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Functional diversity of microorganisms in metal- and alkali-contaminated soils of Central and North-eastern Slovakia

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Abstract: A field-based study and laboratory tests were undertaken to determine the functional diversity of microorganisms in metal- and alkali-contaminated soils in Central and North-eastern Slovakia where iron ore and magnesite have been mined and processed for a long time. To improve the understanding of the functional diversity of microorganisms, we examined the effects of environmental factors on the functional diversity of microorganisms in metal- and alkali-contaminated soils in the emission field of heaps and tailings impoundments of iron ore mines (Central Spiš) and magnesite factories (Jelšava and Lubeník). BIOLOG® Eco Plates were used to determine and assess metabolic profiles of microbial communities. The examined area of Central Spiš showed extremely high values of Hg and Cu and the values of Zn, Cd, Pb and Cr exceeding the permissible limit were determined. Soil reaction was extremely acidic to strongly acidic. The Jelšava and Lubeník area was characterized by alkaline contamination and the soil reaction was slightly acidic to strongly alkaline. The values of Cr, Mn, and Mg exceeding the permissible limit were measured there. The results indicate harmful and even toxic contamination. Our results showed that the diversity of microorganisms was different in the investigated areas and it was significantly influenced by environmental factors such as soil reaction, bulk density, porosity, and heavy metals Hg, Pb, Cr, Zn, Cu, Mn and Mg. Based on the results of the Shannon index, we can conclude that the diversity was low to moderate (2.5–3.3) and medium (3.3–4.0). Correlations between functional diversity of microorganisms and soil reaction, Hg, Cr, and Cu were determined. Our findings are decisive for understanding the microbial diversity in metal- and alkali-contaminated soils and they can be used to assess the quality and health of soil, as well as for scientific applications of remediation techniques.

Keywords: AWCD; bulk density; heavy metal; porosity; Shannon diversity index; soil reaction

Global environmental contamination is one of the most significant environmental challenges of our time that affects all components of the environment. The attention of researchers is focused primarily on hazardous substances (heavy metals), which are hardly degradable in nature and show high persistence and often exhibit toxic effects in the environment (Izah *et al.* 2017). The pollution of the biosphere with toxic heavy metals is a widespread ecological problem resulting from anthropogenic activities like fossil fuel burning, ore mining and smelting, industrial and municipal waste disposal and agricultural activities (Jordan & Abdaal 2013; Fazekašová *et al.* 2016). The most active components of the soil biocoenosis are microorganisms, playing an irreplaceable role in soil ecosystems. Soil biodiversity is probably the

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most important for maintaining ecosystem functions in a disturbed environment and can be measured directly (like species richness) or indirectly, through standardized procedures (various indices). Generally, the evaluation of microbial parameters in soil ecosystems is considered to be the best indicator of soil quality that leads to all changes in soil properties, thus enabling an early detection of soil degradation (Romaniuk *et al.* 2011). Abiotic stress caused by heavy metals in inorganic and organic forms affects the growth, morphology, and metabolism of microorganisms in soils. Numerous studies have demonstrated the adverse effect of different heavy metals on the soil microbial biomass and its activities (Doelman 1985). Heavy metals accumulate in soils; therefore, the amount and activity of the microorganisms may be affected. By applying organic fertilizers and regular liming, this effect can be partially attenuated (He *et al.* 2016). Doelman *et al.* (1994) recommended the microbial community structure as a biological indicator of heavy metal stress. The assay is based on the Biolog system using 31 different carbon sources to produce a metabolic profile of microorganisms (Garland 1997). To improve environmental quality after the end of mining operations, ecological restoration and mine reclamation have become important parts of the sustainable development strategy of many countries (Liao & Xie 2007).

The aim of the study was to determine the influence of environmental factors on the functional diversity of microorganisms in metal and alkaline contaminated soils in the emission field of heaps and tailings impoundments of iron ore mines (Central Spiš) and magnesite factories (Jelšava and Lubeník) in Central and North-eastern Slovakia.

MATERIAL AND METHODS

Study area. The studied area Central Spiš (48°54'53.5''N, 20°52' 6.6''E – KR (Krompachy); 48°52'46.4''N, 20°50'38.8''E – SL (Slovinky); 48°53'02.3''N, 20°43'26.5''E – PR (Poráč); 48°53'03.9''N, 20°40'39.7''E – RD (Rudňany)) is located in the Rudňany-Gelnica area, which belongs according to environmental regionalization among environmentally loaded and unhealthy areas of Slovakia. As a result of long-term and intensive mining and processing of minerals, the area is polluted with heavy metals and landscape has been altered by extensive anthropogenic forms (mining dumps, heaps and tailings impoundments). The dominant soil types are Cambisols from moderate to light and, to a lesser extent, Fluvisols and Pseudogleys are also represented. The soils in this area can be characterized as moderately deep to shallow.

The area Jelšava and Lubeník (48°37'37.0''N, 20°14'33.3''E – JEL; 48°39'17.2''N, 20°11'46.6''E – LUB) with specific alkaline pollutants is one of the most devastated regions of Slovakia showing an alarming degree of environmental damage. Cambisol evolved on this bedrock is slightly skeletal, mostly of medium depth (60–120 cm), and the steeper slopes are prone to erosion. The original soil pH reaction of about 5 was changed to pH about 7.2 to 8.5 caused by magnesite dust contamination. The developed soils are mostly shallow (15–20 cm), loam to loamy clay, predominantly strongly skeletal, and classified as Leptosols, Luvisols and Fluvisols are also represented.

Soil sampling, analyses and statistical analyses. Soil samples were collected from eight permanent research sites in Central Spiš and twelve permanent research sites in the Jelšava and Lubeník area, which are used as permanent grasslands. From each sampling site three samples were collected from the A horizon of the depth of 0.05 m to 0.15 m, in June 2016 (Figure 1).

After homogenization, soil samples were manually crumbled, dried at room temperature, sieved (< 2 mm) and stored in polyethylene bags until their analysis. We studied and evaluated the soil reaction in 1N solution of $CaCl_2$ using the Mettler Toledo FiveEasy™ pH bench meter FE20 (Mettler-Toledo Group ,Switzerland). The total contents of Cd and Hg were determined by atomic absorption spectrometry (AAS) and the total contents of Cu, Pb, Zn, Cr, Mn and Mg were determined by X-ray fluorescence spectrometry following the methodology as devised by Fiala *et al.* (1999). The assessed values of heavy metals in soils were compared with the limit values of Slovak soils (Act No. 220/2004). We studied and measured bulk density (t/m^3) and porosity (%) in the Kopecky physical cylinder with a capacity of 100 cm^3 (Fiala *et al.* 1999). In fresh soil samples, we evaluated the metabolic profiles of microbial communities using Biolog® Eco Plates (Biolog, Hayward, USA). Microtiter plates (Cad. No. 1506, Lot No. 3012201, Hayward, USA) with 31 different organic substrates were incubated for 7 days. The data-normalized parameter AWCD (Average Well Colour Development) was calculated according to Garland (1997) and the functional diversity of soil microbiological communities was calculated for BIOLOG data by

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Figure 1. Location of sampling sites Jelšava and Lubeník (left) and Central Spiš (right)

the classical Shannon diversity index (H') (Shannon 1948):

$$H' = -\sum_{i=1}^{s} \frac{xi}{N} log2 \frac{xi}{N}$$

where:

s – the number of species

xi – individuals of one particular species found

N – divided by the total number of individuals found

i – 1

The results were evaluated based on scales: 1 extremely low (< 0.5), 2 very low (0.5–1), 3 medium-low (1–1.7), 4 low (1.7–2.5), 5 low to moderate (2.5–3.3), 6 medium (3.3–4), 7 moderately high (4–5), 8 high (5–7), 9 very high (7–10), and 10 extremely high (> 10).

The obtained data were processed statistically by means of the Statistica software (Ver. 13, 2015) and PAST (Ver. 3, 2016). The one-way ANOVA test confirmed statistical differences between the investigated areas. The level of significance between soil properties was calculated using Spearman's correlation coefficient. The data were log-transformed before the analysis.

RESULTS AND DISCUSSION

Evaluation of soil reaction. Soil reaction is considered one of the main chemical properties because it affects all biochemical reactions in the soil environment (Hohl & Varma 2010). Mobility, translocation, and toxic effects of risk elements are affected by some soil properties, content of clay, organic matter, and soil reaction (Song *et al*. 2006). The soil reaction of the metal-contaminated area of Central Spiš was 4.80 ± 0.55 (median ± standard deviation). The range of soil reaction values defined the studied soil as extremely acidic to strongly acidic, creating an environment for an easy passage of monitored risk elements and subsequent accumulation of those contaminants in plants.

The soil reaction in the alkali-contaminated areas of Jelšava and Lubeník was 7.95 ± 1.03 (median ± standard deviation). The range of soil reaction values indicated the soil to be slightly acidic to strongly alkaline. Magnesium (Mg) flints with significantly reactive caustic magnesite are aggressive in natural environments; in small quantities and in contact with soil and crop moisture, they form saturated solutions with a high alkaline pH value (Wang *et al*. 2015). The values of soil reaction measured in the investigated areas are listed in Table 1.

Assessment of physical properties of soil. Bulk density as an integral value of soil granularity, humus content, and anthropogenic impacts on soil should not exceed the limits given for individual soil types. Total porosity is closely related to bulk density. Of the total pore volume, the porosity should not fall below 38% for sandy soil and below 48% for clay-loam soil.

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In the Central Spiš area, the bulk density was 1.21 ± 0.17 (t/m^3) and the porosity was 54.21 ± 6.43 (%). In the investigated area of Jelšava and Lubeník, the bulk density values were 1.38 ± 0.22 (t/m^3) with porosity of 48.98 ± 8.37 (%). Considering this parameter, optimum conditions were created for the growth of most arable crops allowed by a general porosity ranging between 55% and 65% (Fazekašová 2012).

Assessment of heavy metal pollution. Extremely elevated concentrations of Hg were recorded in the Central Spiš area in the range of 7.60 ± 16.39 (median ± standard deviation) and Cu 182.50 ± 426.30, which was the result of long-term mining and processing in the investigated area. At the highest concentration of mercury, the limit value was exceeded up to 66 times, and in the case of copper, 21.2 times. Consistently with our results, Šefčík *et al.* (2008) categorized soils in the Central Spiš area as moderately or heavily contaminated by copper. High values were recorded for Zn (160.50 ± 456.34), Cd (0.50 ± 1.56), Pb (30.50 ± 132.53), and Cr (88.00 ± 47.31). The highest observed concentrations exceeded the Zn limit 8.9 times, Cd: 5.7 times, Pb: 5.4, and Cr: 2.6 times. Hronec *et al.* (2008) recorded the values of cadmium, copper, zinc, and arsenic in the Central Spiš area above the permissible limit. He also noted that the contamination of soil environment by arsenic was related not only to anthropogenic impacts but also to the geochemical effects of mineralized zones.

Table 1. Values of selected soil parameters, heavy metals and average well colour development (AWCD) in BIOLOG® Eco Plates in the investigated areas

Area	Soil parameter	Mean	Min	Max	Median	SD
Central Spiš	pH/$CaCl_2$	4.70	4.10	5.40	4.80	0.55
	ρ_d (t/m^3)	1.22	0.98	1.51	1.21	0.17
	Po (%)	54.00	42.88	63.14	54.21	6.43
	Hg (mg/kg)	15.20	0.45	46.40	7.60	16.39
	Cd (mg/kg)	1.39	0.00	4.00	0.50	1.56
	Pb (mg/kg)	107.63	8.00	380.00	30.50	132.53
	Cr (mg/kg)	108.75	53.00	180.00	88.00	47.31
	Zn (mg/kg)	411.88	69.00	1333.00	160.50	456.34
	Cu (mg/kg)	378.75	47.00	1271.00	182.50	426.30
	Mn (mg/kg)	1 900.00	600.00	3 400.00	1 700.00	1 017.00
	Mg (mg/kg)	253.25	101.00	394.00	266.50	82.77
	AWCD	1.36	1.5	1.74	1.35	0.23
	H'	3.24	3.10	3.30	3.25	0.06
	J'	0.95	0.90	0.96	0.95	0.02
Jelšava and Lubeník	pH/$CaCl_2$	7.63	6.20	8.80	7.95	1.3
	ρ_d (t/m^3)	1.31	0.79	1.63	1.38	0.22
	Po (%)	50.71	38.43	70.24	47.98	8.37
	Hg (mg/kg)	0.08	0.04	0.13	0.08	0.03
	Cd (mg/kg)	0.50	0.50	0.50	0.50	0.00
	Pb (mg/kg)	32.42	17.00	45.00	32.00	8.16
	Cr (mg/kg)	231.08	83.00	1055.00	140.00	279.49
	Zn (mg/kg)	88.33	48.00	108.00	88.50	15.17
	Cu (mg/kg)	23.8	11.00	44.00	20.00	9.40
	Mn (mg/kg)	1 575.00	800.00	2 300.00	1 600.00	517.20
	Mg (mg/kg)	49 841.67	7 000.00	197 000.00	26 150.00	59 039.25
	AWCD	1.75	1.25	2.25	1.81	0.29
	H'	3.31	3.00	3.40	3.36	0.11
	J'	0.97	0.90	0.99	0.98	0.03

SD – standard deviation; ρ_d – bulk density; Po – porosity; *H'* – Shannon index diversity for ECO plates; *J'* – equitability for ECO plates

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The chromium contents in the soil in the investigated Jelšava and Lubeník area were in the range 140.00 ± 279.49 (median ± standard deviation) (Table 1). The median level of chromium in the soil of Slovakia was 85 mg/kg in the A horizon (Šefčík *et al.* 2008). However, the data presented by Čurlík and Šefčik (1999) indicated high Cr levels (up to 6096 mg/kg) in both A and C horizons of soils from the Outer Carpathians. Chromium along with cobalt and nickel are considered as metals that come from a geogenic load. Hexavalent chromium (Cr^{6+}) is classified as one of the most important environmental contaminants (Fathizadeh *et al.* 2011). Readily soluble Cr^{6+} in soils is toxic to plants and animals. Therefore, the variability in the oxidation states of Cr in soils is of great environmental concern (Kabata-Pendias 2011). Magnesium is considered the fifth major nutrient for plants. It is located in several primary and secondary minerals. The values of available magnesium content in the topsoil of agricultural land in Slovakia are in the range of 200–400 mg/kg Mg, accounting for a high content of this element in the soil. In the studied area, we found significant soil contamination by magnesium with values in the range of 26 150.00 ± 59 039.25 (median ± standard deviation), which is on average 18 to 493 times in excess of the threshold limit. The highest concentrations of Mg exceeded the high content of this element 492.5 times, which is comparable with Wang *et al.* (2015).

The measured levels of manganese have a similar pattern, and contents in the range of 1600.00 ± 517.20 (median ± standard deviation) (Table 1) were found. The average content of manganese in the soil of the Slovak Republic is in the range of 0.85 to 112.90 mg per kg, indicating a significant spatial heterogeneity of the elements; however, a medium supply of this element dominates the soils. Kabata-Pendias (2011) reported the value of 1500 mg/kg, which shows symptoms of manganese toxicity. Based on the obtained results, it can be stated that the contents of Cd, Pb, and Zn are below the toxic level, but this does not apply to Cr, Mn and Mg. Their significant excess indicates harmful and even toxic contamination.

Assessment of functional diversity of microorganisms. For the analysis of changes in microbial communities, we used BIOLOG® Eco plates. This technique is valued for its sensitivity and quickness and was lately used for the ecotoxicological assessment of contaminated soils (Tischer *et al.* 2008). The BIOLOG technology provides a large amount of data and it can analyse changes in the structure of community dynamics in combination with other methods. Figure 2 shows the results from soil samples. During 168 h, we found differences in the ability of microorganisms to metabolize the substrates. The highest average values of metabolic activities of the community of microorganisms (AWCD) were found in the Jelšava and Lubeník area (1.81 ± 0.29), and the lowest AWCD was found in the Central Spiš area (1.35 ± 0.23). Based on the results of the Shannon index, we can conclude that the diversity in the investigated sites is low to moderate (2.5–3.3) to medium (3.3–4.0) (Table 1). Higher diversity stabilizes the ecosystem functional properties, which are more stable, more productive, and resistant to stress factors and disturbances (Torsvik & Øvreas 2002). Our findings were consistent with the results of Xie *et al.* (2011), who found that clear inhibitory effects on the functional activity of soil microorganisms appeared with an increasing metal content. On this basis, we can conclude that the soil ecosystem in the deteriorated environment is unstable, and its function is impaired. Teng *et al.* (2008) indicated that AWCD increased with decreasing levels of mine tailings, suggesting that the quantity of carbon utilization by soil microorganisms could have increased and more carbon sources were depleted with higher heavy metal levels. According to Konopka *et al.* (1995), even the short-term response to heavy metal contamination is manifested in a significant reduction in microbial activity.

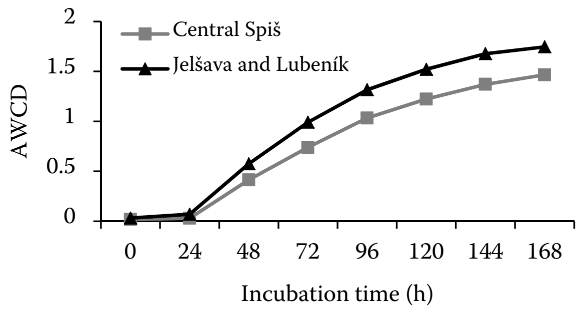

Figure 2. Average well colour development (AWCD) of BIOLOG® Eco Plates in the investigated areas

Statistical analyses. The one-way ANOVA test confirmed statistical differences between the investigated areas ($P < 0.01$) in the soil reaction, Cr, Cu, Mg, and the Shannon index diversity. We reported statistical differences ($P < 0.05$) between the areas in bulk density, porosity, Hg, Mn, AWCD, and equitability. The one-way ANOVA test confirmed no

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Table 2. Results of the one-way ANOVA

Parameter	Sources of variability	df	*F*-ratio	*P*
pH/$CaCl_2$	area	2	17.00	**
ρ_d	area	2	9.00	*
Po	area	2	9.00	*
Hg	area	2	13.33	*
Cd	area	2	5.20	–
Pb	area	2	9.00	*
Cr	area	2	11.83	**
Zn	area	2	10.50	**
Cu	area	2	14.33	**
Mn	area	2	6.72	*
Mg	area	2	22.67	**
AWCD	area	2	6.50	*
H′	area	2	11.67	**
J′	area	2	6.50	*

ρ_d – bulk density; Po – porosity; *H′* – Shannon index diversity for ECO plates; *J′* – Equitability for ECO plates; AWCD – average well color development; df – degrees of freedom; $^{**}P < 0.01$, $^{*}P < 0.05$

Table 3. Correlations between the functional diversity of microorganisms and physical soil parameters, chemical soil parameters, and heavy metals

	AWCD	*H′*	*J′*
pH $CaCl_2$	0.54**	0.26	0.40*
ρ_d	0.17	0.06	–0.01
Po	–0.17	–0.06	0.01
Hg	–0.54**	–0.44*	–0.52**
Cd	–0.29	–0.08	–0.11
Pb	–0.33	–0.1	–0.13
Cr	–0.33	–0.47*	–0.32
Zn	–0.36	–0.13	–0.16
Cu	–0.43*	–0.17	–0.21
Mn	–0.27	–0.28	–0.23
Mg	0.23	0.2	0.28
AWCD	–	0.77**	0.84**
H′		–	0.94**

ρ_d – bulk density; Po – porosity; *H′* – Shannon index diversity for ECO plates; *J′* – Equitability for ECO plates; AWCD – average well color development; $^{**}P < 0.01$, $^{*}P < 0.05$

statistical differences between the areas in Cd content only (Table 2). Many studies confirm our findings (Kizilkaya *et al.* 2004; Angelovicova & Fazekasova 2014). Knight *et al.* (1997) reported that both the metal concentration and the reduced pH values showed significant effects on the BIOLOG pattern of soil microbial communities. The effects of heavy metals on soil microorganisms generally decrease the activity and structure of microbial communities.

Table 3 shows the correlation relationships between the functional diversity of microorganisms and physical soil parameters, chemical soil parameters, and heavy metals. The AWCD was positively correlated with soil pH (R = 0.54, P < 0.01), Shannon index diversity (R = 0.77, P < 0.01) and equitability (R = 0.84, P < 0.01), and negatively correlated with Hg (R = –0.54, P < 0.01) and with Cu (R = –0.43, P < 0.05). There were significant positive correlations between the equitability-pH (P < 0.05) and the equitability-Shannon index diversity (P < 0.01).

Spearman's correlation coefficients confirmed the negative correlation between Shannon index diversity-Hg (P < 0.05), Shannon index diversity-Cr (P < 0.05), and equitability-Hg (P < 0.01). Similar results were obtained by Zhu *et al.* (2017), who found that the Shannon-Wiener index positively correlated with AWCD and negatively with the soil pH value. In the study of Liao and Xie (2007), AWCD values in the BIOLOG plates were not correlated with heavy metal concentrations, although the lowest value was seen in the heavily polluted soil after 4 days of incubation.

CONCLUSION

Based on the research of soil ecosystems present in the emission field of heaps and tailings impoundments in the area of iron ore mines and magnesite factories, we found out significant risks of soil chemical degradation including accelerated acidification, metalization and soil alkalization. Due to long-term mining activities, the Middle Spiš area was polluted with extremely increased Hg and Cu concentrations. We have also recorded high Zn, Cd, Pb and Cr concentrations. The range of the soil reaction values defines the soil as extremely acidic to strongly acidic. The processing of magnesite material in the Jelšava and Lubeník area was accompanied by enormous emission of MgO into the air including heavy metals. The content of Cr, Mn and Mg is above the level of toxicity in the soil but Cd, Pb and Zn were below the level of toxicity. Mg particles with a significant share of reactive caustic magnesite are aggressive in the natural environment, and in contact with soil, they form alkaline solutions with high pH value. The continual magnesite crust covers a part of the soil, influencing ecologically important soil functions.

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For an ecotoxicological assessment of contaminated soils, we used the BIOLOG system (BIOLOG® Eco Plate) which is known for its sensitivity and quickness. Based on the results of Shannon index (*H'*), we can conclude that the diversity at examined sites in Middle Spiš and in the Jelšava and Lubeník area is low to moderate and medium. Our findings confirmed a clear inhibitory effect of increasing the content of heavy metals on the functional activity of soil microorganisms. These results indicate that they are potentially good indices of the soil environmental quality, and that heavy-metal pollution had a significant impact on the microbial community structure.

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