THE IMPACT OF MINING AND MINERAL PROCESSING INDUSTRY ON FLORA BIODIVERSITY AND SOIL ECOSYSTEMS IN METALLIC BURDENED REGION OF CENTRAL SPIŠ (SLOVAKIA)

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Abstract

The paper is focused on assessment of the level of vegetation diversity and functional biodiversity of the soil microbiological communities in metallic burdened region of Central Spiš (Slovakia), where iron and coper ore has been mined and processed for a long time. The research was carried out in the growing season 2014. In the soils of this area the exceedance of limit values of mercury and copper were found. On the basis of phytocenological records the botanical composition of surveyed sites located in immission field of heaps and tailings was determined. The coverage was determined by seven-scale Braun-Blaunqueta (1964) and species diversity was assessed according to Shannon's H'. To assess the functional diversity of soil microbial communities in fresh soil samples, the metabolic profiles of microbial communities was determined, using Biolog® Eco Plates and diversity was assessed by Shannon's H'. The results showed that the diversity of plant species in studied area was very low *0.9 and low to moderate (2.6) and functional diversity of soil microbial communities* was *low to moderate (3.2) and medium (3.4).*

Key words: *soil, heavy metals, functional diversity of soil microbial communities*

1. INTRODUCTION

Contamination of the environment significantly changes vegetation and becomes stressor with strong selectable effects. The environmentally burdened areas are characteristic by reduced diversity of plant communities, their covererage, gene pool of the original vegetation is depleted. Sensitive species suddenly disappear, or are replaced by the more tolerant plants, often weeds that do not belong to the original ecosystem. In consequence, significant qualitative and quantitative change of vegetation appears (Banásová et al., 1993). Contaminated sites pose a significant vegetation stress factor, resulting in death of the plant. Only a small percentage of them are able to adapt to new conditions. There are a group of plants that produce ecotypes specifically adapted to high concentrations of certain elements. Among the higher plants which are most tolerant to heavy metals belongs *Agrostis capillaris*. Well known examples are *Agrostis stolonifera, Festuca rubra,* which tolerate copper. Tolerant to zinc are populations of *Anthoxanthum odoratum* (Lackovičová et al., 1993).

Soil biodiversity is probably the most important for maintaining ecosystem functions in a disturbed environment, and can be measurable directly (e.g. species diversity), or indirectly, using standardized procedures (using various indexes). Functionality of soil processes can be measured by determining the activity of soil enzymes, nutrient mineralization, nitrification potential, soil respiration, etc. (Bradford et al., 2002).

Number of taxons in soil is compared with plant and animal species several times higher, and one gram of soil contains up to ten trillion microorganisms belonging to thousands of different species. A

huge amount of soil microorganisms also features a large diversity at different levels, whether the genetic, taxonomic or functional level (Bending et al., 2000; Sugiyama et al., 2008).

The high spatial variability of physical, chemical and biological properties causes complications in determining the diversity of soil microorganisms (Micheali et al. 2015; Kondrlová et al. 2013; Igaz et al. 2009; Bárek et al. 2008). A significant shift of knowledge about biodiversity of soil microorganisms occurred by introduction of newer methods, of which the most commonly used is method using Phospholipid fatty acid (PLFA) analysis and metabolic potential of the system analysis - BIOLOG® whose identification is carried out at the level of functional groups (Hofman et al., 2004; Gömöryová, 2008).

The aim of the paper is to evaluate the level of vegetation diversity and functional biodiversity of the soil microbiological communities in metallic burdened region of Central Spiš (Slovakia), affected by long term mining and processing of iron and copper ore.

2. MATERIAL AND METHODS

The area is currently classifiable between loaded and hygienically unsafe (Hronec et al., 2008). Phytogeographically this territory belongs to the Western Carpathian flora (*Carpaticumoccidentale*), district of Precarpaticum (*Praecarpaticum*), and the district Slovak Rudohorie. The average altitude of the area is 360-450 m asl.

Nine of contaminated sites central Spiš were monitored (Krompachy - KR, Slovinky - SL Rudňany – RD and Porac – PR) (Fig. 1). Diversity of grassland communities was monitored in the form of plots on the field of 16 m^2 during the growing season 2014. Vegetation was assessed by Braun - Blanquet scale. Terminology is given in accordance with Marhold and Hindák (1998). The determination of species diversity of sites was according to Shannon index $H' = -\sum_{i=1}^{s} \frac{xi}{N} log2 \frac{xi}{N}$, which is sensitive to the different characteristics of plant communities, particularly the number and significance of the coefficient of all kinds. The results were evaluated on the basis of scales: 1 extremely low (<0.5), 2 very low (0.5-1), 3 middle low (1 to 1.7), 4 low (1.7 to 2.5), 5 low to moderate (2.5-3.3), 6 medium (3.3 to 4), 7 half-height (4-5), 8 high (5-7), 9 very high (7-10) and 10 extremely high (> 10).

Soil samples for chemical soil properties and heavy metals content determination were sampled on permanent research sites (Fig. 1), from A horizons the depth of 0.05 m to 0.15 m. We studied and evaluated soil reaction in 1N solution $CaCl_2$ (Fiala et al. 1999). Content of heavy metals – Cu and Hg were determined by atomic absorption spectrometry, after extraction from soil by 1 M NH_4NO_3 and 2M HNO_3.

In fresh soil samples we evaluated metabolic profiles of microbial communities using Biolog® Eco Plates (Garland 1996). Microtiter plates with 31 different organic substrates were incubated with 150 µl of the extract from the sample in 0.9% NaCl, at a 1: 10000 at 27 °C for 7 days. During the incubation of the samples we used a device ELx808 ™Absorbance Microplate Reader to daily determining the value of the absorbance at 590 nm corresponding to the activity of microorganisms on various substrates. Diversity of functional groups of microorganisms was evaluated using the Shannon index.

The obtained data were processed statistically by means of the STATGRAPHIC software and Past.

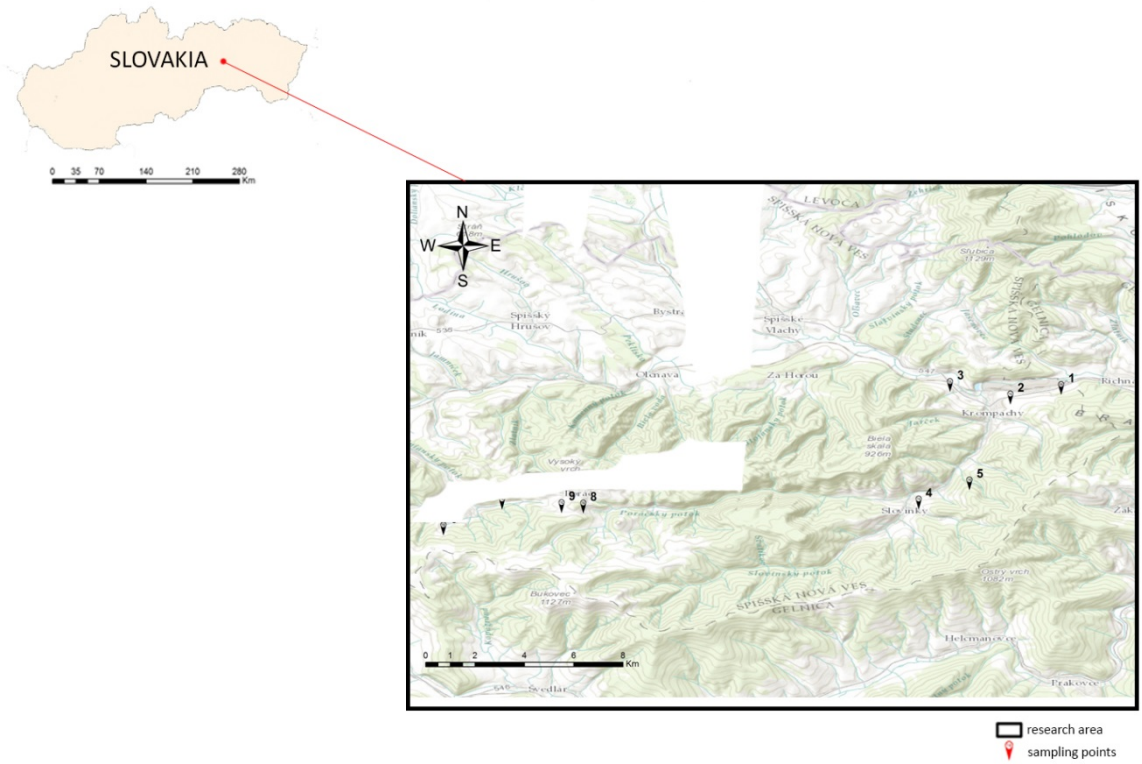


Fig. 1. Location of research sites in the central Spis (Slovakia) (Krompachy, Slovinky, Rudňany, Poráč).

3. RESULTS AND DISCUSSION

Contaminated soil is a very specific habitat for the growth and development of vegetation. Toxicity substrate allows growing only those species that have specific mechanisms necessary to survive in extreme conditions (Ouyang 2002). The content of Cu and Hg in soil samples greatly exceeded the limits set by law (No. 220/2004 Coll. of Laws) (Table 2). Soil contamination is reflected in the species diversity as study sites were mainly covered with species of high ecological valence and resistance to toxic elements. From the group of vascular plants dominated *Tussilago farfara*, *Taraxacum officinale*, *Trifolium pratense*, *Poa pratensis*, *Achillea millefolium*, *Festuca pratensis*, *Silene inflata* and *Geranium pratense*. We recorded the species that often inhabit ruderal habitans as *Acetosa pratensis* and *Solidago gigantea*. Dominant meadow species was *Taraxacum officinale*, which is concluded to the group of hyper accumulators, which are able to receive and store higher quantities of heavy metals in their bodies. *Taraxacum officinale* also represent group of species called bio – indicators of heavy metals (Šačirgič 2011). The results of the phytocenological research are listed in Table 1.

Species diversity of flora was investigated at localities evaluated by Shanonn index H ', which is sensitive to the different characteristics of plant communities, particularly the number of coefficient of significance of all kinds. Among the studied area we did not find significant differences in species diversity of vascular plants. Based on the results of Shanonn index, we can conclude that diversity in investigated sites is very low (0.9) to low and moderate (2.6). Equitability values were in line with the index of diversity (Fig. 2).

Table 1. Phytocenological date of plant communities in central Spiš (Slovakia).

Lokality	Covering (%)	Number of species	Species
1 (KR1)	100%	22	**E_0, E_2, E_3** no species were recorded **E_1:** *Trifolium pratense* 3, *Poa pratensis* 2, *Plantago lanceolata* 2, *Achillea millefolium* 2, *Equisetum pratense* 2, *Acetosa pratenis* 2, *Trifolium hybridum* 2, *Taraxacum officinale* 2, *Plantago major* 1, *Briza media* 1, *Veronica chamaedrys* +, *Capsella bursa-pastoris* +, *Cirsium arvense* +, *Agrimonia eupatoria* +, *Alchemilla vulgaris* +, *Dactylis glomerata* +, *Euphorbia cyparissias* +, *Leucanthemum vulgare* +, *Geranium pratense* r, *Lychnis flos-cuculi* r, *Primula veris* r, *Salvia pratensis* r,
2 (KR3)	100%	27	**E_0, E_2, E_3** no species were recorded **E_1:** *Taraxacum officinale* 3, *Trifolium pratense* 3, *Poa pratensis* 2, *Acetosa pratensis* 1, *Plantago major* 1 *Dactylis glomerata* 1, *Festuca pratensis* 1, *Alchemilla vulgaris* 1, *Geranium pratense* 1, *Galuium aparine* +, *Urtica dioica* +, *Trifolium hybridum* +, *Stellaria media* +, *Achillea millefolium* +, *Cirsium brachycephalum*+, *Campanula patula* +, *Veronica chamaedrys* +, *Plantago lanceolata* +, *Equisetum pratense* r, *Salvia pratensis* r, *Lamium purpureum* r, *Geranium pratense* r, *Capsella bursa-pastoris* r, *Anthyllis vulneraria* r, *Briza media* r, *Origanum vulgare* r, *Rhinanthus minor* r,
3 (KR4)	100%	26	**E_0, E_2, E_3** no species were recorded **E_1:** *Tussilago farfara 3*, *Elytrigia repens* 3, *Trifolium pratense* 3, *Poa pratensis* 2, *Equisetum arvense* 2, *Festuca pratensis* 2, *Taraxacum officinale* 2, *Achillea millefolium* 2, *Dactylis glomerata* 2, *Alchemilla vulgaris* 1, *Plantago major* 1, *Convolvulus arvensis* +, *Capsella bursa-pastoris* +, *Lychnis flos-cuculi* +, *Bellis perennis* +, *Cirsium brachycephalum* +, *Galuium aparine* +, *Urtica dioica* +, *Trifolium hybridum* +, *Leucanthemum vulgare* +, *Ranunculus arvensis* r, *Salvia pratensis* r, *Plantago lanceolata* r, *Primula veris* r, *Veronica chamaedrys* r, *Geranium pratense* r
4 (SL1)	100%	25	**E_0, E_2, E_3** no species were recorded **E1:** *Taraxacum officinale* 4, *Poa pratensis* 3, *Achillea millefolium* 2, *Dactylis glomerata* 2, *Elytrigia repens* 2, *Equisetum arvense* 2, *Festuca pratensis* 2, *Trifolium pratense* 2, *Alchemilla vulgaris* 1, *Capsella bursa-pastoris* 1, *Leucanthemum vulgare* 1, *Lychnis flos-cuculi* 1, *Trifolium hybridum*1, *Plantago lanceolata* 1, *Convolvulus arvensis* +, *Galuium aparine* +, *Geranium pratense* +, *Urtica dioica* +,

			Veronica chamaedrys +, *Bellis perennis* r, *Cirsium brachycephalum* r, *Plantago major* r, *Ranunculus arvensis* r, *Salvia pratensis* r, *Urtica dioica* r
5 (SL4)	100%	12	**E_0, E_2** no species were recorded **E_3 E_1**: *Pinus sylvestris* 3, *Larix decidua* 3, *Corylus avellana* 1, *Dryopteris filix-mas* +, *Campanula patula* +, *Tussilago farfara* +, *Ajuga reptans* r, *Equisetum sylvaticum* r, *Fragaria vesca* r, *Humulus lupulus* r, *Lathyrus sylvestris* r, *Myosotis sylvatica* r, *Platanthera bifolia* r,
6 (RD1)	100%		**E_0, E_2, E_3** no species were recorded **E_1**: *Festuca pratensis* 3, *Trifolium pratense 2, Trisetum flavescens* 2, *Avenella flexuosa* 1, *Briza media* 1, *Campanula patula* 1, *Dactylis glomerata* 1, *Festuca rubra* 1, *Geranium pratense* 1, *Leucanthemum vulgare* 1, *Poa trivialis* 1, *Primula veris* 1, *Ranunculus acris* 1, *Taraxacum officinale* 1, *Leontodon hispidus* +, *Lotus corniculatus* +, *Achillea millefolium* +, *Melandrium album* +, *Silene vulgaris* +.
7 (RD4)	100%	15	**E_0, E_2, E_3** no species were recorded **E_1**: *Silene inflata* 4, *Briza media* 1, *Poa trivilialis* 1, *Taraxacum officinale* 1, *Geranium pratense* 1, *Avenella flexuosa* 1, *Dactylis glomerata* +, *Capsella bursa-pastoris* +, *Achillea millefolium* r, *Plantago lanceolata* r, *Melandrium album* r, *Ranunculus arvensis* r, *Solidago gigantea* r, *Galium aparine* r, *Urtica dioica* r,
8 (PR3)	100%	16	**E_0, E_2, E_3** no species were recorded **E_1**: *Alchemilla xanthochlora* 2, *Poa pratensis* 2, *Hypericum perforatum* 2, *Agrostis capillaris* 1, *Thymus pulegioides* 1, *Trifolium pratense* 1, *Tanacetum vulgare* +, *Acetosa pratensis* +, *Tithymalus cyparissias*+, *Ranunculus acris* +, *Plantago lanceolata*+, *Plantago major* +, *Trifolium rubens* +, *Euphrasia rostkoviana* +,*Equisetum arvense r*, *Cirsium arvense r*,
9 (PR4)	100%	11	**E_0, E_2, E_3** no species were recorded **E_1**: *Geranium pratense* 4, *Taraxacum officinale* 3, *Calamagrostis epigejos* 1, *Poa pratensis* 1, *Alchemilla xanthochlora* +, *Trifolium pratense*+, *Agrostis capillaris* +, *Equisetum arvense* +, *Trifolium rubens* +, *Arctium tomentosum* +, *Cirsium arvense* r, *Symphytum officinale* r,

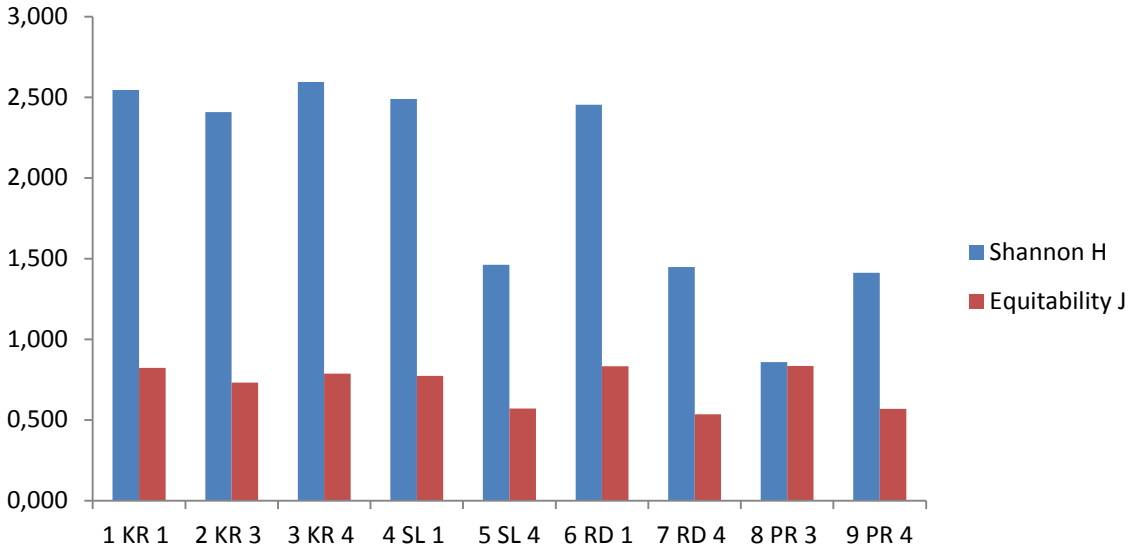


Fig. 2. Diversity of vegetation according to Shanonn index in central Spiš (Slovakia).

Soil microorganisms play a vital and irreplaceable role in many soil processes. Present research has focused on the detection of the number of biomass and activity, while the investigating the diversity of soil organisms and in particular of soil microorganisms was initiated in the last years. Soil organisms are characterized by considerable diversity. In general, the smaller organisms, the greater the diversity, which is related to the extreme heterogeneity of soil environment at all spatial levels and the limited ability of the migration of most of these organisms. Soil microorganisms depend on the metabolic products of primary producers, but it is questionable how the diversity of vascular plants affects diversity of soil microorganisms (Rodríguez-Loinaza et al (2008).

Functional diversity of microbial communities we evaluated using the Biolog Eco Plates. This method is well known for its sensitivity and quickness and was used among other for the ecotoxicological assessment of contaminated soils (Tischer et al. 2008; Hofman et al. 2004). To calculate Shanonn diversity index, we used the absorbance at a given AWCD (Averages Well Color Development) in the samples investigated after 168 hours. We found that the absorbance in the sample contaminated with Cu and Hg reached low values (0.2 to 0.8) (Fig. 3). This correspond to low to moderate (3.2) and medium (3.4) diversity of functional groups of microorganisms (Fig. 4). Our findings were consistent with the results of Xie et al. (2011), who found that with increasing metal content, clear inhibitory effects on the functional activity of soil microorganisms appear. On this basis, we can conclude that the soil ecosystem in deteriorated environment is unstable, and its function is impaired.

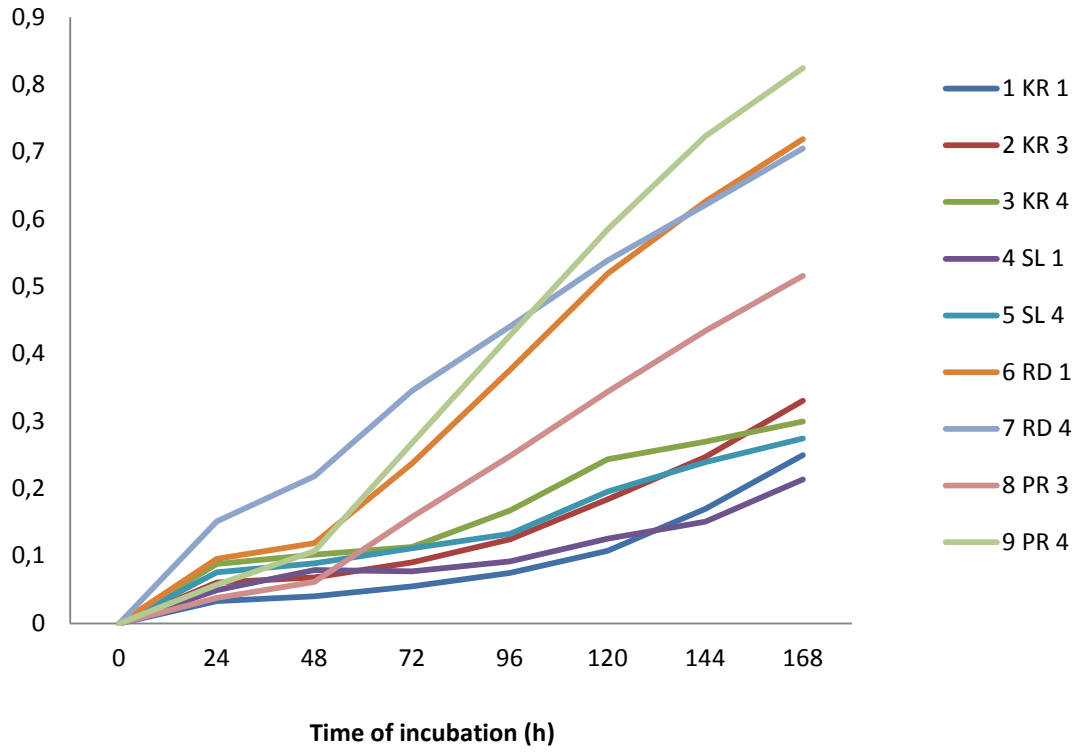


Fig. 3. Averages Well Colour Development (AWCD) of Biolog® Eco Plates (31 substrates) in central Spiš (Slovakia)

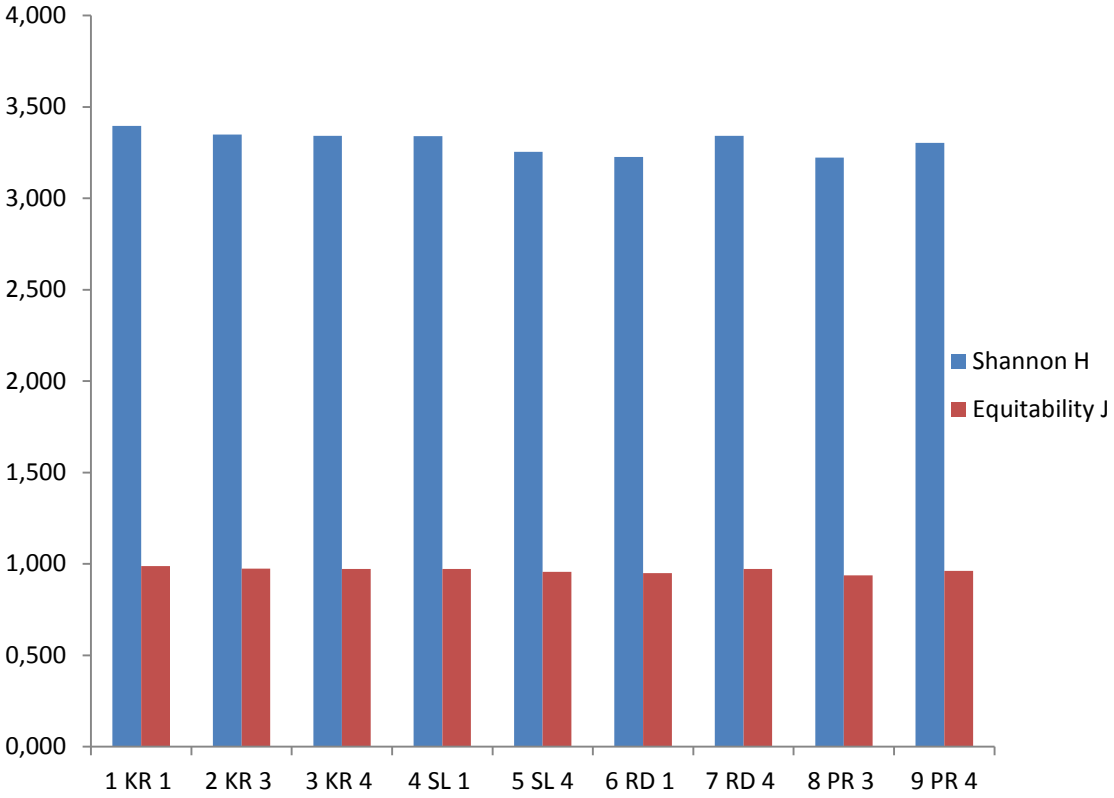


Fig. 4. Shanonn index diversity *H´* for ECO plates.

Table 2. Selected soil parameter and index diversity in central Spiš (Slovakia).

Parameter	Min.	Max.	Mean	Standard error
pH/$CaCl_2$	4.5	6.2	5.3	0.6245
Cu [mg kg^{-1}]	35	1271	347	411.2718
Hg [mg kg^{-1}]	0.35	32.7	8.33	10.6932
Shanonn index *H´* diversity of vegetation	0.9	2.6	2.0	0.6606
Equitability *J* of vegetation	0.54	0.84	0.727	0.1245
AWCD	0.213	0.824	0.459	0.2358
Shanonn index *H´* diversity for ECO plates	3.2	3.4	3.3	0.0608
Equitability *J* for ECO plates	0.94	0.99	0.97	0.0153

Limit value Cu – 60 [mg kg^{-1}] and Hg 0.5 [mg kg^{-1}] Act No. 220/2004 Coll. of Laws

4. CONCLUSIONS

Based on the research conducted in the unhealthy and contaminated environment of Central Spiš (Slovakia), affected by long-term mining and processing of copper and mercury we found high doses of these elements in soil ecosystem. Soil contamination affects the species diversity; as study sites were mainly covered with species of high ecological valence and resistance to toxic elements. Species diversity of flora and functional diversity of soil microorganisms, based on the results Shanonn index was due to strong contamination very low and medium. Equitability of communities in surveyed sites was consistent with the values of species diversity.

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REFERENCES

Act No. 220/2004 Coll. of Laws

Angelovičová, L. and D. Fazekašová, (2014) Contamination of the soil and water environment by heavy metals in the former mining area of Rudňany (Slovakia). In: Soil and Water Research, Vol. 9, no.1, p. 18-24.

Banásová, V., I. Pišút and Z. Holub, (1993) Contamination of soil and vegetation by mercury and other elements of air pollutants steelworks in Rudňany. In Air Quality, Vol. 23, No. 6, 1993 pp. 267-271.

Bárek, V., P. Halaj, and D. Igaz, (2008) The Influence of Climate Change on Water Demands for Irrigation of Special Plants and Vegetables in Slovakia. In Bioclimatology and Natural Hazards. Dordrecht: Springer, 2008, pp. 271-282.

Bending, G.D., C. Putland and F. Rayns, (2000) Changes in microbial community metabolism and labile organic mater fractions as early indicators of the impact of management on soil biological quality. In Biology and Fertility of Soils. Vol. 31, 2000, p. 78-84.

Bradford, M.A. and J.E. Nevington, (2002) With the worms: soil biodiversity and ecosystem functioning. In Biologist, Vol 49, 2002, 127-130.

Braun-Blanquet, J. (1964) Pflanzensoziologie. Grundzüge der Vegetationskunde. 3. edition Aufl. Springer Verlag. Wien., 1964, 865 pp.

Fiala, K., G. Barančikova, V. Brečkova, V. Burik, B. Houškova, A. Chomaničova, J. Kobza, T. Litavec, L. Makovnikova, B. Pechova, and D. Varadiova, (1999) Partial monitoring system – soil, binding methods (in Slovak). Bratislava, VUPOP.

Garland, J.L. (1996) Analysis and interpretation of community-level physiological profiles in microbial ecology. FEMS Microbial Ecology 24: 289-300.

Gömöryová, E. (2008) Diversity of soil microorganisms. In: Piate pôdoznalecké dni:Pôda – národné bohatstvo. Bratislava: VÚPOP, 2008. p. 155-160.

Hofman, J., J. Švihálek, I. Holoubek, (2004) Evaluation of functional diversity of soil microbial communities – a case study. In Plant, Soil and Environment. 2004, Vol. 50, No. 4, pp. 141-148.

Hronec, O., J. Vilček, T. Tóth, P. Andrejovský, P. Adamišin, A. Andrejovská, M. Daňová, E. Huttmanová, M. Vilimová, P. Škultéty, and M. Juhászová, (2008) Heavy metals in soils and plants in Rudniansko-Gelnická loaded area. In: Acta regionalia et environmentalica. Vol. 1, p. 24-28.

Igaz, D., J. Čimo, and L. Maderková, (2009) Wilting point as the indicator for soil drought evaluation . In Journal of International Scientific Publications: Ecology & Safety. Vol. 3, no. 1 (2009), s. 210-216.

Kondrlová, E., D. Igaz, J. Horák and K. Halászová, (2013) Soil texture analysis by optical method laboratory experiment on sample preparation prior to analysis. In Water resources. Forest, marine and ocean ecosystems. International multidisciplinary scientific geoconference SGEM. Sofia: STEP92 Technology, 2013, pp. 677-683.

Lackovičová, A., V. Banásová, and I. Pišút, (1993) Plants bioindicators of environmental pollution. Bratislava: ÚMC MŠV SR, 1993. 65 p.

Marhold, K. and F. Hindák, (1998) The list of lowest and highest plants. Bratislava: Veda, Slovakia, pp 687, 1998.

Michaeli, E., M. Ivanová, and Š. Koco, (2015) The evaluation of anthropogenic impact on the ecological stability of landscape. J. Environ. Biol., 36, 1-7 (2015).

Ouyang, Y. (2002) Phytoremediaton: modeling plant uptake and contaminant transport in the soil-plant-atmosphere continuum. In Journal of Hydrology, 2002, Volume 266, Issues 1–2, pp. 66-82.

Rodrıíguez-Loinaza, G., M. Onaindiaa, I. Amezagaa, I. Mijangosb, and C. Garbisub, (2008) Relationship between vegetation diversity and soil functional diversity in native mixed-oak forests. Soil Biology & Biochemistry, 40 (2008) 49–60.

Šačirgič, S. B. (2011) Taraxacum officinale as heavy metal absorber in phytoremediation of agricultural land. Herbologia, Bosnia and Herzegovina, pp 211-219, 2011.

Sugiyama, S., H.M. Zabed, and A. Okubo, (2008) Relationships between soil microbial diversity and plant community structure in seminatural grassland. In Grassland Science. 2008, 54, p. 117-124.

Tischer, S., H. Tannaberg, and G. Guggenberger, (2008) Microbial parameters of soils contaminated with heavy metals: Assesment for ecotoxicological monitoring. Pol. J. Ecol. 56 (3) 471–479.

Xie, X., M. Liao, A. Ma, and H. Zhang, (2011) Effect of contamination of single and combined cadmium and mercury on the soil microbial community structural diversity and functional diversuty. Chin.J.Geochem. (211)30:366-374.