whereas the Bregalnica River was chosen as non-impacted 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 mining impact on the aquatic life.

2. Materials and methods

2.1. Study area

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

Based on information gathered during existing monitoring programmes (Milevski et al. 2004), Bregalnica was selected as non-impacted river, because it was less contaminated compared to other two selected rivers. It is the longest left tributary of the Vardar River, the principal river in Macedonia. Bregalnica has a length of 225 km and the catchment area of 4,307 km^2. Its water discharge in 2012 was in the range from 1.24-66.30 $m^3 s^{-1}$ (Table 2, data obtained by the courtesy of the Hydrometeorological Service of the Republic of Macedonia). To avoid the influence of the contamination from the Zletovska River, we have chosen as the sampling point at Bregalnica a location 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 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 the catchment area of 460 km^2. Water discharge of the Zletovska River in 2012 was in the range from 0.167-26.55 $m^3 s^{-1}$ (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 the north-eastern Macedonia (Alderton et al. 2005; Dolenec et al. 2005). In the town of Probishtip, there is also a battery factory, as a potential source of contamination (Spasovski and Dambov 2009). As the sampling point at the Zletovska River, we have chosen a site 5-6 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 ($CuFeS_2$), and number of other minerals which occur only sporadically (Alderton et al. 2005; Serafimovski et al. 2006). The mine has started operating in 1940s actively and its production lasts until today, with several short-term interruptions.. Ores from the Zletovo mine, with grades higher than 9% of Pb and 2% of Zn, and with significant presence of Ag, Bi, Cd, and Cu (Alderton et al. 2005), are concentrated by flotation at Probishtip, while tailings are disposed of in two impoundments situated in the adjacent valleys. Two tributaries of the Zletovska River, the Kiselica

River and the Koritnica River 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 River Pčinja, 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 was in the range from 0.08-8.42 $m^3 s^{-1}$ (Table 2). As the sampling point at the Kriva River, we have chosen the site 15-20 km downstream from the Toranica mine. The Toranica deposit is situated in the north-eastern Macedonia, close to the Sasa deposit, but in a separate watershed. The production of Pb and Zn from the Toranica mine have been lasting from 1987, with a few year interruptions which 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 about 10% of Pb+Zn with additional elevated concentrations of Ag, As, Bi, Cd, Cu, Mn, and Sb (Serafimovski et al. 1997). Ore milling and flotation occur at the mine, and there is a tailing dam below the mine with a culvert directing the Toranica River, the Kriva River tributary, beneath the dam (Alderton et al. 2005). During the autumn sampling, the 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').

2.2. Water sampling

The river water was sampled for analyses of dissolved metals and physico-chemical parameters in each river once in the spring period with higher water discharge and once in the autumn period when water discharge was close to annual minimum (Table 1 and 2). On each occasion, the river water samples were collected by grab water sampling in the polyethylene plastic bottles (three bottles of 0.25 L for metal analyses and one bottle of 1.0 L for physico-chemical parameters) which were, prior to sampling, rinsed with acid (for metal analyses: v/v 10% HNO_3, p.a., Merck, Germany; for physico-chemical parameters: v/v 5% HCl, p.a., Merck, Germany) and with Milli-Q water. The samples were stored at +4°C for at most 24 hours 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, Germany), and stored at +4°C.

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

2.3. Physico-chemical parameters

Several physico-chemical 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 physico-chemical parameters were measured subsequently in the river

water samples in the laboratory by the methods listed in Tables 3 and 4. For all the analyses, standardized ISO, APHA (1998) and EPA methods, as well as high purity reagents (Merck and Fluka Analytical) and Milli-Q water (Model Milli-Q 5) were used.

2.4. Dissolved metals and metalloids in the river water

Dissolved trace elements were measured directly in filtered river water samples, whereas macro elements (Na, K, Ca, Mg) were measured in 10 times diluted filtered samples, due to their higher concentrations. The measurements of both trace and macro elements were performed on 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 in medium resolution mode, whereas ^{39}K and ^{75}As were measured in high resolution mode. Indium (1 μg L^{-1}; Indium Atomic Spectroscopy Standard Solution, Fluka, Germany) was added to the samples as an internal standard (Dautović 2006). The 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 Sn (1 g L^{-1}; Analytika, Prague, Czech Republic) were added, and separately for macro elements (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 the samples of the river water). If necessary, the blank corrections of measured trace element concentrations in the river water were made. The accuracy of metal determination was controlled with 100 times diluted quality control sample for trace metals (QC Trace Metals, Catalog number 8072, Lot Number 146142-146143, UNEP GEMS, Burlington, Canada) and with 10 times diluted quality control sample for macro elements (QC Minerals, Catalog number 8052, Lot Number 146138-146139, UNEP GEMS, Burlington, Canada). The limits of detection for trace element measurement in the filtered river water were following (in μg L^{-1}): As (0.005), Ba (0.020), Cd (0.001), Co (0.001), Cs (0.001), Cu (0.010), Fe (0.100), Li (0.010), Mn (0.010), Mo (0.020), Ni (0.020), Pb (0.020), Rb (0.010), Sb (0.001), Sn (0.001), Sr (0.020), Ti (0.020), Tl (0.002), U (0.001), V (0.002), and Zn (0.100) (Dautović 2006; Roje, 2008).

2.5. Bacterial counts in the river water

Microbiological analyses of the river water were performed in the same way as previously described for the marine water by Kapetanović et al. (2013). Total coliform bacteria were identified using Colilert (IDEXX Laboratories, Inc., Westbrook, USA), a defined substrate technology (Edberg et al. 1990). Enterococci were identified similarly, using Enterolert-E (IDEXX Laboratories, Inc., Westbrook, USA). Total coliforms and enterococci were enumerated using the Quantitray2000 (IDEXX Laboratories, Inc., Westbrook, USA), which utilized a 97-test-well system and provided the most probable number of bacteria per 100 mL (MPN/100 mL) (Table 4).

2.6. Data processing and statistical analyses

Statistical program SigmaPlot 11.0 for Windows was applied for graph creating and statistical analyses. Due to 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 the comparison of metal and metalloid concentrations measured in three rivers, separately for each sampling. Mann-Whitney Rank Sum Test was used for the comparison of the results obtained in the spring and autumn, separately for each site. Although some differences were observed between the spring and the autumn samples, they were not statistically significant (p>0.05), due to small number of data per site within each period (n=3).

3. Results and discussion

The assessment of the river water quality presented in this paper was a part of larger study which also encompassed the evaluation of the impact of water contamination on the health of the native fish. Therefore, one of the basic criteria for the sampling site selection was that some essential characteristics of the river water, such as pH and water oxygenation, should have been compatible with possibility for fish surviving. Accordingly, the sampling sites of two mining impacted rivers were selected at locations beeing far enough from the mines to secure the presence of the aquatic life. As a result of the targeted selection of the sampling locations, both pH and dissolved oxygen level in all three rivers in this study (Table 3) were categorized as the 1st class of surface waters (Table 5; GRM 1999). However, as expected, a number of measured parameters did not comply with the requirements for the 1st class water. For example, turbidity in all samples of studied rivers was high, characteristic for 4th class of water (Table 5). Increased turbidity in the vicinity of mines can be result of iron precipitates. It reduces the incidence of the light in water body, impeding photosynthesis, and causing break down of food chains, what finaly results in the biodiversity decline 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 further on separately for each studied watercourse.

3.1. The Bregalnica River

The Bregalnica River receives the water from the Zletovska River, which is directly influenced by the active mine Zletovo (Fig. 1). However, a location was selected at Bregalnica, which is presumably far enough from the source of contamination to be considered as a non-impacted site. Downstream and further away from the mining regions, decrease in dissolved metal concentrations can be expected as a result of a dilution in noncontaminated river water, as well as 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 the 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, and not only in the water, downstream from the contaminant source (Byrne et al. 2012), due to hydraulic sorting (Wolfenden and Lewin 1978), dilution by uncontaminated sediments (Marcus 1987),

hydrogeochemical reactions (Hudson-Edwards et al. 1996), and biological uptake (Lewin and Macklin 1987).

Although within the limits for the 1st class of water (Table 5), pH of the Bregalnica River water was slightly alkaline and comparable to pH values characteristic for the sites with high intensity agricultural activity, which were reported to be around 8 (Cooper and Fortin 2010). In addition, TDS (Table 3) were rather low in the spring samples, but they somewhat increased in the period of low water level in the autumn (Table 5). The levels of nitrates and phosphates were slightly increased compared to the Zletovska River (Table 4). Similarly, the indicators of faecal pollution, the levels of total coliforms and enterococci, which were determined only in the autumn period, were also higher compared to the Zletovska River (~4 and 40 times, respectively), but much below the values obtained for the Kriva River (~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 (CEC 1976; 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 3rd class, or the river water with critical faecal pollution (Table 5; Kavka et al. 2006). Somewhat disrupted physical and organoleptic properties of the water, accompanied by increased level of suspended substances and high microbiological contamination, were already reported for the Bregalnica River under the town of Shtip, as a result of the drainage of sewage from the households, the collection system and the industry (Spasovski 2011).

Interesting finding for the Bregalnica River was several degrees higher water temperature compared to the other two rivers, in both sampling periods (Table 3), which can have a profound influence on the aquatic ecology (Huet 1986), for example by affecting the solubility of gases (Cokgor et al., 2009). The cause of this increase could be found in the geothermal system Kezhovica-Ldzhi, situated nearby, on the right bank of the Bregalnica River, approximately 2 km to the southwest from the center of Shtip. Temperature increase of the river water was possibly the result of the mixing of the river water with the hot water from the deep springs of cracked granites which are the reservoirs of this geothermal system (Spasovski 2012).

Additionally, the concentrations of several dissolved trace elements were moderately increased in the surface water of this river, namely As, Ba, Fe, Mo, Ti, U, V (Fig. 3a,b,g,j,r,t,u). This increase was mostly notable when comparison was made to the Zletovska River, with concentrations being higher about 4 to 30 times. 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 was surpassing freshwater screening benchmark defined by US EPA (Fig. 3b, Table 6). The concentrations of other six elements have not exceeded either US EPA benchmarks (http 1) 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 observable adverse effects on aquatic ecosystems over the long term (http 2).

The specificity of these moderate changes of the river water quality observed at the Bregalnica River was that they could be usually seen in the rivers flowing through agriculturally developed regions. In the Sutla River in Croatia, for example, 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 and Seifert 2004; V, Vachirapatama et al. 2002) that might contaminate nearby rivers (Nash et al. 2003; Bolan et al. 2004; Cooper and Fortin 2010). Increased concentrations of As, Cu, and Zn have been previously associated with the poultry litter (Jackson and Bertsch 2001; Jackson et al. 2003), and therefore increase of As concentration could be also associated to the presence of a poultry farm in the town of Shtip (Spasovski 2011). On the other hand, increase of U concentrations at agriculturally impacted sites can occur due to its complexation with the 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). And finally, increased level of phosphorus and nitrogen in the aquatic ecosystems was also reported in association to agricultural activities, as well as increased number of enterococci in the river water, which indicates the water contamination with manure, since enterococci are present in the faeces of warm blooded animals (Carpenter et al. 1998; Lata et al. 2009; Dragun et al. 2011; http 3).

The concentrations of several elements (Cu, Mn, Pb, Ti; Fig. 3f,i,l,r), otherwise present in rather low concentrations, were somewhat increased in the spring compared to the autumn samples (2-8 times), which could be also connected to leaching from agriculturally utilized soils during rainy period. Previous analyses of the sediments indicated that the effects of the Zletovo mine extended all the way to the Bregalnica River, since even at the confluence between the Zletovska and Bregalnica, the sediment still contained Zn in concentration of 990 mg/kg (Alderton et al. 2005). However, the effect of mining was not visible in the river water downstream from the confluence of these two rivers, even during the spring period of higher water discharge, when sediment resuspension and consequent increase in metal concentrations could be anticipated (Neal et al. 2000; Dragun et al. 2009).

3.2. The 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 redox potential (pE), the highest water hardness, as well as the highest conductivity and TDS (Table 3), classified as 3rd and 5th class of water, in the spring and autumn, respectively (Table 5). Total hardness, the concentration of $CaCO_3$ (Table 3) and the concentrations of macro elements (Na, K, Ca, Mg; Fig. 2a-d) were also the highest in that river, with Ca concentration even higher than US EPA benchmark (Table 6). Since conductivity is especially sensitive to sulphate ions (Jiménez et al. 2009), the concentrations of SO_4^{2-} were accordingly higher in this river compared to Bregalnica and Kriva, 8-15 times in the spring and 20-35 times in the autumn (Table 4). Observed increase of Cl^- concentrations in the Zletovska River was

somewhat less pronounced (Table 4). The sulphate concentrations were approximately 2-4 times above 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 selected sampling site at the Zletovska River. It is because sulphur is not easily adsorbed and thus can migrate further than heavy metals: its migration can represent the biggest 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 and Morgan 1981; Lapakko 2002), which is the reason why an increase in pyrite content, such as characteristic for the Zletovo mine, commonly results in higher acidity (Alderton et al. 2005), as well as in the formation of soluble metal sulphates in the waste dumps (Aykol et al. 2003). Consequently, the first indicator of sulphide mineral oxidation is presence of sulphates, as the dominant anions, and H^+ in mine drainage waters (Nordstrom and 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 and Robinson 1995). Such acidic, metal-rich waters, that flow from the abandoned or active mines can contaminate streams and rivers far downstream from the drainage source, and consequently can have toxic effects on the biota (Aykol et al. 2003).

With increasing distance from the contamination source the acidity of the mine water is generally buffered to a higher or lower degree by dilution (Aykol et al. 2003). Relatively high pH of the river water (above 6.1) favours the incorporation of metals into the particulate phase through the processes of sorption (Bird et al. 2010). However, even then the 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, Cr, Cu, Fe, Mn, Pb, Zn) were reported as 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. 3c,d,e,f,h,i,k,m,o,p,s,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 (EPCEU 2008; Table 6). Comparison with the Canadian guidelines and US EPA benchmarks (Table 6) indicated 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 was already 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 and Dambov 2009). The increase of Cu and Mn concentrations in the water of the Kalnistanska River was also observed, where Cu increase was a

result of chalcopyrite presence associated with the Pb/Zn minerals (Spasovski and Dambov 2009). The concentrations of few metals (Cd, Mn, Ni, Pb, Tl, and Zn) were especially high in October, during low water discharge. Specifically, dissolved Cd was around 10 times above its EQS and 20 times above Canadian recommendations, whereas dissolved Mn and Zn were as much as 12 and 50 times above Canadian limits, respectively (Fig. 3c,i,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 sphalerite (ZnS) and galena (PbS) are present in equal amounts in the Pb-Zn mines (Barnes 1979), such as Zletovo. This could be explained by higher solubility of ZnS compared to PbS (Barnes 1979), as well as the fact that Pb is readily adsorbed by Al and Fe oxide phases in sediment (Lee et al. 2002) resulting in 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 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, Tl) were also found in highly elevated concentrations in the paddy soil samples in the vicinity of the Zletovska River (Dolenec et al. 2005). It was undoubtedly the consequence of the discharge of untreated acid mine water and effluents from tailings, rich in Zn and Cd (39 mg L^{-1} and 176 mg L^{-1}, respectively), into the river water which 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 has to be considered as a source of high trace element concentrations in the Zletovska River water, since the previous studies also reported high metal levels upstream of the mine (e.g. Zn 330 µg L^{-1} 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 the faecal pollution. The levels of total coliforms and enterococci were lower than 10,000 and 400 MPN/100 mL (Table 4), respectively, indicating good microbiological water quality, according to the European recommendations (CEC 1976; EPCEU 2006). If classification by Kavka et al. (2006) was applied, this river would belong to the 1st and 2nd class, which would indicated weak or moderate faecal water pollution (Table 5). The level of nutrients was also lower compared to 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 the trace elements in the Zletovska River (Aykol et al. 2003).

3.3. The Kriva River

The Kriva River water had slightly alkaline pH and negative redox potential, same as Bregalnica (Table 3). However, conductivity, TDS and alkalinity were lower (Table 3), indicating lower concentrations of various salts in this river, that was also confirmed by low levels of macro elements (Fig. 2a-d). Accordingly, although both rivers had higher concentrations of the same trace elements compared to the Zletovska River, being characteristic for agricultural type of 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 the Kriva River water (Fig. 3b,g,j,u). Furthermore, it would be expected to find similar type of

contamination in the Kriva River as in the Zletovska River, since the sampling location was situated downstream from the mine Toranica, which has exploited similar Pb- and Zn-rich minerals to those at Zletovo and exhibited 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 shown even in the previous studies (Alderton et al. 2005). As explained by Alderton et al. (2005), it was probably caused by lack of pyrite in the ore compared to Zletovo, as well as buffering of the acid waters by carbonate host lithologies (limestone), which kept metal concentrations low. Similar finding was reported for the 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, the acid formed in the oxidation of sulphides can be neutralized by carbonate rock, such as limestone and dolomite, resulting in slightly alkaline surface water and retarding the migration of heavy metals (Holmstrom et al. 1998; Zhang et al. 2004). This could explain low dissolved metal concentrations in the surface water of the Kriva River, as well as 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. 3c,l) were found in the river water in the spring period, which could be associated with the impact of the nearby mine. The concentration of Cd in the spring was above its EQS , whereas Pb concentration, although increased, was still lower than its EQS, approximately 4 times (Table 6). In the autumn period, however, Cd and Pb concentrations decreased at the selected sampling site, but closer to the mine they were still rather high and comparable to spring values (at Zhidilovo: Cd, 210 ng L^{-1}; Pb, 1.95 μg L^{-1}).

The contamination event observed in the autumn referred to increased number of faecal bacteria, accompanied by the increase of NH_4^+, PO_4^{3-}, total nitrogen and total phosphorus (Table 4). The levels of total coliforms and enterococci were far above 10,000 and 400 MPN/100 mL, respectively, indicating unsatisfactory microbiological water quality (CEC 1976; EPCEU 2006). In the autumn period, the Kriva River was classified as the 4th class (Table 5), which pointed strong faecal water pollution (Kavka et al. 2006). The concurrent presence of high level 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 and 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 the gardens and cultivated land, which could be associated with the presence of the faecal bacteria and high level of nutrients in the river water.

However, with exception of the continuous slight increase of several trace elements characteristic for agricultural use (Ba, Fe, Mo and V; Fig. 3b,g,j,u), it seemed that these occasional contamination events were not the consequence of the continuous leaching either from the mine tailings or the agricultural soil, considering that they were rather extreme, but of short duration. Therefore, it could be hypothesized that the contamination of the Kriva River was caused by periodic waste input directly into the river water,

either from the mine or the manure from the farms. In the spring period, it is even more probable that sediment resuspension as a consequence of higher water discharge has resulted in the concentration increase of elements sequestered in the sediment, such as Cd and Pb, as often reported for trace elements in the river water (Neal et al. 2000; Dragun et al. 2009). The contamination of the river sediments has been reported in the most metal mining regions of the world, with metal concentrations usually being several orders of magnitude higher than that in the water column (Macklin et al. 2006). The sediments of the Toranica River, the tributary of the Kriva River, were reported as highly contaminated with Pb, Zn, and S; although the metal concentrations decreased after the confluence with the Kriva River, they were still significantly elevated (Alderton et al. 2005). Similarly, in the Twymyn River in the UK, in the vicinity of the former Pb/Zn mine, Pb concentrations in the sediment were up to 100 times higher than levels reported to have deleterious impacts on the 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, disturbance and oxidation of sediments during the flood flows (Byrne et al. 2010). Therefore, even when the concentrations of toxic elements in the water decrease due to dilution or precipitation and the pH is not very low, the river sediments still can be the source of high pollution level and high toxicity (Sarmiento et al. 2011).

4. Concluding Remarks

The results of this survey have confirmed the appropriateness of the sampling site selection for the study of the mining impact on the aquatic life in the freshwater ecosystems of the north-eastern Macedonia. It was demonstrated that the selected sampling site on the Bregalnica River could serve as a non-impacted site in such study, since it has not got water contamination characteristic for the mining impacted rivers, but only weak contamination by several trace elements and faecal bacteria, characteristic for the agriculturally developed regions. On the contrary, both the Zletovska and Kriva rivers exhibited clear signs of the water contamination as a result of the 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 the comparison with previously published data. Further study on these two rivers will, thus, enable the investigation of the effect of mining on freshwater fish as a result of two different ways of exposure: exposure through the water and exposure originating from the sediment.

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Figure captions

Fig. 1 The map of the sampling area with marked sampling sites; the black arrows point to studied Pb/Zn mines Zletovo and Toranica

Fig. 2 The concentrations (mg L^{-1}) of dissolved macro elements (a) Na, b) K, c) Ca, and d) Mg) in the river water of three rivers in the north-eastern Macedonia (1 – Bregalnica; 2 – Zletovska River; 3 – Kriva River); the results are presented as mean values and standard deviations (n=3); the statistically significant differences between sites according to Kruskal-Wallis one way analysis of variance on ranks (levels of significance indicated in the figure) followed by *post-hoc* Dunn's test ($p<0.05$) were indicated by different letters (lower case letters for spring, and upper case letters for autumn); legend: light gray bars – spring; dark gray bars – autumn

Fig. 3 The concentrations (µg L^{-1}) of dissolved trace elements in the river water of three rivers in the north-eastern 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 and v) Zn; the results are presented as described in the caption of Fig. 2

Table 1. The list of the sampling sites with coordinates, known pollution sources, and the dates of the samplings.

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

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

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

Sampling period	**Bregalnica River**	**Zletovska River**	**Kriva River**
Spring	2.90	9.51	0.933
Autumn	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

Table 3. General physico-chemical parameters determined in the river water of the Bregalnica River, the Zletovska River and the Kriva River in the spring and autumn of 2012.

	Bregalnica River		**Zletovska River**		**Kriva River**		**Measurement method**
	Spring	**Autumn**	**Spring**	**Autumn**	**Spring**	**Autumn**	
General physico-chemical parameters							
T (ºC)	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 2130 B)
Conductivity (µS cm^{-1})	390	595	1490	2020	211	347	measured on site
TDS (mg L^{-1})	316	510	987	1568	153	261	gravimetrically (APHA 2540 C)
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 (ºdH)	10.43	14.27	37.60	70.00	5.92	8.62	EDTA titrimetric method (APHA 2340 C)

For parameters measured on site, applied equipment is described in the section *2.3. Physico-chemical parameters*.

Table 4. The concentrations of nutrients and other anions, as well as microbiological parameters determined in the river water of the Bregalnica River, the Zletovska River and the Kriva River in the spring and autumn of 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})*	<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 (EPA 325.2 Chloride)
Number of bacteria (MPN/100 mL)							
Total coliforms		15214±543.2 (n=3)		3864±254.6 (n=2)		435170 (n=1)	
Enterococci		1004.9±174.6 (n=3)		23.5±11.8 (n=3)		15653±7837 (n=2)	

*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 different way of the result expression in two seasons

**microbiological analyses were performed only in the autumn sampling; methods are described in the section *2.5. Bacterial counts in the river water*

Table 5. The permitted ranges and upper limits of several physico-chemical parameters (GRM 1999) and faecal indicator bacteria (Kavka et al. 2006) 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	TDS	DO	Total coliforms	Enterococci
			NTU	mg L^{-1}	mg O_2 L^{-1}	*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	1,500	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
Classification of each river							
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

Table 6. The environmental quality standards (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 the surface waters, and freshwater screening benchmarks defined by US EPA for dissolved trace and macro elements.

	EQS[a]	Canadian guidelines[d]	US EPA[g]
As / µg L^{-1}	-	5	-
Ba / µg L^{-1}	-	-	4
Cd / µg L^{-1}	0.08-0.25[b]	0.031-0.112[e]	0.25[h]
Co / µg L^{-1}	-	50[f]	23
Cu / µg L^{-1}	8.2[c]	2.25-4[e]	9[h]
Fe / µg L^{-1}	-	300	-
Li / µg L^{-1}	-	2500[f]	14
Mn / µg L^{-1}	-	200[f]	120
Mo / µg L^{-1}	-	73	-
Ni / µg L^{-1}	20.0	91.3-150[e]	52[h]
Pb / µg L^{-1}	7.2	2.95-7.00[e]	2.5[h]
Sb / µg L^{-1}	-	-	30
Sn / µg L^{-1}	-	-	73
Sr / µg L^{-1}	-	-	1500
U / µg L^{-1}	-	15	2.6[h]
V / µg L^{-1}	-	100[f]	20
Zn / µg L^{-1}	-	30	120[h]
Na / mg L^{-1}	-	-	680
K / mg L^{-1}	-	-	53
Ca / mg L^{-1}	-	-	116
Mg / mg L^{-1}	-	-	82

[a] European Parliament and the Council of the European Union (EPCEU) (2008);
[b] EQS for Cd depends on the concentration of $CaCO_3$ in the river water; for the Kriva River, EQS for Cd is 0.150 µg L^{-1}, and for the Zletovska and Bregalnica rivers, 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 the water: the lower limit given in the table is based on the lowest $CaCO_3$ level 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 the 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 equal to 100.

Figure 1.

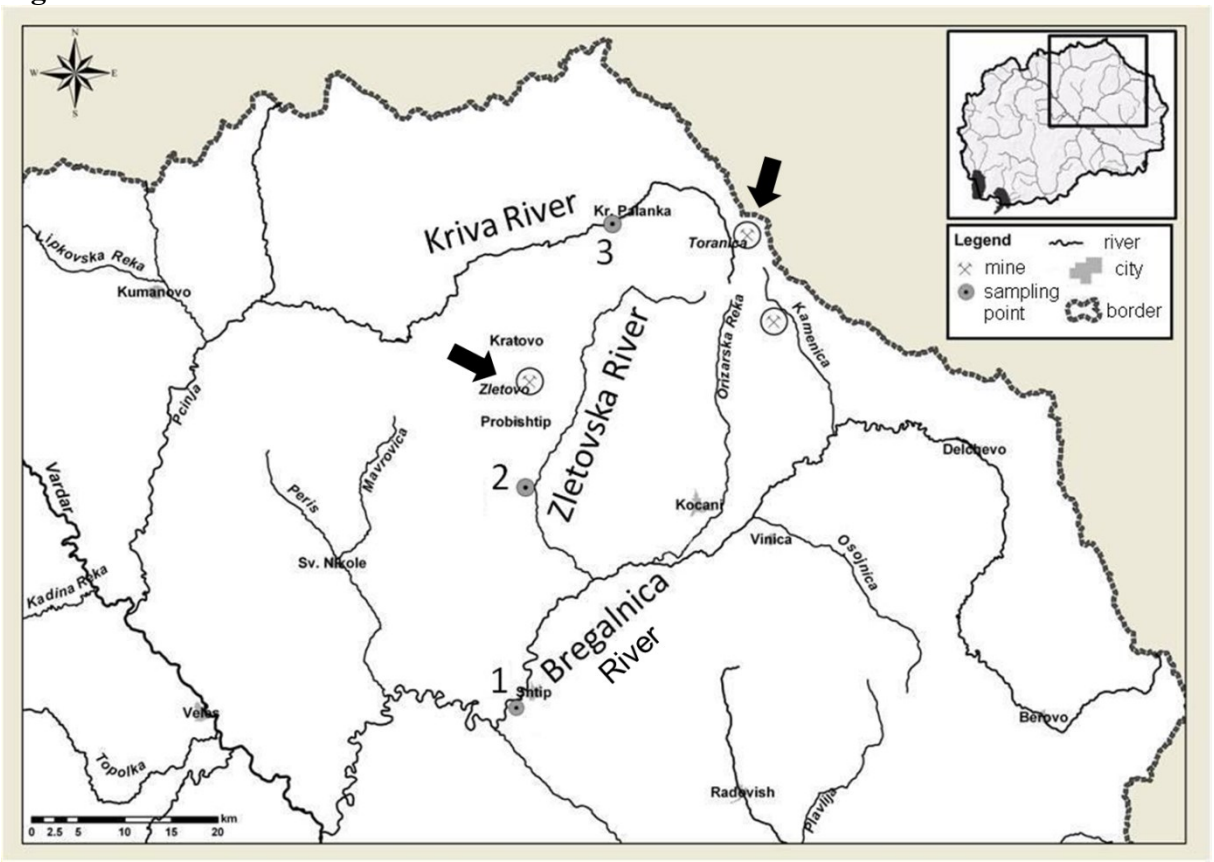

Kriva River
Kr. Palanka
Toranica
3
Lipkovska Reka
Kumanovo
Kratovo
Zletovo
Probishtip
Zletovska River
Onzarska Reka
Kamenica
Pcinja
Mavrovica
Peris
Vardar
2
Kocani
Delcevo
Vinica
Sv. Nikole
Kadina Reka
Osojnica
Bregalnica River
1
Shtip
Veles
Topolka
Berovo
Radavish
Plavilla
0 2.5 5 10 15 20 km
Legend
river
mine
city
sampling point
border

Figure 2.

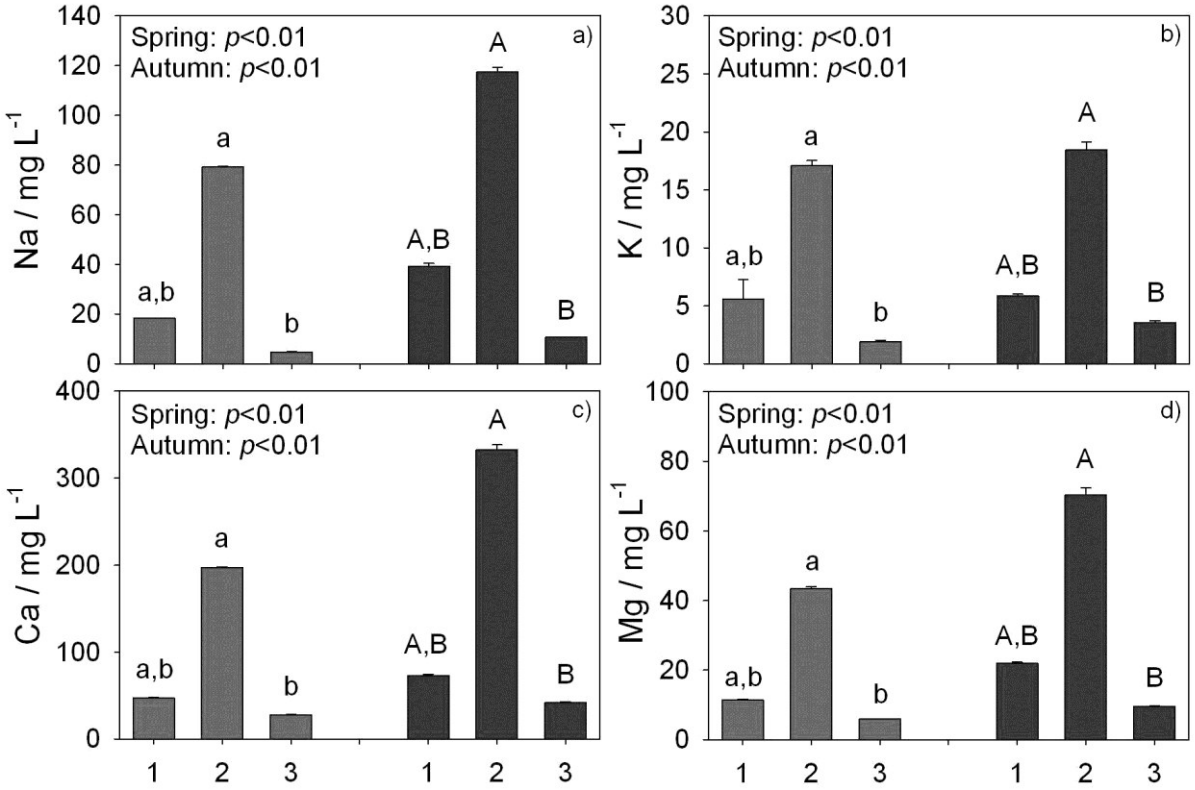
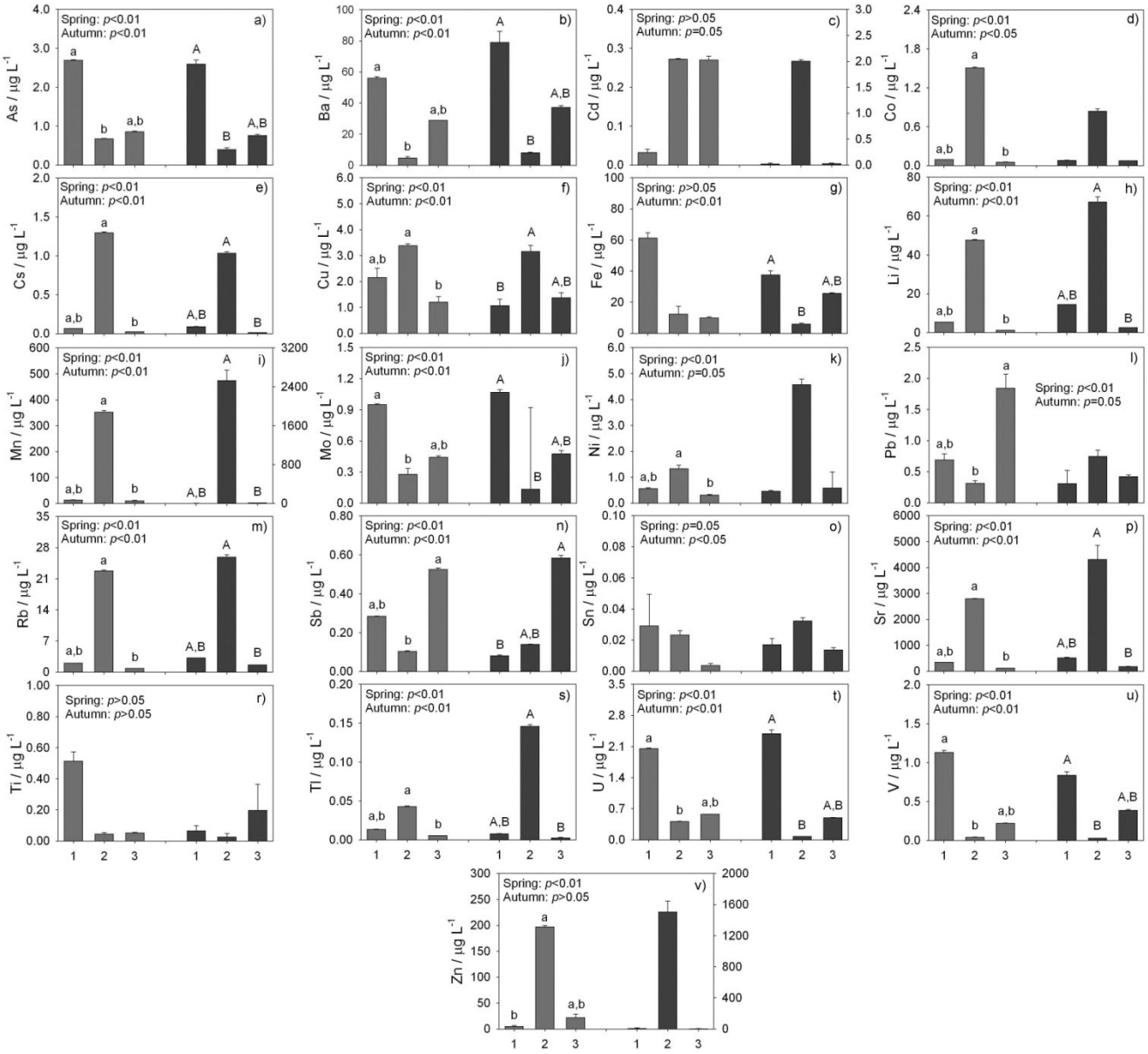

Spring: p<0.01
Autumn: p<0.01
a)
Na / mg L-1
b)
K / mg L-1
c)
Ca / mg L-1
d)
Mg / mg L-1
a,b
a
b
A,B
A
B
1
2
3

Figure 3.


a) As / µg L-1 Spring: p<0.01 Autumn: p<0.01
b) Ba / µg L-1 Spring: p<0.01 Autumn: p<0.01
c) Cd / µg L-1 Spring: p>0.05 Autumn: p=0.05
d) Co / µg L-1 Spring: p<0.01 Autumn: p<0.05
e) Cs / µg L-1 Spring: p<0.01 Autumn: p<0.01
f) Cu / µg L-1 Spring: p<0.01 Autumn: p<0.01
g) Fe / µg L-1 Spring: p>0.05 Autumn: p<0.01
h) Li / µg L-1 Spring: p<0.01 Autumn: p<0.01
i) Mn / µg L-1 Spring: p<0.01 Autumn: p<0.01
j) Mo / µg L-1 Spring: p<0.01 Autumn: p<0.01
k) Ni / µg L-1 Spring: p<0.01 Autumn: p=0.05
l) Pb / µg L-1 Spring: p<0.01 Autumn: p=0.05
m) Rb / µg L-1 Spring: p<0.01 Autumn: p<0.01
n) Sb / µg L-1 Spring: p<0.01 Autumn: p<0.01
o) Sn / µg L-1 Spring: p=0.05 Autumn: p<0.05
p) Sr / µg L-1 Spring: p<0.01 Autumn: p<0.01
r) Ti / µg L-1 Spring: p>0.05 Autumn: p>0.05
s) Tl / µg L-1 Spring: p<0.01 Autumn: p<0.01
t) U / µg L-1 Spring: p<0.01 Autumn: p<0.01
u) V / µg L-1 Spring: p<0.01 Autumn: p<0.01
v) Zn / µg L-1 Spring: p<0.01 Autumn: p>0.05