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RESEARCH PAPER

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Environmental impact of mining activity in Bor area as indicated by the distribution of heavy metals and bacterial population dynamics in sediment

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Abstract – The environmental impact of inorganic pollution is pronounced in water adjacent to Bor Copper Smelter Complex (RTB Bor, Serbia), with Cu, Zn, Pb, and As being the main determinants of aquatic pollution pattern. Communities of microorganisms present in the sediments are mainly affected by heavy metal pollution. Some groups of bacteria can be considered pollution bio-indicators, due to their sensibility and ability to bioaccumulate heavy metals, thus contributing to reducing pollution. This study investigates the relationships between trace element accumulation and heterogeneity in sediment bacteria community structure found in water streams adjacent to the Bor Copper Smelter Complex (RTB Bor, Serbia). Our results showed no contamination with copper, zinc, nickel, iron, and chromium, but did show a low to moderate contamination with lead and a moderate to high contamination with arsenic in aquatic sediments within the area of interest. Spatial heterogeneity in sediment-associated bacterial communities did not relate significantly to location of sampling sites, except for iron reducing bacteria. Iron reducing bacteria and nitrifying bacteria were the best distinguishing groups of bacteria. However, only iron reducing bacteria were significantly influenced by sampling locations. The iron reducing bacteria has correlated negatively with the degree of sediment contamination with lead, and therefore, we suggest that this group of bacteria could serve as potential bio-indicators of inorganic water contamination in Bor RTB area.

Key-words: bacterial community / sediments / heavy metals pollution

Résumé – L'impact environnemental de l'exploitation minière aux alentours de Bor comme indiqué par la distribution de métaux lourds et la dynamique de population bactérienne dans le sédiments. L'impact environnemental de la pollution inorganique est détecté dans l'eau adjacente à la fonderie de cuivre de la ville de Bor (RTB Bor, Serbie), le Cu, Zn, Pb, et As étant les principaux déterminants du schéma de pollution aquatique. Les communautés de micro-organismes présents dans les sédiments sont les plus touchés par la pollution des métaux lourds. Certains groupes de bactéries peuvent être considérés comme bio-indicateurs de pollution par leur sensibilité, leur capacité à bio-accumuler d'autres métaux et à contribuer à réduire la pollution. Cette étude s'est penchée sur la relation entre l'accumulation d'oligo-éléments et l'hétérogénéité dans la structure du sédiment de la communauté bactérienne, dans les courants d'eau à proximité de la fonderie de cuivre de la ville de Bor (RTB BOR, Serbie). Nos résultats n'ont montré aucune contamination par le cuivre, zinc, nickel, fer et chrome, mais une contamination faible à modérée par le plomb, et une contamination modérée à forte par l'arsenic dans les sédiments aquatiques des zones d'intérêts. L'hétérogénéité spatiale dans les communautés bactériennes associées aux sédiments n'a pas de lien significatif avec l'emplacement des sites de prélèvements, à l'exception des bactéries réduisant le fer. Les bactéries réduisant le fer et les bactéries nitrifiantes étaient les meilleurs groupes distinctifs de bactéries ; ils étaient fortement influencés par l'emplacement de sites d'échantillonnage. Les bactéries réduisant le fer sont négativement corrélées au degré de contamination par le plomb, et par conséquent nous proposons que ce groupe de bactéries serve comme potentiel bio-indicateur de contamination inorganique dans l'eau adjacente à RTB Bor.

Mots-clés : communauté bactérienne / sédiments / pollution avec les métaux lourds

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1 Introduction

Sources of water pollution in the Bor area are: waste and drainage water from on-going mining activity, drainage water from the flotation tailings which are no longer in function, overburden dumps from old inactive mine, city-urban wastewater, which is discharged without treatment directly into the Bor River. These wastewaters are a direct source of pollution for the Bor and Krivelj River, which then flow into the Bela River. The on-going mining activity (production of sulphuric acid, copper electrolytic refining, production of copper sulphate and processing of slime anode) has the biggest pollution impact on the Bor River (Bugarin *et al.*, 2013; Marinkovć *et al.*, 2014).

The copper mining and smelting operations located in Bor (Serbia) are among the largest nonferrous metal processing plants in Europe (Nikolic *et al.*, 2010). As a result, the Bor River, which collects the water from most water streams in this region, is one of the most contaminated rivers in Europe (Simić, 2007; Petrović *et al.*, 2013) being exposed to long-term inorganic contamination (Petković, 2009).

The Borska Reka River crosses the region of Bor, which is well known for the extensive soil, water, and air pollution with inorganic compounds caused by non-ferrous metal mining, smelting, and processing operations located in this area (Dragićević *et al.*, 2011). The basin of Bela River includes: Krivelj River with its tributaries; Cerova River with its tributaries; Bor River with its tributaries; Ravna River with its tributaries (Obradović *et al.*, 2012).

The Ravna River is located relatively far from the major current pollution sources within the Bor area, such as Cerovo, Bor, or Krivelj open pit mines. By contrast, the Kriveljska River collects the highly polluted waters of the Cerova River and passes next to the Bor open pit mine (Stevanović *et al.*, 2009). Together with additional sources of polluted waters, such as wastewaters from the "Jama Bor" underground mine or other types of industrial waters, it is estimated that almost 1285 tons of iron (Fe), 502 tons of copper (Cu), 52 tons of zinc (Zn), 6 tons of manganese (Mn), 2 tons of lead (Pb), 1.5 tons of nickel (Ni), 0.5 tons of arsenic (As), and 0.3 tons of cadmium (Cd) are being discharged every year, in the hydrographic basin of the Bor River (Panias, 2006).

Several studies reported on elevated level of various trace elements (TE), such as copper (Cu), lead (Pb), zinc (Zn), nickel (Ni), chromium (Cr), and arsenic (As) accumulate in aquatic sediments within the Bor River Basin (Nikolic *et al.*, 2010; Milijašević *et al.*, 2011).

In specific literature, there is a study based on the use of the water pollution index (WPI) presenting a geographical approach on the assessment of water quality within the Timok River Basin. They also reveal a general evaluation of the rivers' health, tacking into account many biotic parameters and abiotic factors, such as: dissolved O_2, O_2 saturation, pH, suspended sediments, BOD_5, Cod_{Mn}, nitrites, nitrates, orthophosphates, ammonium, metals (Fe, Mn, Hg, Cu, Pb, Zn, Cd), sulphates and coliform germs. According to the calculated WPI values, the Borska Reka is classified into Class VI, meaning heavily polluted (Milijašević *et al.*, 2011).

A recent study revealed that these water streams accommodate bacterial life although higher organisms are poorly represented in native aquatic ecosystems (Simić, 2007). Therefore, one can conclude that sediment microbial communities may serve as potential bioindicators to assess the impact of inorganic contamination on environmental health in the Bor area. This approach may overcome the drawbacks encountered when environmental quality is being assessed using various geochemical bioindicators, such as the enrichment factor (ER) or the geoaccumulation index (I_{geo}). Such bioindicators accurately measure sediment contamination (Abrahim and Parker 2008; Shafie *et al.*, 2013), but do not provide relevant information concerning its biological implications.

Microbial communities are key players for metal mobility in soils or sediments, and as a consequence, the spatial and temporal heterogeneity of native bacterial biota is frequently associated with the extent of inorganic pollution (Thiyagarajan *et al.*, 2010). It was found that bacterial biota responds differently to metal exposure, depending on environmental conditions (Gillan *et al.*, 2012). Thus, trace metals were shown to inhibit microbial biomass and diversity in certain environments (Muyzer *et al.*, 2008; Gillan *et al.*, 2012), whereas in other environments even elevated levels of trace metals had no effect on bacterial biomass and diversity (Bouskill *et al.*, 2010; Pringault *et al.*, 2010).

Studies on the soil/sediment highlights the impact of metal contamination on the groups of bacteria involved in the nitrogen cycle. Pb contamination of the soil affects the bacteria involved in the nitrogen cycle, nevertheless denitrifying bacteria seem to adapt to high concentrations of Pb (Sobolev and Begonia, 2008). Other studies report decreases in activity and the number of ammonifying and denitrifying bacteria in soil contamination with Zn, Pb, Cu and Hg (Li *et al.*, 2009; Liu *et al.*, 2010, 2014; Ruyters *et al.*, 2010; Magalhães *et al.*, 2011). Significant decreases are being registered in sediment activity and in the number of denitrifying bacteria in case of contamination with Cd, Cu or Zn (Sakadevan *et al.*, 1999). Another study analyses the effect of pollution with sulphate reducing bacteria. The results of this study suggest that SRB from metal-contaminated environments have a markedly higher metal tolerance than those enriched from uncontaminated environments (Jin *et al.*, 2007). The contamination with Cu resulted in an increased time reduction of Fe (III) and a reduction in the rate of metabolism. The study demonstrates that Cu toxicity impedes anaerobic carbon oxidation and bacterial reduction of hydrous ferric oxide (Markwiese and Colberg, 2000).

Five bacterial groups (sulphate reducing bacteria (SRB), iron reducing bacteria (IRB), ammonifying bacteria (AMB), denitrifying bacteria (DNB) and nitrifying bacteria (NB)) were considered in the present work based on their ecophysiological role in aquatic ecosystems and potential application for environmental biomonitoring and bioremediation. To the extent of our knowledge, this is the first study that addresses the shifts induced by inorganic water contamination to the dynamics of bacterial communities in aquatic bed sediments from water streams adjacent to the Bor Copper Smelter Complex (RTB Bor, Serbia). The results of this study are important, because the Bor River poses a serious threat to many other river-based communities in the Western Balkans and to the Lower Danube Basin.

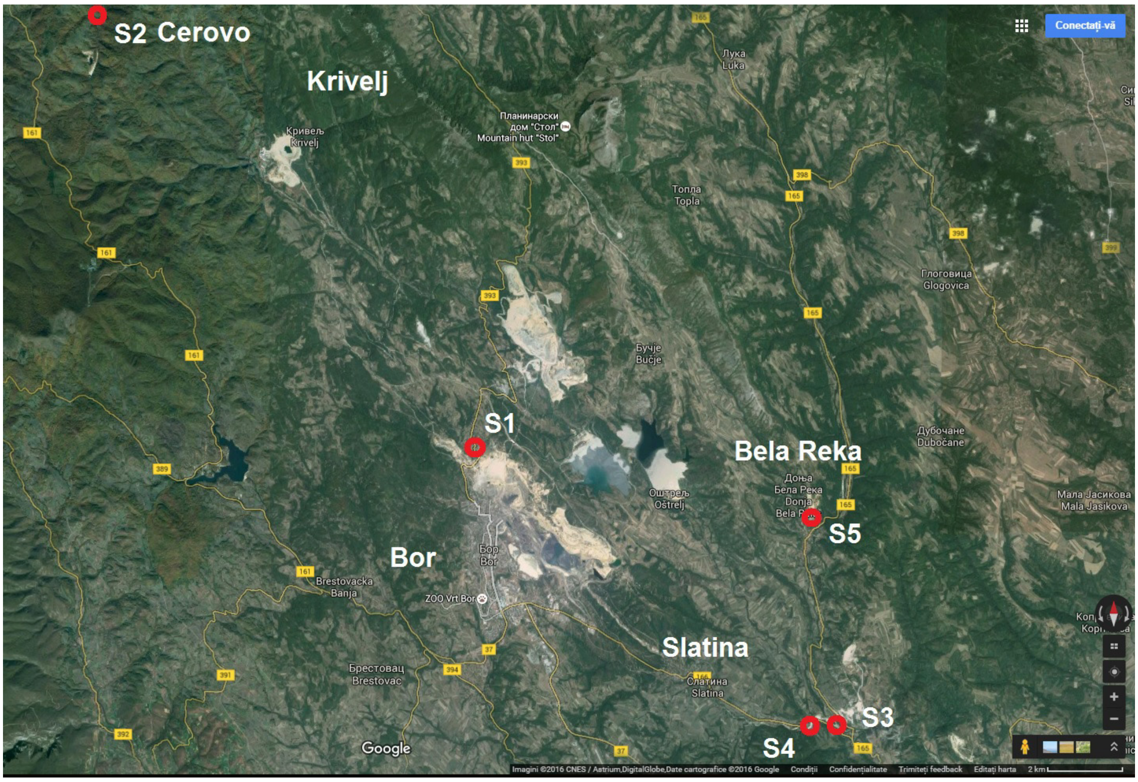

Fig. 1. Map showing the location of sampling sites - Legend. S1, site S1 (44.5368° lat. N, 22.5403° long. E); S2, site S2 (44.1018° lat. N, 22.1548° long. E); S3, site S3 (44.1512° lat. N, 22.1238° long. E); S4, site S4 (44.1466° lat. N, 22.1229° long. E); S5, site S5 (44.4593° lat. N, 22.1385° long. E).

The aim of this study was to determine the impact of mining activities on surface waters, based on the level of water contamination by heavy metals found in the Bor mining area, and determines the bioaccumulation index and impact of heavy metals on the dynamics of bacterial communities living in the sediment.

2 Materials and methods

2.1 Study areas

In this study, five tapping points have been chosen to collect water and sediment samples that were representative for the hydrologic system surrounding the city of Bor (Figure 1). All sampling sites were located within a 15 km radius of the Bor city, and at different distances from actual sources of inorganic contamination, such as the Bor, Cerovo, or Krivelj open pits (Figure 1). The first tapping point (S1) is located near the "Jama Bor" underground mine. The second tapping point is the Lutarica River, which passes near the Cerovo pit mine (closed in 2002 and then reopened in 2012). The third tapping point (S3) and the fourth tapping point (S4) lie close to each other and near the confluence of the Bor and Kriveljska rivers. Both rivers flow north to south, passing nearby major sources of inorganic contamination; the Bor River passes nearby the Bor open pit mine and the Kriveljska River passes nearby the Krivelj open pit mine. The fifth sampling point (S5) is the Ravna River; although this water stream was not affected by anthropic pollution during the past decades or so, there is strong evidence to suggest this site was also exposed to inorganic contamination during the Roman Empire (Petković, 2009).

2.2 Chemical and microbiological analysis

To provide relevant results, the sediment samples were collected in four different campaigns (summer 2011, autumn 2011, spring 2012, and summer 2012) from five sites that were placed at different distances from the main sources of inorganic contamination around the city of Bor. The samples were collected in triplicate from the top layer of aquatic bed sediments, were placed inside sterile bottles, preserved in a cooler bag (at a temperature of +4 °C), and transported to the Microbiology Laboratory from West University of Timisoara (Romania).

To determine the levels to which trace elements accumulate in aquatic sediments, the samples (100 mL/each sample) were oven dried at a temperature of 105 °C, for 48 h, and weighed using an analytical balance to the nearest 0.01 mg. Ultrapure nitric acid (HNO_3 65%, $\rho = 1.39$ g·cm^{-3}) was purchased from Sigma-Aldrich Chemie GmbH (Buchs, Switzerland) to prepare the necessary digestion solutions. After burning inside a muffle furnace, the resulting ash was dissolved in 20 mL of 0.5 N HNO_3 solution and then filtered through ash-free filter paper. For each sample, the volume was brought to 50 mL with 30 mL of 0.5 N HNO_3 solution. Trace metal analyses were conducted by using flame atomic absorption spectrophotometry with an acetylene-nitrous oxide flame (Perkin-Elmer 403 AAS). Percentage recoveries for trace elements ranged from 85% to 105%. The concentration of trace elements in aquatic sediments was expressed as microgram per gram dry weight ($\mu g \cdot g^{-1}$ dry wt).

Serial decimal dilutions were prepared from sediments (10^{-1}–10^{-6}) for each sample using double-distilled, sterile water, and were inoculated in selective culture medium. The number of bacteria was estimated according to the multiple-tube method.

Sulphate reducing bacteria (SRB) were cultivated on a Starkey's medium, with the following chemical composition: Na_3PO_4 (5 g), NH_4Cl (1 g), K_2HPO_4 (0.5 g), $MgSO_4.7H_2O$ (2 g), Na_2SO_4 (0.5 g), $CaCl_2.2H_2O$ (0.1 g), $(NH_4)Fe(SO_4)_2.6H_2O$ (0.01 g) (Domagala *et al.*, 1992). Incubation was done at 28 °C for 14 days. Positive tubes are considered to have a black precipitate at the bottom (iron sulphide).

In order to determine the iron-reducing bacteria (IRB) the following Ottow culture media with modified chemical composition was used: glucose (20 g), peptone (5 g), yeast extract (0.5 g), $MgSO_4.7H_2O$ (0.2 g), K_2HPO_4 (3 g), KH_2PO_4 (0.8 g), KCl (0.2 g), $Fe_2O_3.3H_2O$ (1 g). The samples were incubated for 14 days, at 28 °C. Fe^{2+} ions, produced by iron-reducing bacteria, can be highlighted by adding 0.1 mL of reactive α, α-dipiridil to 0.7 mL of culture. The red or pink color indicates the presence of Fe^{2+} ions (Drăgan-Bularda, 2000).

The culture medium for ammonifying bacteria (AMB) had the following chemical composition: NaCl (0.5 g), peptone (2 g), distilled water (1000 mL). The samples were incubated for 14 days, at 28 °C. AMB number was assessed based on the reaction between ammonia and Nessler reagent $[K_2(HgI_4)]$; the Alexander table was used as screening benchmark (Drăgan-Bularda, 2000).

The culture medium for nitrifying bacteria (NB) consisted of standard saline traces (50 mL), $(NH_3)SO_4$ (0.5 g), $CaCO_3$ (1 g), and distilled water (950 mL). Samples were incubated for 21 days, at 28 °C. Tubes containing nitrate were identified with diphenylamine sulphuric acid. A blue colour reaction showed that nitrite and nitrate were formed, and therefore, the tube was scored positive (Cappucino and Sherman, 2008).

The denitrifying bacteria (DNB) were grown in selective culture medium, which contained: standard saline solution (50 mL), KNO_2 (20 g), glucose (10 g), KCO_3 (5 g), oligoelement solution (1 mL), and distilled water. The samples were incubated at 28 °C, for 14 days, and diphenylamine-sulphuric acid was added in each test tube. Positive samples were colourless due to nitrate metabolization by DNB (Dunca *et al.*, 2007).

2.3 Statistical analysis

The geoaccumulation Index (I_{geo}), was calculated in order to determine the degree of metal accumulation in sediments by using the formula:

$$I_{geo} = \log_2 C_n / 1.5 B_n \tag{1}$$

where C_n defines the concentration of element in sediments, B_n the geochemical background value, and the factor 1.5 accounts for possible variation in background data due to lithogenic effect (Yisa *et al.*, 2012). When the measured values did exceed 0.5, a non-parametric analysis was conducted to assess possible differences among sampling sites (Mood's Median test). A One-Way Analysis of Similarity (Anosim) using the Bray-Curtis similarity measure was performed, in order to determine whether there is a significant difference between the sampling units or not.

This non-parametric test generates a global value of R that ranges between +1 and –1; a value of 0 shows no differences among the samples. Comparison of pair-wise R values defines on a scale of 0 (indistinguishable) to 1 (all similarities within groups are less than any similarity between groups) differences existing between groups: $R > 0.75$ as well separated groups; $R > 0.5$ as overlapping groups; $R < 0.25$ as barely separable groups (Clarke and Gorley, 2001). The p-values were corrected for multiple testing by a Bonferroni correction. Next, a Similarity Percentage Analysis (Simper) was conducted to identify which ecophysiological groups of bacteria accounted for most spatial dissimilarities observed in the dynamics of bacterial population dynamics.

The spatial differences in population's dynamics for each bacteria group were then compared statistically using Kruskal-Wallis tests. Spearman's rank-order correlation coefficients were finally computed to determine the existing relationships between the dynamics of bacterial population and metal concentrations in aquatic bed sediments.

Statistical analysis was performed using Past (Hammer *et al.*, 2001) and Statistica 10 software packages. All data is presented as the median ± SE, as the samples were processed in triplicate. A p value <0.05 was considered significant.

3 Results

Table 1 contains trace element concentrations ($\mu g \cdot g^{-1}$) and Table 2 contains geoaccumulation indices (I_{geo}) in aquatic sediments, depending on site location and sampling season. The measured values for Cu, Zn, Ni, and Cr indicated no contamination with these trace elements in the study area. There was a low to moderate contamination with Pb, and a moderate to high contamination with As in aquatic bed sediments. In the majority of heavy metals cases, the highest mean I_{geo} values for these metals were reported at the sites S2 and S3, whereas the lowest values were found for at the site S1. The site location had no influence on the degree of lead contamination (Mood's Median test, $p = 0.199$), but significantly influenced the arsenic accumulation in aquatic sediments (Mood's Median test, $p = 0.040$).

The population dynamics for different groups of sediment bacteria are described in Table 3. The assessment by Anosim revealed no significant spatial differences in bacterial population structure among different locations (*Global* $R = 0.106$, $p = 0.065$). The population dynamics were similar at the sites S1 and S2 ($R = 0.020$, $p = 1.000$), and S1 and S3 ($R = 0.020$, $p = 1.000$). Identical results were found between the sites S1 and S5 ($R = 0.104$, $p = 1.000$), and S2 and S4 ($R = 0.104$, $p = 1.000$). High similarities in quantitative population dynamics were also reported at the sites S1 and S4 ($R = 0.062$, $p = 1.000$), S2 and S3 ($R = 0.041$, $p = 1.000$), S3 and S4

Table 1. Trace element concentrations in aquatic bed sediments from water streams adjacent to the Copper Smelter Complex Bor (RTB Bor, Serbia) depending on site location and sampling season.

	Trace element concentration					
Site	Cu (μg·g^{-1})	Pb (μg·g^{-1})	Zn (μg·g^{-1})	Ni (μg·g^{-1})	Cr (μg·g^{-1})	As (μg·g^{-1})
1.1.	497.0 ± 261.2	45.32 ± 10.2	87.82 ± 42.5	4.62 ± 0.92	34.21 ± 12.90	96.21 ± 23.08
1.2.	1491 ± 190.2	125.6 ± 23.2	137.5 ± 54.2	4.695 ± 0.23	13.593 ± 12.09	182.70 ± 76.14
1.3.	1097.5 ± 319	160.9 ± 39.9	140.4 ± 72.9	3.272 ± 1.274	10.722 ± 5.894	259.3 ± 172.07
1.4.	1748 ± 424.7	64.90 ± 17.0	106.7 ± 43.7	5.424 ± 0.254	15.985 ± 7.439	24.127 ± 12.99
1.5.	324.1 ± 281.5	43.12 ± 9.34	222.1 ± 83.2	7.29 ± 1.712	13.907 ± 12.09	13.723 ± 5.096
2.1.	363.6 ± 120.5	47.38 ± 12.7	231.2 ± 31.5	7.539 ± 0.925	13.563 ± 2.055	17.693 ± 12.85
2.2.	3770.8 ± 501	262.9 ± 42.3	2294 ± 305	11.22 ± 2.157	77.88 ± 54.737	333.72 ± 154.4
2.3.	4433.8 ± 320	400.1 ± 59.6	3241 ± 577	12.871 ± 2.81	120.3 ± 48.862	523.1 ± 187.96
2.4.	3982.5 ± 448	360.1 ± 41.7	2923 ± 219	11.13 ± 1.997	121.02 ± 51.99	450.72 ± 152.2
2.5.	6283 ± 1218	480.1 ± 39.2	7150 ± 1023	17.528 ± 3.06	211.17 ± 79.45	858.59 ± 302.6
3.1.	515.6 ± 257.6	66.22 ± 13.0	221.7 ± 51.0	4.229 ± 0.416	20.652 ± 13.63	92.754 ± 43.02
3.2.	2851.6 ± 403	211.7 ± 51.2	1170 ± 286	5.203 ± 0.684	40.944 ± 28.78	309.94 ± 152.2
3.3.	1537.7 ± 284	120.8 ± 27.3	1735 ± 359	14.85 ± 1.274	15.229 ± 8.502	257.57 ± 104.4
3.4.	2142.1 ± 430	81.55 ± 9.09	1470 ± 477	4.772 ± 0.619	113.38 ± 73.95	223.32 ± 38.86
3.5.	4348.4 ± 385	115.4 ± 17.2	7190 ± 1301	16.81 ± 3.219	125.5 ± 174.09	721.8 ± 302.94
4.1.	501.7 ± 131.0	56.1 ± 16.05	152.7 ± 72.9	5.44 ± 1.441	23.61 ± 13.05	32.74 ± 21.623
4.2.	2585.6 ± 494	151.6 ± 25.0	2170 ± 567.0	7.202 ± 1.723	54.9 ± 24.1	219.5 ± 132.03
4.3.	3237 ± 1020	220.8 ± 6.25	1873.5 ± 510	8.896 ± 1.529	103.2 ± 75.02	335.8 ± 152.44
4.4.	2945.2 ± 306	218.5 ± 26.5	1773.5 ± 490	6.71 ± 1.204	117.32 ± 37.94	237.89 ± 87.70
4.5.	4847.4 ± 881	215.9 ± 30.0	6372 ± 1756	13.80 ± 3.71	178.53 ± 16.09	681.2 ± 172.90

Note. Trace element concentrations (values - the median ± SE), 1.1., site S1 (July 2011); 1.2., site S2 (July 2011); 1.3., site S3 (July 2011); 1.4., site S4 (July 2011); 1.5., site S5 (July 2011); 2.1., site S1 (November 2011); 2.2., site S2 (November 2011); 2.3., site S3 (November 2011); 2.4., site S4 (November 2011); 2.5., site S5 (November 2011); 3.1., site S1 (March 2012); 3.2., site S2 (March 2012); 3.3., site S3 (March 2012); 3.4., site S4 (March 2012); 3.5., site S5 (March 2012); 4.1., site S1 (July 2012); 4.2., site S2 (July 2012); 4.3., site S3 (July 2012); 4.4., site S4 (July 2012); 4.5., site S5 (July 2012).

Table 2. Geoaccumulation indices in aquatic bed sediments from water streams adjacent to the Copper Smelter Complex Bor (RTB Bor, Serbia) depending on site location and sampling season.

Site	Cu	Pb	Zn	Ni	Cr	As
1.1.	0.087	0.367	0.054	0.016	0.024	2.091
1.2.	0.103	0.465	0.060	0.016	0.018	2.385
1.3.	0.099	0.489	0.060	0.012	0.016	2.545
1.4.	0.105	0.401	0.057	0.018	0.019	1.457
1.5.	0.081	0.362	0.066	0.021	0.018	1.199
2.1.	0.084	0.371	0.066	0.022	0.018	1.313
2.2.	0.116	0.536	0.094	0.026	0.030	2.661
2.3.	0.118	0.576	0.098	0.027	0.033	2.867
2.4.	0.117	0.566	0.097	0.026	0.033	2.799
2.5.	0.123	0.594	0.108	0.031	0.037	3.094
3.1.	0.088	0.403	0.066	0.015	0.021	2.074
3.2.	0.112	0.515	0.086	0.018	0.025	2.627
3.3.	0.103	0.461	0.091	0.029	0.019	2.542
3.4.	0.108	0.423	0.089	0.017	0.032	2.477
3.5.	0.118	0.457	0.108	0.030	0.033	3.014
4.1.	0.087	0.387	0.061	0.018	0.022	1.597
4.2.	0.111	0.483	0.094	0.021	0.028	2.469
4.3.	0.114	0.519	0.092	0.023	0.032	2.664
4.4.	0.113	0.518	0.091	0.020	0.033	2.506
4.5.	0.120	0.517	0.107	0.028	0.036	2.988

Note. 1.1., site S1 (July 2011); 1.2., site S2 (July 2011); 1.3., site S3 (July 2011); 1.4., site S4 (July 2011); 1.5., site S5 (July 2011); 2.1., site S1 (November 2011); 2.2., site S2 (November 2011); 2.3., site S3 (November 2011); 2.4., site S4 (November 2011); 2.5., site S5 (November 2011); 3.1., site S1 (March 2012); 3.2., site S2 (March 2012); 3.3., site S3 (March 2012); 3.4., site S4 (March 2012); 3.5., site S5 (March 2012); 4.1., site S1 (July 2012); 4.2., site S2 (July 2012); 4.3., site S3 (July 2012); 4.4., site S4 (July 2012); 4.5., site S5 (July 2012).

Table 3. Bacteria population dynamics in aquatic bed sediments from water streams adjacent to the Copper Smelter Complex Bor (RTB Bor, Serbia) depending on site location and sampling season (no bacteria/ 1 g sediment).

	SRB	IRB	AMB	NB	DNB
1.1.	1712.32 ± 173	4522.85 ± 603	2429.76 ± 306	66812.98 ± 5091	2291.08 ± 365
1.2.	610.86 ± 234	5401.21 ± 774	2312.40 ± 120	25971.87 ± 3825	2002.05 ± 196
1.3.	1703.98 ± 14	3598.82 ± 451	3228.83 ± 298	5921.95 ± 20	1725.87 ± 304
1.4.	422.20 ± 56	2476.99 ± 102	3875.09 ± 104	6984.25 ± 413	2183.81 ± 424
1.5.	1405.92 ± 253	4414.65 ± 705	3217.74 ± 297	8156.09 ± 20	1788.33 ± 56
2.1.	2082.04 ± 384	66275.20 ± 3012	43074.35 ± 3683	1073.81 ± 292	2473.86 ± 534
2.2.	1204.55 ± 185	1739.87 ± 87	2328.63 ± 95	820.02 ± 124	810.10 ± 163
2.3.	2087.28 ± 128	4028.93 ± 564	3656.99 ± 225	1786.78 ± 612	937.75 ± 128
2.4.	1728.25 ± 421	1128.38 ± 241	8137.84 ± 1223	1725.08 ± 312	1934.02 ± 493
2.5.	2403.90 ± 104	3627.27 ± 201	4548.08 ± 177	4812.95 ± 423	1727.62 ± 305
3.1.	2389.98 ± 309	4078.72 ± 809	2803.05 ± 402	3724.13 ± 582	424.97 ± 103
3.2.	3862.76 ± 127	1898.98 ± 427	2321.81 ± 305	1427.86 ± 203	2244.88 ± 93
3.3.	2116.11 ± 149	2157.73 ± 525	740.90 ± 266	1746.35 ± 241	2105.22 ± 387
3.4.	1773.87 ± 209	0	2879.25 ± 455	14811.97 ± 3816	1736.65 ± 405
3.5.	1211.21 ± 138	2226.76 ± 305	2472.36 ± 254	28613.62 ± 3149	1474.86 ± 287
4.1.	2233.40 ± 364	4675.30 ± 398	3291.07 ± 402	2458.76 ± 413	1809.24 ± 439
4.2.	1765.29 ± 259	1728.07 ± 102	2582.91 ± 243	2146.97 ± 398	1736.97 ± 325
4.3.	1512.12 ± 243	2663.93 ± 405	3891.01 ± 412	1863.80 ± 241	1293.86 ± 298
4.4.	1329.07 ± 98	1627.27 ± 306	4200.08 ± 805	2153.64 ± 103	1423.33 ± 142
4.5.	1873.08 ± 565	3825.72 ± 298	4812.97 ± 561	7828.98 ± 429	1598.97 ± 238

Note. SRB – sulfate-reducing bacteria; IRB – iron reducing bacteria; AMB – ammonifying bacteria; DNB – denitrifying bacteria; NB – nitrifying bacteria; 1.1., site S1 (July 2011); 1.2., site S2 (July 2011); 1.3., site S3 (July 2011); 1.4., site S4 (July 2011); 1.5., site S5 (July 2011); 2.1., site S1 (November 2011); 2.2., site S2 (November 2011); 2.3., site S3 (November 2011); 2.4., site S4 (November 2011); 2.5., site S5 (November 2011); 3.1., site S1 (March 2012); 3.2., site S2 (March 2012); 3.3., site S3 (March 2012); 3.4., site S4 (March 2012); 3.5., site S5 (March 2012); 4.1., site S1 (July 2012); 4.2., site S2 (July 2012); 4.3., site S3 (July 2012); 4.4., site S4 (July 2012); 4.5., site S5 (July 2012).

($R = 0.083$, $p = 1.000$), S3 and S5 ($R = 0.197$, $p = 0.869$), and S4 and S5 ($R = 0.166$, $p = 1.000$), respectively. The greatest dissimilarity was observed between sites S2 and S5 ($R = 0.364$, $p = 1.000$).

The results of Simper analysis showed that IRB and NB were the best discriminating groups of bacteria, accounting for 63.20% of the overall average dissimilarity among study in the studied areas (Table 4). However, the population dynamics of sediment bacteria was shown to be significantly different among sites only for IRB (Kruskal-Wallis test, $p = 0.022$), but not for SRB, AMB, ND, and DNB (Kruskal-Wallis test, $0.112 < p < 0.779$). Among sediment bacteria groups, only the population dynamics of IRB and DNB were significantly related to the degree of sediment contamination (Table 5). Thus, IRB were reversely associated with I_{geo} for Cu, Pb, and Cr, whereas DNB was negatively correlated with I_{geo} for Zn and Cr.

4 Disscusion

Studies regarding the level of water pollution in Bor area had included several biotic and abiotic parameters. The general conclusion is that the values of some parameters that indicate organic pollution (BOD_5, ammonium, coliform germs, etc.) and the presence of metals (Fe, Mn, Cu) are far above the permitted limits, indicating severe organic and inorganic pollution in this area. This is a consequence of the activities of various subjects. The main sources of Timok River Basin pollution are untreated communal wastewater and the wastewater from the Bor mining industrial complex. The technological processes related to copper ore exploitation and processing in the Bor mining industrial complex generate important quantities of wastewater. The wastewaters from the mining process, as well as the wastewater from metallurgical and chemical processes in industrial plants are both being discharged directly into the Borska Reka. The solution to the problem of water quality and environmental protection lies in the construction of appropriate wastewater treatment plants. The progress in environmental protection and water pollution reduction is the area must be analyzed: in the Bor mining industrial complex the construction of wastewater purification plant was undertaken in 2005; it will enable to separate copper and iron for further processing and to reduce the discharge of untreated water into the Borska Reka River (Brankov *et al.*, 2012).

Despite the high levels of heavy metals and low pH of the water, this river still accommodates bacterial life. However, no field survey has investigated the effects of extensive inorganic pollution towards sediment bacterial communities. This type of pollution is generally associated with changes in physico-chemical properties of the water and enrichment of aquatic sediments with heavy metals (UNESCO/WHO/UNEP, 1996).

Despite being exposed to long-term impact of mining and smelting activities (Serbula *et al.*, 2010), the aquatic bed sediments from water streams adjacent to the Bor Copper Smelter Complex (RTB Bor, Serbia) showed no contamination with either Cu, Zn, Ni, or Cr. In our study the values measured for

Table 4. Results of Simper analysis with taxon contributions to dissimilarities among sites.

Taxon	Contribution	Cumulative %	Mean abund. S1	Mean abund. S2	Mean abund. S3	Mean abund. S4	Mean abund. S5
IRB	2.197	34.520	4.030	3.370	3.470	2.410	3.520
NB	1.862	63.750	3.790	3.450	3.370	3.630	3.980
AMB	0.896	77.840	3.740	3.370	3.380	3.640	3.560
SRB	0.875	91.600	3.310	3.170	3.000	3.040	3.220
DNB	0.534	100.000	3.140	3.200	3.150	3.240	3.200

Note. Mean abund., mean abundance; I_{geo}, geoaccumulation index. Values marked with asterisks (*) define significant correlations (Spearman's correlations, $p < 0.05$).

Table 5. Correlations between the population dynamics of sediment bacteria and geoaccumulation index of trace elements.

	I_{geo} Cu	I_{geo} Pb	I_{geo} Zn	I_{geo} Ni	I_{geo} Cr	I_{geo} As	I_{geo} Fe
IRB	–0.508*	–0.452*	–0.439	–0.191	–0.469*	–0.416	–0.320
NB	–0.179	–0.438	–0.278	–0.292	–0.012	–0.137	–0.278
AMB	0.214	0.209	0.232	0.232	0.301	0.086	–0.059
SRB	0.069	0.071	0.201	0.192	0.226	0.092	0.127
DNB	–0.433	–0.405	–0.477*	–0.194	–0.465*	–0.403	–0.259

concentrations of heavy metals (Pb, Cu, Zn, Ni, Cr, As) present in the sediments, have exceed the reference values in most cases. Since our study only determined the sediment metal concentrations and abundant groups of bacteria, with no other parameters, we cannot calculate WPI and classify the water in one of the 6 classes. Further study and analysis of several other parameters will allow us to determine water quality according to WPI in the future. Arsenic was found to be the most important factor determining the pattern of inorganic contamination in aquatic bed sediments within the area of interest. High As levels have been reported as suspended particulate matter in the industrial waste waters generated in the primary copper smelter at Bor, Serbia (Giannopoulou and Panias, 2008; Serbula *et al.*, 2010). Since almost 300 tons of inorganic arsenic (As) are being discharged every year in the hydrographic basin of the Bor River (Kovačević *et al.*, 2010), our results are not surprising at all. These findings reflect the risks posed by As contamination on the native aquatic ecosystems.

We observed that the population structure of sediment bacteria biotopes was homogeneous irrespective of location. It was therefore hypothesized that long-term exposure to As-rich sediments resulted in an increased As-resistance in these bacterial biotopes. Such mechanisms are related to the ability of bacteria to methylate soluble toxic inorganic arsenic to the less toxic, organic form (Tamaki and Frankenberger, 1992). Elevated I_{geo} values for arsenic that are found at the site S5 are of particular interest since this water stream was not affected by anthropic activities during the past decades or so. These results are thought to be associated with the disaffected Roman mines from the Ravna River basin and the upper course of the Pek River, such as those uncovered at Donja Bela Reka (Petkovic, 2009), rather than being related to recent contamination events.

A recent study reported elevated levels of accumulated Pb in vegetation (*Tilia* spp., *Pinus* spp.) sampled around the copper smelter in Bor (Serbula *et al.*, 2013). Serbula *et al.* (2012) investigated Pb accumulation in different parts of *Robinia pseudoacacia* L. (*Fabaceae*), and showed that leaves and branches acted as excluders, not as indicators of Pb. In this study, by contrast, IRB dynamics was significantly associated with the degree of Pb contamination. The natural chalcopyrite ($CuFeS_2$) ores from the Bor metallogenic zone is rich in lead (Panias, 2006).

The processing of such ores is therefore expected to result in their accumulation in aquatic sediments from water streams adjacent to the Copper Smelter Complex Bor (RTB Bor, Serbia). As a result, IRB are potential bioindicators of inorganic contamination within the area of interest.

Another major threat to human and environmental health in the Bor area is the out-dated processing technology (*i.e.*, classic pyrometallurgical processing) uses sulphur dioxide gas (SO_2) to produce sulphuric acid (H_2SO_4) with less than 50% degree of utilization (Nikolic *et al.*, 2010). This variable was not considered in our study, and as a consequence, future studies should comprehensively examine its impact on native aquatic ecosystems since over 200 000 tons of sulphur dioxides are being released to the environment from the Copper Smelter Complex Bor alone (Nikolic *et al.*, 2010).

Heavy metals present in high concentrations are manifest potentially toxic to microorganisms and affect their community structure by reducing their number, diversity, and biochemical activity. At the same time, metal exposure results in the establishment of metalotolerant/resistant microbial populations. Due to selective pressure from the metals in the growth environment, indigenous microorganisms have evolved and developed various mechanisms to resist heavy metal stress and may play a significant role in the restoration of contaminated environments (Rathnayake *et al.*, 2009; Bajkić *et al.*, 2013).

The bacteria can have genes that encode for metal tolerance in the bacterial chromosome, and genes encoding tolerance to metals in the plasmid. Bacteria is capable of ensure the transfer of genetic material between species of plasmids and characters such as resistance to heavy metals can be transferred between related species (Gogartin *et al.*, 2002). The bacteria can tolerate heavy metals by controlling the transport in and out of their cells or divide them into cells. Also other mechanisms such as the production of siderophores, are known to detoxify heavy metals in bacteria (Boyd and Rajakaruna, 2013). Substrates with high content of heavy metals represent environments with high levels of metal pollution and provide habitat for bacteria resistant to metal or respective metals. Previus studies have shown that surface waters around mining exploitations contain a high concentration of heavy metals, as well as certain species of bacteria resistant to this kind of contamination, as for example bacteria found in waters contaminated with residues from the uranium mine (Choudhary *et al.*, 2012). Heavy metals can not be degraded, so the only way to remove them from the environment is to use a bioremediation strategy based on the use of microorganisms. Bioremediation is the most effective and least expensive method of treating metal contaminated environments (Wei *et al.*, 2009).

Other studies highlight the possibility to use phytoremediation (hyperaccumulator plants) which in some cases are more efficient than microbial treatment. Decontamination and pollution reduction organic and inorganic at the water column and sediment may be achieved with the aid phytoplanktonului; structure (*e.g. Lemna minor, Lemna gibba, Spirodela polyrhiza*) and its functions being the decisive factor (Lafabrie *et al.*, 2013; Baudo *et al.*, 2015). At soil level of heavy metal pollution reduction is made using hyperaccumulator plants (*Rumex crispus, Poa trivialis, Brassica napus, Allium sativum, Zea mays, Lolium perenne, Polygonum convolvulus, Vicia faba, Sonchus oleraceus*, etc.) and green filamentous algae (*Lindernia dubia, Chara vulgaris, Cladophora vagabunda, Spirogyra* sp., etc.) (Tangahu *et al.*, 2011; Muller *et al.*, 2013; Chibuike and Obiora, 2014).

The concentration of heavy metals and bacterial community dynamics detected in the sediments, indicate the existance of inorganic pollution of aquatic environment due to mining and smelting in the Bor area. Therefore, further studies will be of great importance to identify the potential bio-indicators for heavy metal pollution and new groups of microorganisms capable to tolerate high concentrations of such metals.

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