*GEOLOGICA CARPATHICA, 50, 5, BRATISLAVA, OCTOBER 1999*
*389–394*

# MICROBIAL ACTIVITY IN SOILS UNDER THE INFLUENCE OF PYRITE WEATHERING

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*(Manuscript received February 12, 1998; accepted in revised form December 9, 1998)*

**Abstract:** The chemical and microbial characteristics of soils at seven research sites — four affected by an acid sulphate weathering and three intact, undisturbed have been investigated. The extremely low soil pH, the increased electrical conductivity and high exchangeable aluminium contents are characteristic features of the affected soils. These chemical changes were accompanied by the inhibition of the soil respiration and enzyme activities denoted the microbial activity in soils. The small microbial biomass and the abundance of bacteria and microscopic fungi indicated the worsening of living conditions for the soil microorganisms. In spite of this some representatives of microscopic fungi (Deuteromycotina) isolated from the damaged soils gave evidence of their adaptability to the extreme conditions.

**Key words:** acid sulphate weathering, soil acidification, microscopic fungi, respiration and enzyme activity changes.

## Introduction

The quartzite quarring has been practiced on the Šobov hill near Banská Štiavnica since the year 1956. The waste material containing pyrite has been accumulated on a dump pile next to the quarry. The weathering of pyrite leads to the production of extremely acid and mineralized water solutions within the dump pile. The acid solution with high concentrations of iron and sulphatic ions has damaged soils and the vegetation downslope. The soil acidification, the precipitation of the characteristic secondary minerals, the destruction of the soil structure, and consequently the absence of the plant cover and the strong erosion of topsoils are the degradational features on the slope of Šobov hill.

The first symptoms of the damage appeared in 1978. The area of about 15 ha is influenced at present. The geological, chemical and biological aspects of the affected territory has been studied by a number of authors (Holub et al. 1991; Holub et al. 1993; Križáni et al. 1994a; Križáni et al. 1994b; Forgáč et al. 1995; Šimonovičová et al. 1996; Šimonovičová et al. 1997; Dlapa et al. 1997; Šucha et al. 1997). The processes of the acid sulphate weathering in the volcanic complex in Vtáčnik Mts. was described by Čurlík & Forgáč (1983).

The aim of this paper is to compare the microbiological characteristics of the original topsoils with those affected by the acid and mineralized water originating from the pyrite oxidation. The soil microbial characteristics, like the soil respiration or enzyme activities, inform about the microbial activity in the soils. In addition, the microbial biomass, the abundance of bacteria and microscopic fungi, the occurrence of microscopic fungi species and genera testify to the quality of the soil environment.

## Material and methods

The Šobov hill is situated NE of Banská Štiavnica town in the Štiavnické vrchy region. The soils are Eutric Cambisol (FAO) with transitions to Planosol, in depressions. Detailed mapping of soils was done in the area affected by the water leaking out from the dump pile. Seven sampling sites were studied — four influenced (P1, P2, P3, P4) and three without degradational features (N5, N6, N7). The samples were taken from the topsoils (0–20 cm) for the chemical and the microbiological analyses.

Soil samples were air-dried and fraction < 2 mm was taken for chemical analyses. Soil pH values ($H_2O$, 1 M KCl) and exchangeable Al were determined using a soil : solution ratio of 1 : 2.5. Total organic carbon was measured by oxidation with $K_2Cr_2O_7$–$H_2SO_4$ and titration of non-reduced chromate. Electrical conductivity was determined in the saturation soil extracts, after allowing the pastes to stand for 24 h.

The microbiological investigation was realized with the fresh soil samples. The soil fraction < 2 mm was used for laboratory experiments.

The microbiological analyses include:

— the $CO_2$-evolution (the soil respiration) — basal and potential after adding 1 % of glucose (Kopčanová et al. 1990);

— the enzyme activities: carboxymethylcellulase (CMC), xylanase and saccharase (Schinner et al. 1993);

— the amount of carbon in microbial biomass ($C_{bio}$) (Schinner et al. 1993);

— the abundance of bacteria and microscopic fungi (Kopčanová et al. 1990);

— the composition of microscopic fungi species and genera (Fassatiová 1979).

The number of repeated samplings (3–10) was chosen in accordance with the methods used. The arithmetical means and the mean deviations have been calculated and illustrated in the Figs. 1–4.

Figures on Pls. I–II were taken on Scanning Electron Microscopy (SEM) model Tesla BS 301 by J. Blahutiaková (Institute of Experimental Phytopathology and Entomology, Slovak Academy of Sciences, Ivanka pri Dunaji). Before that, the samples were air-dried and coated with gold.

## Results and discussion

The chemical properties of investigated topsoils reveal degradation processes as follows from Table 1. A drop in the soil pH to about 3 is typical. The soil buffering mechanisms are insufficient and almost ineffective because acid water entering the soil profiles contains high concentrations of iron and sulphatic ions (in contrast to acid rains with only very low concentrations of salts). The salinity of saturated soil extracts increases due to the dissolution of secondary minerals which are formed during the interaction of acid water with the original soil. Exchangeable base cations ($Ca^{2+}$, $Mg^{2+}$, and $K^+$) are displaced by $Al^{3+}$ (Dlapa et al. 1997). The low nutrient status and aluminium toxicity will cause the inhibition of all biological activities.

**Table 1:** Basic analytical data of the studied soils (P1–P4 influenced, N5–N7 not influenced by acid sulphate weathering).

| Sample No. | pH ($H_2O$) | pH (KCl) | $C_{ox}$ % | EC µS . $cm^{-1}$ | Al mg . $kg^{-1}$ |
|---|---|---|---|---|---|
| P1 | 3.0 | 2.8 | 2.6 | 1300 | 727 |
| P2 | 3.1 | 2.8 | 1.4 | 1000 | 506 |
| P3 | 3.1 | 2.9 | 1.7 | 2500 | 585 |
| P4 | 3.3 | 3.2 | 2.2 | 3600 | 832 |
| N5 | 6.0 | 5.0 | 2.1 | 300 | 18 |
| N6 | 5.4 | 4.5 | 3.2 | 500 | 22 |
| N7 | 6.1 | 5.2 | 2.6 | 400 | 6 |

EC — electrical conductivity of saturated soil extract
Al — exchangeable aluminium (1M KCl).

The study of carbon cycling is necessary to understand the functioning of the ecosystems. The carbon fluxes from the soil and litter compartments to the atmosphere (collectively referred to as soil respiration) are major pathways of the carbon cycle (Redmann 1978). The evolution of $CO_2$ is an indicator of intensified processes of organic matter decomposition in the environments (Kubicka 1973). The carbon dioxide output of soil samples reflects the overall metabolic activity of the microflora in soil (Gray & Williams 1971). The highest values of $CO_2$-evolution (112.0 and 73.8 mg $CO_2 \cdot kg^{-1}$ soil $\cdot$ (24 h)$^{-1}$) were measured at the chemically undisturbed places N6 and N7 (Fig. 1). The potential soil respiration (the respiration after adding of glucose as the source of energy) was more intensive than the basal soil respiration in all samples (Fig. 1). Such results testify to the fact, that the quantities of available nutrients had a direct and the greatest influence on the activity of microorganisms.

The soil microorganisms play a very important role in the decomposition of dead organic matter. The chemical pathway from the plant or litter bioorganic compounds to the colloidal humic substances is very complex involving a number of degradative and condensation reactions. These reactions involve numerous enzymes, since non-enzymatic reactions in soils are very slow (Lähdesmäki & Piispanen 1988). The first components to be removed, by the combined action of microbes and leaching, are water-soluble compounds, such as sodium and potassium salts and sugars. After these, more complex substances such as starch and proteins are attacked. Whereas many of these components can be decomposed during a few months, more complex structural ones, such as lignin or keratin, may persist in soils for several years (Gray & Williams 1971). The enzyme activities — saccharase, xylanase and carboxymethylcellulase (CMC) — from the carbon cycle of organic matter circulation in the natural environment, were clearly detected. It has been observed that the highest saccharase and xylanase activities (5 170.4–8 694.6 µg GLC$\cdot g^{-1}$ dry soil $\cdot$ (3 h)$^{-1}$ and 648.0–3 522.5 µg GLC $\cdot g^{-1}$ dry soil $\cdot$ (24 h)$^{-1}$) are at the places N5–N7, that is at the places without degradational attributes (Fig. 2). The saccharase

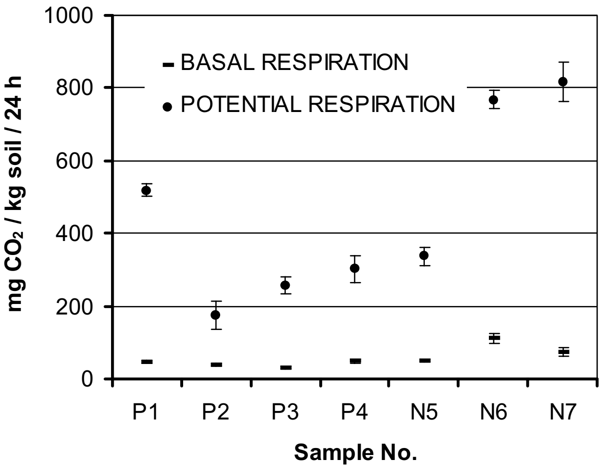


**Fig. 1.** Basal and potential respiration of microorganisms.

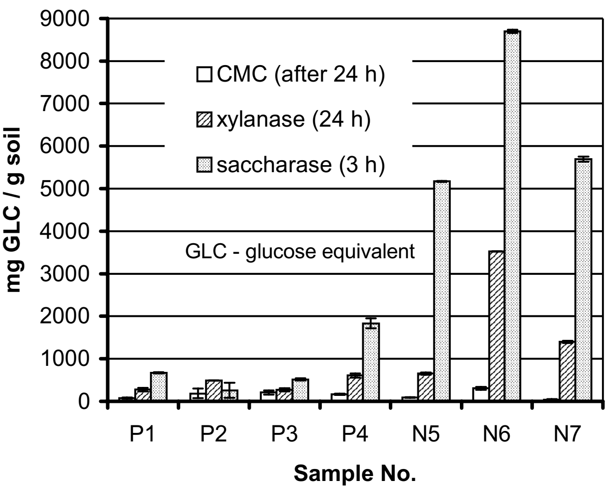


**Fig. 2.** Saccharase, xylanase and carboxymethylcellulase activities.

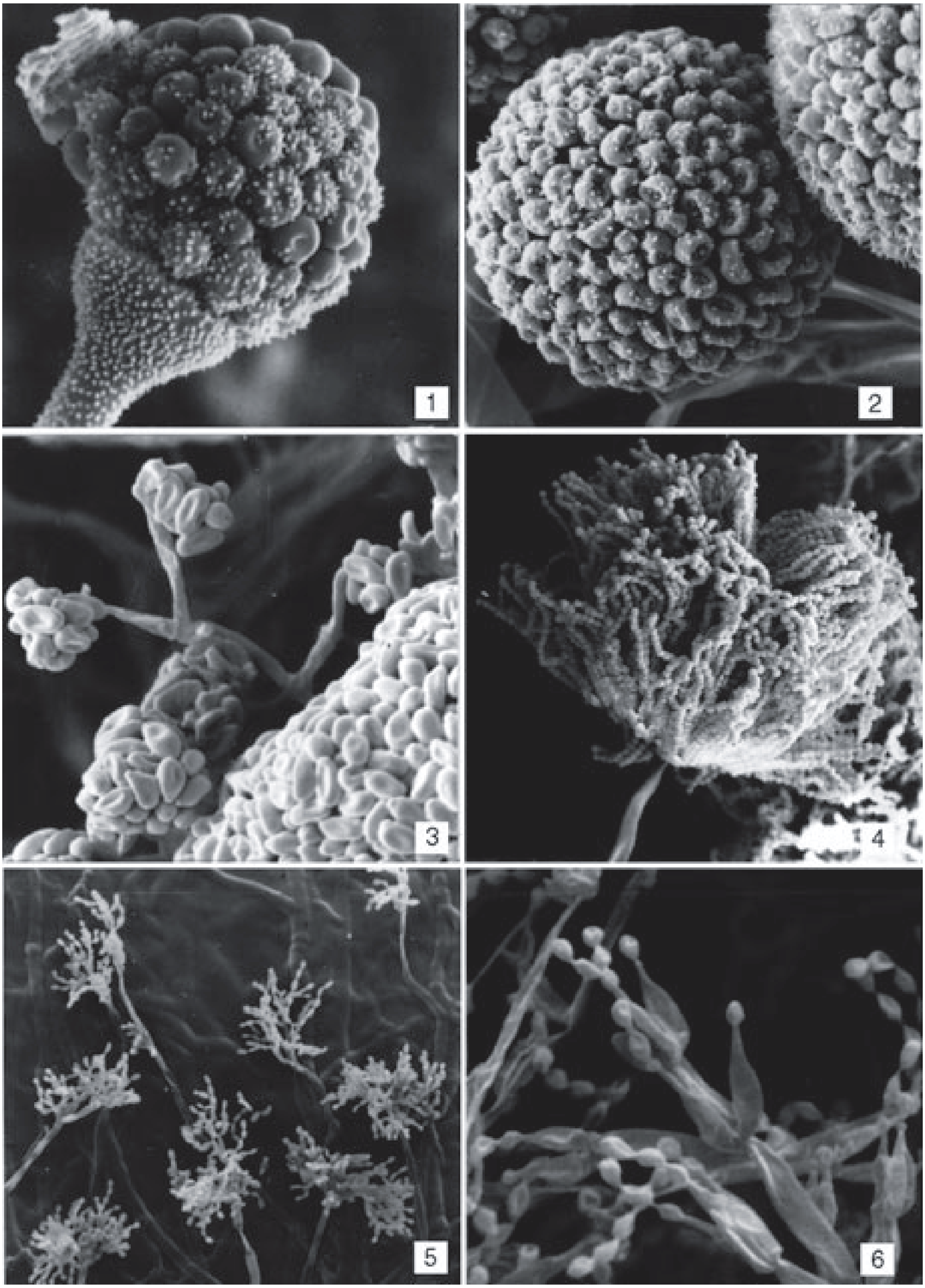


**Plate I: Fig. 1.** *Absidia* sp., (Zygomycotina), 3900×. **Fig. 2.** *Zygorhynchus* sp., (Zygomycotina), 1300×. **Fig. 3.** *Acremonium* sp., (Deuteromycotina), 250×. **Fig. 4.** *Aspergillus* sp., (Deuteromycotina), 640×. **Fig. 5.** *Cladosporium cladosporioides*, (Deuteromycotina), 440×. **Fig. 6.** *Paecilomyces* sp., (Deuteromycotina), 2200×.


**Plate II: Fig. 1.** *Penicillium* sp., (Deuteromycotina), 1600×. **Fig. 2.** *Penicillium vulpinum*, (Deuteromycotina), 40×. **Fig. 3.** *Chaetomium* sp., (Ascomycotina), 90×.

and xylanase activities were explicitly different between the groups of the unaffected and the affected topsoils. Their measured values were higher than those of CMC activity. Low CMC activities were observed at all places (37.4–303.9 μg GLC · $g^{-1}$ dry soil · $(24\ h)^{-1}$). The differences between the studied groups of undamaged and devastated soils were not ascertained (Fig. 2). It is known that the decomposition of cellulose is rather slow in the soil, whereas, sugars, carbohydrate polymers as well as starch, pectin, and hemicellulose decompose more rapidly (Lähdesmäki & Piispanen 1988).

A large amount of energy within the soil is accumulated in the form of humic substances, plant tissue residues, and as microbial biomass, too. The microbial biomass is important in the cycling of matter and energy and it determines the character and the intensity of biological processes within the soil (Bernát et al. 1984). The amount of carbon in microbial biomass ($C_{bio}$ 7.2–14.3 mg · $(100\ g)^{-1}$ dry soil, Fig. 3) and the abundance of bacteria (0.0–1.4 · $10^4$ CFU · $g^{-1}$ dry soil,

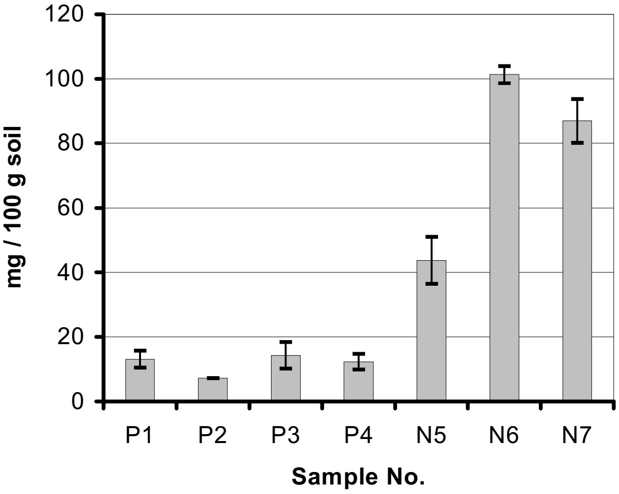


**Fig. 3.** The amount of carbon in microbial biomass.

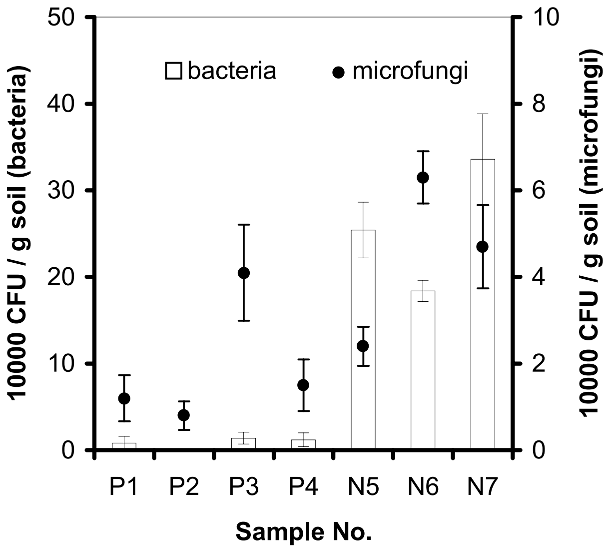


**Fig. 4.** Bacteria and microfungi abundances.

Fig. 4) determined at the affected places (P1–P4) were of lower values in comparison with those at unaffected places ($C_{bio}$ 43.7–101.3 mg· $(100g)^{-1}$ dry soil, the bacteria abundance 18.4–33.6 · $10^4$ CFU · $g^{-1}$ dry soil). The abundance of microscopic fungi (Fig. 4) was comparable in all samples (0.8– 6.3 · $10^4$ CFU · $g^{-1}$ dry soil). The sensitivity of microscopic fungi to decreased soil pH is lower than the sensitivity of bacteria (Holub et al. 1993).

Fifteen genera and species of microfungi were isolated and identified in the studied topsoils (Table 2, Pls. I–II). The highest number of genera and species was isolated at the places N5 (6), N6 (6), and N7 (8). The representatives of classes ZYGOMYCOTINA (*Absidia* sp. Tiegh., *Mucor* sp. P. Micheli ex St.-Amans, *Zygorhynchus* sp. Vuill.), DEUTEROMYCOTINA (*Acremonium* sp. Link ex Fresen., *Aspergillus* sp. P. Micheli ex Fresen., *Aureobasidium* sp. Viala ex Boyer, *Cladosporium cladosporioides* (Fresen.) N.F. de Vries, *Cladosporium macrocarpum* Preuss, *Geotrichum* sp. Link ex Léman, *Paecilomyces sp.* Bainier, *Paecilomyces lilacinus* (Thom) Samson, *Penicillium* sp. Link ex Fresen., *Penicillium vulpinum* (Cooke et Massee) Seifert et Samson, *Verticillium* sp. Nees ex Link) and ASCOMYCOTINA (*Chaetomium* sp. Kunze ex Fresen.) were determined. The representatives of the class Zygomycotina exist in the topsoils and they require sufficient amounts of easily decomposable organic substances. Their absence reflects the insufficiency of soil organic matter and the deterioration of decomposing processes at the places P2 and P3. *Penicillium* sp. and *Cladosporium cladosporioides* of the class Deuteromycotina were found at almost all affected places. The survival of the representatives of DEUTEROMYCOTINA under the physiologically extreme conditions confirms their high adaptability to the adverse soil environment.

**Table 2:** Species and genera of soil micromycetes observed in the studied soils.

| Micromycetes | Sample No. | | | | | | |
|---|---|---|---|---|---|---|---|
| | P1 | P2 | P3 | P4 | P5 | P6 | P7 |
| ZYGOMYCOTINA | | | | | | | |
| *Absidia* sp. | | | | | + | + | + |
| *Mucor* sp. | + | | | + | | | |
| *Zygorhynchus* sp. | | | | | | + | |
| DEUTEROMYCOTINA | | | | | | | |
| *Acremonium* sp. | | + | | | | | + |
| *Aspergillus* sp. | | | | + | | | |
| *Aureobasidium* sp. | + | | | + | | + | + |
| *Cladosporium cladosporioides* | + | + | + | + | + | | |
| *Cladosporium macrocarpum* | | | | | + | | |
| *Geotrichum* sp. | | | | | + | | |
| *Paecilomyces* sp. | | | + | | | + | + |
| *Paecilomyces lilacinus* | | | | | | | + |
| *Penicillium* sp. | + | | + | + | | + | + |
| *Penicillium vulpinum* | | | | | | | + |
| *Verticillium* sp. | + | | | | + | | + |
| ASCOMYCOTINA | | | | | | | |
| *Chaetomium* sp. | | | | | + | + | |

## Conclusions

The observed degradation processes represent the extreme case of soil acidification. It has been found that the chemical deterioration within the investigated soils (extremely low pH, high contents of exchangeable aluminium) was accompanied by significant changes in the soil's microbiological properties. The inhibition of the microbial activity, as the soil respiration or the enzyme activities, was observed in the soils influenced by the acid sulphate weathering. The microbial biomass, the abundance of microorganisms, as well as the diversity of microscopic fungi genera were reduced in the affected soils, too. Only tolerant groups of microorganisms survive in the chemically degraded soils on the slope of Šobov hill.

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*Note:* This paper was presented at the Conference of the IGCP Project #405 — “ENVIVEATH”, held in Bratislava from 24th to 26th November, 1997