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TOXICITY OF HEAVY METALS TO SOIL BIOLOGICAL AND CHEMICAL PROPERTIES IN CONDITIONS OF ENVIRONMENTALLY POLLUTED AREA MIDDLE SPIŠ (SLOVAKIA)

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Abstract: Long-term mining and subsequent processing activities predominantly focused on copper and mercury became the main source of soil pollution in the Middle Spiš area. The natural environment of former mining areas is significantly disrupted by toxic elements, which enter the soil and water ecosystem. Total content of toxic elements (Cu, As, Pb, Cd, Zn, Hg), biological (activity of urease (URE), acid phosphatase (ACP), alkaline phosphatase (ALP) and catalase (CAT) and chemical (soil reaction (pH), organic carbon (C_{ox}), available content of nutrients (P, K, Mg) soil properties were determined. The research was carried out at the heaps and grasslands in the cadastres of three villages (Krompachy, Rudňany, Slovinky). Extremely high and about the limit value of Cu, As and Hg were determined at all sampling sites. Activity of soil enzymes observed in our study proved, their ability to reflect soil degradation caused by heavy metal pollution. Activity of URE, ACP, ALP decreased with increasing of heavy metal content in soils. Significantly acidic environment on the heaps of waste material was found, compared to the grasslands. Heaps showed lower values of organic carbon and nutrients, but no direct influence of soil pollution to the organic carbon, soil pH and nutrients was found.

Keywords: heavy metals; enzyme activity; mining area; soil properties; Slovakia

1. INTRODUCTION

Pollution of soil environment by toxic elements has become a serious problem. Until recently soil was regarded as environmental filter ensuring the quality of both water and atmosphere (Trasar-Cepeda et al., 2000). Now is recognized that soil is not only an effective de-contaminant of pollutants because its chemical, biological and physical quality must be maintained (Parr et al., 1992). Polluted soil is not capable of producing healthy and abundant crops, it contaminates water passing though and emitting gases in quantities detrimental to the environment.

Environmental degradation with varying degrees of devastation is negatively reflected in the reduced quality of the ecosystem as well as the deterioration of the health status of the population. The full scale of the problem depends on size of the polluted area, depth at which pollutants penetrate soil and chemical composition of polluting substances (Bálintová & Ľuptáková, 2012). Soil contamination comes from industry, agricultural practices, combustion of fossil fuels (Gülser & Erdoğan, 2008) and mining activities (Zhang et al., 2011), which together with mining related industries became the largest sources of environmental pollution by heavy metals. The most of metals are naturally present in soil, but the human activities disrupted their natural balance in environment (Lyubenova & Schrőder, 2010). Despite the importance of mineral resources for human progress, extraction of the minerals has caused a lot of environmental problems. The most intensive development of mining activity in Slovakia was recorded at 12 - 13 century, when the extraction of surface ores in the oxidation zone was profitable and productive (Špaldon et al., 2006). Great range of surface mining lasted a long time as evidenced by the large heap of waste material. After the exhaustion of surface material, deepwater extraction began, which brought the improvement of mining techniques, but also significantly greater disturbance of the landscapes as evidenced by tens of miles of mining tunnels (Singh et al., 2006). In the process of mining activity, heavy metals enter into soils accompanied with dust, which have caused environmental pollution and endangered the health of human beings (Liu et al., 2005). Understanding the behavior of heavy metals in the soil system is one of the most important tasks of environmental sciences. To the group of risk elements belong biologically irreplaceable elements (Cu, Zn, Mn and others), as well as numerous non-essential chemical elements (Cd, Pb, Hg and others) (Bálintová et al., 2013). A large amount of copper, lead, zinc and related elements, such as cadmium, have been released into the environment due to mineral processing activities (Zhang et al., 2011). These elements have accumulated in toxic levels in soil, water and biota. Removing of heavy metals from the soil environment is not easy because of their irreversible immobilization within different soil component such as humidified organic matter.

In recent decades there has been increasing interest on long-term effect of heavy metals at high concentration in the environment due to their persistent in the soil for tens of thousands years (Ho & Tai., 1988). Physical, chemical and biological soil properties are very important for its behavior and fertility. Characteristic of this behavior should focus on the properties that are the most sensitive to environmental stress. Microbiological characteristics such as soil enzymatic activity are good indicators of long–term contamination of soils by heavy metals and could be useful for monitoring changes in agricultural ecosystems (Karaca et al., 2010). Many researchers have used the soil enzymatic activities as bio-indicators to determine toxicological influences of various pollutants on soil quality (Shen et al., 2005). Soil enzymatic activity is reliable indicator reflecting biological state of soil and it is possible very quickly obtains reliable results of pollution influence to the biological properties (Wyszkowska et al., 2002). Yang et al., (2007) reported that the inhibition of soil enzyme activities caused by metal contamination negatively affect soil fertility. Soil enzymes are natural molecules that catalyze soil microbial reactions and mainly originate from microorganisms and plants. Urease is one of the most common enzymes in microbial, animal and plant cells. In the soil, as the part of soil solution is tightly bound to soil organic matter or clay particles (Yang et al., 2006). Urease catalyzes the hydrolysis of urea into ammonia or ammonium ion depending on soil reaction and carbon dioxide phosphatase is hydroles compounds of organic phosphorus and transforms them into different forms of inorganic phosphorus, which are assimilable by plants (Amador et al., 1997; Andrews et al., 1989; Byrnes & Amberger, 1989). Phosphatases are very important for organic phosphorus transformation into organic forms, which are suitable for plants (Gao et al., 2010). Apart from being good indicators of soil fertility, phosphatase enzyme plays a key role in the soil system (Eivazi & Tabatabai, 1977; Dick et al., 2000). Cathalase is a kind of oxidoreductase which can accelerate the degradation of hydrogen peroxide and protect organism from the toxicity of hydrogen peroxide (Cang et al., 2008; Guo et al., 2012). The objective of this study was to investigate levels of heavy metals (Cu, Pb, Cd, Zn, Hg) and As in the fields of environmentally loaded area of Middle Spiš, and assess their impacts to the soil quality which can be reflected by changes of enzymatic activities and some other soil properties. Similar studies have not been previously conducted in this area, in terms of the influence of heavy metals to the soil properties.

2. MATERIAL AND METHODS

2.1 Study area

The research was conducted in the Middle Spiš area, which is according environmental regionalization included to the environmentally loaded and unhealthy areas in Slovakia. Area covers 375 km^2 and is located in the Hornad river valley. Altitude varied from 372 to 1082 meters above sea level. In the terms of pedology view, cambisols mostly moderate to slightly heavy are typical. Region is known by long-time mining and smelting activities, predominantly focused on copper and mercury. In the time of intensive growth of mining activities, ores included high proportion of metals and production costs were low. Surface mining has great range and lasted a long time which is evidenced by the large heaps of waste material. For the research investigation the cadastres of Krompachy village [48°54'741"N; 20°52'420"E], Rudňany village [48°53'870"N; 20°39'960"E] and Slovinky village [48°55'304"N; 20°50'918"E] were selected. Processing plant for copper production situated in the Krompachy village cadastral area and for mercury production in Rudňany village cadastral area represents the main sources of pollution. Heaps of mining waste material are stored along a Slovinsky and Poracsky creek and represent the source of toxic elements, which contaminate components of the environment. Flotation pond localized in the Slovinky village cadastral area, contains sewage sludge, which originate as by-product of the manufacture and processing of copper.

2.2 Soil assays

Total set of 33 soil samples (3 samples of each field) were taken in 2012 from 5 heaps of waste material and 6 grasslands in environmentally loaded area of Middle Spiš (Fig. 1). Characteristics of all sampling points are given in table 1.

Soil samples were homogenized, dried at room temperature, sieved through 2-mm sieve opening and stored in plastic bags until analyzes. Total content of toxic elements (Cu, Cd, Pb, Zn, As) were determined in accredited laboratory by AAS (Atomic Absorption Spectrometry) and XFS (X-Ray Florescence Spectrometry) method. Total content of Hg was determined by DMA 80 (Direct Mercury Analyzer). Soil reaction was determined as follows: 5 g of soil was mixed with 25 mL of 0.01 M $CaCl_2$. Soil reaction was measured using inoLab pH 720-WTW. Organic carbon was measured by Joblbauer method (Peterburskij, 1963) and nutrient content (P, K, Mg) was determined in accredited laboratory. Urease activity and catalase activity were measured using a method described by Chazijev (Chazijev, 1976). Acid and alkaline phosphatases were measured according to a method described by Chazijev, modified method by Grejtovsky (Grejtovský, 1991).

2.3 Statistical analysis

Statistica 10 software was used for all data analysis. The significance level between the heavy metals and other soil properties was calculated by Spearman's correlation coefficient.

3. RESULTS AND DISCUSSION

3.1. Heavy metal pollution

Except the industrial areas, pollution of soil environment by heavy metals in Slovak republic occurs in areas with long-term mining and smelting activities. Polluted soils are no longer appropriate for agricultural production because they lose very common biochemical soil properties which may cause the reduction of soil fertility and inhibition of plant growth. Measured data of heavy metals (Table 2) were compared with permissible limit values for Slovak soils (Act No. 220/2004 Coll of Laws).

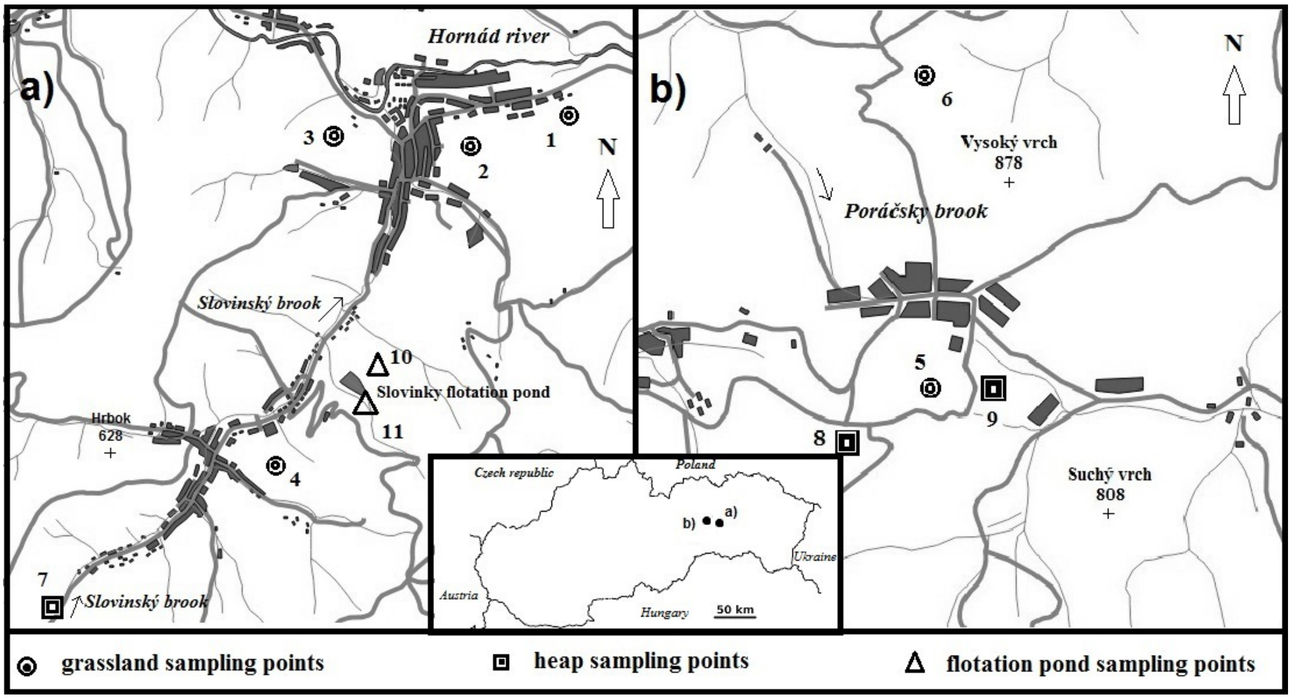

Figure 1. Sampling points in the a) Krompachy and Slovinky village and b) Poráč village as the part of former mining area of Middle Spiš

Table 1. Field, soil and climate characteristics of sampling points.

	GPS coordinate	field type	village cadastre	soil type	climate region* (average January temperature)
1.	[48°55´19.4´´N; 20°53´58.1´´E]	grassland	Krompachy	Shallow Cambisols on the crystalline rocks, moderate heavy	slightly warm, slightly wet (-2 - -5 °C)
2.	[48°55´6.8´´N; 20°52´51.6´´E]	grassland	Krompachy	Shallow Cambisols on the crystalline rocks, moderate heavy	slightly warm, slightly wet (-2 - -5 °C)
3.	[48°55´23.3´´N; 20° 51´32.3´´E]	grassland	Krompachy	Gley Fluvisols, moderate heavy	slightly warm, slightly wet (-2 - -5 °C)
4.	[48°52´48.9´´N; 20°50´51.1´´E]	grassland	Slovinky	Cambisols on the crystalline rocks, moderate heavy to light soils	slightly cold, slightly wet (3 - -6 °C)
5.	[48°52´43.6´´N; 20°43´30.7´´E]	grassland	Poráč	Cambisols on the crystalline rocks, moderate heavy to light soils	very cold, wet (-5 - -6 °C)
6.	[48°52´44.3´´N; 20°43´2.3´´E]	grassland	Poráč	Shallow Cambisols on the crystalline rocks, moderate heavy	very cold, wet (-5 - -6 °C)
7.	[48°55´24.1´´N; 20°53´59.2´´E]	heap	Slovinky	-	slightly cold, slightly wet (3 - -6 °C)
8.	[48°52´44.6´´N; 20°43´33.4´´E]	heap	Poráč	-	very cold, wet (-5 - -6 °C)
9.	[48°52´45.2´´N; 20°43´36.8´´E]	heap	Poráč	-	very cold, wet (-5 - -6 °C)
10.	[48°53´19.6´´N; 20°52´4.0´´E]	flotation pond	Slovinky	-	slightly cold, slightly wet (3 - -6 °C)
11.	[48°53´14.0´´N; 20°51´57.8´´E]	flotation pond	Slovinky	-	slightly cold, slightly wet (3 - -6 °C)

*[Džatko, 1989]

Table 2. Toxic element contents on the sampling fields of Middle Spis area (average ± standard deviation).

	Cu [$mg.kg^{-1}$]	As [$mg.kg^{-1}$]	Cd [$mg.kg^{-1}$]	Pb [$mg.kg^{-1}$]	Zn [$mg.kg^{-1}$]	Hg [$mg.kg^{-1}$]
locality	grasslands					
1.	718±3.74	168±12.02	2.9±0.16	218±5.35	857±9.89	4.5±0.45
2.	436±5.35	119±9.89	2.7±0.42	143±6.48	463±7.07	3.3±0.29
3.	1271±41.23	170±6.98	4.0±0.61	380±5.72	1333±31.51	6.7±0.35
4.	89±4.96	36±3. 56	0.5±0.08	25±4.54	126±5.89	0.45±0.03
5.	104±4.32	74±5.65	0.5±0.0	26±5.65	69±9.27	18.9±1.46
6.	257±10.61	62±3.27	0.5±0.0	27±4.97	126±4.55	32.7±1.93
	heaps of waste material					
7.	1209±29.42	181±9.1	57±5.09	377±5.89	1019±12.03	1.9±0.49
8.	405±16.57	90±5.71	0.5±0.0	26±3.56	165±5.72	98.7±4.75
9.	1287±18.49	108±7.79	0.5±0.0	102±4.55	832±14.98	33.5±2.94
10.	4226±111.3	461±16.57	1.1±0.21	1928±54.26	1725±12.02	0.8±0.14
11.	166±4.97	20±2.16	0.5±0.0	52±4.08	132±4.92	0.43±0.04
limit value	**60**	**25**	**0.7**	**70**	**150**	**0.7**

The heavy metal content on the grasslands and heaps ranged far above permissible limit values. Values of copper exceed permissible limit value (60 mg/kg) at all sampling fields, on average more than 7 times on the grasslands and almost 60 times on the heaps. Consistently with our results, Šefčík et al., (2009) categorized soils in Middle Spiš area as moderate or heavily contaminated by copper. Level of arsenic ranged high above the limit value (25 mg/kg) at all sampling fields (96.5±453.5) on the grasslands; 181±4282 on the heaps). Hronec et al., (2008) recorded above the permissible limit values of cadmium, copper, zinc and arsenic in the Middle Spiš area. He also noted, that contamination of soil environment by arsenic is related not only to anthropogenic impact but also to the geochemical effects of mineralized zones. Limit value of cadmium (0.7 mg/kg), lead (70 mg/kg) and zinc (150 mg/kg) was not exceeded at all sampling fields, but in general we reported higher heavy metal contents on the heaps of waste material compared to the grasslands. Presence of sludge from the copper and zinc production in the dumps of waste material was reported repeatedly (Michaeli & Boltižiar, 2010). The solid waste of dumps also contains residue of lead, arsenic and cadmium (Klinda & Lieskovská, 2012), which was confirmed by our results.

Extremely high and above the limit values of mercury (0.7 mg/kg) were measured at all sampling fields. Average level of mercury exceeds permissible limit value more than 42 times on the grasslands and 132 times on the heaps. Pollution of soil environment with heavy metals also negatively influences soil microbial properties such as enzyme activities (Kucharski & Wyszkowska, 2004). Enzyme activities are good indicators of the long-term soil contamination by heavy metals and could be useful for monitoring changes in agricultural ecosystems (Kizilkaya et al., 2004).

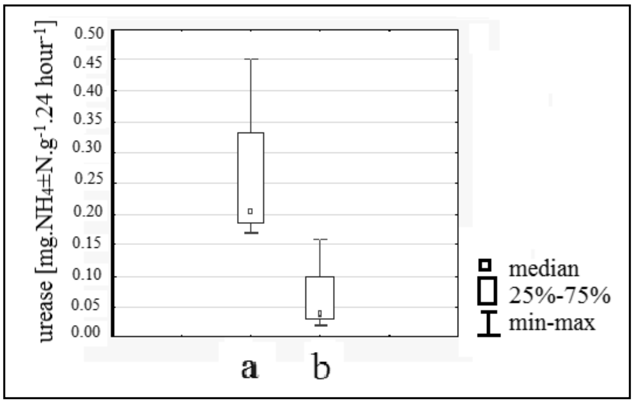

Figure 2. Activity of soil urease on grasslands (a) and heaps (b) expressed by descriptive statistics.

Activity of urease (Fig. 2) varied depending on the field type. High polluted heaps of waste material had in general lowest activity of urease (0.04±0.055) relative to the grasslands (0.205±0.11). Effects of soil pollution on enzyme activities are complex. Reaction of different enzymes to the same pollutant may vary greatly and the same enzymes may respond differently to different pollutants (He et al., 2003). We reported that activity of enzymes decreased with increasing heavy metal content for all studied enzymes, what is in consistence with the authors (Kizilkaya et al., 2004; Karaca et al., 2002). Some of them have observed differences in the changes of soil enzyme activities under the heavy metal pollution. Qu et al., (2011) and Gao et al., (2010) observed the highest inhibition effect of heavy metals on urease in comparison to other enzymes. Our findings are in agreement with these authors, because the activity of urease decreased by 98 percent of the most polluted field compared to the least polluted.

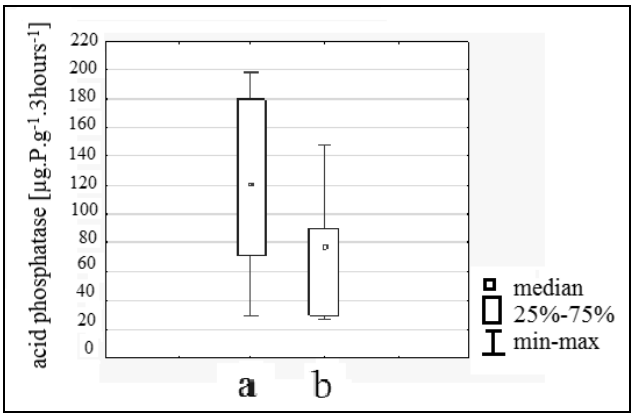

Figure 3. Activity of soil acid phosphatase on grasslands (a) and heaps (b) expressed by descriptive statistics.

Activity of acid, alkaline phosphatase and catalase (Fig. 5) decreased by 90, 85 and 79 percent, respectively, of the most polluted field compared to the least polluted. Activity of acid (Fig. 3) and alkaline phosphatase (Fig. 4) was changing depending to the soil pollution, what correspond with the findings of Liu et al., (2005), who reported significant negative correlation between phosphatases and heavy metal content.

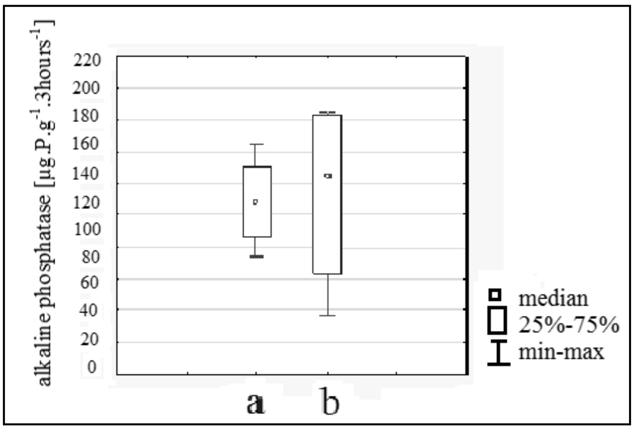

Figure 4. Activity of soil alkaline phosphatase on grasslands (a) and heaps (b) expressed by descriptive statistics.

On the heaps of waste material compared to the grasslands was activity of acid and alkaline phosphatase lower, on average about 74 % and 87 %, responsively. Determination of soil catalase in relation to the impact of heavy metals on their activity is compared to the other enzymes is more rarely. The main reason is the higher stability of catalase in the soil environment (Ladd, 1978).

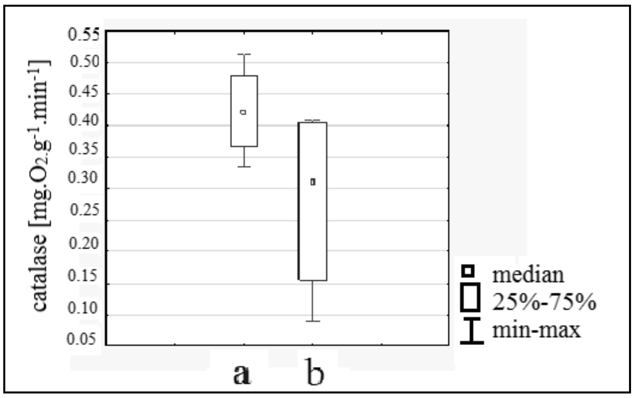

Figure 5. Activity of soil catalase on grasslands (a) and heaps (b) expressed by descriptive statistics.

3.2. Correlations between heavy metals

All studied heavy metals (except mercury) gave significant positive correlation between them, with exception the relationship between total content of copper and cadmium. If there is a significant correlation between elements, there is a high probability they come from the same pollution source (De Wolf & Rashid, 2008). Production of copper is localized in the environmental plant, which belong to the Krompachy village cadastre. Solid waste from the production of copper, which is concentrate in the heaps of waste material next to the copper processing plant, contains the residues of lead, arsenic and zinc (Michaeli & Boltižiar, 2010). It explains the significant correlations between heavy metal contents. We found no significant correlation between mercury and other heavy metals. The main source of mercury in the Middle Spiš area is plant for mercury production localized in the cadastre of Rudňany village. Production of mercury gets into the environment accompanying metals such as chlorine, barium, iron and antimony, which weren't observed in our study. Relationships between heavy metals are listed in table 3.

3.3. Correlation between enzymes

The data in table 3 shows there is a significant negative correlation between activity of soil enzymes and some heavy metals. As the numerous studies show (Kendeler et al., 2012; Mikanová, 2006; Šimon, 1999), heavy metals inhibit the activity of soil enzymes linking to the substrate, creating complexes with substrate reaction blocking-functional groups reacting with enzymes or enzyme-substrate complex. Therefore the influence of each heavy metal on soil enzymatic activities was greatly different. Urease gave significant negative correlation only with total content of arsenic ($P<0.01$). We found no significant bud negative correlation between acid phosphatase and heavy metals but significant negative correlation between alkaline phosphatase and copper, arsenic, lead and zinc. Gülser & Erdoğan (2008) determined influence of heavy metals to the alkaline phosphatase, and their findings agree with ours. Alkaline phosphatase gave Brehuv et al., (2005) who examined the impact of mining loads on the watercourses contamination noted that on the heaps of waste material, tailings or sludge from the mine, the value of soil reaction ranges is mostly alkaline. Although they were no significant statistically, soil pH gave positive correlations with all heavy metals and negative correlation with enzyme activities, organic carbon and nutrients. Taylor et al., (2002) approvingly reported negative correlations between soil pH and enzyme activities. A large number of published papers deal to the relationship between soil enzyme activity and soil parameters, such as organic carbon. Our results coincide with the findings of Badiane et al., (2001) who found no direct relationship between enzyme activity and organic carbon, which is probably the result of quality of organic inputs to the soil.

Table 3. Correlation between heavy metals and enzyme activities.

	Cu	As	Cd	Pb	Zn	Hg
URE	ns	-0.939**	ns	ns	ns	ns
ACP	ns	ns	ns	ns	ns	ns
ALP	-0.924**	-0.94**	ns	-0.924**	-0.916**	ns
CAT	- 0.742*	ns	ns	-0.742*	ns	ns
Cu		0.881**	ns	0.893**	0.871**	ns
As			0.750**	0.893**	0.912**	ns
Cd				0.752**	0.843**	ns
Pb					0.908**	ns

** Correlation in significant at the 0.01 level
*Correlation in significant at the 0.05 level
ns – not significant
URE: urease, ACP: acid phosphatase, ALP: alkaline phosphatase, CAT: catalase

Values of organic carbon on the grassland (3.4±1.1) and heaps (2.0±3.7) were not significantly different. Zhang et al., (2010) and Friedlová (2010) found no significant correlation between organic carbon and soil total heavy metal content, what is the same as our findings. Levels of nutrients varied depending to the type of the field. Obtained results were compared with the optimum values for medium-heavy soils of Slovakia. They ranged from 196 to 250 $mg.kg^{-1}$ for phosphorus, from 381 to 490 $mg.kg^{-1}$, for potassium and 281 to 360 $mg.kg^{-1}$ for magnesium. Values of phosphorus and potassium were higher on the grasslands compared to heaps. Levels of magnesium changed contrary than other nutrients and highest values were measured on the heaps (282±108) compared to grasslands (124±116.7). Hinojosa et al., (2004) observed that lead had non-significant correlation with urease, acid and alkaline phosphatase. Contrary to this claim, we reported significant negative correlation ($P<0.05$) between lead and alkaline phosphatase. In the case of catalase we reported significant negative correlation with copper ($P<0.05$) and lead ($P<0.05$). Soil enzyme activities changed in dependence of heavy metal pollution. Our results showed that enzyme activities were in general altered by heavy metal pollution, what agrees with the results of Trasar-Cepeda et al., (2000) who suggest the possibility of using these enzymes as indicators of soil pollution. Therefore the urease, acid phosphatase, alkaline phosphatase and catalase can be considered as biochemical indexes that reflect the degree of soil heavy metal pollution. A large number of published papers deal to the relationship between enzyme activities. In our study, all enzymes gave significant positive correlation ($P<0.01$) among them. In the work of Khan et al., (2010) significant positive correlation between urease and acid phosphatase was reported.

3.4 Chemical soil properties

The values of chemical soil properties measured on the Middle Spiš area are listed in table 4. In the group of soil basic physicochemical soil properties, soil pH is a very important factor, because it influences the formation, composition and physiological characteristics of enzymes (Cang et al., 2008). Values of soil reaction on the grasslands (4.9±0.48) were in general lowest compared to the heaps of the waste material (7.1±0.08).

4. CONCLUSIONS

In this paper we presented the results of the study carried out in former mining area of Middle Spiš, which is highly polluted by heavy metals. Increased content of heavy metals in the soil environment is a serious problem that adversely affects soil characteristics, resulting in a reduction of soil productivity as well as its environmental function. Measured values of heavy metals on permanent grassland and heaps of waste material, which ranged above the permitted limit values shows that the conditions for quality and healthy land in this former mining region are not met.

According to the statistical testing and evaluating the impact of toxicity on biological and chemical soil properties in spatial and horizon, we can conclude that biological properties are much more sensitive to environmental stress, which resulted mainly reduced activity of soil enzymes in the localities polluted heavy metal. Biological soils properties are suitable indicators of soil health and quality, because respond very quickly to environmental stress and their monitoring may help the early detection of degradation of the soil environment.

Table 4. Chemical soil properties on the grasslands and heaps of Middle Spiš area

	pH	**C_{ox} [%]**	**P [$mg.kg^{-1}$]**	**K [$mg.kg^{-1}$]**	**Mg [$mg.kg^{-1}$]**
grasslands					
average	5	3.5	70.8	152	171
min	4.6	2.1	15	25	101
max	6	5.5	179	374	266
st.deviation	0.48	1.11	56.72	116.7	65.9
heaps					
average	7.1	3.7	2.2	17	268
min	7	2.8	1.6	15	130
max	7.2	4.9	2.9	21	394
st.deviation	0.08	0.89	0.51	2.82	108.1

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Urease, acid phosphatase, alkaline phosphatase and catalase can be considered as biochemical indicators, which reflect the degree of soil contamination with heavy metals.

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REFERENCES

Act No. 220/2004 Coll. Of Laws on protection and use of agricultural land. 38 p.

Amador, J.A., Glzcksman, A.M., Lyons. J.B. & Gores J.H., 1997. *Spatial distribution of soil phosphatase activity within a riparian forest*. Soil Science, 162, 11, 808-825.

Andrews, R.K., Blakeley, R.L. & Zerner, B., 1989. Urease: a Ni (II) metalloenzyme. In: Lancaster JR (ed) *The bioinorganic chemistry of nickel*, VCH, New York, pp 141-166.

Badiane, N.N.Y., Chotte, J.L., Pate, E., Masse, D. & Rouland, C., 2001. *Use of soil enzyme activities to monitor soil quality in natural and improved fallows in semi-arid tropical regions*. Applied Soil Ecology, 18, 3, 229-238.

Bálintová, M. & Ľuptáková, A., 2012. *Treatment of acid mine waters*. Technical University in Košice, Košice. 131 p.

Bálintová, M., Holub, M., Singovszká, E., 2013. *Study of iron, copper and zinc removal from acidic solutions by sorption***.** Chemical Engineering Transactions, 35, 865–870.

Brehuv, J., Bobro, M., Hančuľák, J., Špaldon, T. & Slančo, P., 2005. *Effect of old environmental loads to contamination of flows which estuary into the reservoir dam "I Ružín" selected elements in 2004*. Acta Montanistica Slovaca, 10, 322 – 328.

Byrnes, B.H. & Amberger, A., 1989. *Fate of broadcast urea in a flooded soil when treated with N- (n-butyl) thiophaspheric triamide, a urease inhibitor*. Fertilizer Research, 18, 3, 221-231.

Cang, L., Zhou, D.M., Wang, Q.Y. & Wu, D.W., 2008. *Effect of electrokinetic treatment of heavy metal contaminated soil on soil enzyme activities*. Journal of Hazardous Materials, 172, 2-3, 1602-1607.

Chazijev F.CH., 1976. *Fermentativnaja aktivnosť počv*, Nauka, Moscow. 106-120.

De Wolf, H. & Rashid, R., 2008. *Heavy metal accumulation in Littoraria scabra along polluted and pristine mangrove areas of Tanzania*. Environ Pollution, 152, 3, 636-643.

Dick, W.A., Chengm L. & Wang, P., 2000. *Soil acid and alkaline phosphatase activity as pH adjustment indicators*. Soil Biology and Biochemistry, 32, 13, 1915-1919.

Džatko, M., 1989. *Pedoecological, productive and environment aspects of the land use classification in Slovakia. Soil conservation and environment,* Proceedings of VIIth Czechoslovak soil science conference, 208-210.

Eivazi, F. & Tabatabai, M.A., 1977. *Phosphates in soils*. Soil Biology and Biochemistry, 9, 167-172.

Friedlová, M., 2010. *The influence of heavy metals on soil biological and chemical properties*. Soil and Water Research, 5, 1, 21-27.

Gao, Y., Zhou, P., Mao, L., Zhi, Y. & Shi, W., 2010. *Assessment of effect of heavy metals combined pollution on soil enzyme activities and microbial community structure modified ecological dose-response model and PCR-RAPD.* Environmental Earth Sciences, 60, 3, 603-612

Grejtovský, A., 1991. Effect of soil improve processes to enzymatic activity of heavy alluvial soil. Rostlinná Výroba, 37, 289-295.

Gülser. F. & Erdoğan, E., 2008. *The effect of heavy metal pollution on enzyme activities and basal soul respiration of roadside soils*. Environmental Monitoring and Assessment, 145, 1-3, 127-133.

Guo, X.L., Gu, J., Chen, Z.X., Gao, H., Qin, Q.J., Sun, W. & Zhang, W,J., 2012. *Effects of heavy metals pollution on soil microbial communities metabolism and soil enzyme activities in coal mining area of Tongchuan, Shaanxi Province of Northwest China*. Chinese Journal of Applied Ecology, 23, 3, 798-806.

He, Z.I., Xu, Z.H. & Hughes, J., 2003. *Analyses of soil fungal communities in adjacent natural forest and hoop pine plantation ecosystems as revealed by molecular approaches based on 18S rRNA genes*. FEMS Microbiology Letters, 247, 1, 91-100.

Hinojosa, M.B., Carreira, J.A., Garcia-Ruiz, R. & Dick, R.P. 2004. *Soil moisture pre-treatment effect on enzyme activities as indicators of heavy metal-contaminated and reclaimed soils*. Soil Biology and Biochemistry, 36, 10, 1559-1568.

Ho, Y.B. & Tai, K.M., 1988. *Elevated levels of lead and other metals in roadside soil and grass and their use to monitor aerial metal depositions in Hong Kong.* Environmental Pollution, 49, 1, 37-51.

Hronec, O., Vilček, J., Tóth, T., Andrejovský, P., et al., 2008. *Heavy metals in soils and plants of contaminated area "Rudňany – Gelnica"*. Acta regionalia et environmentalica. 1, 24-28.

Karaca, A., Cetin, S.C., Turgay, O.C. & Kizilkaya, R., 2010. Effects of heavy metals on soil enzyme activities. In: Sherameti, I., Varna, A. (ed), *Soil heavy metals*, Springer, New York, pp 237-262

Karaca, A., Neseby, D.C. & Lynch, J.M., 2002. *Effect of cadmium contamination with sewage sludge and phosphate fertilizer amendments on soil enzyme activities, microbial structure and available cadmium*. Biology and Fertility of Soils, 36, 6, 428-434.

Kendeler, E., Kampichler, C. & Horak, O., 2012. *Influence of heavy metals on the functional* diversity of soil microbial communities*. Biology and Fertility of Soils, 23, 3, 299-306.

Khan, S., Heshamel, L., Qiao, M., Rehman, S. & He, J.Z., 2010. *Effects of Cd and Pb on soil microbial community structure and activities*. Environmental Science and Pollution Research, 17, 2, 288-296.

Kizilkaya, R., Aşkin, T., Bayakli, B. & Sağlam, M., 2004. *Microbiological characteristics of soil contaminated with heavy metals*. European Journal of Soil Biology, 40, 2, 95-102.

Klinda, J. & Lieskovská, Z., 2012. *Report on the State of the Environment of the Slovak Republic in 2004*. Ministry of Environment of Slovak republic, Bratislava. 204 p.

Kucharski, J. & Wyszkowska, J., 2004. *Interrelationship between number of microorganisms or spring barley yield and degree of soil contamination with copper*. Plant, Soil and Environment 50, 243 – 249.

Ladd, J.N., 1978. Origin and range of enzymes in soil. In: Burns, R.G., (ed), *Soil Enzymes*, Academic Press, New York, pp 51-96.

Liu., H,Y., Probst, A. & Liao, B.H., 2005. *Metal contamination of soils and crops affected by the Chenzhou lead/zinc mine spill (Hunan, China)*. Science of Total Environment, 339, 1-3, 153–166.

Lyubenova, L. & Schröder, P., 2010. *Uptake and effect of heavy metals on the plant detoxification Cascade un the presence and absence of organic pollutants.* Soil Heavy Metals, 19, 66-85.

Michaeli, E. & Boltižiar, M., 2010. *Selected localities of environmental loads in environmentally loaded areas in Slovakia*. Geographical studies, 1, 18-47.

Mikanová, O., 2006. Effect of heavy metals on some soil biological parameters. Journal of Geochemical Exploration, 88, 1-3, 220-223.

Parr, J.F., Papendick, R.I., Hornick, S.B. & Meyer, R.E., 1992. *Soil quality: attributes and relationship to alternative and sustainable agriculture*. American Journal of Alternative Agriculture, 7, 1-2, 5-11.

Peterburskij, A.V., 1963. *Praktikum po agronomičeskoj chimii.* Izd. Seľskochozjejstvennoj literatury, žurnalov a plakatov, Moskva. 591 p.

Qu, J., Ren, G., Chen, B. & Fan, J., 2011. *Effects of lead and zinc mining contamination on bacterial community, diversity and enzyme activities of vicinal cropland*. Environmental Monitoring and Assessment, 182, 1-4, 597-606.

Šefčík, P., Pramuka, S. & Gluch, A., 2009. *Assessment of soil contamination in Slovakia according index of geoaccumulation.* Agriculture, 54, 3, 119-130.

Shen, G., Gao, L., Lu, Y. & Hong, J., 2005. *Influence of phenanthrene on cadmium toxicity to soil enzymes and microbial growth*. Environmental Science and Pollution Research, 12, 5, 259-263.

Šimon, T., 1999. *The effect of increasing rates of nickel and arsenic on the growth of radish and soil microflora.* Rostlinná výroba, 45, 421-430.

Singh, R., Chavan, S.L. & Sakale, P.H., 2006. *Heavy metal concentration in water, sediments and body tissues of Red Worm (Tubifex SPP) collected from Natural Habitats in Mumbai, India.* Environmental Monitoring and Assessment, 129, 1-3, 471-481.

Špaldon, T., Brehuv, J., Bobro, M., Hančuľák, J. & Šestinová, O., 2006. *Mining development the Spiš-Gemer pre location*. Acta Montanistica Slovaca, 11, 375-379.

Taylor, J.P., Wilson, B., Mills, M.S. & Burns, R.G., 2002. *Comparison of microbial numbers and enzymatic activities in surface soil and subsoils using various techniques*. Soil Biology and Biochemistry, 34, 3, 387-401.

Trasar-Cepeda, C., Leirós, M.C., Seoane, S., Cil-Sotres, F., 2000. *Limitations of soil enzymes as indicators of soil pollution.* Soil biology and Biochemistry, 32, 13, 1867-1875.

Wyszkowska, J., Kucharski, J. & Waldowska, E., 2002. *The influence of diesel contamination on soil enzymes activity*. Roslinná výroba, 48, 58-62.

Yang, Y.G., Shu, W.S. & Lab, C.Y., 2007. *Heavy metal concentration and plant ability assessment of edible vegetables in Lechang lead-zinc mine area*. Metal Mine, 12, 126-127.

Yang, Z., Liu, S., Zheng, D. & Feng, S., 2006. *Effects of cadmium, zinc and lead on soil enzyme activities*. Journal of Environmental Sciences, 18, 1135-1141.

Zhang, F.P., Li, CH.F., Tong, L.G., Youe, L.X., Li, P., Ciren, Y.J. & Cao, C.G., 2010. *Response of microbial characteristics to heavy metal pollution of mining soils in central Tibet, China.* Applied Soil Ecology, 45, 3, 144-151.

Zhang, X., Yang, L., Li, Y., Li, H., Wang, W. & Ye, B., 2011. *Impact of lead/zinc mining and smelting on the environment and human health in China*. Environmental Monitoring and Assessment, 184, 4, 2261-2273.

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