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Effect of copper, zinc and lead acetates on microorganisms in soil

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The impact of a mixture of Cu, Zn and Pb acetates on several soil microbial groups in model columns filled with natural soil monoliths was investigated. Moraine sandy loam on clay loam *Calcari-Endohypogleyic Luvisol (LVg-n-w-cc)* 0–100 cm deep taken from the site located 350 meters from the roadside of Vilnius–Kaunas–Klaipëda highway (Kaunas distr., Giraitë) was used for the investigation. Metal acetates reduced the counts of all investigated microbial groups: organotrophic, spore-forming, mineral nitrogen assimilating, oligonitrophilous bacteria, actinomycetes and micromycetes. Actinomycetes, mineral nitrogen assimilating and oligonitrophilous bacteria were most susceptible to metals. The highest tolerance towards metal acetates was shown by micromycetes and spore-forming and cellulose-degrading bacteria. The influence of the test metals was most negative in the Btkg horizon 56–61 cm deep, where no actinomycetes, mineral nitrogen assimilating bacteria and oligonitrophylic bacteria were found.

Key words: soil, microorganisms, heavy metals

INTRODUCTION

Heavy metals are known as harmful pollutants in soil having a negative effect on soil biota including microorganisms. Some heavy metals, *e.g.* Pb, even at low levels are toxic, while others such as zinc and copper at low concentrations are essential for microorganisms (Hughes, Poole, 1989; Morselt et al., 1986; Ross, 1975). Nevertheless, high metal concentrations of the latter metals can exert a harmful effect on microorganisms. The physico-chemical properties of a particular environment determine metal speciation and consequently their biological availability and toxicity (Bååth et al., 1998; Babich, Stotzky, 1982; Duxbury, 1985; Martino et al., 2000; Vangronsfeld, Clijsters, 1994; Winkelmann, 1992). The presence of organic matter, other ions, clay minerals may reduce the toxicity of heavy metals. On the other hand, toxicity may be exerted also by many inorganic and organic metal complexes (Bewley, Stotzky, 1983). Additionally, mixtures of heavy metals often show a more pronounced effect on microorganisms (Vargha et al., 2002).

Heavy metals affect microorganisms in soil multisidedly: they shift the structure of microbial populations, impoverish their diversity, affect species composition, reproduction and activity of indigenous microorganisms (Arnebrant, Bååth, 1987; Frostegrad et al., 1996; Gadd, 1986, 1993). A general reduction in fungal numbers was notices in soils polluted with Cu, Pb, Zn and other metals (El-Sharouny et al., 1988; Gadd, 1993; Nordgren et al., 1983; Ruhling et al., 1984). Nordgren et al. (1983) reported that along a gradient of Cu and Zn in soil towards a brass mill, fungal biomass decreased by 75%. The numbers of bacteria *Rhizobium leguminosarum* were reduced by several orders of magnitude in soil polluted with Zn, Cu, Ni and Cd (McGrath et al., 1995). A harmful influence of heavy metals was observed on the physiological activity of microorganisms in natural habitats. A considerable reduction was reported in litter decomposition in sites polluted with zinc and other heavy metals (Freedman, Hutchinson, 1980; McGrath et al., 1995). They found an evident inhibition of N_2 fixation by free-living heterotrophic bacteria in soil polluted with Zn, Pb, Cd and Ni.

Many works deal with the effect of metals on microorganisms in the upper soil horizon, while not much investigations concern the metal impact on microbial groups in deeper horizons. The aim of the work was to evaluate the effect of the combined effect of Zn, Cu and Pb acetates on the survival of microbial groups in different soil horizons.

MATERIALS AND METHODS

Investigation site. Moraine sandy loam on clay loam *Calcari-Endohypogleyic Luvisol* (*LVg-n-w-cc*) was selected for the investigation. The representative experimental plot (10 × 10 m) was 350 m far from the roadside of Vilnius–Kaunas–Klaipëda highway (Kaunas distr., Giraitë). Soil samples from 0–10, 20–25, 39–43, 56–61, 78–83 and 95–100 cm layers (reflecting the main horizons of the soil) were taken for the assessment of microorganism distribution *in situ*. The description of the soil profile from the soil pit is presented in Table 1.

Table 1. **Description of the soil profile (350 meters from the roadside of Vilnius–Kaunas–Klaipëda highway, Kaunas distr., Giraitë)**

Horizons	Description of soil
Ap 0–30 cm	Dark grayish brown with yellow shade (10YR 4/2), fluffy dampish sandy loam with medium crumby structure
Ael 30–37 cm	Yellowish brown (10YR 5/4), fluffy dampish sandy light loam with medium cloddy structure
ElBt 37–50 cm	Brownish yellow (10YR 6/6), hard dampish sandy light loam with medium nutty structure
Btkg 50–72 cm	Yellowish brown (10YR 5/6), hard dampish sandy light loam with slaty–nutty structure
Bkg 72–93 cm	Brown with white spots and bluish fibres (7.5YR 5/3), hard dryish medium loam with medium prismatic structure
BCkg 93–120 cm	Brown with white spots and bluish fibres (7.5YR 5/4), hard dryish sandy clay loam with nutty–slaty structure

The soil Ap horizon pH was 6.9, base saturation 98.2%, organic C and total N content 1.47% and 0.14% phosphorus and potassium 113.0 and 71.0 mg kg^{-1} respectively, gleyic properties occurred in deeper layers – from the Btkg horizon >72 cm.

Arrangement and assessment of metal sorption in soil. To investigate metal sorption and metal impact on soil microorganisms, special columns were constructed. *In situ* four columns were filled with natural monoliths of soil and taken to a laboratory. Soil in three columns was treated with a mixture of Zn, Pb and Cu acetate solutions (1 g of each metal/l), and to the soil in the control column distilled water was added. The treatment was performed when a full sorption of metals in soil was achieved. Measurements of metals were conducted using an atomic absorption spectrometer (Perkin Elmer M403). Soil samples for detection of the total metal content were treated with HF, HNO_3 acids, and for mobile phase of metals 1N CH_3COONH_4 was used for extraction.

Isolation of microorganisms. The prepared suspension of soil (10 g/90 ml 0.9% NaCl), when necessary, was diluted with H_2O to concentrations of microorganisms proper for plating. Soil suspensions were plated on growth media suitable for specific microorganism groups in Petri dishes. The following microbial groups were investigated: organotrophic, spore-forming, mineral N assimilating, cellulose degrading and oligonitrophilous bacteria, actinomycetes and micromycetes. To cultivate these microorganisms, the following media were used: nutritive peptone agar (Oxoid) for organotrophic bacteria, starch–ammonia agar for bacteria assimilating mineral nitrogen and actinomycetes (Ñåãè, 1983), Eshbi medium for oligonitrophilous bacteria, a medium with cellulose as the only C source for cellulose-degrading bacteria (Ñåãè, 1983) and wort agar for micromycetes. To investigate spore-forming bacteria, soil suspensions were heated at 80 °C in a water bath for 10 min and plated on nutrient peptone agar (Oxoid).

RESULTS AND DISCUSSION

Distribution of microorganisms in the soil *in situ*. Results of the investigation of microorganisms distribution in the soil showed the highest numbers of microorganisms at a depth of 0–25 cm with a gradual decrease in deeper layers (Table 2).

Organotrophic bacteria represent a huge group of bacteria that use organic substances as a source of carbon and nitrogen. The total content of these bacteria was 2.12 mill. in the upper layer. A very similar number of these bacteria was found at a depth 20–25 cm, while in deeper layers bacterial count decreased significantly. A particularly sharp decrease (100 times) was noticed from the BCkg-56-61 to BCkg-78-83 layers. The count of organotrophic bacteria was lowest in the deepest 95–100 cm layer (5.78 thousand cfu/g dry soil).

Spore-forming bacteria in the investigated soil profile showed approximately by one order of magnitude lower counts than organotrophic bacteria. The highest counts (410–420 thousands cfu/g) were found at a depth of 0–25 cm while the lowest (600–900 cfu/g) at a depth of 78–100 cm.

Bacteria assimilating mineral nitrogen were detected in huge amounts in the Ap horizon. In the upper 0–10 cm layer their count was 108 mill. cfu/

Table 2. **Counts of microorganisms of different groups in the soil in model columns after treatment with copper, zinc and lead acetates**

Group of micro-organisms	Depth of soil samples, cm	Count of micro-organisms in natural soil, $\times 10^3$	Count of microorganisms in soil in model columns after 6-month exposure, $\times 10^3$				
			Control column, soil treated with H_2O	Soil treated with heavy metals			Percentage of counts (average) compared with the control, %
				1 column	2 column	3 column	
Organotrophic bacteria	0–10	2128.0 ± 680.5	7157.0 ± 596	266.0 ± 11.5	83.31 ± 24.74	155.5 ± 24.5	2.35
	20–25	2117.0 ± 895.0	1736 ± 388	57.70 ± 11.4	85.79 ± 19.70	114.6 ± 21.9	4.94
	39–43	327.7 ± 81.0	302.5 ± 36.0	23.90 ± 4.4	0.63 ± 0.25	47.50 ± 6.8	7.93
	56–61	129.8 ± 13.7	469.0 ± 89.9	4.8 ± 0. 8	0.18 ± 0.02	0.42 ± 0.05	0.38
	78–81	6.2 ± 0.6	212.4 ± 14.5	0.37 ± 0.0 3	0.18 ± 0.05	2.4 ± 1.7	0.98
	95–100	5.8 ± 1.6	296.4 ± 40.7	0.24 ± 0.01	0.27 ± 0.1	1.19 ± 0.76	1.38
Spores-forming bacteria	0–10	410.24 ± 31.8	6145 ± 1565	1476.0 ± 84.0	50.99 ± 5.46	27.20 ± 17.36	12.25
	20–25	420.49 ± 79.9	2312 ± 405	9945.0 ± 1622.0	2.89 ± 0.26	5.2 ± 2.6	144.53
	39–43	9.28 ± 2.0	89.1 ± 14.9	16.10 ± 1.63	0.12 ± 0.005	0.05 ± 0.004	603.40
	56–61	1.4 ± 0.5	59.4 ± 7.2	0.22 ± 0.03	54.0 ± 10.0	0.06 ± 0.002	1.85
	78–81	0.6 ± 0.07	6.30 ± 1.0	0.21 ± 0.05	0.21 ± 0.03	0.002 ± 0.007	23.8
	95–100	0.9 ± 0.1	4.70 ± 0.2	0.12 ± 0.01	0.11 ± 0.01	1.74 ± 0.34	138.30
Mineral nitrogen assimilating bacteria	0–10	108570.0 ± 2300.0	318500 ± 23300	3964.0 ± 181.0	2226.0 ± 900	2188.0 ± 216.0	0.88
	20–25	78310.0 ± 3000.0	163300 ± 14200	945.0 ± 108.0	1353.0 ± 71.0	2349.0 ± 175.0	9.5
	39–43	138.3 ± 66.0	1400 ± 160	90.0 ± 4.4	153.2 ± 19.2	0	5.79
	56–61	12.9 ± 0.3	41.00 ± 1.60	0	0	0	0
	78–81	0.3 ± 0.2	1888 ± 156	0.035 ± 0.003	0	0.47 ± 0.01	0.95
	95–100	0.4 ± 0.1	2447 ± 170	0.33 ± 0.036	0	0.058 ± 0.008	0.05
Oligonitrophylic bacteria	0–10	46.1 ± 13.3	16.53 ± 2.04	0.229 ± 0.024	0.183 ± 0.008	0.131 ± 0.011	1.09
	20–25	41.1 ± 14.0	8.46 ± 0.66	0.188 ± 0.013	0.117 ± 0.013	0	1.46
	39–43	4.0 ± 1.2	1.20 ± 0.3	0.084 ± 0.005	0	0.072 ± 0.0065	2.33
	56–61	3.7 ± 0.8	1.0 ± 0.2	0	0	0	0.63
	78–81	0.67 ± 0.2	0.08 ± 0.01	0	0.015 ± 0.003	0.050 ± 0.0045	6.13
	95–100	0.09 ± 0.01	0.087 ± 0.015	0.011 ± 0.0015	0.0097 ± 0.0015	0	0

Table 2 (continued)

Cellulose utilising bacteria	0–10	6420.0 ± 148.3	0.047 ± 0.002	0.0029 ± 0.001	0.0055 ± 0.001	0.005 ± 0.0024	9.57
	20–25	3720.0 ± 87.1	0.022 ± 0.001	0	0	0.0033 ± 0.0005	5.0
	39–43	850.0 ± 110.0	0.017 ± 0.002	0	0.0008 ± 0.0006	0	15.89
	56–61	380.0 ± 9.6	0.0045 ± 0.0016	0.004 ± 0.0005	0	0	29.33
	78–81	120.0 ± 40.0	0.005 ± 0.0009	0.004 ± 0.0008	0.0033 ± 0.0005	0	43.00
	95–100	25.7 ± 10.3	0.008 ± 0.002	0.005 ± 0.0006	0.0012 ± 0.0006	2.69 ± 2.34	36.25
Actinomycetes	0–10	236230 ± 115.0	4480 ± 180	0	0	0	0
	20–25	14819.3 ± 208.0	14300 ± 370	0	0	0	0
	39–43	914.3 ± 17.3	15.0 ± 1.0	0	12.4 ± 3.5	0	0.27
	56–61	120.7 ± 15.3	44.2 ± 32.2	0	0	0	0
	78–81	0.9 ± 0.1	0.47 ± 0.17	0	0	0	0
	95–100	1.3 ± 0.1	0	0.032 ± 0.009	0	0	–
Micromycetes	0–10	3140.0 ± 464.0	255.6 ± 91.9	56.29 ± 2.56	31.81 ± 2.55	5.82 ± 0.27	12.25
	20–25	2240.0 ± 231.0	72.8 ± 42.4	8.04 ± 1.41	33.47 ± 8.13	17.49 ± 0.4	27.02
	39–43	64.8 ± 14.7	2.115 ± 0.49	0.21 ± 0.06	0.18 ± 0.03	0.072 ± 0.017	4.78
	56–61	16.35 ± 2.3	0.49 ± 0.14	0.048 ± 0.017	0.081 ± 0.014	23.5 ± 5.8	10.82
	78–81	5.9 ±.2.0	0.388 ± 0.057	0.04 ± 0.0068	0.014 ± 0.006	0.59 ± 0.12	13.66
	95–100	5.6 ± 1.1	0.206 ± 0.072	0.02 ± 0.0046	0.85 ± 0.28	0.12 ± 0.008	160.19

g, while starting from the depth of 39–43 cm their counts decreased and were lower than those of organotrophic bacteria. The lowest counts of bacteria assimilating mineral nitrogen (32–35 thousand cfu/g) were found at a depth of 78–100 cm.

Oligonitrophylic bacteria able to grow under conditions of nitrogen deficit were detected in low amounts: 46 thousand cfu/g in the upper layer and only 90 cfu/g at a depth of 95–100 cm.

Cellulolytic bacteria able to decompose cellulose were most abundant in the 0–10 cm horizon (6.4 mln. cfu/g). At a depth of 95–100 cm, the count of cellulolytic bacteria decreased by 10^2 – 25.7 thousand cfu/g.

The distribution of actinomycetes and bacteria in soil was similar. Very high numbers of these microorganisms (236 mill. cfu/g) was found in the upper layer. In deeper layers the count of actinomycetes decreased significantly; the decrease being especially sharp in BCkg-78–83 cm layer (only 900 cfu/g were detected).

Micromycetes in soil comprised from 3.14 mill. cfu/g at a depth of 90–10 cm to 560 cfu/g at 95–100 cm.

It should be mentioned that the decrease of counts of microorganisms from the upper to the lowest soil horizon differed among the groups of microorganisms. The highest decrease in counts was shown by actinomycetes and bacteria assimilating mineral nitrogen (10^6 times), while counts of the other groups of microorganisms decreased 10^2–10^3 times.

Sorption of heavy metals in the soil. The experiment on accumulation of zinc, lead and copper in soil showed that the highest amounts of metals were sorbed in the upper soil horizon rich in organic matter. The total content of zinc, lead and copper at a depth of 0–10 cm was 844.24, 838.76 and 773.38 mg/kg soil respectively (Fig. 1). An evident decrease in the total amount of metals started in the ElBt 39–43 cm horizon, and the lowest concentrations were detected in the BCkg 95–100 cm horizon –

410.69 (Zn), 278.33 (Pb) and 428.89 (Cu). The content of the mobile phase of the metals was much lower than their total concentrations. In Ap 0–10 cm horizon zinc, mobile lead and copper were detected at concentrations of 247.87, 239.17 and 209.93 mg/kg, while in the BCkg 95–100 cm horizon their amounts decreased to 111.67, 72.70 and 112.80 respectively. The total and mobile amounts of these metals were similar; only the concentrations of total and mobile lead were significantly lower than those of other metals in 39–43 and 95–100 cm soil horizons.

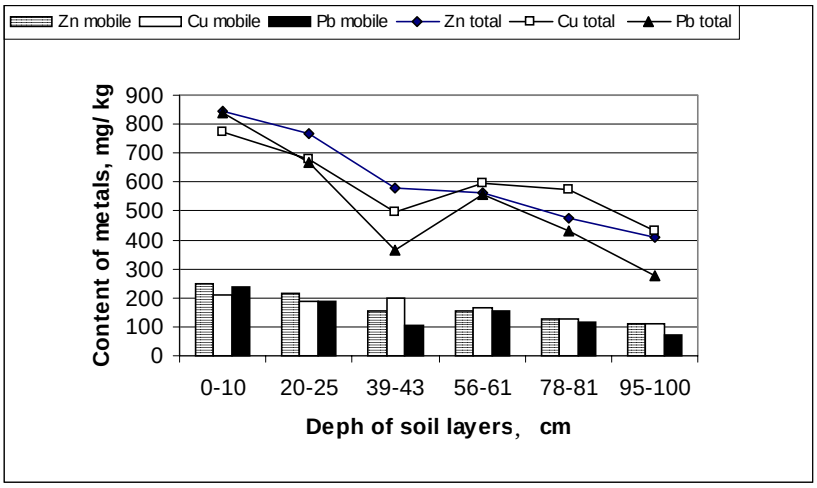

Fig. 1. Total and mobile copper, zinc and lead in soil layers in model columns (mg/kg)

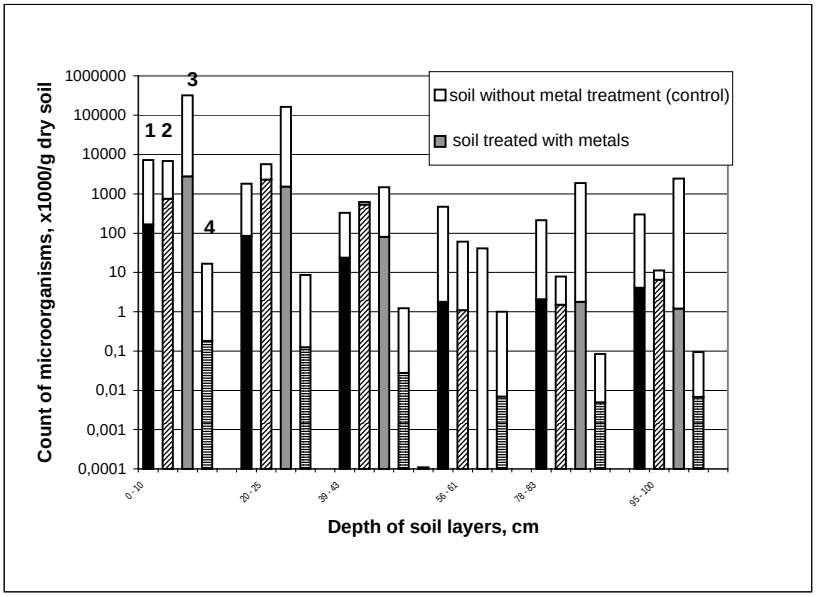

Fig. 2. Counts of organotrophic (*1*), spore forming (*2*), mineral nitrogen assimilating (*3*) and oligonitrophylic (*4*) bacteria in soil treated and untreated with copper, zinc and lead acetates

Impact of metals on microorganism counts in the soil in model columns. Exposure of microorganisms to copper, zinc and lead acetates resulted in a significant count decrease of all test groups of microorganisms. It should be noted that the counts of microorganisms in the control column during exposure also decreased in comparison with those in the natural environment.

In the soil of the control column treated with distilled H_2O the count of organotrophic bacteria reached from 7157.0 to 296.4 thousands cfu/g dry soil (Table 2). Copper, lead and zinc acetates reduced the amounts of organotrophic bacteria (Fig. 2). At a depth of 0–25 cm, bacterial counts after treatment with the metals reached only 2.35–4.94% of their counts in the control column. A slightly lower decrease of organotrophic bacteria as compared to the control was observed at a depth of 39–43 cm: they amounted to 7.93%. A more severe decrease in the counts was detected at a depth of 56–100 cm, where bacterial counts were reduced even by 3 orders of magnitude and reached only 0.38–1.38%. The differences between bacterial counts in different columns were more evident than in the upper layers; this could be related to differences in soil composition as well as the abundance of initial communities of indigenous microorganisms *in situ*.

Spore-forming bacteria in the control column were not affected negatively during the exposure; on the contrary, their counts were found about 10 times higher than those detected *in situ* and equaled to 6.1 mill. – 4.7 thousand cfu/g (Table 2). Under the influence of heavy metals, reduction of spore-forming bacteria was mostly noticed in soil layers at a depth of 0–10, 56–61 and 78–83 cm (Fig. 2). The lowest number of spore-forming bacteria was found at a depth of 56–61 cm – only 1.85% *versus* with the control. Meanwhile in the other soil layers an enhancement of bacterial counts was detected. A particularly high increase (up to 603.40%) of spore-forming bacteria was found in the 39–43 cm layer. Rather high amounts of these bacteria were found the 20–25 and 95–100 cm layers (144.53% and 138.30%, respectively).

Bacteria assimilating mineral nitrogen were severely affected by heavy metals (Table 2, Fig. 2). The most significant reduction in their counts was noticed in the upper 0–10 cm soil layer and in deeper horizons 56–100 cm deep. The content of these

bacteria in the 0–10 cm layer was only 0.88% in comparison with the control. The highest numbers of bacteria assimilating mineral nitrogen were found in the 20–43 cm layer (9.50–5.79%). These bacteria were totally absent in the Btkg 56–61 cm horizon; in the BCkg 95–100 cm horizon their count was reduced 10^4 times and in the soil treated with the metals decreased significantly (Table 2). The greatest reduction in their counts under the influence of heavy metals was found in the Btkg 56–61 cm layer, where bacteria were inhibited absolutely (Fig. 2). A rather significant reduction in the counts of these bacteria was noticed in the 0–43 cm layers where only 1.09–2.33% of bacteria survived. The survival of oligonitrophylic bacteria was higher at the depth 78–83 cm (6.13%), while in the 95–100 cm layer their amount decreased to 0.78%.

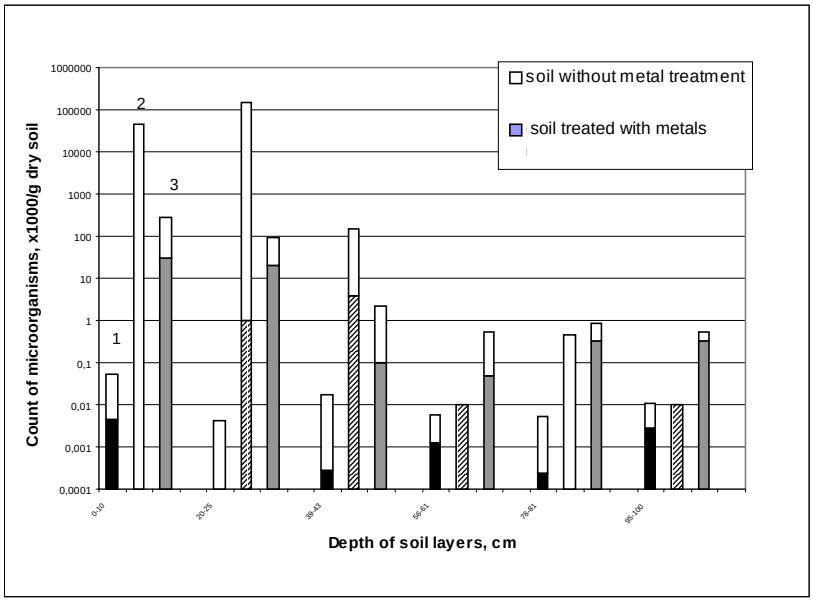

Fig. 3. Counts of cellulose degrading bacteria (*1*), actinomycetes (*2*) and micromycetes (*3*) in soil treated and untreated with copper, zinc and lead acetates

The number of cellulolytic bacteria in the columns was not high even in the soil treated with water (Fig. 3). Evidently conditions for this group of bacteria were unfavorable. Under the influence of heavy metals the number of cellulolytic bacteria decreased even more. The most significant reduction in the counts of these bacteria was found in the upper soil layers (0–25 cm), where they reached 9.57–5.0% in comparison with the control (Table 2). However, in the deeper soil horizons cellulolytic bacteria were comprised 15.89–43.0%.

Actinomycetes after treatment with heavy metals were inhibited most severely; they were not found in most of soil horizons (Fig. 3). These microorganisms were found only in two soil samples. In the 39–43 cm layer of soil the number of actinomycetes decreased to 0.27% in comparison with the control. In the 95–100 cm layer the count of actinomycetes was low (32 cfu/g in one column), while in the control column actinomycetes were not detected (Table 2). Occurrence of microorganisms in the lowest soil layer in higher counts than in the above layers could be due to a constant percolation of metal solutions through the soil profile, and microorganisms could have been carried down to the lowest layer.

Fungi were affected by metals not so significantly as many bacteria and actinomycetes (Fig. 3). Their number decreased to 4.78–27.02%, except the lowest layer where the fungal count was higher than in the control (Table 2). The most evident influence of metals on fungi was noted at a depth of 39–61 cm, while just above this horizon (at 20–25 cm) the survival percentage of micromycetes was rather high.

Microbial survival after metal treatment in different soil horizons. The metal impact on microorganisms in different horizons of the soil varied. In the Ap horizon 0–10 cm, the inhibitory effect of metals on microorganism groups was high and manifested in two patterns: a very high effect was exerted on actinomycetes and mineral nitrogen assimilating, oligotrophic and organotrophic bacteria (survival 0–2.35%), and quite a high inhibitory effect was exhibited on cellulose-degrading and spore-forming bacteria as well as on fungi (9.57–12.25%) (Table 2). In the Ap horizon 20–25 cm, much higher counts of spore-forming bacteria and fungi were detected, while other microorganism groups differed significantly less from those in the upper layer (Fig. 2). The Ap horizon is rich in humus, plant residue and other organic matter, which can chelate high amounts of metals (Bååth et al., 1995; Gadd, 1993). Both the total amount and the amount of the mobile phase of the test metals were very high in this horizon (Fig. 1). In the 0–10 cm layer, the metal levels were somewhat higher than in the 20–25 cm layer and could have resulted in a more severe inhibition in the upper layer. In the ElBt horizon 39–43 cm deep, the response of microorganism groups to the metals was rather similar. Notably the occurrence of actinomycetes, which were inhibited absolutely in most layers, was observed in this horizon (Fig. 3). The total amount of metals and of their extractable phase (especially Pb) was much lower than in the Ap horizon. However, poorer nutritive resources, which could have probably been bound with heavy metals, did not allow microorganisms to develop successfully and reach a higher abundance in this lay-

er. The most evident negative effect of metals on microbes was noticed in the Btkg horizon 56–61 cm deep. Microorganisms of even three bacterial groups were inhibited absolutely, and organotrophic bacteria were detected in negligible numbers. Nevertheless, other microorganisms such as spore-forming and cellulose degrading bacteria and fungi were more tolerant and survived in higher numbers (43.0–27.2%). The total amounts of metals and mobile Pb in this layer were higher (except Zn) than in the 39–43 cm layer. Thus, high metal contents in a soil poor in organic matter resulted in an absolute inhibition of some groups of microorganisms. In the Bkg 78–83 cm and BCkg 95–100 cm horizons, the inhibition of the most groups of microorganisms was lower than in the higher 56–61 cm layer; metal contents in these deepest layers were lower, too.

The inhibition of microorganisms in soil horizons could have been determined by several factors. The toxic effect of metals depends on metal species and concentrations, the organism and physico-chemical factors of the environment (Gadd, 1993). Toxicity of a metal may be exerted by different forms of the metal. Free metal ions can directly affect microbial cells, and a multiplicity of interactions can occur between microbial cells and heavy metal ions (Chaterjee et al., 1990; Gadd, 1986; Gadd, White, 1989). Nevertheless, organometals are generaly more toxic towards microorganisms than the corresponding free metal ions, and their toxicity varies with the number and identity of the organic groups (Cooney et al., 1989; Gadd, 1993). Reduction in viable counts of microorganisms indicates that essential functions have been affected or even damadge of microbial cells occured. Metals exert an effect on several functions of microorganisms at the same time resulting in the influence on the whole metabolism (Chaterjee et al., 1990; Gorbunova, Terekhova, 1995). Toxic effect of metals include blocking of functional groups of biologically important molecules, the displacement and substitution of essential metal ions from biomolecules, conformation, modification, denaturation and inactivation of enzymes and disruption of cellular and organellar membrane integrity (Gadd, 1993; Tomsett, 1993). Microbial survival in the presence of toxic metals depends on availability of metal speciation, which is greatly determined by physical and chemical properties of the environment (Joho et al., 1990; Roane, Pepper, 2000). Additionaly, sorbed metals to soil matter also influence indirectly survival of microorganisms eventualy, since they bind to soil elements, which serve as nutritive sources for microbes and, thus, make them hardly available (Morley, Gadd, 1995). Microbial survival in the presence of toxic metals depends on availability of metal speciation, intrinsic biochemical and structural properties, physiological and/or genetical adaptation (Joho et al., 1990; Roane, Pepper, 2000; Tomsett, 1993).

To sum it up, our results indicate that the inhibitionary effect of the test metals on the survival of microorganisms was high almost in all microbial groups. Actinomycetes were the most susceptible among investigated microorganisms: they were almost totally inhibited by heavy metals. A very high suppression of survival after metal impact was found in oligonitrophylic and mineral N assimilating bacteria, despite the fact that their counts in control colums differed very much. The percentage of survived organotrophic bacteria was also similar, however, these microorganisms, contrary to those discussed above, were found in all soil horizons studied. Cellulose-degrading bacteria withstood the impact of metals rather well. Nevertheless, it should be noted that very low numbers of them remained in the control column. In comparison with other microorganisms, the survival of micromycetes was rather high. Quite a high fungal tolerance towards heavy metals is related to the intrinsic properties of fungi that can determine their survival, such as possession of impermeable pigmented cell walls, extracellular polysaccharide and metabolite exretion, which lead to binding or precipitation of metals (Caesar-Tonthat et al., 1995; De Groot, Woodward, 1999). The response of spore-forming bacteria to metals differed from that of the other microorganisms: it varied from clear inhibition to high enhancement of their counts.

Such a variable response of microbial groups to heavy metals could be predetermined not only by their constitutional biological structure and functions but also by defence mechanisms, which include extracellular precipitation, complexation and crystallisation, transformation of metal species by oxidation, reduction, methylation, dealkylation, biosorption to cell walls, decreased transport or impermeability, efflux, intracellular compartmentation and sequestration (Atlas, Bartha, 1993; Gadd, 1993; Gadd, White, 1989; Joho et al., 1990; Martino et al., 2000; Ono et al., 1988). Adapting peculiarities (physiological or genetic) are very important in microbial survival strategy under stress conditions. Continuous exposure of microorganisms to heavy metals selects for resistant strains that adapt to the presence of metals and develop metal resistance mechanisms by inducing physiological or genetic changes in the organisms (Margesin, Schinner, 1996; Tomsett, 1993).

To conclude, our investigation shows that the impact of heavy metal acetates on microbial groups is related to metal concentrations and the properties of soil as well as to the biological peculiarities of microbes of different groups. The high susceptibility of such microorganisms as actinomycetes and oligonitrophylic and mineral N assimilating bacteria suggest low adapting possibilities of these microorganisms, while fungi and spore-forming bacteria seem to possessing much more effective defence mecha-

nisms leading to higher survival under environmental stress. Such different response of microorganisms to heavy metals indicates a shift in the ratio of microbial groups and suggests changes in the structure of particular microbial communities directed to selection of more tolerant microorganisms.

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VARIO, CINKO IR ÐVINO ACETATØ POVEIKIS DIRVOÞEMIO MIKROORGANIZMAMS

S a n t r a u k a

Tirtas bendrasis Cu, Zn ir Pb acetatø poveikis kelioms dirvoþemio mikroorganizmø grupëms modelinëse kolonose, uþpildytose natûraliais dirvoþemio monolitais. Tyrimams pasirinktas 0–100 cm gylio karbonatingasis giliau glëjiðkas iðplautþemis (IDg4-k), paimtas ið tyrimø vietos, esanèios 350 m nuo automagistralës Vilnius–Kaunas–Klaipëda (Giraitë, Kauno r.). Nustatyta, kad metalø acetatai sumaþino visø tirtø mikroorganizmø grupiø – organotrofiniø, sporiniø, mineralinà azotà asimiliuojanèiø ir oligonitrofiliniø bakterijø, aktinomicetø bei mikromicetø – gausumà. Jautriausiai á metalø poveiká reagavo aktinomicetai, mineralinà azotà asimiliuojanèios ir oligonitrofilinës bakterijos. Atspariausi metalø acetatams buvo mikromicetai, sporinës ir celiuliozæ skaidanèios bakterijos. Ryðkiausias tirtø metalø neigiamas poveikis pastebëtas Btkg horizonte 56–61 cm gylyje, kur visiðkai iðnyko aktinomicetai, mineralinà azotà asimiliuojanèios ir oligonitrofilinës bakterijos.

Raktaþodþiai: dirvoþemis, mikroorganizmai, sunkieji metalai