JOURNAL OF ENVIRONMENTAL SCIENCE AND HEALTH, PART A
https://doi.org/10.1080/10934529.2019.1567158

Activity of the soil enzymes and moss and lichen biomonitoring method used for the evaluation of soil and air pollution from tailing pond in Nižná Slaná (Slovakia)

Lenka Demková[a], Július Árvay[b], Lenka Bobuľská[a], Martin Hauptvogl[c], and Miloslav Michalko[d]

[a]Department of Ecology, Faculty of Humanities and Natural Sciences, University of Prešov, Presov, Slovakia; [b]Department of Chemistry, Faculty of Biotechnologies and Food Sciences, Slovak Agricultural University in Nitra, Nitra-Chrenová, Slovakia; [c]Department of Suitable Development, Faculty of European Studies and Regional Development, Slovak Agricultural University in Nitra, Nitra-Chrenová, Slovakia; [d]Department of Geography and Applied Geoinformatics, Faculty of Humanities and Natural Sciences, University of Prešov, Presov Slovakia

ABSTRACT

The surrounding of the poorly maintained tailing ponds is endangered by the toxic substances and represents a serious risk for the health of the local population. The aim of the study was to determine the soil pollution by the hazardous elements (As, Cr, Cd, Cu, Fe, Mn, Ni, Pb, Zn) around the tailing pond using contamination factor (C_f), degree of contamination (C_d) and pollution load index (*PLI*). The health and the condition of soil were evaluated by soil enzyme activity (urease, acid and alkaline phosphatase, florescein diacetate, and ß-glucosidase). The spreading of the airborne hazardous elements from the body of the tailing pond was evaluated by moss and lichen bag technique and relative accumulation factor was used for the result expression. Cd, Fe, and Mn in soils reached above the limit values at all sampling sites. According to the degree of contamination (C_d), the soils at the sampling area were very high contaminated by As, Cd, Cu, Fe, Mn, Ni, and Pb. The most part of the assessed area was according to the *PLI* values extremely polluted. The air pollution was the most serious around the tailing pond, but serious levels of some hazardous elements were determined also in the remote distances.

ARTICLE HISTORY
Received 23 October 2018
Accepted 5 January 2019

KEYWORDS
Air pollution and soil pollution; tailing pond; environmental pollution indices; soil enzyme activity; moss and lichen bag technique

Introduction

The inappropriate storage, inadequate reclamation and releasing of hazardous elements from mining bodies into the surrounding environment is still an up-to-date problem at former mining areas in Slovakia. Spreading of inorganic toxic substances from these secondary sources of pollution could affects soils, waters, and the atmosphere.[1] Especially the surface of the tailing ponds covered by small particles of a dust consistency could be easily eroded by wind and particles transported to a long distance.[2]

According to the information system for the disposing of mining waste, which was created as the implementation of the provisions of the act on management of the waste, 338 repositories of mining material of which 28 are high risk are registered in the Slovak Republic.[3] There are 56 tailing ponds of various levels and types used for ash material, ore material and other industrial material storing.[4] The level of re-cultivation or storing safety is inadequate and poses an immensely treat in terms of environment protection[4] and human health.

There has been repeatedly shown that the soils in former mining areas contain hazardous elements such as Cd, Cr, Hg, Pb, Zn, sometimes in dangerous quantities[5–7] exceeding the limit values set by law.[8]

High pollution levels impact soil fertility, influence food production[9] and through the food chain may affect animal and human health.[10] Polluted soils lose very common biochemical properties which are necessary for the functioning of the ecosystem. Compared to the other soil characteristics, soil enzymes react quickly to the environmental stress and are reliable indicators reflecting the biological state of soil.[11] Because of their easy, rapid, and precise determination, they are often used as bioindicators of soil quality.[12] Mining activities and mining-related industries contribute significantly to the air pollution,[13] which is reflected by soil contamination, changes in plant community structure[14] or human health problems.[7] Moss and lichen bag technique introduced by Ref. [15] is a useful tool for biomonitoring air pollution in different types of urban areas.[16,17] Numerous advantages of the moss and lichens, such as geographical widespread, a high abundance in different geographical conditions and the lack of root system, what prevents the uptake of the hazardous elements from mineral substances, make them an ideal biomonitors for air pollution. Additionally, compared to traditional air pollution monitoring methods, mosses and lichens are low-cost, independent of power supply and able to evaluate the whole range of the hazardous elements at the same time.[18]

CONTACT Lenka Demková lenka.demkova@unipo.sk Department of Ecology, Faculty of Humanities and Natural Sciences, University of Prešov, Presov, Slovakia.

Present study focused on the evaluation of the environmental quality in the surrounding of the tailing pond Nižná Slaná (Eastern Slovakia). The aim of the study was to (i) determine the concentration of the hazardous elements in soils in different distances from the tailing pond and assess the impact of soil pollution on biological characteristics (activity of soil enzymes), (ii) to evaluate the ecological risk of the hazardous elements by applying contamination factor (C_f), degree of contamination (C_d) and the pollution load index (*PLI*), (iii) evaluate the spreading of the hazardous elements in air, from the tailing pond down the valley, using moss and lichen bag technique, (iii) compare accumulation abilities of different moss and lichen taxa.2.

Materials and methods

Study area

Nižná Slaná village is situated in the Eastern Slovakia in Slovenské rudohorie hills, surrounded by three national parks – Muránska planina plain, Slovenský raj, and Slovenský kras karst area. From the climatological point of view, area belongs to the moderately warm – moderately wet climate region, with the mean January temperature −2 to −5 °C.[19] Nižná Slaná village was established in the 14th century as the mining village focused to the iron ores and precious metals mining. During the 18th and the 19th century, the biggest industrial and mining development was recorded. Accompanied processing activities were performed in the smelter provided by three blast furnaces.[20] Mining and ore processing activities were stopped in 2008, but abandoned mining pits, heaps of waste material, huge abandoned smelter area and the tailing pond stayed the source of toxic substances endangering surround environment.[21] Tailing pond Nižná Slaná [48°44′36.90″; 25°25′51.24″] with an area of 20.6 ha is situated in the 464 m a.s.l. and 7 million tons of the sewage sludge is stored there. Overall high of the dam is 100 m. Because of inadequate remediation, the tailing pond is unstable and represent serious environmental treat for the region.[22]

Samples of the soil taken in the surrounding of the tailing pond are according to the International Union of the Soil Science[23] characterized as technosols – new referential soil formed by the material of technogenic origins. In our case, the proportion of soil and the material of technical origin was changing with the distance from the tailing pond – that's why we will use the term "soil" for all the soil/technosols samples.

Soil sample collection and preparation

Topsoil samples (10–20 cm) were sampled at 11 sampling sites distributed at a distance 0, 50, 100, 150, 200, 300, 400, 500, 700, 1000, 1100 m from the main pollution source – the dam of the tailing pond. The localization of the sampling sites regarding the relief is shown in Figure 1. The sampling sites are localized in the direction of the predominant wind (east-west direction) which flow from the tailing pond, down to the valley.[24] At each sampling site, 500 g of the soil was sampled, stored in the plastic bag, and transported to the laboratory, where the samples were air-dried at a room temperature for 2 weeks. Subsequently, the samples were manually crushed, cleaned of rough particles and dead parts of the plants, and sieved through a mesh sieve (2 mm).

Soil enzyme activity and pH determination

Soil reaction (pH) was determined as follows: 20 g of the soil was mixed with a 50 mL $CaCl_2$ ($c = 0.01$ mol L^{-1}) (Sigma-Aldrich, spol. s r.o., Bratislava, Slovakia). After the 20 minutes of shaking in Unimax 2010 horizontal shaker (Heidolph Instruments, GmbH, Schwabach, Germany) the samples were filtered through the filter paper Filtrak 390 (Munktell&Filtrak, GmbH, Bärenstein, Germany) and subsequently measured by pH meter Metrohm 691 (Metrohm AG, Herisau, Switzerland).[25]

Activity of acid (ACP) and alkaline phosphatase (ALP) was colorimetrically determined as a phenol release after the incubation (for 3 h at 37 °C) of soil samples with phenyl phosphate solution and acetate buffer (for acid phosphatase) and acetate buffer (for alkaline phosphatase).[26] Phenol release was measured by the spectrometer at 510 nm. Activity of urease (URE) was colorimetrically determined as an ammonia release after the incubation (for 24 h at 37 °C) of soil samples with urea solution.[27] Ammonium determination was measured by spectrometer at 410 nm. Fluorescein diacetate activity (FDA) was spectrophotometrically determined using fluorescein diacetate as a soil and incubated at the temperature of 30 °C for 1 h after the soil hydrolysis.[28] Activity of ß-glucosidase (BG) was determined as a p-nitrophenol release after the incubation (for 1 h at 37 °C) of the soil samples with 4-Nitrophenyl glucopyranoside.[29]

Contamination factor, degree of contamination and the pollution load index

To determine the hazardous elements pollution in soil samples, the contamination factor (C_f^i)[30] was calculated as follows Equation 1:

$$C_f^i = \frac{C_{0-1}^i}{C_n^i} \tag{1}$$

where, C_{0-1}^i is the measured concentration of the hazardous element and C_n^i is the background level of the hazardous element in upper Earth's crust according to Čurlik and Šefčík[31] and Kabata-Pendias.[32] The background level (C_n^i) of As, Co, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn in natural soils were considered as 25, 0.3, 20, 10, 20, 530, 20, 530, 10, 20, 40 mg kg^{-1} respectively.[31,32] Hakanson[30] recognized four classes according to the contamination factor values: (i) low contamination factor (if $C_f^i < 1$), (ii) moderate contamination factor (if $1 \leq C_f^i < 3$); (iii) considerable contamination factor (if $3 \leq C_f^i < 6$) and (iv) very high contamination factor (if $C_f^i \geq 6$).

Degree of contamination (C_d) is a degree of overall contamination in a sampling site calculated as follows Equation

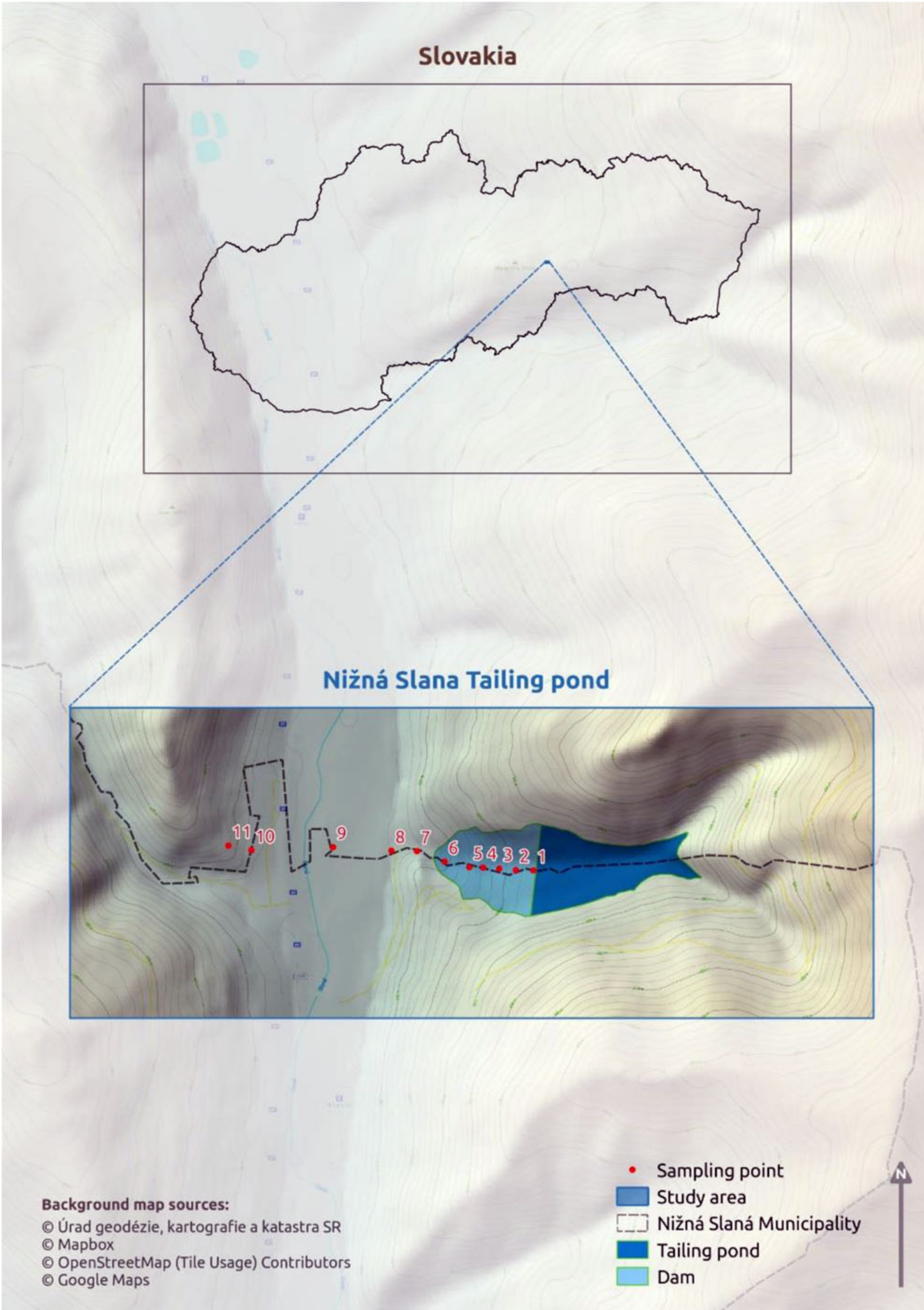

Fig. 1. The localization of the sampling sites selected for soil sampling and M/L bags exposition.

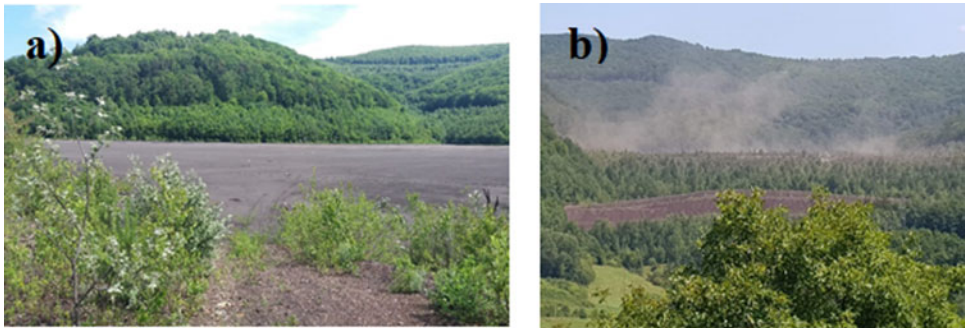

Fig. 2. The area of the Nižná Slaná tailing pond (a) and the spreading of the dust particles from the tailing pond (b).

2:

$$C_d = \sum C_f^i \quad (2)$$

The final C_d values were divided by Hakanson[30] into four categories as follows: (i) low degree of contamination (if $C_d < 8$), (ii) moderate degree of contamination (if $8 \leq C_d < 16$), (iii) considerable degree of contamination (if $16 \leq C_d < 32$), (iv) very high degree of contamination (if $C_d \geq 32$).

For the comparative assessment of the level of hazardous elements pollution at each sampling site, pollution load index (*PLI*) proposed by Tomlinson et al.[33] was used. *PLI* was calculated as follows Equation 3:

$$PLI = (C_{f1} \times C_{f2} \times C_{f3} \times \ldots \times C_{fn})^{1/n} \quad (3)$$

where n – is number of assessed metals, C_f is a contamination factor of individual pollutants. The *PLI* values were divided into four categories according to Wang et al.[34] as follows: (i): no pollution ($PLI < 1$), (ii) moderate pollution ($1 \leq PLI < 2$), (iii) heavy pollution ($2 \leq PLI < 3$), (iv) extreme pollution ($PLI \geq 3$).

One lichen (*Hypogymnia physodes* (L.)) and three moss taxa (*Dicranum* spp., *Hypnum* spp., *Polytrichum* spp.) were sampled during the June 2016 in Slanské vrchy hills (Eastern Slovakia). Sampling localities were selected at least 500 m from the forest road and at least 1000 m from the main road. Approximately 500 g of each taxon was sampled. Moss and lichen (M/L) samples were stored in the paper bags and transported to the laboratory conditions where they were manually cleaned from the soil particles and needles, separated from the brown tissue, homogenizes and washed tree times (approximately 10 L water per 100 g of moss dry weight lasting 5, 10, 20 minutes) in deionized water. Subsequently were M/L samples hand squeezed and air-dried in an oven at 60 °C for 24 h (Venticell 111, BMT, a.s., Czech Republic).

Moss and lichen sampling and preparation

About 5 g of each M/L taxa was packet into the nylon net (2 mm mesh size) cut at pieces 10 × 10 cm. Two bags of each taxon were exposed to 11 sampling points (same as the soil sampling sites) (Fig. 2), hanging on the trees in the height of 2 m. One part of each taxon was stored as the background sample (not – exposed) to determine initial hazardous elements values. A total set of 88 M/L bags were exposed for 6 weeks. After the exposure, M/L bags were collected and stored in plastic bags, at −20 °C prior to analysis.

Soil and moss and lichen hazardous element determination

The homogenized M/L samples and soil samples were prepared by milling in a laboratory grinder IKA A10 basic (IKA Works, Wilmington, USA). The homogenized samples were stored in closed plastic bags until the next treatment step. For pressure microwave digestion, approximately 0.20 g (with a precision to 4 decimal places) of samples was weighed into PTFE digestion tubes. Consequently, 5 mL of HNO_3 and 1 mL of H_2O_2 (trace purity) were purchased from Lambda Life spol. s r. o., Bratislava, Slovakia (producer: Sigma-Aldrich Chemie GmbH, Steiheim, Germany), was added directly to the PTFE vessels. The digestion procedure was carried out using pressure microwave digestion system ETHOS-One (Milestone, Srl., Italy). The mineralized sample solutions were filtered through a quantitative Munktell filter paper No. 390 (Munktell & Filtrak, Bärenstein, Germany) into 50 mL volumetric flasks and filled with deionized water (ddH_2O) to the final volume. Sample solutions were stored in polyethylene tubes until ICP-OES analysis. Each sample was prepared in three replicates. Ultra-pure water – ddH_2O (18.2 MΩ cm^{-1}, 25 °C) was treated in a Simplicity 185 purification (Millipore SAS, Molsheim, France) and was used in all cases.

Elemental analysis was carried out on an Agilent ICP-OES spectrometer 720 (Agilent Technologies Inc., Santa Clara, CA, USA) with axial plasma configuration and with an auto-sampler SPS-3 (Agilent Technologies, GmbH, Germany). Detailed experimental conditions were set as follows: RF power 1.45 kW; plasma gas flow 16.0 L min^{-1}; auxiliary gas flow 1.50 L min^{-1} and nebulizer gas flow 0.85 L min^{-1} and CCD detector temperature −35 °C. Signal accusation time 3 s for three replicates. In total, 88 M/L samples and 11 soil samples were analyzed for concentration of nine elements (As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn). Calibration of the analytical method ICP-OES was realized using mixed standard TraceCert® ICP 5 (Sigma Aldrich, GmbH, Steiheim, Nemecko), which was diluted to the three calibration levels (I.: 0.0475 mg kg^{-1}; II.: 0,950 mg kg^{-1}; III.:

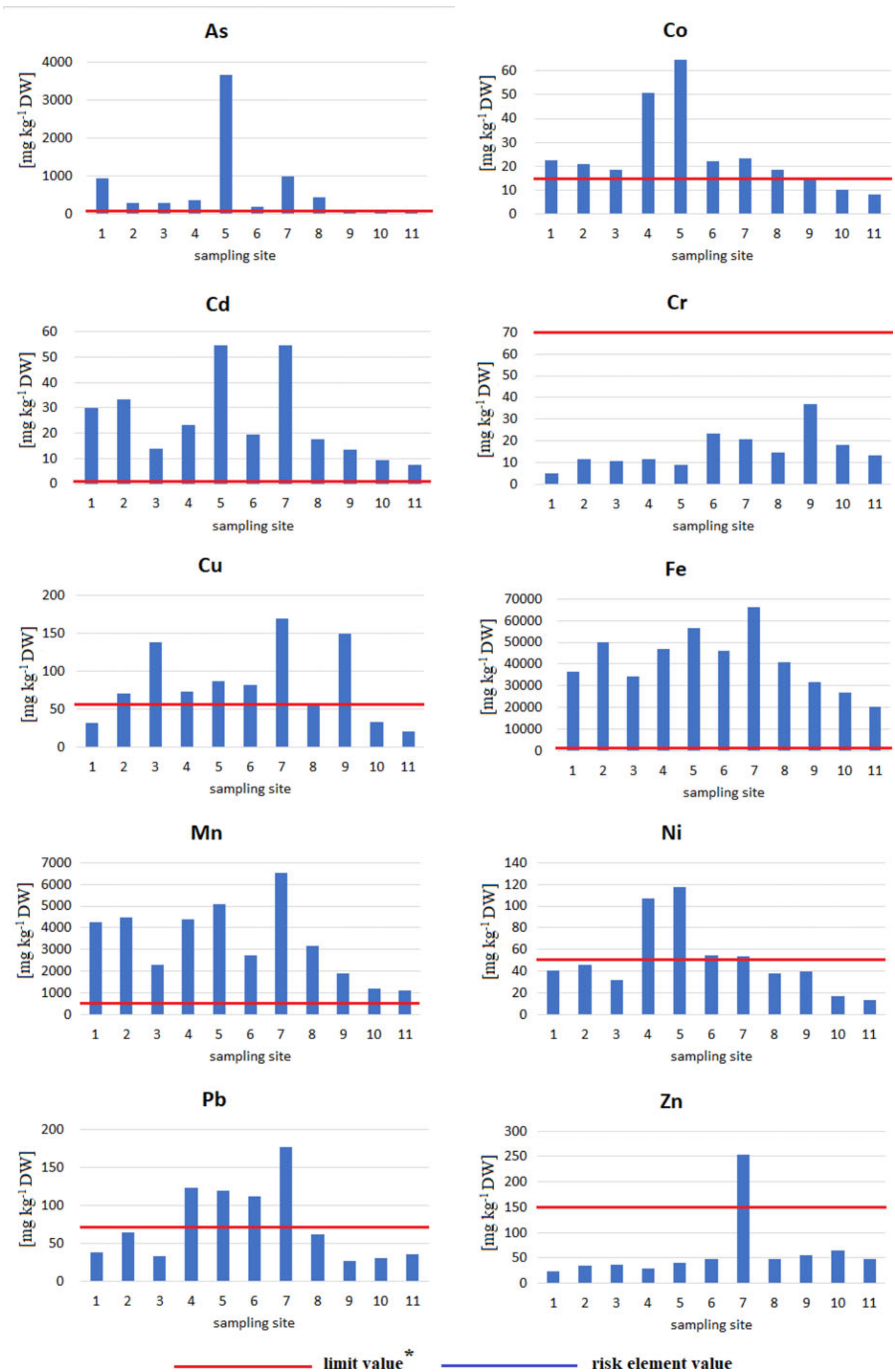

Fig. 3. The content of the hazardous elements determined in soils sampled in different distance from the tailing pond and the limit values determined by the National Council of the Slovak Republic no. 220/2004 Coll. of Laws.[8]

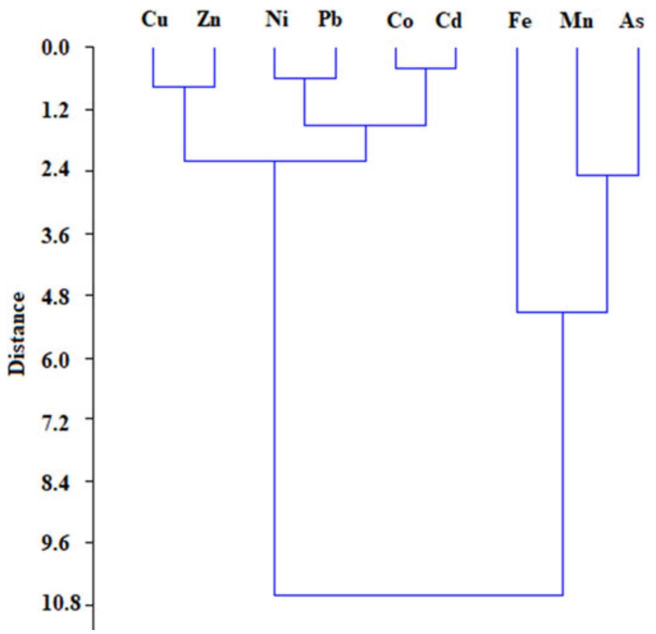

Fig. 4. Dendrogram obtained by cluster analysis for hazardous elements content in soil samples.

0,190 mg kg^{-1}). Argon and carbon were used as internal standard elements. ERM®-CE278k (mussel tissue; IRMM, Belgium) was used for quality measurements control. Their recovery values (regarding the water volume), for all determined elements, ranged between 85 and 118%. Following spectral lines were used for quantitative and qualitative elements determination: As: 188.980 nm; Cd: 226.502 nm; Cr: 267.716 nm; Cu: 324.754 nm; Fe: 234.350 nm; Mn: 257.610 nm; Ni: 231.604 nm; Pb: 220.353 nm and Zn: 206.200 nm. All hazardous elements data were calculated to the mg kg^{-1} DW. A total content of the hazardous elements was determined also in the reference material – M/L samples not exposed. The final values of hazardous elements in M/L bags were computed as the measured (exposed) valued minus reference values.

Relative accumulation factor

Relative accumulation factor (RAF) was used to assess the content of each hazardous element in the exposed moss and lichen species (Equation 4) as follows:

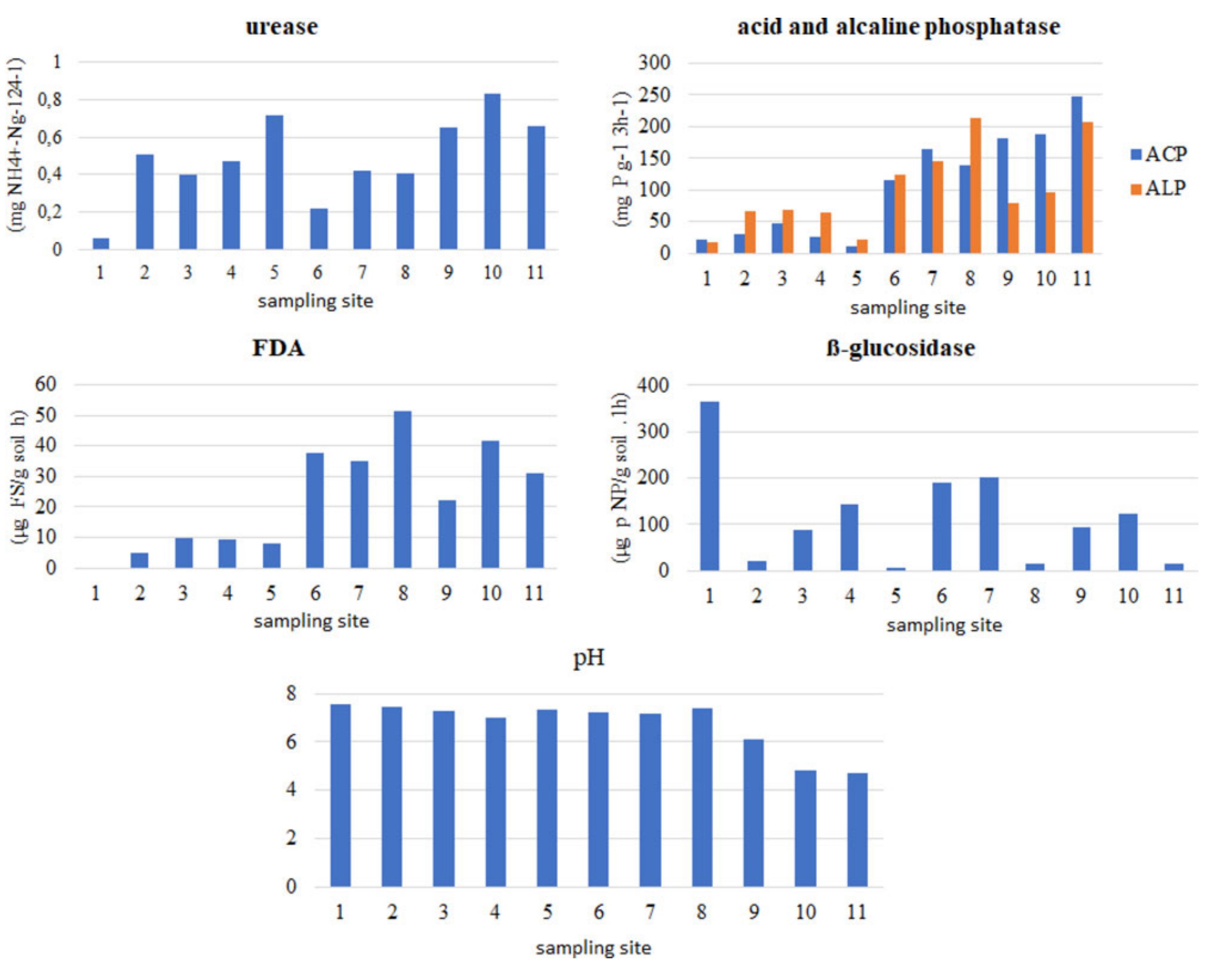

Fig. 5. The activity of soil urease (URE) acid phosphatase (ACP), alkaline phosphatase (ALP), FDA and ß-glucosidase (BG) at sampling sites localized near Nižná Slaná tailing pond.

Table 1. Correlation relationship (Spearman's correlation) between hazardous elements, activity of soil enzymes and soil pH (URE – urease, ALP -alkaline phosphatase, BG - ß-glucosidase).

	Cd	Cu	Fe	Mn	Ni	Pb	Zn	As	URE	ACP	ALP	FDA	BG	pH
Co	0.77**	0.39	0.77**	0.79**	0.96**	0.70*	−0.19	0.82**	−0.16	−0.80	−0.57	−0.37	−0.07	0.69*
Cd		0.46	0.92**	0.95**	0.78**	0.74**	0.21	0.90*	−0.28	−0.62*	−0.49	−0.39	0.01	0.75**
Cu			0.63	0.47	0.54	0.37	0.40	0.35	−0.02	−0.09	−0.04	0.12	0.17	0.54
Fe				0.93**	0,83**	0.84**	0.28	0.79**	−0.26	−0.48	−0.26	−0.14	0.06	0.79
Mn					0,81**	0.75**	0.11	0.88**	−0.43	−0.62	−0.42	−0.42	0.08	0.85
Ni						0.73**	−0.10	0.76**	−0.19	−0.69*	−0.49	−0.29	0.01	0.73**
Pb							0.36	0.62*	−0.15	−0.28	0.01	0.12	−0.01	0.50
Zn								−0.01	0.23	0.57	0.51	0.61*	0.14	−0.15
As									−0.34	−0.74**	−0.58	−0.46	−0.12	0.78**
URE										0.24	0.26	0.48	−0.55	−0.63
ACP											0.86**	0.79*	0.12	−0.62*
ALP												0.85**	−0.12	−0.40
FDA													−0.19	−0.45
BG														0.16

*$P < 0.05$, **$P < 0.01$.

$$RAF = (C_{exposed} - C_{initial}) / C_{initial} \quad (4)$$

where $C_{exposed}$ is the content of the hazardous element after exposure, $C_{initial}$ is the content of the hazardous element before exposure.

Statistical evaluation and the map preparation

Spearman's correlation coefficient was used to determine the relationship between hazardous element concentrations, soil enzymes activity and soil pH in the soil samples. Cluster analysis, Wards method was used to highlight the similarity/distinction between hazardous elements determined in soil samples. One-way ANOVA test followed by Turkey's multiple comparison test was used to find out the differences in hazardous element concentrations between moss/lichen taxa at the $P < 0.01$ and $P < 0.05$ level. All statistical analyses were performed in R studio.[35]

For the reached data visualization and the map preparation geographic information system QGIS was used. As an analytical base maps OpenStreetMap (OSM); GoogleMaps; Geodesy, Cartography and Cadastre Authority of Slovak Republic and Mapbox layers where used.

Results and discussion

Total content of hazardous elements in soil samples

The values of the hazardous elements determined in soil samples at different distance from the main pollution source - tailing pond and the limit values of the hazardous elements[31,32] are listed in Figure 3. The values of Cd, Fe, and Mn reached above the limit values[8] at all sampling sites and their average value was 36, 75, and 6 times higher than the acceptable limit value, respectively. The similar course with the highest values at the site 5 and the below limit values at the last sampling site, were determined for As and Co. Pb and Ni exceeded the limit values only in the middle part of the sampling area. The whole research area was unpolluted by Cr. According to the earlier researches on the study area, the serious content of As, Fe, and Mn in the atmospheric deposition was determined.[21] Analyses focused on the hazardous element content in sediments around Nižná Slaná tailing pond have also confirmed extremely high levels of As, Fe, and Mn.[20] A close relationship between mentioned hazardous elements was confirmed by cluster analysis (Fig. 4).

Table 2. The values of the hazardous elements contamination factor (C_f), with highlighted the highest values and the degree of contamination (C_d) of evaluated hazardous elements (*distance from the dam of the tailing pond).

Sampling site	Distance* (m)	As	Co	Cd	Cu	Fe	Mn	Ni	Pb	Zn
1	0	37.5	1.12	99.9	1.6	68.5	8.04	4.04	1.88	0.57
2	50	12.0	1.04	110	3.56	94.4	8.44	4.63	3.21	0.89
3	100	11.9	0.92	46.8	6.90	65.1	4.32	3.16	1.63	0.91
4	150	14.7	2.57	77.6	3.67	88.6	8.26	10.73	6.14	0.71
5	200	**146**	**3.24**	181	4.33	107	9.63	**11.8**	5.95	1.00
6	300	7.22	1.10	65.2	4.11	87.1	5.14	5.45	5.61	1.22
7	400	40.0	1.15	**182**	8.48	**124**	**12.4**	5.38	**8.86**	**6.36**
8	500	17.1	0.94	59.2	2.78	76.9	5.97	3.78	3.10	1.21
9	700	3.03	0.71	44.5	7.47	60.2	3.57	4.01	1.34	1.38
10	1000	1.94	0.52	31.2	1.63	50.6	2.23	1.75	1.55	1.60
11	1100	1.83	0.40	24.9	1.03	38.5	2.07	1.33	1.80	1.18
C_d	–	293	13.7	924	45.6	861	70.0	56.0	41.1	17.0

As it shown in Figure 3, the highest pollution was determined in the middle part of the sampling area in the distance from 250 to 400 m from the tailing pond body. It should relate to the wind force and the transmitted particle size. Especially particle size has been reported to influence or control many critical factors including atmospheric distance and deposition rate.[36] Niu et al.[37] have found, that hazardous elements such as Mn, Ni, Cu, Zn, and Cd are usually bounded to the nanoparticles, the elements such as Fe and Pb are bounded to the fine-size particles. Despite the assumption that smaller particles will be transported over longer distances, no significant differences between hazardous elements were determined.

Soil pH and the activity of the soil enzymes

Soil pH at the sampling sites ranged between 4.72 and 7.54 (Fig. 5). The highest values were determined at the body of the tailing pond and decreased down the valley. According to Čurlik and Šefčik classification[31] soil samples were determined as slightly alkaline to strongly acid. There has been repeatedly reported,[38,39] that pH in heavily polluted soils uses to reach low values. In our case the opposite trend

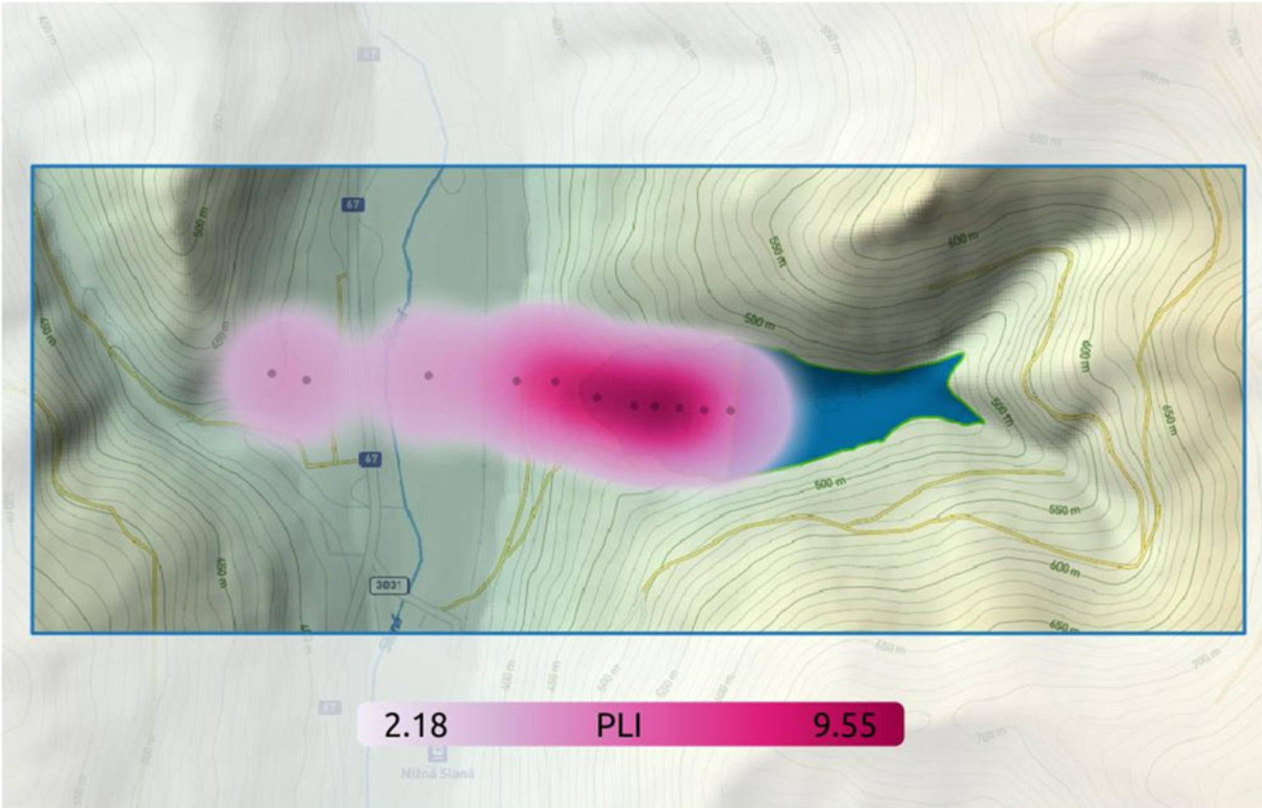

Fig. 6. The values of pollution load index (*PLI*) determined at the sampling sites localized near tailing pond.

Table 3. The hazardous elements content (post-exposed minus pre-exposed) determined in four different taxa after the 6 weeks exposure near the Nižná Slaná tailing pond (min – minimum, max – maximum, ave – average, std – standard deviation, med – median).

M/L taxa		Hazardous elements [mg kg^{-1} DW]								
		As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Dicranum spp.	min	1.75	0.80	2.44	12.1	1413	134	2.14	33.9	37.1
	max	76.5	2.22	4.35	16.6	5919	495	11.2	42.0	41.6
	ave	12.9	1.10	3.30	13.5	2239	199	4.50	37.2	39.5
	std	20.7	0.38	0.72	1.23	1209	98.8	2.31	2.27	1.73
	med	4.68	0.97	3.21	13.5	1833	155	3.73	37.1	39.5
Hypnum spp.	min	1.41	1.20	5.15	15.6	2479	188	4.68	46.8	75.6
	max	118	3.57	15.8	23.7	9875	1030	16.7	71.3	97.8
	ave	182	1.71	9.71	18.5	3895	459	8.49	57.8	90.8
	std	32.4	0.63	3.21	2.03	1990	222	4.02	6.31	6.27
	med	5.49	1.49	10.8	18.2	3321	451	7.04	59.1	92.6
Polytrichum spp.	min	0.99	0.33	1.52	10.8	561	212	1.37	15.8	19.9
	max	51.1	1.33	3.70	14.7	3758	459	6.83	23.6	28.5
	ave	8.27	0.55	2.29	11.9	1120	298	3.20	18.9	25.5
	std	13.8	0.25	0.72	1.01	848	61.4	1.50	1.94	2.33
	med	3.60	0.50	2.18	11.6	895	288	3.03	19.0	25.6
Hypogymnia physodes	min	0.64	1.72	2.06	6.65	452	70.9	2.65	11.8	56.9
	max	69.4	3.11	4.96	12.7	4512	416	11.1	20.9	76.6
	ave	12.8	2.19	2.95	8.87	1175	148	5.11	15.0	68.9
	std	18.9	0.38	0.94	1.48	1122	93.8	2.48	2.53	5.68
	med	5.41	2.12	2.53	8.85	786	114	4.09	14.1	69.5

was determined what could be influenced by the low activity of soil organisms. Javoreková et al.[40] have found that the soil organisms could influence the formation of the soil acidity. A composition of the material stored in the tailing pond could affect soil pH.

The values of the soil enzymes activity are shown in the Table 1. The lowest values of URE, ALP and FDA were determined at the body of the tailing pond and increase with the distance from the tailing pond body. The lowest values of ACP and BG were determined at the 5th sampling site (in the middle of the slope). It has been repeatedly shown that accumulation of the hazardous elements in soils reduce the content of microbial biomass, limiting the functional diversity of ecosystem.[41,42] On the other hand, the effect of the hazardous elements on soil microorganisms is not always identical, it depends on many physical and chemical characteristics,[43] what explain the different development of various enzymes activity in our case. Alcaic pH values use to suppress the activity of acid phosphatase,[40] what was confirmed in our study. Hu et al.[44] and Yang et al.[45] also recorded that urease and acid phosphatase react significantly to the environmental stress.

Correlation relationship between hazardous elements and soil properties

Several studies had confirmed that a positive correlation among hazardous elements suggest their common origin.[46,47] The results of the Spearman's correlation between hazardous elements are listed in Table 1. Significant positive correlations ($P < 0.01$; $P < 0.05$) were confirmed between As, Co, Cd, Fe, Mn, Ni, and Pb to each other. In addition, these elements significantly positively correlated with pH (except Pb, which gave only positive, no significant correlation, probably because the Pb pollution wasn't as serious as pollution by other hazardous elements). Zinc gave no correlation with other elements. Although, zinc pollution is usually associated with mining activities (among others),[48] around

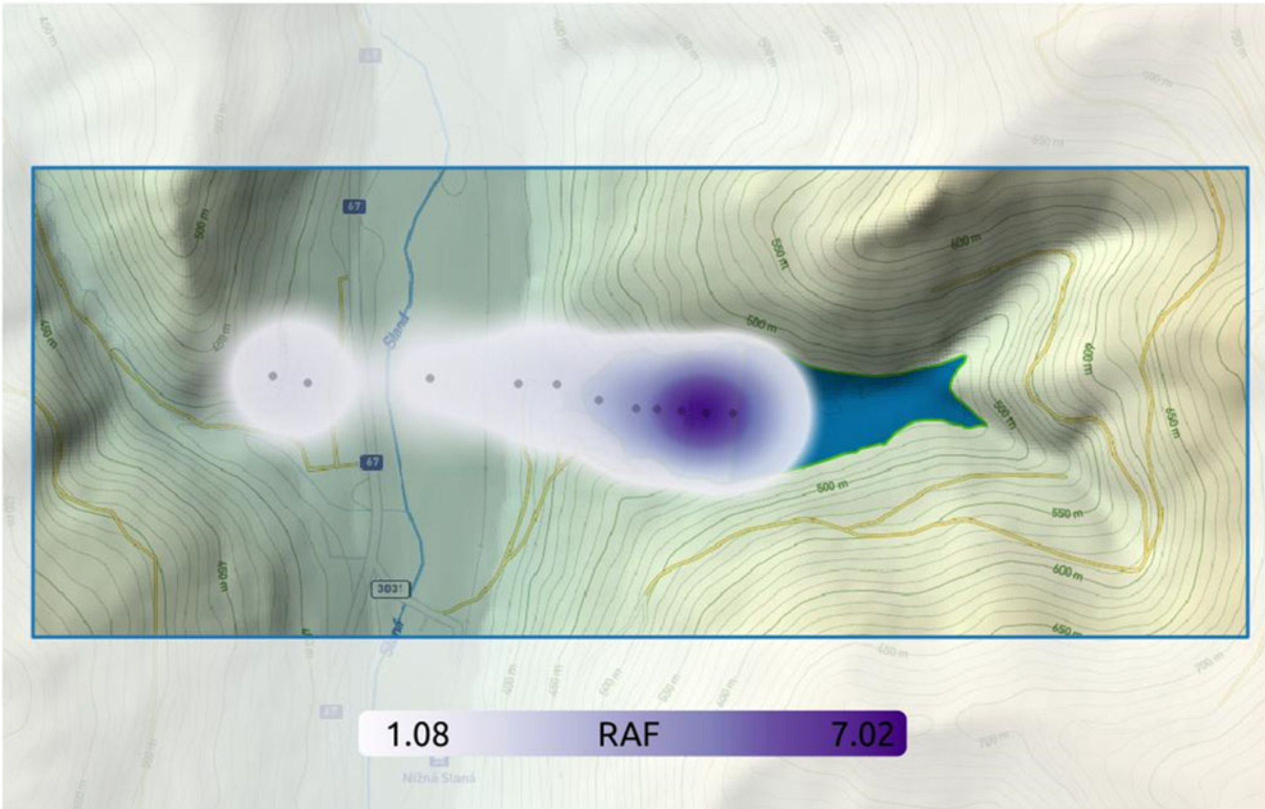

Fig. 7. The average RAF values (regardless the taxa) at the sampling sites in different distance from the tailing pond.

Nižná Slaná tailing pond wasn't as serious comparing other elements.

In the areas with a high soil pollution, negative correlation between hazardous elements and soil pH was commonly confirmed.[5,49] In this study, hazardous elements significantly positively correlated with soil pH, what could be the result of the sludge from tailing pond, which usually reach higher (alkaline) pH values.[50] Soil acidification enhances the mobilization of metals in soils, resulting in increased uptake by plants[5] On the other hand, the bioavailability of heavy metals in soils decreases above the pH 5.5–6.[51]

Soil enzymes activity, which is considered useful tools for environmental stress bioindication,[52] react differently to different types of toxic substances. Hazardous elements such as As, Co, Cd, Mn, and Ni gave significant negative correlation with ACP. Soil enzymes URE, ALP, and FDA gave negative (almost in all cases) correlation with all evaluated hazardous elements (Table 1). Only BG was not influenced by hazardous elements toxicity (Table 1). It has been repeatedly shown, that soil pollution influence negatively the microbial activity what resulted in decreasing soil enzyme values and ultimately the soil fertility.[53,54] A significant positive correlation was found between ACP-ALP, FDA-ACP, FDA-ALP. All evaluated enzymes correlated negatively (significantly in the case of URE and ACP) with soil pH. Taylor et al.[55] and Angelovičová et al.[42] approvingly reported negative correlations between soil pH and enzyme activities.

Cluster analysis

Cluster analysis, Ward method, was used to identify similar hazardous elements in soils. Results are shown in dendrogram (Fig. 4). Elements belonging to the same cluster get used to having a strong correlation among themselves and may originate from a common source.[56] In our case two groups of hazardous elements were identified (i) Cu, Zn, Ni, Pb, Co, and Cd (ii) Fe, Mn, and As. The pollution by second group elements (Fe, Mn, and As) in Nižná Slaná was already determined as serious (above the limit values) by previous studies.[20,21] A close relationship between these elements was already verified by the Spearman's correlation (Table 1).

Contamination factor and the degree of contamination in soils

Contamination factor (C_f), degree of contamination (C_d) and the pollution load index (*PLI*) were calculated for a comprehensive soil pollution assessment in the study area. The results are listed in Table 2 and Figure 6. The highest RAF values were determined between the 5th and 7th sampling site, what represents the bottom part of the tailing pond and the distance 250–400 m from the tailing pond body.

Based the average C_d results we can conclude, that the soils of the research area are moderately contaminated by Co, considerably contaminated by Zn, and very high

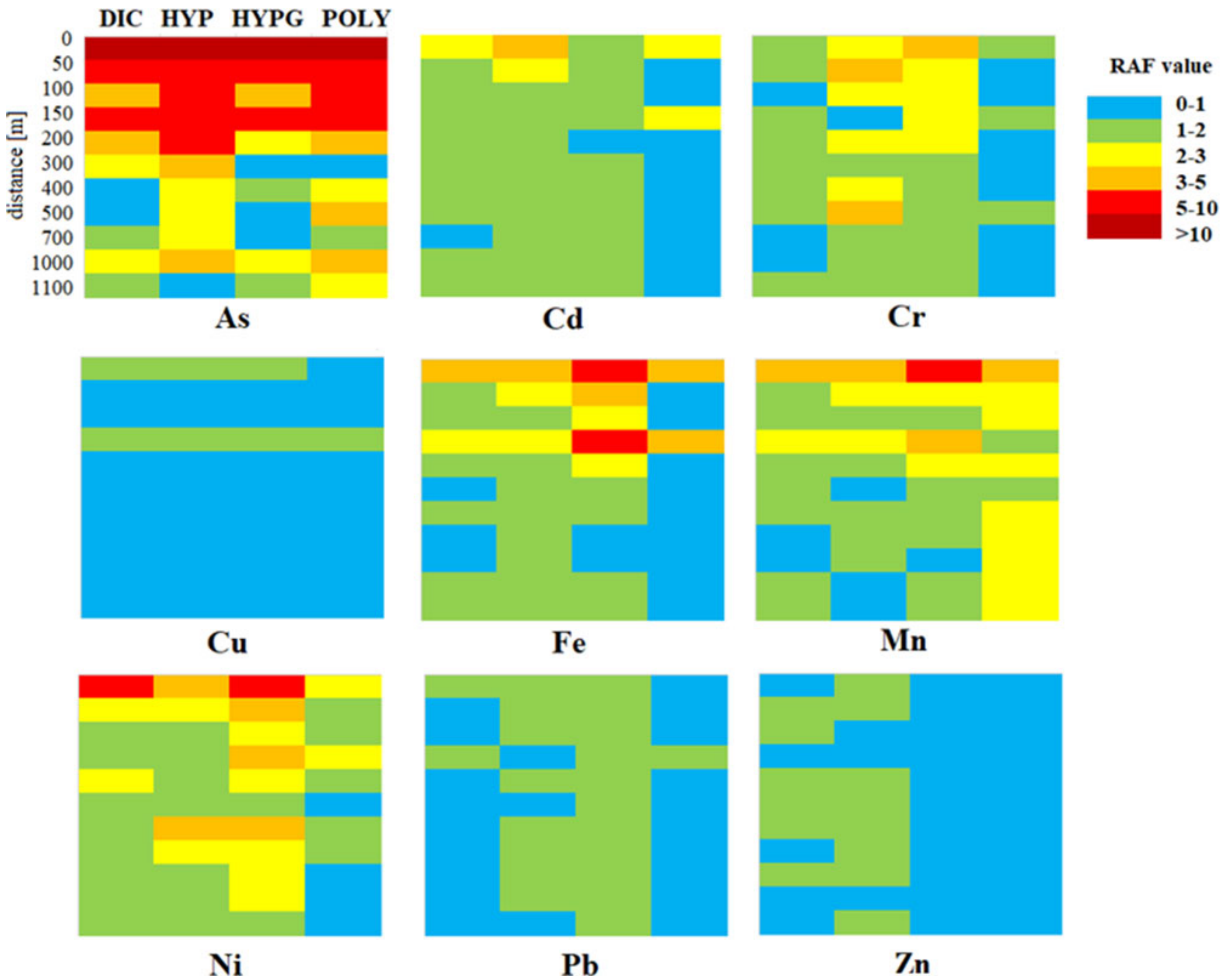

Fig. 8. RAF values of individual hazardous elements determined in four M/L taxa depending the distance from the main pollution source (HYPG - *Hypogymnia physodes*, DIC - *Dicranum* spp., HYP - *Hypnum* spp., POLY - *Polytrichum* spp).

contaminated by As, Cd, Cu, Fe, Mn, Ni, and Pb. *PLI* was calculated separately for each sampling site to assess the quality of soils at the sampling sites.[34] According to the *PLI* values (Fig. 6), all sampling sites (except no. 10 and 11) are considered extremely polluted ($PLI \geq 3$), and sampling sites localized in the biggest distance from the pollution source (10, 11) are considered as heavily polluted.

Hazardous elements in the moss and lichen taxa

Hazardous element values (post-exposed minus pre-exposed) determined in different taxa are listed in Table 3. The highest average values of all hazardous elements were determined in the *Hypnum* spp. tissues, except the average value of Cd, which reached the highest average value in *Hypogymnia physodes*. *Hypnum* spp. is widely used as a bioindicator in the bioaccumulating studies, due to its wide-ranging distribution and very good accumulation abilities.[57]

The average RAF values of the hazardous elements, regardless of the taxa, are listed in Figure 7. The highest values were determined at the first sampling sites and decreased down the valley. Serious pollution is along the length of the dam, and the lowest values are reached at the last sampling site.

Based the results (Fig. 8) showing RAF values of individual elements in different taxa and different sampling sites, we can conclude, that the pollution by all evaluated hazardous elements is spreading from the main source - tailing pond down the valley. Wind is an important factor influencing the spreading the pollution.[58] Some studies deal with the ability of different size particles to accumulate hazardous elements.[59] Smaller particles could be transferred for a longer distance, but no significant differences in their ability to accumulate hazardous elements were confirmed.[56] The content of the hazardous elements (regardless the taxa) at the evaluated area decrease in the following order: As > Ni > Mn > Fe > Cr > Cd > Pb > Zn > Cu.

The highest concentrations of the hazardous elements in M/L taxa expressed by RAF (except Cu, Pb, and Zn) were assessed at the tailing pond dam, or at close sampling sites, what do not correspond with the values of the hazardous elements determined in soil samples. Soil pollution (Fig. 3) was the most serious in the middle part of the tailing pond dam. It has been stated by several authors that also physicochemical mechanism of the

Table 4. One-way ANOVA results for the comparison of values of hazardous elements RAF values between taxa.

RAF values		Df	*F* value	*P*-value
As	Between taxa	3	0.42	0.74
Cd		3	4.05	0.00072**
Cr		3	4.83	9.95e − 06 **
Cu		3	11.9	0.0058**
Fe		3	2.25	0.09
Mn		3	2.30	0.09
Ni		3	2.41	0.026*
Pb		3	27.9	6.56e − 10**
Zn		3	149	2e − 16**

**$P < 0.01$; *$P < 0.05$.

mosses could influence the accumulation of the individual hazardous elements.[60,61]

Study area was seriously polluted also by Fe, Mn, and Ni. Extremely high RAF values of As were measured in all evaluated taxa at sampling sites close to the tailing pond (approximately to the distance 200 m from tailing pond). As mentioned above, in earlier studies focused on the sediments and atmospheric deposition pollution near Nižná Slaná tailing pond serious pollution by As, Mn, and Fe was confirmed.[20,21] The RAF values of Cu, Pb, and Zn was not as serious comparing others.

The ability of M/L taxa to entrap the hazardous elements, expressed by RAF increases in the order: *Hypnum* spp.>*H. physodes*>*Dicranum* spp.>*Polytrichum* spp.

One-way ANOVA test followed by Tukey post hoc test (Table 4) was used to detect significant differences in the accumulation abilities between M/L taxa. The evaluated taxa did not differ in their ability to accumulate As. *Polytrichum* spp. showed significantly lowest ability to accumulate Cu ($P < 0.01$) but based the scheme (Fig. 8) also the lowest (not statistically) average values of Cd, Cr, Fe, Ni, and Pb were measured in their tissues comparing other M/L taxa. In the study conducted around tailing pond in Slovinky (Slovakia).[62] *Polytrichum* spp. identically showed the lowest hazardous elements accumulation abilities. The ability to accumulate Pb and Zn significantly differ between evaluated taxa (only *Hypnum* spp. and *Dicranum* spp. showed similar results). In the case of Cd, differences were found only between *Polytrichum* spp. and *Hypnum* spp. ($P < 0.01$). Spaqnuolo et al.[63] confirmed better resistance to environmental stress for lichens comparing mosses, additionally better preserving, or recovering vitality during bio-monitoring. On the contrary, higher uptake ability for mosses was confirmed in the studies of Vingiani et al.[64] and Giordano et al.[65] In this study, *H. physodes* accumulate significantly higher volumes of Fe and Ni comparing *Polytrichum* spp., but statistical differences for other hazardous elements and other taxa wasn't confirmed.

Conclusions

The hazardous elements determined in the soil samples sampled at different distances from the tailing pond reached above the limit values predominantly in the central part of the tailing dam. The degree of contamination (C_d) confirmed for the soils around tailing pond, moderate contamination by Co, considerable contamination by Zn and very high contamination by As, Cd, Cu, Fe, Mn, Ni, and Pb. Based on the results of pollution load index, all sampling sites (except last two) are considered extremely polluted. Serious soil pollution negatively (significantly in some cases) influence activity of soil enzymes expressing the fertility and the health of the soil. The ability of three moss and one lichen taxa, to accumulate hazardous elements from air, expressed by RAF increased in the order: *Hypnum* spp.>*H. physodes*>*Dicranum* spp.>*Polytrichum* spp. One-way ANOVA confirmed differences in accumulation abilities between taxa, what points to the need of using different taxa to reach the complex results about pollution.

Funding

This research was funded by the project Ministry of Education, Science, Research and Sport of the Slovak Republic VEGA 1/0326/18.

ORCID

Miloslav Michalko http://orcid.org/0000-0003-2537-0297

References

[1] Pascaud, G.; Leveque, T.; Soubrand, M.; Boussen, S.; Joussein, E.; Dumat, C. Environmental and Health Risk Assessment of Pb, Zn, As and Sb in Soccer Field Soils and Sediments from Mine Tailings: Solid Speciation and Bioaccessibility. Environ. *Environ. Sci. Pollut. Res.* **2014**, 21, 4254–4264. DOI: 10.1007/s11356-013-2297-2.

[2] Zanuzzi, A.; Arocena, J. M.; van Mourik, J. M.; Faz Cano, A. Amendments with Organic and Industrial Wastes Stimulate Soil Formation in Mine Tailings as Revealed by Micromorphology. *Geoderma* **2009**, 154, 69–75. DOI: 10.1016/j.geoderma.2009.09.014.

[3] Act no. 514/2008 Coll. Act on the Management of Waste from Extractive Industries and on Amendments and Supplements to Some Laws (in Slovak).

[4] Bosák, M.; Hajduová, Z.; Majerník, M.; Andrejovsky, P. Experimental-Energy Combustion of Biomass Combined with Coal in Thermal Power Plants. *Polish J. Environ. Stud.* **2015**, 24, 1517–1523.

[5] Árvay, J.; Demková, L.; Hauptvogl, M.; Michalko, M.; Bajčan, D.; Stanovič, R.; Tomáš, J.; Hrstková, M.; Trebichalský, P. Assessment of Environmental and Health Risks in Former Polymetallic Ore Mining and Smelting Area, Slovakia: Spatial Distribution and Accumulation of Mercury in Four Different Ecosystems. *Ecotoxicol. Environ. Saf.* **2017**, 144, 236–244. DOI: 10.1016/j.ecoenv.2017.06.020.

[6] Demková, L.; Bobul'ská, L.; Árvay, J.; Jezný, T.; Ducsay, L. Biomonitoring of Heavy Metals Contamination by Mosses and Lichens around Slovinky Tailing Pond (Slovakia). *J. Environ. Sci. Health - Part A Toxic/Hazardous Subst. Environ. Eng.* **2017**, 52, 30–36. DOI: 10.1080/10934529.2016.1221220.

[7] Li, Z.; Ma, Z.; van der Kuijp, T. J.; Yuan, Z.; Huang, L. A Review of Soil Heavy Metal Pollution from Mines in China: Pollution and Health Risk Assessment. *Sci. Total Environ.* **2014**, 468–469, 843–853.

[8] Act No. 220/2004 on Protection and Agricultural Land Use. MPSR – Ministry for Land Management, 2004 (in Slovak).

[9] Kelepertzis, E. Accumulation of Heavy Metals in Agricultural Soils of Mediterranean: Insights from Argolida Basin, Peloponnese, Greece. *Geoderma* **2014**, 221–222, 82–90. DOI: 10.1016/j.geoderma.2014.01.007.

[10] Kowalska, J. B.; Mazurek, R.; Gąsiorek, M.; Zaleski, T. Pollution Indices as Useful Tools for the Comprehensive Evaluation of the Degree of Soil Contamination–A Review. *Environ. Geochem. Health* **2018**, 40, 2395–2420.

[11] Wyszkowska, J.; Kucharski, J.; Waldowska, E. The Influence of Diesel Contamination on Soil Enzyme Activity. *Rostl. Vyroba* **2002**, 48, 58–62.

[12] Demková, L.; Bobuľská, L.; Fazekašová, D. Toxicity of Heavy Metals to Soil Biological and Chemical Properties in Conditions of Environmentally Polluted Area Middle Spiš (Slovakia). *Carpathian J. Earth Environ. Sci.* **2015**, 10, 193–201.

[13] Collins, M. J.; Williams, P. L.; MacIntosh, D. L. Ambient Air Quality at the Site of a Former Manufactured Gas Plant. *Environ. Monit. Assess.* **2001**, 68, 137–152.

[14] Pandey, B.; Agrawal, M.; Singh, S. Assessment of Air Pollution around Coal Mining Area: Emphasizing on Spatial Distributions, seasonal Variations and Heavy Metals, using Cluster and Principal Component Analysis. *Atmos. Pollut. Res.* **2014**, 5, 79–86. DOI: 10.5094/APR.2014.010.

[15] Goodman, G. T.; Roberts, T. M. Plants and Soils as Indicators of Metals in the Air. *Nature* **1971**, 231, 287–292.

[16] Alsbou, E. M. E.; Al-Khashman, O. A. Heavy Metal Concentrations in Roadside Soil and Street Dust from Petra Region. *Jordan Environ. Monit. Assess.* **2018**, 190, 48.

[17] Bozkurt, Z. Determination of Airborne Trace Elements in an Urban Area Using Lichens as Biomonitor. *Environ. Monit. Assess.* **2017**, 189, 573.

[18] Barandovski, L.; Frontasyeva, M. V.; Stafilov, T.; Šajn, R.; Ostrovnaya, T. M. Multi-element Atmospheric Deposition in Macedonia Studied by the Moss Biomonitoring Technique. *Environ. Sci. Pollut. Res.* **2015**, 22, 16077–16097. DOI: 10.1007/s11356-015-4787-x.

[19] Bonitované pôdno-ekologické jednotky 1:5,000 [Rated soil-ecological units 1:5,000] http://www.podnemapy.sk/portal/verejnost/bpej/bpej.aspx, **2013**. (in Slovak).

[20] Brehuv, J.; Špaldon, T.; Šestinová, O.; Slančo, P.; Hančuľák, J.; Bobro, M. Contamination of the Water and Sediment Load from the Drainage Basin of the Slaná River by Influence of Former and Present Mining Activities. *Acta Fac. Ecol.* **2007**, 16, 91–100.

[21] Hančuľák, J.; Fedorová, E.; Šestinová, O.; Špaldon, T.; Matik, M. Influence of Iron Ore Works in Nizna Slana on the Atmospheric Deposition of Heavy Metals. *Acta Montan. Slovaca* **2011**, 16, 220–228.

[22] Michaeli, E.; Boltižiar, M. Selected Localities of Environmental Loads in Environmentally Loaded Areas in Slovakia. *Geografické štúdie* **2010**, 1, 18–74. DOI: 10.17846/GS.2010.14.1.18-48.

[23] WRB. World Reference Base for Soil Resources **2014**.

[24] European Centre for Medium-Range Weather Forecasts (ECMWF). Advancing Global NWP through International Collaboration. URL: https://www.ecmwf.int/

[25] Árvay, J.; Tomáš, J.; Hauptvogl, M.; Kopernická, M.; Kováčik, A.; Bajčan, D.; Massányi, P. Contamination of Wild-grown Edible Mushrooms by Heavy Metals in a Former Mercury-mining Area. *J. Environ. Sci. Heal. - Part B Pestic. Food Contam. Agric. Wastes* **2014**, 49, 815–827. DOI: 10.1080/03601234.2014.938550.

[26] Grejtovsky, A. Effects of Improvement Practices on Enzymatic Activities of Heavy-textured Alluvial Soil. *Rostl. Vyroba* **1991**, 1, 299–307.

[27] Khaziev, F. K. *Soil Enzyme Activity*, Nauka: Moscow, **1976**, 152–180 (in Russian).

[28] Green, V. S.; Stott, D. E.; Diack, M. Assay for Fluorescein Diacetate Hydrolytic Activity: Optimization for Soil Samples. *Soil Biol. Biochem.* **2006**, 38, 693–701. DOI: 10.1016/j.soilbio.2005.06.020.

[29] Eivazi, F.; Tabatabai, M. A. Glucosidases and Galactosidases in Soils. *Soil Biol. Biochem.* **1988**, 20, 601–606. DOI: 10.1016/0038-0717(88)90141-1.

[30] Hakanson, L. An Ecological Risk Index for Aquatic Pollution Control. A Sedimentological Approach. *Water Res.* **1980**, 14, 975–1001. DOI: 10.1016/0043-1354(80)90143-8.

[31] Čurlík, J.; Šefčík, P. *Geochemical Atlas of Slovakia - Part V. Soils*. Bratislava, Slovakia: Ministry of the Environment of the Slovak Republic GS of SR, **1999**.

[32] Kabata-Pendias, A. *Trace Elements in Soils and Plants*. Boca Raton: CRC Press, Taylor & Francis Group **2011**, pp. 528–530.

[33] Tomlinson, D. L.; Wilson, J. G.; Harris, C. R.; Jeffrey, D. W. Problem in the Assessment of Heavy-metal Levels in Estuaries and the Formation of a Pollution Index. *Helgolander. Meeresunters.* **1980**, 33, 566–578. DOI: 10.1007/BF02414780.

[34] Wang, X.; He, M.; Xie, J.; Xi, J.; Lu, X. Heavy Metal Pollution of the World Largest Antimony Mine-affected Agricultural Soils in Hunan Province (China). *J. Soils Sediments* **2010**, 10, 827–837.

[35] R Core Team. R: A Language and Environment for Statistical Computing. R Found Stat Comput. **2016**.

[36] Allen, G.; Nemitz, E.; Shi, J. P.; Harrison, J. P.; Greenwood, J. C. Size Distributions of Trace Metals in Atmospheric Aerosols in the United Kingdom. *Atmos. Environ.* **2001**, 35, 4581–4591. DOI: 10.1016/S1352-2310(01)00190-X.

[37] Niu, J.; Rasmussen, P. E.; Hassan, N. M.; Vincent, R. Concentration Distribution and Bioaccessibility of Trace Elements in Nano and Fine Urban Airborne Particulate Matter: Influence of Particle Size. *Water. Air. Soil Pollut.* **2010**, 213, 211–225. DOI: 10.1007/s11270-010-0379-z.

[38] Hohl, H.; Varma, A. S. The living matrix. In *Soil Heavy Metals*; Sherameti, I.; Varma, A., Eds.; New York: Springer, **2010**, pp. 1–19.

[39] Alloway, J. B. *Heavy Metals in Soils: Trace Metals and Metalloids in Soils and Their Bioaviability*. London: Springer, **2010**, pp. 78–92.

[40] Javoreková, S.; Králiková, A.; Labuda, R.; Labudová, S.; Maková, J. *The Biology of the Soil in Agrosystems*. Nitra: Agricultural University in Nitra, **2011**, pp. 86–92. (in Slovak).

[41] Kandeler, F.; Kampichler, C.; Horak, O. Influence of Heavy Metals on the Functional Diversity of Soil Microbial Communities. *Biol. Fertil. Soils* **1996**, 23, 299–306. DOI: 10.1007/BF00335958.

[42] Angelovicová, L.; Lodenius, M.; Tulisalo, E.; Fazekasová, D. Effect of Heavy Metals on Soil Enzyme Activity at Different Field Conditions in Middle Spis Mining Area (Slovakia). *Bull. Environ. Contam. Toxicol.* **2014**, 93, 670–675. DOI: 10.1007/s00128-014-1397-0.

[43] Chen, M.; Xu, P.; Zeng, G.; Yang, C.; Huang, D.; Zhang, J. Bioremediation of Soils Contaminated with Polycyclic Aromatic Hydrocarbons, Petroleum, Pesticides, Chlorophenols and Heavy Metals by Composting: Applications, Microbes and Future Research Needs. *Biotechnol. Adv.* **2015**, 33, 745–755. DOI: 10.1016/j.biotechadv.2015.05.003.

[44] Hu, X. F.; Jiang, Y.; Shu, Y.; Hu, X.; Liu, L.; Luo, F. Effects of Mining Wastewater Discharges on Heavy Metal Pollution and Soil Enzyme Activity of the Paddy Fields. *J. Geochem. Explor.* **2014**, 147, 139–150.

[45] Yang, X.; Liu, J.; McGrouther, K.; Huang, H.; Lu, K.; Guo, X.; He, L.; Lin, X.; Liu, X.; Che, L.; et al. Effect of Biochar on the Extractability of Heavy Metals (Cd, Cu, Pb, and Zn) and Enzyme Activity in Soil. *Environ. Sci. Pollut. Res.* **2016**, 26, 974–984. DOI: 10.1007/s11356-015-4233-0.

[46] Li, W. X.; Zhang, X. X.; Wu, B.; Sun, S.; Chen, Y.; Pan, W.; Zhao, D.; Cheng, S. A. Comparative Analysis of Environmental Quality Assessment Methods for Heavy Metal-Contaminated Soils. *Pedosphere* **2008**, 18, 344–352. DOI: 10.1016/S1002-0160(08)60024-7.

[47] Lu, X.; Wang, L.; Li, L. Y.; Lei, K.; Huang, L.; Kang, D. Multivariate Statistical Analysis of Heavy Metals in Street Dust of Baoji, NW China. *J. Hazard Mater* **2010**, 173, 744–746. DOI: 10.1016/j.jhazmat.2009.09.001.

[48] Furini, A. *Plants and Heavy Metals*. New York: Springer; **2012**, pp. 66–76.

[49] Demková, L.; Árvay, J.; Bobuľská, L.; Tomáš, J.; Stanovič, R.; Lošák, T.; Harangozo, O.,Ľ.; Vollmannová, A.; Bystrická, J.; Musilová, J.; Jobbágy, J. Accumulation and Environmental Risk Assessment of Heavy Metals in Soil and Plants of Four Different Ecosystems in a Former Polymetallic Ores Mining and Smelting Area (Slovakia). *J. Environ. Sci. Heal. A* **2017**, 52, 479–490. DOI: 10.1080/10934529.2016.1274169.

[50] Debosz, K.; Petersen, S. O.; Kure, L. K.; Ambus, P. Evaluating Effects of Sewage Sludge and Household Compost on Soil Physical, chemical and Microbiological Properties. *Appl. Soil. Ecol*. **2002**, 19, 237–248. DOI: 10.1016/S0929-1393(01)00191-3.

[51] Tangahu, B. V.; Sheikh Abdullah, S. R.; Basri, H.; Idris, M.; Anuar, N.; Mukhlisin, M. A Review on Heavy Metals (As, Pb, and Hg) Uptake by Plants through Phytoremediation. *Int. J. Chem. Eng*. **2011**, 2011, 1–31.

[52] Rao, M. A.; Scelza, R.; Acevedo, F.; Diez, M. C.; Gianfreda, L. Enzymes as Useful Tools for Environmental Purposes. *Chemosphere* **2014**, 107, 145–162.

[53] Xian, Y.; Wang, M.; Chen, W. Quantitative Assessment on Soil Enzyme Activities of Heavy Metal Contaminated Soils with Various Soil Properties. *Chemosphere* **2015**, 139, 604–608. DOI: 10.1016/j.chemosphere.2014.12.060.

[54] Jin, Z.; Li, Z.; Li, Q.; Hu, Q.; Yang, R.; Tang, H.; Li, M.; Huang, B.; Zhang, J.; G. Canonical, L. Correspondence Analysis of Soil Heavy Metal Pollution, Microflora and Enzyme Activities in the Pb–Zn Mine Tailing Dam Collapse Area of Sidi Village. *Environ. Earth Sci*. **2015**, 73, 267–274.

[55] Taylor, J. P.; Wilson, B.; Mills, M. S.; Burns, R. G. Comparison of Microbial Numbers and Enzymatic Activities in Surface Soils and Subsoils Using Various Techniques. *Soil Biol. Biochem*. **2002**, 34, 387–401. DOI: 10.1016/S0038-0717(01)00199-7.

[56] Yildirim, G.; Tokalioğlu, Ş. Heavy Metal Speciation in Various Grain Sizes of Industrially Contaminated Street Dust Using Multivariate Statistical Analysis. *Ecotoxicol. Environ. Saf*. **2016**, 124, 369–376.

[57] Aničić Urošević, M.; Vuković, G.; Vasić, P.; Jakšić, T.; Nikolić, D.; Škrivanj, S.; Popović, A. Environmental Implication Indices from Elemental Characterisations of Collocated Topsoil and Moss Samples. *Ecol. Indic*. **2018**, 20, 529–539. DOI: 10.1016/j.ecolind.2018.03.048.

[58] Tembo, B. D.; Sichilongo, K.; Cernak, J. Distribution of Copper, Lead, Cadmium and Zinc Concentrations in Soils around Kabwe Town in Zambia. *Chemosphere* **2006**, 63, 497–501.

[59] Han, X.; Lu, X.; Zhang, Q.; Wuyuntana Hai, Q.; Pan, H. Grain-size Distribution and Contamination Characteristics of Heavy Metal in Street Dust of Baotou, China. *Environ. Earth Sci*. **2016**, 75, 468.

[60] Varela, Z.; Fernández, J. A.; Real, C.; Carballeira, A.; Aboal, J. R. Influence of the Physicochemical Characteristics of Pollutants on Their Uptake in Moss. *Atmos. Environ*. **2015**, 102, 130–135. DOI: 10.1016/j.atmosenv.2014.11.061.

[61] Aboal, J. R.; Fernández, J. A.; Boquete, T.; Carballeira, A. Is It Possible to Estimate Atmospheric Deposition of Heavy Metals by Analysis of Terrestrial Mosses?. *Sci. Total. Environ*. **2010**, 408, 6291–6297. DOI: 10.1016/j.scitotenv.2010.09.013.

[62] Demková, L.; Baranová, B.; Oboňa, J.; Árvay, J.; Lošák, T. Assessment of Air Pollution by Toxic Elements on Petrol Stations Using Moss and Lichen Bag Technique. *Plant. Soil Environ*. **2017a**, 63, 355–361.

[63] Spagnuolo, V.; Zampella, M.; Giordano, S.; Adamo, P. Cytological Stress and Element Uptake in Moss and Lichen Exposed in Bags in Urban Area. *Ecotoxicol. Environ. Saf*. **2011**, 74, 1434–1443.

[64] Vingiani, S.; De Nicola, F.; Purvis, W. O.; Concha-Graña, Muniategui-Lorenzo, S.; Lópes-Mahía, P.; Giordano, S.; Adamo, P. Active Biomonitoring of Heavy Metals and PAHs with Mosses and Lichens: A Case Study in the Cities of Naples and London. *Water Air Soil Pollut*. **2015**, 226, 240.

[65] Giordano, S.; Adamo, P.; Spagnuolo, V.; Tretiach, M.; Bargagli, R. Accumulation of Airborne Trace Elements in Mosses, lichens and Synthetic Materials Exposed at Urban Monitoring Stations: Towards a Harmonization of the Moss-bag Technique. *Chemosphere* **2013**, 90, 292–299. DOI: 10.1016/j.chemosphere.2012.07.006.