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Kovacevic V., Antunovic M., Bukvic G., Rastija M., Kadar I.: Vplyvy pôdy a genotypu na stav ťažkých kovov v kukurici.

Na vápnitej fluvizemi (caFL) a stagnickej bielej luvizemi (stAB) sme počas dvoch vegetačných období v štyroch opakovaniach sledovali desať kukuričných hybridov, ktoré v terénnych podmienkach rástli na dvoch pôdach.

Obe pôdy ležia v údolí Drávy a od seba sú vzdialené asi na 2 km. Koncentrácie kadmia, olova, chrómu a niklu v šúpolí a v pôde sme namerali technikou ICP-AES po mikrovlnovom vylúhovaní pri použití koncentrovaného HNO₃+H₂O₂. Výnos kukurice na stAB bol o 25% nižší ako na caFL. Podobné rozdiely sme zistili aj medzi hybridmi. Vo všeobecnosti sme nízke koncentrácie ťažkých kovov našli v kukurici s významnými rozdielmi medzi hybridmi (mg.kg¹ v sušine): od 0,112 do 0,224 (Cd), od 0,73 do 1,04 (Pb) a od 0,303 do 0,391 (Pb), kým hodnoty Ni boli podobné (stredná 1,58). Tieto hodnoty z aspektu škodlivých vplyvov na prostredie sú veľmi nízke. Preto produkcia zdravých potravín je v Chorvátsku možná. Vypestovaním genotypu charakterizujúceho nízky príjem ťažkých kovov, hlavne v kontaminovaných podmienkach, možno zmierniť environmentálny problém.

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Abstract

Šimonovičová A., Krnáčová Z., Pavličková K., Beňová A.: Microbiological characterization of the soil influenced by the negative anthropization. Ekológia (Bratislava), Vol. 23, No. 1, 71-79, 2004.

In the contribution we have dealt with a long-term negative influence on anthropogenic activities on microbial character of soil in the studied territory – Nováky region. The affected area belongs to region with the most important energetic raw in Slovakia, where one of the biggest fuel – energetic complex is build. The main environmental problems of the region are: the air contamination, the surface water contamination, and also the problematic of arsenic. The source of arsenic is the brown coal from Nováky mine. The samples for microbiological analysis were sampled from the Novaky mine – from the brown coal and mound in the depth 0-5 cm, 20-30 cm. The species from kind Aspergillus, Penicillium and Paecilomyces were dominant. Species Paecilomyces lilacinum and Scopulariopsis brevicaulis have the ability of methylation As from inorganic into the organic form.

Key words: environment, Nováky region, arsenic, soil microscopic fungi, methylation

Introduction

In accordance to the geomorphologic classification of Slovakia the studied territory belongs to Hornonitrianska basin (Upper Nitra basin), exactly to its partial basin – Prievidza basin. Hornonitrianska basin forms branched ditched founder between the arch of cored mountains (Strážov mountain, Small Fatra, Žiar, Tríbeč) and volcanic mountains of Slovak upland (Kremnica upsides, Vtáčnik). Toward cored mountain it is limited with heavy faults,

passage into volcanic mountains is partially erased by widespread block drifts. It is divided to four geomorphologic subunits. The bed of basin is filled with neogene and post – tertiary accumulation with seams of coal and lignite. In the region the most important sources of energetic raw of Slovakia are founded. They are coal deposition in Nováky and Handlová. Both deposits consist the most valuable raw in the region.

Within the studied region we are able to identify several expressional georelief elements, which predetermined soil – parent materials complexes:

- Fluvial flat of flood plain of river Nitra (part of Hornonitrianska basin) with fluvial Sandy Gravel non-calcaric sediments and with clay loam overlay with above all developed Eutric Fluvisols and Gleyic Eutric Fluvisols which passes along the whole affected territory of the region. It is relatively most productive region in the territory.
- It is in the climate area with following characteristics: warm, dry, uplands, TS ≥ 10°C: 2800-2500, td ≥ 5°C days: 231, moisture characteristic to VI-VIII in mm: 150-100.
- Moderately, medium or strong dissected uplands (proluvial fluvial upland, part of Hornonitrianska basin) with polygenetic and loess sediments (loess clays and loess polygenetic so mixture clay – loam sediments, etc.) with dominant occurring of Eutric Planosols, with local presence of Haplic Gleysols. Within the studied territory it is relatively low productive soil stand.
- It is in the climate area with following characteristics: from warm, dry, uplands, TS ≥ 10°C: 2800-2500, td ≥ 5°C days: 231, moisture characteristic to VI-VIII in mm: 150-100 to moderately warm, moderately dry, TS ≥ 10°C: 2500-2200, td ≥ 5°C days: 215, moisture characteristic to VI-VIII in mm: 100-0.
- Strong dissected uplands build up by ash rocks with layers of tuffs and pyroxenic andesites (Vtáčnik High Vtáčnik) overlaid by mixture of deluvial sediments with above all occurrence of Dystric Cambisols, Stagnic Cambisols and Luvic Cambisols, local with Planosols. They are medium or less productive stands.

It is in the climate area with following characteristics: moderately warm, moderately dry, TS \geq 10°C: 2500-2200, td \geq 5°C days: 215, moisture characteristic to VI-VIII in mm: 100-0.

(Explanations: TS \geq 10°C – the sum (value) of average annual temperatures above 10°C td \geq 5°C – the length of the season with temperatures above 5°C in days to VI- VIII – climate index of irrigation under Budyk calculated for Slovakia by Tomlain, 1980 (the difference between potential evaporation and precipitation in mm.)

Affected region is otherwise natural, seattle and internal non-homogeneous, but in its planar greater part is heavy influenced by strong concentration of residential areas, industrial areas, areas of animal production and local dense communications. These factors are leading to its significant degradation of the quality of the environment with its negative impacts on its partial components so on residential and working occupation in municipalities, or agriculture, forest or recreational landscape outside of mucipality.

Generally, from the point of you of its quality 5 classes of environment are differential in Slovakia. The most part of analyzed region follows under the worst levels – 4 and 5, so under the region with strongly or extremely disturbed environment (Ministry for the Environment of the Slovak Republik, 2000).

Upper Nitra has very important economical position. One of the most important fuel—energetic complexes in Slovakia based on extraction and burning of brown coal is situated here. Many other industrial and manufacture branches are linked on it. Fundamental causes of the changes of quality are human activity within particular industrial activities. Impacts are displayed in many forms as air contamination, surface and underground water contamination, soil contamination, actions on relief, whether open or underground extraction, degradation of landscape, deforestation, etc. Collapse areas in regions of old mining activities with closed mining yield together with present coal extraction basically influence area between Prievidza and Novice towns. Negative changes in the state of environment elements are reflecting aggravate state of human health.

Main environmental problems of the territory

Important and well-known environmental problem of the territory is contamination of air, what is clear by the fact, that under former Declaration of the Ministry for Environment of the Slovak Republic No. 112/1993 was Upper Nitra the region with special protection of air. Even if in present it is 5-multipoind decreased of emissions from the enterprise Slovak Power Station situated in Zemianske Kostol'any, it is still the greatest source of sulfur oxide in Slovakia (18% of state emissions in 2000 year). In last two decades was followed decrease of region. In Upper Nitra is also found high air dust pollution. High dustiness has the origin in landfills of loose materials, sludge, build and mining activities, agriculture, traffic and small sources. Special case of air contamination is ground ozone which critical level used to be exceeded here (Ondrušová et al., 2002).

Another important environmental problem of the affected area is contamination of surface water, which shows the strong demonstration of anthropogenic contamination, which is produced by the greatest contaminators. They have in the region practically all indexes in worst classes – in IV and V class of quality, even if this situation in some indexes is improving (oxygen regime, biological indexes).

It is not possible to ship other environmental problem – the problem of arsenic. The source of arsenic in the whole region is the brown coal from Nováky mine. Anthropogenic source of arsenic is indirectly caused by coal extraction a directly by mass burning (it is certain reserve in improving the present technologies, which could be reached also by loading the products after burning).

Soils contaminated by As are relatively frequent. In region of Upper Nitra was growth of skin diseases and trouble of digestive system in 60-ies as a result of an air contamination with high presence of arsenic caused by coal burning (Čurlík, Ševčík, 1999).

Content of arsenic in soils exceeds an allowed limit. As demonstration of them could be shown results from geochemical monitoring of soils in Slovakia in 1991-1995 years (Čurlík, Ševčík, 1999) (Table 1). Limits and allowed thresholds of element/matter concentration in soil environment are based on the Declaration of Ministry for Land Management No. 531/1994-540 on the greatest allowed thresholds for some risk chemical elements in agriculture

Table 1. Total content of arsenic in studied territory

| Element | Present state [mg.kg ⁻¹] A horizon | Reference value A [mg.kg ⁻¹] | Reference value A ₁ [mg.kg ⁻¹] | Indicator value B [mg.kg ⁻¹] |
|---------|--|--|---|---|
| As | 16.2 – 58.3 (7.2-9.9) | (29.0) | 5.0 | 30.0 |

A – reference value – soil is not contaminated, if the concentration of the element is below this value; A_1 – reference value in relation to value A for determination of risk matters in 2M HNO₃ extractant; B – indicator value means, that contamination of the soil is analytically demonstrated

soils of Slovakia and on determination of competent institutions to find out actual values of these matters.

The toxicity of arsenic is connected with its mobility in soil—parent complex. On the basis of evaluation of the soil vulnerability according to arsenic contamination we could assumed to which measure abiotic characteristics are causing the toxicity of studied element.

At evaluation of the soil vulnerability according to their contamination it is necessary to consider two main groups (Čurlík, Fiala, 1991):

• soil – substrate complexes with low capacity of accumulation as it is in sand acid soils

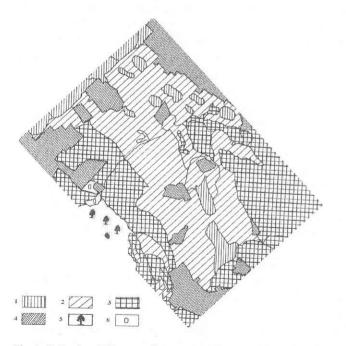


Fig. 1. Soil vulnerability according to arsenic contamination. Legend: 1 - high vulnerable soils, 2 - medium vulnerable soils, 3 - little vulnerable soils, 4 - municipality, 5 - forest, 6 - uncovering.

with law content of organic matters, law sorptive and buffer capacity

• soil—substrate complexes with high capacity of accumulation as it is in loam and clay-loam soils, with high content of organic matters, high buffer capacity; as higher is this capacity, so more chemical components could be bounded, what could on the other hand caused release of this element into the environment as a result of environmental changes.

On the Fig. 1 we are presenting placement of main soil types in the affected territory according to their vulnerability according to the arsenic contamination.

Arsenic and selected microbiological characterization of the soil

As it was written above, the source of arsenic in the whole territory is a brown coal from Nováky mine. Ondrušová et al. (2002) confirm that average content of arsenic in dry matter was 900 ppm in the brown coal. Anthropogenic source is localized in region Chalmová – Bystričany – Dolné Lelovce – Zemianske Kostoľany with values of As from 51 ppm till 388 ppm. Other region is Cigeľ – Koš – Nováky with values of arsenic from 49.8 ppm till 256 ppm (Ondrušová et al., 2002). From ecological point of view contamination of arsenic in rock milieu for its hygienically – toxicological impact on human population has the greatest importance. By using the suitable technology it is possible to eliminate this impact.

The brown coal in Nováky contains (in mg.kg⁻¹): Cr 27, Cd 21, As 587, V 16, Sr 74, Pb < 8 and brown coal from Cigel' contains: Cr 81, Cd < 2, As 43, V 68, Sr 68 and Pb 17. So according to arsenic, the ash from Nováky coal after burning in electric power station is for the environment more risk as from the Cigel' coal (Bedrna, 2002).

Methods

Methods used for chemical analysis: pH (H₂O and KCl) (Hraško et al., 1962); %C_{ox} by Walkey-Black method modified by Novák and Pelíšek (Klika et al., 1954); %N_{tot} analysed by Jodlbauer (Kopčanová et al., 1990); % of organic matter (Sotáková, 1982). Total contents of metals and phosphorus were determined by flamed AAS at Perkin-Elmer 1100 apparatus (USA) and by TMA-254 (Tesla-VÚCHT, ČR). All analysis were made at Department of analytical chemistry (Ing. V. Streško, PhD.).

Methods used for microbiologiacl analysis: abundance of bacteria and microscopic fungi by dilution plate method (10⁴ CFU) (Samson et al., 1996) cultivated on Sabouraud Maltose Agar (Himedia-Bombay) in the dark by 25°C; identification of microscopic fungi by Domsch et al. (1980).

Results and discussion

For the microbial analysis we had sampled patterns from the mound in Nováky mine area in the depth of 0-5 cm (mound 1) and in the depth of 20-30 cm (mound 2). Mound matter presents spoil which is overlaid by ground. Other sample represents the brown coal from Nováky mine. Patterns were sampled in 2002 year, when the actual contents of metals were analyzed (Table 2).

Both samples from the mound distinguished by acid right to neutral reaction, from the coal it is strong acid. High amount of C_{ox} especially from the coal appears from its organic origin (Table 3).

Heavy metals and toxic elements significantly influence the existence of live organisms – microorganisms as in positive so in negative importance. Their mechanism and character is changed in dependence of concentration, chemical binding and inter-relationship (Šimonovičová et al., 1997). Even if analyzed samples do not have soil character, they contain enough organic matter, which is a source for the existence of heterotrophic micro-

T a b l e 2. Actual contents of metals and phosphorus from coal and mound

| Sample | As [mg/kg] | Cd [mg/kg] | Pb [mg/kg] | P [%] | Hg [mg/kg] |
|-------------------|------------|------------|------------|--------|------------|
| Coal | 400.0 | < 1 | 6.8 | < 0.01 | 0.1 |
| Mound 1: 0-5 cm | 269.0 | < 1 | 15.1 | < 0.01 | 0.1 |
| Mound 2: 20-30 cm | 215.0 | < 1 | 18.6 | < 0.01 | 0.1 |

| Sample | Zn [mg/kg] | Cu [mg/kg] | Mn [mg/kg] | Fe [mg/kg] | V [mg/kg] |
|-------------------|------------|------------|------------|------------|-----------|
| Coal | 21.4 | 8.4 | 302.4 | 1.7 | 49.2 |
| Mound 1: 0-5 cm | 60.8 | 18.8 | 1163.0 | 2.6 | 104.0 |
| Mound 2: 20-30 cm | 70.5 | 18.8 | ,1775.0 | 4.6 | 116.5 |

T a b l e 3. Basic chemical characteristics of study samples

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| Sample | pH H ₂ O | pH KCl | C _{ox} [%] | N _{tot} [%] | C:N | % organic matter |
|-------------------|---------------------|--------|---------------------|----------------------|------|------------------|
| Coal | 3.3 | 2.9 | 39.0 | 0.6 | 63.9 | 67.2 |
| Mound 1: 0-5 cm | 6.9 | 6.55 | 8.4 | 0.2 | 40.0 | 14.5 |
| Mound 2: 20-30 cm | 6.8 | 6.9 | 8.2 | 0.2 | 35.6 | 14.1 |

T a b l e 4. Abundance of microorganisms in 10⁴.10 % C_{ox} in study samples

| Sample | В | MF | B:MF | ΣΜΟ |
|------------------|------|------|------|------|
| Coal | 15.3 | 33.1 | 0.5 | 48.4 |
| Mound 1: 0-5cm | 36.9 | 22.8 | 1.6 | 59.7 |
| Mound 2: 20-30cm | 55.5 | 39.0 | 1.4 | 94.5 |

B - bacteria, MF - microscopic fungi, B:MF - ratio of bacteria and microscopic fungi, ∑ MO - total amount of microorganisms

organisms - bacteria and micromycetes. Cu, Zn and As belong to biogene microelements, Cd, Hg and Pb belong to abiogene microelements (Wolf et al., 1985).

The ratio of bacteria and microbial fungi (B:M) in the coal is very balanced. In this sample, with acid nature, significantly are dominating acidotolerant micromycetes. Vice versa, mound matter with neutral soil reaction is considerable enriched by bacteria, the amount of microorganisms is increasing with the depth (Table 4).

Together 10 genera and 16 species of soil microscopic fungi were isolated from the samples of coal and mound (Beňová, 2003), (Table 5). They are systematically listed in class Mitosporic fungi. In particular, species of genera Aspergillus, Penicillium, Paecilomyces and Doratomyces stemonitis are dominant in analyzed samples, what is corresponding with works of other authors as in Slovakia (Bernát et al., 1984; Výbohová et al., 1997, 1999), so in the abroad (Marfenina, Mircink, 1988; Zhou, Kiff, 1991).

Many of mentioned metals (e.g. Pb, Cd, Cu and Hg) cause on te microscopic fungi in repressive way, they produce the changes in the growth of mycelium, they inhibit the production of biomass (Chorvátová, Vizárová, 1999), they cause deformations in reproducing structures (Azab et al., 1986: Ševc, Šimonovičová. 1999), and suchlike. But microscopic fungi are well-known as biosorbents of different chemical elements including heavy metals and toxic elements (Gharieb et al., 1999; Sag et al., 2000; Šimonovičová et al., 2002).

Mound Mound Coal Soil microscopic fungi 0-5 cm 20-30 cm Alternaria tenuis Aspergillus flavus Aspergillus niger Cladosporium cladosporioides Doratomyces stemonitis Paecilomyces sp. Paecilomyces lilacinus Penicillium sp. Penicillium sp.(Biverticillata) Penicillium chrysogenum Penicillium simplicissimum Scopulariopsis brevicaulis

9

11

Table 5. Compositions of microscopic fungi in study samples

* occurrence of soil microscopic fungi

Stachybotrys chartarum

Trichoderma sp.

Trichurus sp.

Together: 16

Trichoderma viride

Directly in the biomass of micromycetes mycelia isolated from the coal and mound we had determined the amounts of heavy metals and toxic elements (Table 6).

Inspite of the values of arsenic in mound and coal highly increased the value C = 100 mg.kg-1, which needs sanitation transgression, its accumulation in the biomass of micromycetes mycelia is very law (Table 6). In this case, as at one hand the arsenic is in unavailable form for micromycetes (as auripigment As, S, in coal ash and as scorodit FeAsO4 in the mound), so at the other hand arsenic also liable to the wide competition of other

Table 6. Amounts of metals and phosphorus in mg/kg⁻¹ and in % in biomasss of microscopic fungi

| Sample | As [mg/kg ⁻¹] | Cd [mg/kg ⁻¹] | Pb [mg/kg ⁻¹] | P [%] | Hg [mg/kg ⁻¹] |
|---------------------------|------------------------------|------------------------------|------------------------------|----------|------------------------------|
| Biomass – coal | 0.04 | < 1 | 4.9 | < 0.01 | 0.7 |
| Biomass – mound 1: 0-5 cm | 0.45 | < 1 | 8.29 | < 0.01 | 7.0 |
| Biomass – mound 1: 0-3 cm | 0.23 | < 1 | 7.9 | < 0.01 | 0.8 |

| Sample | Zn [mg/kg ⁻¹] | Cu [mg/kg ⁻¹] | Mn [mg/kg ⁻¹] | Fe [mg/kg ⁻¹] | V [mg/kg ⁻¹] |
|-----------------------------|------------------------------|------------------------------|------------------------------|---------------------------|-----------------------------|
| Biomass – coal | 56.9 | 0.80 | 1.36 | 63.2 | 0.5 |
| Biomass – mound 1: 0-5 cm | 59.3 | 1.09 | 1.46 | 142.0 | 0.3 |
| Biomass – mound 2: 20-30 cm | 100.8 | 1.91 | 1.94 | 180.0 | 0.3 |

metals and elements, which are relatively light accumulated in the biomass of micromycetes (f.e. Pb > 50%, Zn - almost 100%, also Cu and Fe) (Šimonovičová et al., 2002). Bioaccumulation of heavy metals and toxic elements is decreasing in order: $Zn \rightarrow Hg \rightarrow Pb \rightarrow Cu \rightarrow Fe \rightarrow Mn \rightarrow As \rightarrow Cd \rightarrow V$.

By the species of microscopic fungi as *Scopulariopsis brevicaulis* and *Paecilomyces lilacinus* is confirmed their ability to methylating of As, it means, the ability to transformate inorganic form of As into the organic form, which is accessible for live organisms.

Summary

Biological accumulation is the process of binding the heavy metals and toxic elements from the environment into the biological matter of different origin, which could be micromycetes. After their adjustment – after their accumulation on suitable sorbate it is possible to assume their using in decontaminating an environment protection as a whole.

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V práci sa zaoberáme dopadom dlhodobého negatívneho pôsobenia antropogénnych aktivít na mikrobiologické vlastnosti pôdy v okolí mesta Nováky. Skúmané územie je najvýznamnejším zdrojom energetických surovín na Slovensku a taktiež je tu vybudovaný jeden z najvýznamnejších palivovo-energetických komplexov. K hlavným environmentálnym problémom územia patrí znečistenie ovzdušia, povrchových vôd a problematika arzénu, ktorého zdrojom je v celom území hnedé uhlie z Novák. Vzorky sme odoberali v areáli banského podniku z uhlia a z haldy (v hĺbke 0-5 cm a 20-30 cm). Z mikroskopických húb vo vzorkách dominovali predovšetkým druhy rodu Aspergillus, Penicillium a Paecilomyces, pričom druhy Paecilomyces lilacinus a Scopulariopsis brevicaulis sú schopné metylovať As z anorganickej formy na formu organickú.