THE MICROBIAL ACTIVITY IN SOIL FROM AN ABANDONED INDUSTRIAL AREA IN POLAND

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Abstract
The area around closed (since mid 90 ties) Chemical Plant in Tarnowskie Gory has been qualified as a zone of high contamination (contaminated industrial megasite). For decades soil had been polluted with above 1,7 mln. tons of hazardous waste containing: Ba, B, Zn, As, Sr, and many others. This paper presents the investigations focused on the activity of microbial communities naturally occurring in these soils. The samples of soil were taken from 21 points (16 from polluted area and 5 at a distance of ca. 1 km from dumping sites). The total number of aerobic bacteria (psychrophiles), fungi, ascomycetes, and enzymatic activities (dehydrogenase, cellulase, amylase and protease) were analyzed under laboratory conditions. Simultaneously, some abiotic parameters (humidity, pH, Eh, sorption capacity, contents of humic acids), and contents of heavy metals were measured. The results indicate that soils in vicinity of the chemical plant (as compared to non-polluted soil) are generally characterized by comparable values of moisture, pH, Eh, sorption capacity, but lower contents of humic acids, number of colonies of microorganisms and enzymatic activities. However, a direct correlation between high contents of heavy metals and low activity of microorganisms was not classified.

Introduction
From different elements of biosphere, soils occupy a special position. They are not only the center of accumulation and transformation of many chemical and biological compounds, but also a protective filter against migration of different compounds to groundwater. The multifunctional character causes opposite biological processes to run simultaneously, e.g. aerobic and anaerobic processes, synthesis and decomposition, oxidation and reduction. Due to specifically properties (every pellet is a special biotop with different constitution), soil is a place of existence of microorganisms such as: bacteria, fungi, algae, actinomycetes. Only the healthy soil is able to play an essential role in functioning of natural ecosystems, including microorganisms, plants, animals and humans. Because soil environment is complex, the impacts of pollutants can be extremely diverse, ranging from a directly toxic effect to particular genus of soil-inhabiting microorganisms, plants or animals, to many indirect effects, which determine biochemical processes in soils.
The relative effects of heavy-metal contamination on biogeochemical cycles can be determined by comparing microbial biomass and activity in contaminated and not/less contaminated soils. Different studies have reported reductions in microbial biomass and/or enzyme activities for acidic forest soils exposed to the long-term deposition of metals from smelters (1), or for agricultural soils treated with metal-containing sewage sludge (2).
Soil enzymes have been proposed as useful indicators of soil quality, because of their essential role in soil biochemistry, a relative ease to estimate and rapid response to changes of soil management (3). All biochemical transformations are closely related to, and even dependent on enzymatic activities. Such investigations may also show the differences in the microbial dynamic and population (4). However, not many studies are carried out on microbial communities and their activities with long-term exposures for heavy metals present in soils at high contents (5, 6).

This paper presents the investigations focused on evaluating the activity of microbial communities naturally occurring in vicinity of a chemical plant in Tarnowskie Góry. The total number of aerobic bacteria, fungi, ascomycetes, and the enzymatic activities (amylase, cellulase, dehydrogenase and protease), were analyzed under laboratory conditions. Simultaneously, some abiotic parameters (moisture, pH, Eh, sorption capacity, content of humic acids), and contents of Zn, Ba Fe were also measured.

Materials and methods

Site description and soil collection: The soil samples were taken at a depth below 100 cm from highly Zn, Ba and Fe contaminated soil in vicinity of the chemical plant in Tarnowskie Gory (South Poland): 16 samples came from directly contaminated places around dumping sites, and 5 were taken at a distance of ca. 1 km from the chemical plant (reference/control soil).

Soil sample were thoroughly mixed and passed through a 2 mm sieve. Fresh soils were used to microbiological analysis, measurements of moisture, Eh and sorption capacity. A part of each sample was air-dried and stored at 4^oC before the following analyses were carried out: pH (soil-to-water ratio of 1:2.5), humic acid contents, enzyme assays and heavy metals contents.

Chemical analyses: The following methods were used: the gravimetric method to estimate humidity, the pycnometric method for measuring pH and Eh in H_2O, the GAAS to analyze heavy metal contents and the sequential extraction method for humic acid contents.

Microbiological analyses: Microorganisms were isolated using the dilution plate method: 1 ml of 10^3 dilution was plated on Petri plates containing the selective media: Plate count agar (Scharlau) for isolating of bacteria, Sabouraud dextrose agar containing gentamicin-chloramphenicol (Scharlau) for isolating of fungi, and Actinmomycete agar (Scharlau) for isolating of actinomyctes.

Enzyme assays: Dehydrogenase activity was determined according to Brzezińska et al. (7), protease and amylase according to Tabatabai (8), and cellulase according to Criquet (9).

Results

The pH of analyzed samples varied from 4.67 to 7.59 in polluted soils, and from 5.26 to 6.36 in control soils. The moisture was very different: from 3.1 to 29.5% (w/w). The Eh values varied from 422 to 658 mV. The sorption capacity ranged from 3.39 to 11.69 mmol/100g in polluted soils, and from 3.1 to 5.83 mmol/100g in control soils. The Zn, Ba and Fe contents were much higher in polluted soil as compared to a control.

The content of humic acids in polluted soils was even 10 times lower as compared to a control (Fig. 1); the lowest noted value in polluted soils was of 0.09%, whereas in a control of 1.29%.

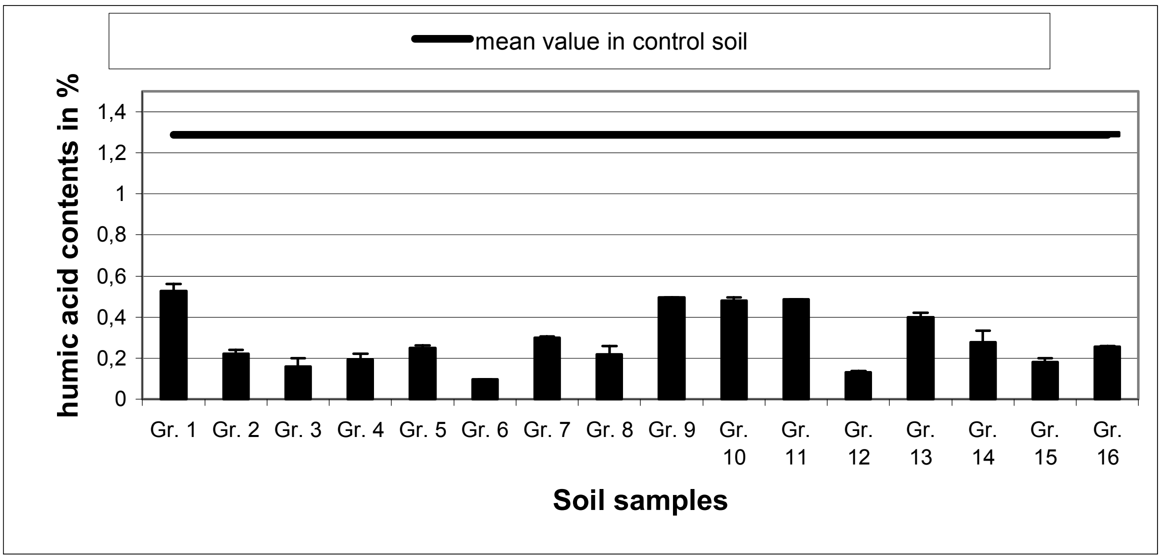

Fig. 1. The humic acid contents in polluted samples, as compared to a control

Similarly, in most cases the number of all analyzed microorganisms was lower, as compared to a control (Fig. 2). The number of psychrophiles colonies varied from 70×10^3 to 312×10^3 CFU, whereas in a control it was of 323×10^3 CFU. The number of actinomyces colonies varied from 0.70×10^3 to 18.2×10^3 CFU, as compared to the mean value of 10.7×10^3 CFU in a control. The number of fungi colonies estimated on the Sabouraud medium varied from 0 to 8.0×10^3 CFU (with the mean value equal to 1.8×10^3 CFU) in polluted soils, as compared to 7.7×10^3 CFU in a control. The fungal CFU noted on the Czapek-Dox medium ranged from 0 to 8.8×10^3 (the mean value was of 2.34×10^3 CFU), as compared to a control – 107.7×10^3 CFU.

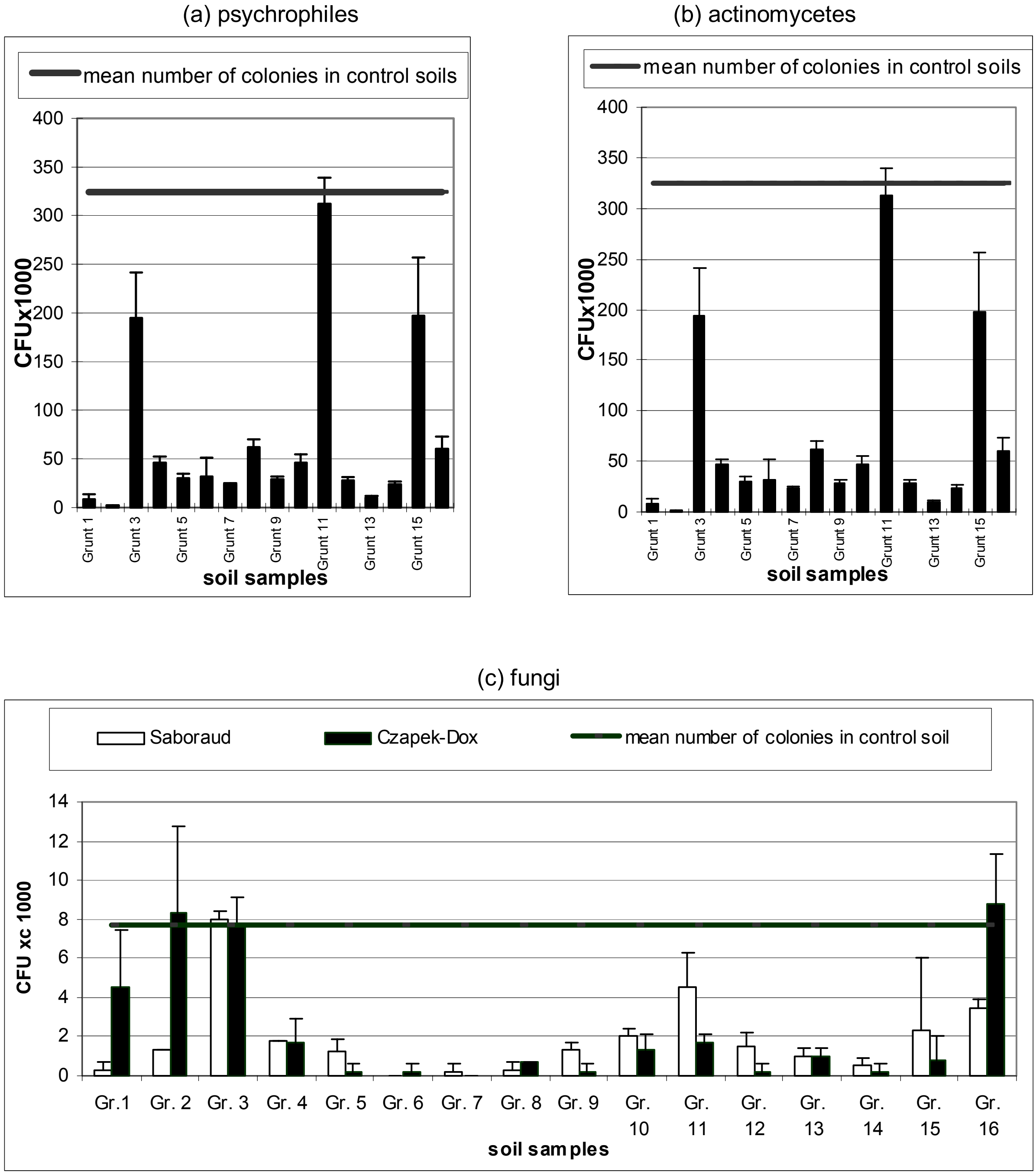

Fig. 2. The number of colonies of (a) psychrophiles, (b) actinomycetes, (c) fungi in soil samples

The enzyme assays (Fig. 3) generally showed lower enzymatic activities in polluted soils as compared to a control. In the case of amylase only one sample was characterized by a higher activity (10.2µg glucose/1g soil) than in a control (8.3µg glucose/1g soil), (Fig. 3d). The protease activity ranged in polluted soils from 4 to 495 µg amine nitrogen/1g soil, whereas in a control it was of 336 µg amine nitrogen/1g soil (Fig 3a). The dehydrogenase activity in polluted soil varied from 0.6 to 3.6 mgTF (only for one sample the value was much higher and equal to 29 mgTF). The mean activity in control soils was of 3.84 mgTF (Fig. 3b). In the contrary, the cellulase activity in polluted soils was comparable and sometimes even higher (up to 6.5µg glucose/1g soil) than in a control (4.06 µg glucose/1g soil), (Fig. 3c).

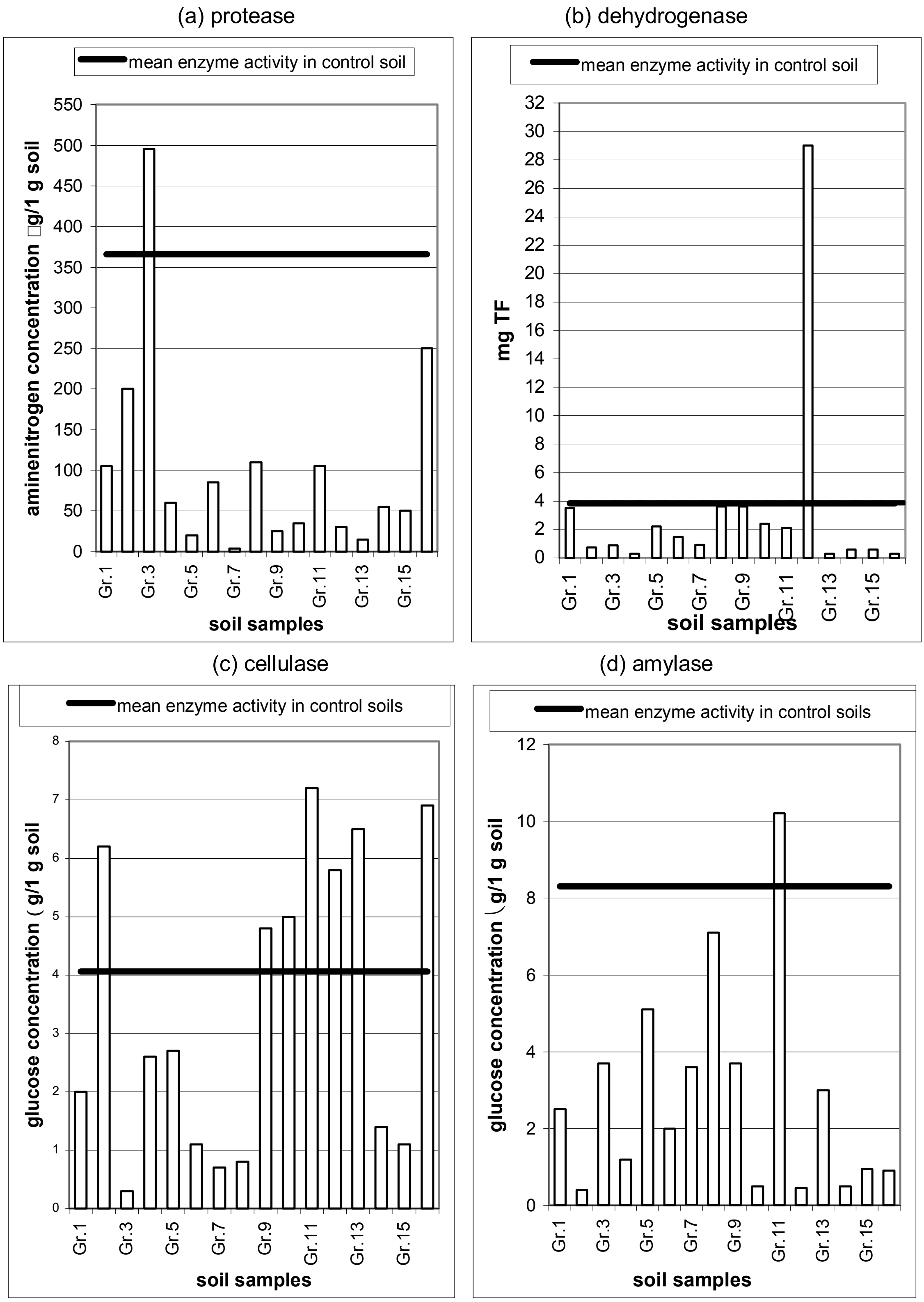

Fig. 3. The activity of (a) proteases, (b) dehydrogenases, (c) cellulases and (d) amylases in soil samples

Discussion

Microorganisms play a significant role in soil, though their biomass contributes less than 5% to the soil organic material. The results of investigation showed that polluted soils in vicinity of the chemical plant contain less number of microorganisms, as compared with a control. As an average, in polluted soils the number of psychrophiles, fungi and actinomycetes, was of 68%, 32% and 60% lower, respectively, as compared to a control. These results confirm that high metal concentrations negatively affect the frequency of microorganisms in soil. Metal ions may inhibit enzyme reactions by complexing the substrate, reacting with the protein-active groups of enzymes, or with the enzyme-substrate complexes (10). The negative effect of Zn, Ba and Fe (at high contents) on correct functioning of enzymes was also observed in presented investigations. Generally, in polluted soils compared to a control, lower activities of 78% (proteases), 75% (amylases) and 8% (dehydrogenases and cellulases) were observed. As reported by Belistyna et al. (11), a decrease of dehydrogenase activity was observed with increasing contents of Pb, Zn and Cd. In presented results however, a direct correlation between high contents of heavy metals and low enzyme activities was not clear.

Conclusion

Long-term influence of chemical wastes deposited in soil caused the decrease of activity of indigenous microorganisms. As compared to non-contaminated soil, the lower number of psychropiles, fungi and actinomycetes, and lower amylase, cellulase, dehydrogenase and protease activities were observed.

Acknowledgements

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References:

1. A. NORDREGEN, T. KAURI, E. BÅÅTH and B. SÖDERSTRÖM, Soil microbial activity, mycelial lengths and physiological groups of bacteria in a heavy metal polluted area. Environmental Pollution **41**, 89-100 (1986).
2. K. CHANDER, J. DYCKMANS, R.G. JOERGENSEN, B. MEYER and M. RAUBUCH, Different souces of heavy metals and their long-term effects on soil microbial properties. Biol. Fertil. Soils. **34**: 241-247 (2001).
3. R.RIFFALDI, A.SAVIOZZI, LEVI-MINZI and R. CARDELLI, Biochemical properties of a Mediterranean soil as affected by long-term crop management systems. Soil & Tillage research **67**: 109-114 (2002).
4. A.K.BANDICK and R.P. DICK, Field management effects on soil enzyme activities. Soil Biol. Biochem. **31**: 1471-1479 (1999).
5. J.L. BADER, G. GONZALEZ and P.C. GOODELL, Chromium-resistant bacterial populations from a site heavily contaminated with hexavalent chromium. Water, Air, Soil Pollut, **109**: 263-276 (1999).
6. W. SHI, M. BISCHOFF, R. TURCO and A. KONOPKA, Long-term effects of chromium and lead upon the activity of soil microbial communities. Applied Soil Ecology **21**: 169-177 (2002).
7. M. BRZEZIŃSKA, Z. STĘPNIEWSKA and W. STĘPNIEWSKI., Soil oxygen status and dehydrogenase activity. Soil Biol. Biochem. **30** (13): 1783-1790 (1998).
8. M.A. TABATABAI, Soil enzymes. In: Page A.L., Miller R.H., Keeney D.R. (Eds.) Methods of soil analysis. Part 2. Chemical and Microbiological Properties. Soil Science. Society of America. Madison. WI, pp. 907-943 (1997).
9. S. CRIQUET, Measurement and characterisation of cellulose activity in sclerophyllous forest litter. J. Microb. Met. **50**, 165-173, (2002)
10. S.P.DENG and M.A.TABATABAI, Cellulase activity of soils: effect of trace element. Soil Biol. Biochem. **27(7):** 977-979 (1995).
11. G.D. BELISTYNA, N.Y. DRONOWA, I.N. SKVORVTOVA and L.N. TOMILINA, Alteration of certain indices of soil biological activity under antropogenic stress. Sov. Soil Sci. 21, 40-44 (1989).