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IMPACT OF AGRICULTURAL ACTIVITY ON SUBTERRANEAN ECOSYSTEMS IN CASE OF PONICKÁ CAVE (SLOVAKIA)

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ABSTRACT

Cave ecosystems, which represent a very specific environment for living organisms, are largely influenced by the external environment. These systems are among the most vulnerable ecosystems because they are extremely sensitive to any environmental changes. The main aim of this study was to analyse the biological and chemical indices of soil samples collected in the Karst region. Soil organic carbon, total nitrogen content, soil pH, activity of selected soil enzymes (FDA, β-glucosidase, urease, phosphatases), and content of risk elements were determined at nine sites around Ponická Cave. Enzymatic activity reduces its function under the influence of inappropriate agricultural activity as well as the high content of risk elements. Ponická Cave is characterized by a close hydrological connection with the earth's surface, thus representing a very vulnerable habitat, which deserves more attention in the protection of natural elements of the landscape. Very high concentration of toxic elements (As, Cd, Co, Cu, Fe, Mn, Pb and Zn) was recognized on localities that are close to the cave entry, which in higher concentrations cause contamination of underground ecosystems. The results showed that risk elements have an impact on important soil parameters, such as the enzymatic activity of the microbial population and chemical properties. In view of the risks associated with water erosion and rainwater runoff from non-karst lands, agricultural activity in these soils should be completely excluded.

Keywords: *Cave system, Risk elements, Soil microbial activity, Ponická Cave, Pollution*.

INTRODUCTION

Cave ecosystems represent a very specific and pristine environment for living organisms that is largely influenced by the external environment (Lunghi *et al.*, 2020). Cave geosystems consist of underground spaces that are completely or largely bounded by a cave georelief. They are characterized by several specific physiognomic features of the components of the geographical sphere, spatial organization, and natural processes. They are characterized by the absence of light, a relatively stable temperature, and a specific composition of cave biota (Bella, 2005). These systems are among the most vulnerable ecosystems on Earth because they are extremely sensitive to change (Tomova *et al*., 2019). Cave systems respond sensitively to anthropogenic influences, which can significantly affect energy availability by increasing the proportion of organic matter and nutrients in caves. These new organic resources are primarily used by microorganisms, which can indirectly affect the cave climate (e.g., reducing the availability of oxygen) and thus change the diversity and abundance of the cave biota. Soil and hydrological disturbances caused by contaminated rainwater entering cave spaces occur mainly because of intensive agricultural production, deforestation, urbanization, and waste disposal in its immediate vicinity (Neill *et al*., 2004). The biggest problem with agriculturally treated soil is the input and long-term accumulation of heavy metals in the soil environment, which at higher concentrations causes contamination of underground ecosystems (Gilli, 2015). From an environmental point of view, such places represent an environmental burden and potential source of water and soil pollution. An increased amount of hazardous substances in the environment can permanently change the stability and structure of living organisms, thereby degrading the overall state of ecosystems (Demková *et al*., 2020). Wastewater, agricultural and industrial waste, various chemical leaks, and microbial pollution are also responsible for high levels of pollution in underground ecosystems (Simon, 2019; Slay, 2019). These harmful factors can directly or indirectly change the internal conditions of cave systems and significantly change the composition of living organisms (including microbial communities).

The aim of this study was to determine the enzymatic activity of the microbial community near the surface karst of Ponická Cave. We hypothesize that, as a result of long-term agricultural activity, the function of the microbial community is significantly altered, and this disproportionate intervention results in contamination of the underground system.

MATERIAL AND METHODS

Site description

Ponická Cave is located southeast of the city of Banská Bystrica in the village of Poniky (Slovakia) (48°42'47.6"N 19°15'43.8"E), which is part of the karst area called Ponický karst. Ponická Cave was declared to be a natural monument in 1994. However, this natural monument is located in an area significantly affected by human activity. Subsequently, the connection of the Ponická Cave with the Oravecká exsurgence was established by a dye test, on which the initial survey began (Belička, 1981), which mainly focused on monitoring the water quality of the underground hydrological system.

Soil sampling and analyses

Eight sampling points were selected in the vicinity of Ponická Cave on summer season 2020. Four sampling points were located near the sinkholes at the cave entrance (S), three sampling points were located near the Oravecká exsurgence (E), and one was located at the former manure pit (near the sinkholes at the cave entrance) (MP), which represents potential pollution in this area. Three random samples were collected from each sampling point. All the soil samples were transferred to a laboratory in plastic bags, sieved with a mesh size of 2 mm, and stored in a refrigerator before analysis. Part of the samples were air dried at room temperature to determine the selected chemical parameters.

The soil reaction (pH) was determined in a mixture of soil and $CaCl_2$ solution (c=0.01 mol/L) in a ratio of 1:3 using a digital pH meter. The activity of the soil enzymes β-glucosidase (Eivazi and Tabatabai, 1988), FDA (Green *et al.*, 2006), phosphatases (acid and alkaline) (Grejtovský, 1991) and urease (Khaziev, 1976) were determined using specific substrates, buffer solutions and conditions of each enzyme. For each soil enzyme activity, a corresponding control was performed with the same analytical analysis but without the addition of substrate. Determination of the content of organic carbon (Cox) and total nitrogen (Ntot) in the soil samples was carried out using the ISO10694 method. The content of monitored elements (As, Cd, Co, Cu, Fe, Mn, Ni, Pb, Zn) was determined by OES-ICP (Agilent 720, Agilent Technologies, USA) and (Hg) CV-AAS (AMA-254; AlTec, Prague), using methodological procedures published in the work Demková *et al.*, 2019.

Statistical evaluation of the obtained data was performed using the STATISTICA 12. Before the analysis, all data were log+1 transformed.

RESULTS AND DISCUSSION

Despite the fact that agricultural activity in this area has decreased at the present time, the threat to the underground spaces and waters of Ponický Karst persists. The biggest problem is considered to be a manure pit located near active sinkholes, whose organic content can be floated directly into the karst massif, and inappropriate agricultural practices such as the use of artificial industrial fertilizers. Another possible threat in this area could be the remains of mining activities that took place in the past, emerging illegal solid waste dumps, etc.

Evaluation of soil chemical properties

The soil reaction at the monitored locations near Ponická Cave ranged from 6.2 to 7.2 (Table 1), from which we can conclude that it is a neutral to slightly alkaline subterranean environment. Table 1 shows that the highest values of organic carbon content (Cox) (22.82%) and total nitrogen content (Ntot) (1.91%) at the monitored locations were recorded at the manure pit, which is located near the diving entrances to Ponická Cave itself.

Table 1. Average values of soil chemical properties

Locality	pH/0.01M $CaCl_2$	Cox (%)	Ntot (%)
S	6,6±0,3	5,75±0,2	0,47±0,01
MP	6,6±0,1	22,82±0,3	1,91±0,02
E	7,1±0,2	3,90±1,9	0,40±0,18

A significant positive correlation ($P < 0.01$) between Cox and Ntot was observed at the monitored sites (0,94**). Cox and Ntot were significantly negatively correlated with soil pH ($p < 0.05$). This trend in the chemical parameters has been confirmed in several studies (Li *et al*., 2014; Bobuľská *et al*., 2015).

As, Pb, Zn, Cu, and other toxic elements are pollutants in the soil, the sources of which are anthropogenic activities such as industry, mining, metallurgy, and agriculture. Because of their negative effects on the ecological functions of the soil, this represents a serious environmental problem. Table 2 shows the average values of risk substances (As, Cd, Co, Fe, Hg, Mn, Ni, Cu, Zn, Pb) at individual locations together with the limit values for soils in the territory of Slovakia defined in Act NR SR 220/2004 Coll. whose exceeded values indicate pollution and endangerment of the soil and the surface environment, respectively.

Table 2. Average values of heavy metals on selected localities

Locality	As [mg/kg]	Cd [mg/kg]	Co [mg/kg]	Cu [mg/kg]	Fe [mg/kg]	Hg [mg/kg]	Mn [mg/kg]	Ni [mg/kg]	Pb [mg/kg]	Zn [mg/kg]
S1	120±25	7,1±3,5	27,6±2,1	216±54	24917±123	0,06±0,0	2849±138	23,6±7,0	3113±154	878±62
MP	7,5±3,3	2,3±0,3	23,7±3,0	35,7±4,4	12445±147	0,06±0,0	738,4±75	15,2±4,0	225,9±34	231±24
E1	23,3±4,2	4,04±0,2	42,1±2,8	37,4±4,5	24532±168	0,06±0,0	1260±56	28,7±4,2	447,7±37	318±37
Limit values	**30**	**1**	**20**	**70**	**550**	**0,75**	**550**	**60**	**115**	**200**

According to the limit values, the maximum values were exceeded for several risk elements. The results indicated an increased risk of contamination in this karst area. Near the entrance of Ponická Cave, we noticed an almost 7 times increase in As content, which should not exceed 30 mg/kg of dry matter. An increased amount of Cd was detected at all sampling sites. A 5 times increase in the limit values of Cu content (70 mg/kg) was also recorded near the entrance of the Ponická Cave. In our study, a high content of risk substances was confirmed, mainly in terms of Fe, Mn, Pb, and Zn. Their several-fold increase was mostly confirmed near the entrance to the cave. Exceeding the limit values of Hg and Ni was not recorded for any sample. The results showed multiple contamination of the soil with heavy metals, which may have a negative impact on the underground ecosystems located in their vicinity. Most hazardous substances occur naturally in the soil; however, human activity can greatly disturb the natural balance in the environment. Large amounts of Pb, Zn, Cu, Cd, and other substances are released into the environment as a result of activities related to the processing of mineral raw materials or agricultural activities (Atafar *et al*., 2010). These elements accumulate at highly toxic levels in soil, water, and biota, and threaten underground ecosystems. The deterioration of the environment with varying degrees of devastation has a negative impact on the quality of the ecosystem, with the entire extent of the problem depending on the size of the polluted area, the depth of the soil into which the pollutants penetrate, and the chemical composition of the pollutants. Understanding the behaviour of heavy metals in soil systems is one of the most important tasks in environmental science (Angelovičová *et al*., 2015).

Evaluation of soil enzymatic activity

The average values of soil enzyme activity are listed in Table 3. The activity of the soil enzymes was relatively low at the monitored locations. Comparing enzymatic activity with other scientific works devoted to the study of soil enzymes in different ecosystems (Vinhal-Feitas *et al*., 2017; Bobuľská *et al*., 2019), we found that the values of phosphatases and urease were significantly lower.

Table 3. Average values of soil enzymes (average ± SD)

Locality	**β-glukosidase (μg pNP/g soil.1h)**	**FDA (μg FS/g soil.1h)**	**Acid phosphatase (mg P/g soil.3h)**	**Alkaline phosphatase (mg P/g soil.3h)**	**Urease (ug NH_4 /g soil.1h)**
S1	19,08±3,6	20,23±1,5	88,69±6,3	178,57±10,3	4,49±0,3
MP	22,13±3,4	19,12±0,9	116,32±6,7	164,21±8,7	4,43±0,2
E1	18,14±2,7	13,60±2,4	62,46±8,0	162,20±10,9	9,83±1,3

The highest values of soil phosphatases, β-glucosidase, and FDA activity were found in locations near the entrances to Ponická Cave; in the case of urease, the opposite trend was observed. Some studies have reported that phosphatases do not change significantly due to a slight increase in the content of heavy metals in the soil environment (Yeates *et al*., 1994), but only a significantly high content of these toxic elements has an inhibitory effect on their activity (Brookes, 1995). Analysis of the activity and diversity of microbial communities adjacent to industrialized sites revealed that these soils showed lower enzymatic function with higher soil metal content, as well as lower microbial community diversity. Kandeler *et al*. (2000) in their study found lower activity of soil urease, alkaline phosphatase and xylanase in soils that were contaminated with risk elements (Zn, Cu, Ni, V and Cd). However, in some studies (Brookes, 1995; Demková *et al*., 2020) it was noted that a high load of metals in the soil was associated with high enzymatic activity or changes in the composition and functioning of microbial communities. These results highlight the complexity of the effects of heavy metal contamination on enzymatic activity and the need to consider the experimental details of each study. Based on the increased values of heavy metals, we can conclude that the monitored locations of Ponická Cave and Oravecká exsurgence represent a significantly vulnerable karst cave system. We can consider unreasonable agricultural practices and grazing, which take place in the immediate vicinity of this cave system, as probable pollution of the underground water and cave spaces of Ponický Karst. The biggest problem is the manure pit, which is inappropriately located near the underwater entrances and sinkholes into the cave, through which the seeping limestone enters the cave geosystem and causes organic pollution of the groundwater. The highest values of heavy metal content were also measured at these sampling points.

CONCLUSIONS

The results of our study confirmed that soils loaded with high levels of hazardous substances can inhibit the activity of the microbial community by reducing the activity of soil enzymes. Higher contamination with risk elements in the monitored locations reduced the activity of microbial enzymes and other biologically important soil properties that are important for the stability of underground ecosystems. Although most risk elements occur naturally in the environment, human activities (industry, agricultural practices, burning of fossil fuels, or mining activity) disturb the stability and interactions between individual components of underground ecosystems. Based on the proven highest contamination by risk elements in the area of the dives, we can conclude that they represent the most vulnerable part of karst systems. Despite the reduction in the negative impacts caused by agricultural activity by several steps that partially helped to protect these unstable ecosystems in the past, protection is needed in the future, including the protection of both subsurface and surface environments, in such a way that this ecosystem maintains its natural load capacity. Therefore, it is very important to pay attention to regular monitoring and prevent any further accumulation of substances, thereby ensuring the reduction of risk factors affecting the ecosystem.

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