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Effect of long-term industrial pollution on soil microorganisms in deciduous forests situated along a pollution gradient next to a fertilizer factory

1. Abundance of bacteria, actinomycetes and fungi

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Soil fungi, bacteria and actinomycetes were studied in seven unequally polluted forest plots situated at different distances from a fertilizer factory in Lithuania. Average numbers of colony forming units in 1 g of dry weight soil (CFU g^{-1}) were determined by plate count on appropriate media: fungi on malt extract agar (MEA), bacteria on nutrient agar (NA), and actinomycetes on starch-casein agar (SCA). During the investigation period (2001–2002), the concentration of viable fungi varied from 23.3 to 387.9 thousand, bacteria – from 380.2 thousand to 9.02 million, and actinomycetes – from 0.11 to 7.58 thousand CFU g^{-1} of soil. Negative correlation between the fungal and actinomycetal concentrations and the distance from the pollution source ($r = -0.56$ and $r = -0.05$, respectively) was determined. Content of some heavy metals (Cu, Cr, Cd, Ni and Zn) in soil positively correlated with the humus content, but did not correlate with the distance from the pollution source. The highest concentration of heavy metals was determined in the soil of the plot situated at a distance of 5 km from the pollution source. Significant and high positive correlation between the fungal concentration and nitrogen ($r = 0.77$), phosphorus ($r = 0.88$) and humus ($r = 0.74$) content in soil was determined. The lowest number of fungal genera was determined in the forest plots where contents of the heavy metals (especially Pb, Cr, Zn and As), nutrients (nitrogen, phosphorus), and humus in soil were the highest. The results showed that abundance of bacterial and fungal populations increased within factory-induced soil pollution, but the diversity of species was decreased.

Key words: soil pollution, heavy metals, microbial counts, bacteria, fungi, actinomycetes

INTRODUCTION

Chemical compounds coming to ecosystem from various human activities are accumulated in soil and water reservoirs. That is why the soil may be regarded as a long term reservoir of environment contaminants from which these compounds enter the terrestrial food chains and / or underground water. Soil pollution with metal ions near pollution sources or smelters is a constant process having a toxic effect on plants and on soil microorganisms, which participate in soil biochemical processes, and directly or indirectly modify their environment. The capability of soil microorganisms to multiply even under undesirable environmental conditions relatively quickly, signalizes the high degree of their susceptibility to either positive or negative effects, e. g. those caused by pollution (Adriaens et al., 2001). These organisms sometimes affect soil environment more quickly than an abiotic process can do (Titljanova and Tesarova, 1991). Thus, microbial community structure may be useful as a highly sensitive bioindicator of soil disturbance and progress of remediation (Gremion et al., 2004). Several researchers have demonstrated that heavy metal contamination can cause shifts in microbial populations (Doelman et al., 1994; Roane and Kellogg, 1996; Ellis et al., 2001; Kelly et al., 2003; Lugauskas et al., 2005). The predominance of gram-negative bacteria over gram-positive bacteria has

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been found in metal contaminated soils (Frostegård et al., 1993) and decrease in the fungal community in metal contaminated soils was observed (Kelly et al., 2003), especially in micorrhizal fungi (Pennanen et al., 1996; Kelly et al., 2003; Hinojosa et al., 2005). According to the literature, it is evident that fungi are more resistant to heavy metals than bacteria (Bååth, 1989; Hiroki, 1992; Brokes, 1995; Müller et al., 2001; Rajapaksha et al., 2004; Dirginčiutė, Pečiulytė, 2007; Stefanowicz et al., 2008).

The aim of this study was to investigate the impact of long-term industrial pollution (enlarged amount of nutrients, heavy metals) on the abundance and diversity of soil fungi, bacteria and actinomycetes in deciduous forests situated at different distances from a chemical factory in Lithuania. The first product of this chemical enterprise was obtained in 1963. At present, the company's major products are nitrogen phosphorus fertilizers. The main emission products are NO_X, CO, sulphur anhydride, apatite and fluorine. Hard pollutants emitted from the chimneys make up about 140 t a year (data provided by the Ministry of the Environment of the Republic of Lithuania).

MATERIALS AND METHODS

Study sites

This study examined soil from a forest located near the city of Kėdainiai in Middle Lithuania (70 m above sea level). The average annual precipitation and average air temperature were 550 mm and 6.4 °C. Seven 30 × 30 m study plots situated at different distances from the pollution source were chosen. Care was taken to choose plots with similar vegetation along the gradient of pollution. The study area was occupied by deciduous forest stands (Stankevičienė, Pečiulytė, 2004). Dominant tree species were *Fraxinus excelsior* L., *Populus tremula* L., *Padus avium* Mill., and *Corylus avellana* L. The nearest plots Juodiškis ("2" – 55°16' N, 24°01' E) and Zabieliškis-Šilainėliai ("1" – 55°14' N, 24°01' E) were at a distance of about 0.7–3 km in the eastern and southeastern directions, respectively, Vilainiai ("3" – 55°18' N, 24°01' E) and Pašiliai ("7" – 55°03' N, 23°58' E) forests – about 4 km in northeastern and 5 km southwestern directions, Berunkiškis ("6" – 55°13' N, 24°08' E) and Stebuliai ("4" – 55°19' N, 24°06' E) – about 8 km in southeastern and 9 km northeastern, and Lančiūnava ("5" – 55°20' N, 24°12' E) forest – about 15 km in the eastern direction from the emission source (Fig. 1).

Soil sampling and observation of microorganisms

Seven sites were common to the two samplings in May and September, 2001 and 2002. At each site, three replicate bulk samples were taken, consisting of 18–20 randomly collected sub-samples from the surface soil (0–10 cm horizon, after removal of litter). The samples were transported to the laboratory, stored overnight at 4 °C, air-dried at room temperature and sieved (2-mm mesh) prior to further use in the experiment. Total counts of heterotrophic aerobic bacteria, actinomycetes and fungi were evaluated on appropriate selective media. The total number of fungi in 1 g of dry weight soil was determined on malt extract agar (MEA) plates (2% Oxoid malt extract, 1.5% agar, and 30 μg chloramphenicol g^{-1}), using the dilutions of 10^{-2}, 10^{-3}, and 10^{-4}. The total number of bacteria was determined on the nutrient agar (NA) medium (beef extract 3 g, peptone 10 g, NaCl 5 g, agar 15 g in one liter of medium; pH 7.2–7.4) using the dilutions of 10^{-5}, 10^{-6}, 10^{-7}. Isolation and enumeration of actinomycetes were performed by soil dilution plate technique using Starch-casein agar (Starch 10, casein 0.3, KNO_3 2, NaCl 2, K_2HPO_4 2, Mg $SO_4.7H_20$ 0.05, $CaCO_3$ 0.02, $FeSO_4.7\ H_20$ 0.01 and agar 18 g/l). Nystatin (50 μg ml^{-1}) was added to avoid fungal contamination. Concentration of viable bacteria, fungi and actinomycetes in soil expressed as colony-forming units (CFU) in g of dry weight soil were determined after 2–3, 7–14 and 7 days, respectively, after incubation at 22 ± 2 °C (fungi), 32 ± 2 °C (bacteria) and 28 ± 2 °C (actinomycetes) in the dark. Most of the 2,000 fungal isolates were transferred on the potato dextrose agar, corn meal agar and Czapek's agar and were identified to genus. The microscopic characterization of actinomycetes was done by cover slip method. The mycelium

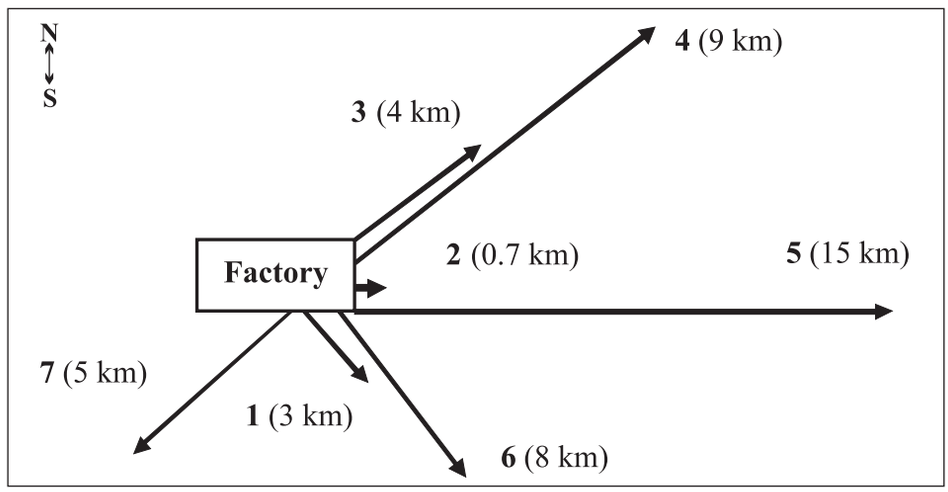

Fig. 1. Direction and average distance from the factory of the forests in which investigation plots were chosen

structure, color and arrangement of conidiophores and athrospores on the mycelium were observed through the oil immersion (×1000). The observed structure was compared with Bergey's Manual of Determinative Bacteriology (Williams et al., 1989).

Soil analysis

The pH_{KCl} was measured with a glass electrode using a mixture of soil with a 1.0 M KCl solution. The concentration of nitrogen and phosphorus was determined with the photometer "SPECOL11", that of potassium by applying flame photometer "FLAPHO41", and content of humus calorimetrically (Mineev, 1989). Heavy metal content was digested by concentrated HNO_3 and HCl (1 : 3, v/v) (aqua regia). Quantity of the metals (As, Cd, Cr, Cu, Ni, Pb, and Zn) in soil was studied by electrothermal atomic absorption spectrophotometry (EAAS) using a Perkin-Elmer-Zeeman 3030 spectrophotometer.

The results concerning the number of bacteria and fungi were statistically analysed, and the significance of differences was determined on the basis of Tukey's confidence intervals. Data analysis was also carried out employing the methods of statistics (Zar, 1999) using *Statistica* 4.5 software.

RESULTS

Chemical characteristics of forest soil

The highest concentration of nutrients (N, P, K) was determined in the 2nd and 7th investigation plots located at a distance of 0.7 and 5 km from the pollution source, respectively (Table 1). The lowest amount of nitrogen and phosphorus was found in the 6th plot and that of potassium in the 4th and 5th plots, which were distanced 8 km, 9 km and 15 km, respectively. Negative correlation was observed between potassium concentration in the forest soil and distance from the pollution source. Concentration of nitrogen and phosphorus positively correlated with concentration of humus maximum value, which was determined in forests at a distance of 0.7 and 5 km. The lowest value of pH was determined in the soil of forests situated next to the factory. Correlation between pH and the distance from the factory was not significant. Five of the seven investigated forest sites can be characterized as soil with conditionally acid reaction (pH < 5.0).

The highest concentration of heavy metals was determined in the soil of the 7th plot situated at a distance of 5 km from the factory, and in the soil of the 1st and 3rd (3 and 4 km, respectively) plots and the lowest – in the soil of the 6th plot (8 km) (Table 2). Forests situated at a distance of 0.7, 9 and 15 km (2nd, 4th and 5th plots) took the intermediate position. Evaluation of the distribution of various metals in different investigated forests revealed a negative correlation between the distance and concentration of Pb and Cu (r = –0.82 and –0.52, respectively). Thus, the forests situated at a distance of about 3–5 km from the factory were the most polluted by heavy metals. The highest concentrations of Zn, As, Cr and Ni distinguished these forests. Content of some heavy metals (Cu, Cr, Cd, Ni and Zn) in the soil samples positively correlated with the humus content in soil (r = 0.46). One of the functions of humus in soil is accumulation of the metals as well as other pollutants. Content of N, P and K in the soil of the investigated forests also correlated with humus content in it. That correlation was well observed in the 2nd and 7th investigated forest sites (Table 1).

Table 1. **Chemical analysis of soils, applied from 2001 to 2002 at the seven forest plots (mean ± standard error) (n = 18)**

Plots (distance from the factory)	N (% ds)	P (% ds)	K (mg/kg)	Humus (% ds)	pH (KCl)
2 (0.7 km)	0.86 ± 0.45	0.106 ± 0.024	133.02 ± 68.23	9.89 ± 3.46	4.01 ± 0.2
1 (3 km)	0.39 ± 0.05	0.052 ± 0.015	143.3 ± 35.61	6.23 ± 1.06	4.66 ± 0.23
3 (4 km)	0.42 ± 0.08	0.049 ± 0.01	117.7 ± 31.12	7.03 ± 0.98	6.02 ± 0.83
7 (5 km)	0.74 ± 0.09	0.076 ± 0.019	146.07 ± 52.46	9.06 ± 1.72	5.27 ± 0.11
6 (8 km)	0.25 ± 0.03	0.033 ± 0.012	129.05 ± 42.77	4.57 ± 1.02	4.85 ± 0.35
4 (9 km)	0.43 ± 0.06	0.051 ± 0.014	105.47 ± 22.78	6.76 ± 0.67	6.09 ± 0.15
5 (15 km)	0.41 ± 0.08	0.051 ± 0.022	109.7 ± 39.87	6.75 ± 0.93	5.03 ± 0.82

ds – dry weight soil.

Table 2. **Concentration of heavy metals (mg kg^{-1} dry soil) in soils of the investigated forest plots (1–7)**

Plots	Pb	Cd	Ni	Cr	Cu	Zn	As
2 (0.7 km)	13	0.14	6	10.5	6.5	16	0.8
1 (3 km)	20.5	0.12	5.7	12.5	4.82	19.5	1.2
3 (4 km)	12.5	0.16	6.5	14.5	5.8	23	1.6
7 (5 km)	11.2	0.24	11.5	21	8.5	25	1.3
6 (8 km)	8.5	0.06	5.6	11.5	3.6	16	1.02
4 (9 km)	10.4	0.12	5.7	12	4.35	18.5	1.1
5 (15 km)	10.5	0.17	6.4	13	4.82	18	1.25

Abundance and diversity of microorganisms

Bacteria. A dilution plate technique measures only a small portion of the total soil community, nevertheless, it is a useful tool for studying the relative abundance of cultivable populations and the changes in population density which occur depending on the medium used. Abundance of bacteria was evidently different in separate investigation plots ($P < 0.5$). Average amounts of viable on nutrient agar (NA) medium bacteria during the investigation period in different forests varied from 380 thousand to 9.02 million CFU g^{-1} dry weight soil (Fig. 2). This parameter was higher in the second year of investigation and did not correlate with the distance from pollution source ($r = 0.16$ and 0.26, in 2001 and 2002, respectively) (Table 4). The highest numbers of viable on nutrient agar bacteria were determined in the forest situated at a distance of about 5 km from the pollution source in 2002 and

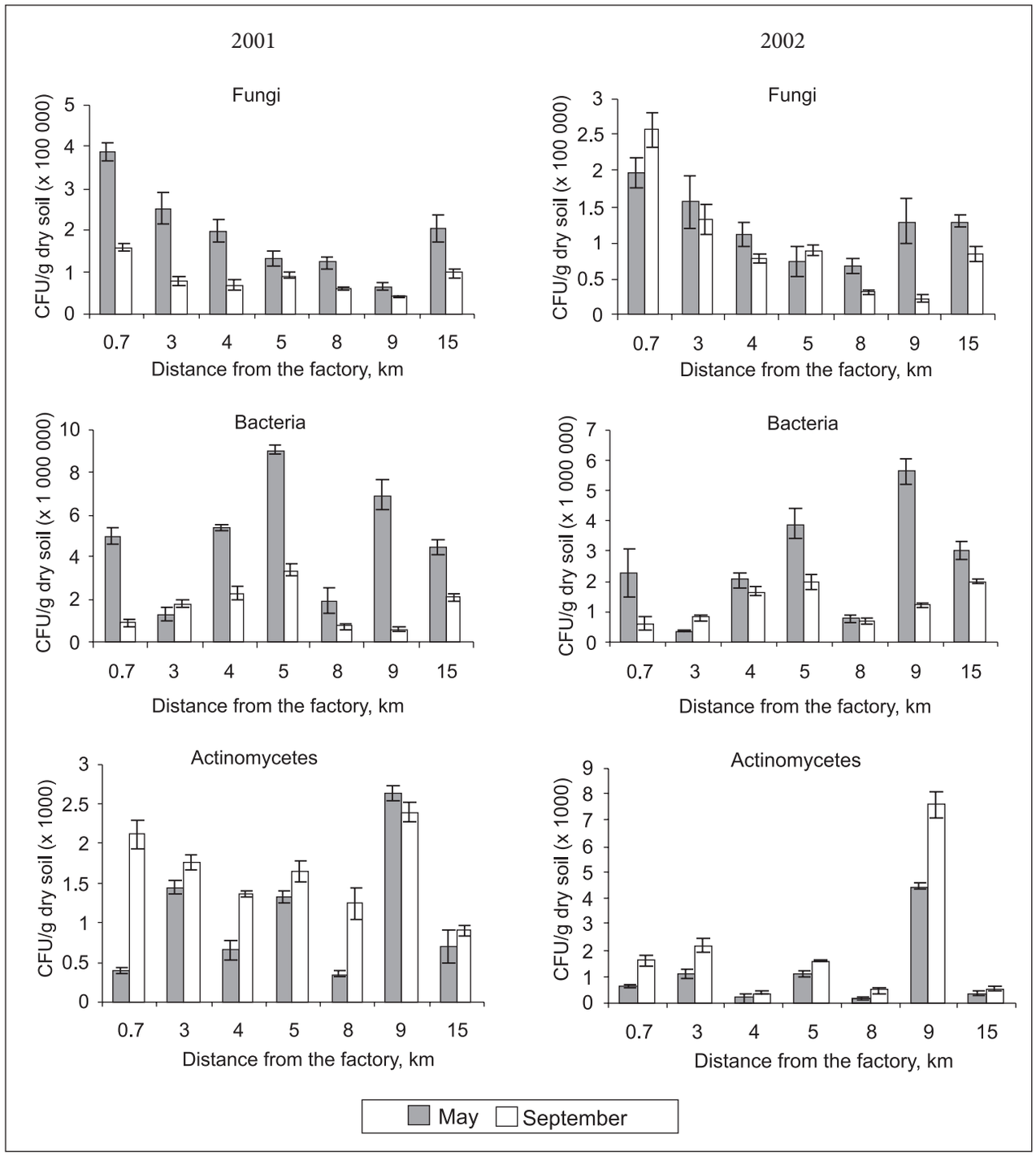

Fig. 2. Total counts of colony forming units (CFU g^{-1} of dry weight soil) of microorganisms in the soil of the forest plots located at a different distance from the fertilizer factory in 2001 and 2002. Error bars in figures denote standard error (n = 3)

in the forest situated at a distance of 9 km from the pollution source in 2002. Those values significantly ($P < 0.05$) differed from the bacterial counts in the soil samples collected in other investigated forests. Soil samples of the forest located at a distance of about 5 km were characterized by the highest amount of pollutants. Total number of the bacteria in the 2nd, 3rd and 7th plots (0.7, 3 and 7 km, respectively) was 2–3 times higher in 2001 than in 2002. The lowest numbers of the bacteria were determined in the 1st (3 km from the factory) and 6th (8 km) plots where the lowest humus content was determined. The positive correlation between the bacterial counts and the humus content in the soil was observed throughout the investigation period ($r = 0.55$) (Table 4). Unexpected positive correlation between the amount of bacteria determined by plate count method and amount of metals Cd, Ni, Cr, Cu and Zn was discovered ($r = 0.88$, 0.84, 0.88, 0.66 and 0.75, respectively). Bacterial populations were not investigated in detail to species composition. However, it must be noted that approximately 80% of the strains were gram negative at higher metal concentration and the rest were either gram positive or gram variable. Commonly, gram positive bacteria at higher concentration belonged to genus Bacillus (data not shown).

Actinomycetes. The plate viable counts of the actinomycetes in the seven plots of soil ranged between 0.11 and 7.58 thousand CFU/g of soil (Figs. 2, 3; Table 3). Actinomycetes mainly inhabit the lower part of litter of the forest biomass as well as the upper soil horizon. The total counts of the actinomycetes in 1st, 2nd, 3rd and 6th plots of the investigated forest were higher in September than in May. The 4th plot, located at a distance of 9 km from the pollution source, distinguished itself by the abundance of the actinomycetes during all the period of the investigation (Fig. 2). Total counts of the actinomycetes were 2–4 times higher in 2002 than in 2001. Their lowest concentrations were determined in the 3rd, 6th and 5th plots. Soil of the 5th plot, located at a distance of 15 km from the pollution source, statistically differed ($P < 0.05$) in the viable counts of the actinomycetes from the soil of the other plots investigated. Their concentration in the soil of the 5th plot was statistically different from those in

Table 3. **Average CFU values (per d dry soil) of counted groups of microorganisms in May and September, 2001–2002 (n = 6)**

Plot (Distance from the factory)	Total counts of fungi ($\times 10^5$)		Total counts of bacteria ($\times 10^6$)		Total counts of actinomycetes ($\times 10^3$)	
	May	September	May	September	May	September
2 (0.7 km)	2.92 ± 0.21cdefg	2.07 ± 0.24bcdefg	3.64 ± 0.81bdef	0.74 ± 0.20cdg	0.50 ± 0.06bdf	1.88 ± 0.21cdefg
1 (3 km)	2.04 ± 0.38defg	1.05 ± 0.21aef	0.83 ± 0.05aedfg	1.28 ± 0.07cdeg	1.27 ± 0.21acfg	1.97 ± 0.30cdefg
3 (4 km)	1.54 ± 0.17efg	0.73 ± 0.06aef	3.71 ± 0.26bdef	1.37 ± 0.16abdefg	0.43 ± 0.17bdf	0.86 ± 0.05abdf
7 (5 km)	1.02 ± 0.25abg	0.91 ± 0.08aef	6.46 ± 0.51abceg	2.69 ± 0.27abef	1.21 ± 0.13acefg	1.46 ± 0.04abcfg
6 (8 km)	0.95 ± 0.11abg	0.45 ± 0.04abcdg	1.36 ± 0.11acdfg	0.70 ± 0.07bcdg	0.67 ± 0.07bdf	1.05 ± 0.10abf
4 (9 km)	0.98 ± 0.30abg	0.32 ± 0.05abcdg	6.31 ± 0.42abceg	0.92 ± 0.09cdg	3.55 ± 0.12abcde	4.41 ± 0.51abcdeg
5 (15 km)	1.669 ± 0.09abf	0.90 ± 0.11aef	3.74 ± 0.28bdef	2.02 ± 0.07abef	0.52 ± 0.07bc	0.71 ± 0.12abdf

a, b, c, d, e, f, g – different letters in one column show statistically significant differences between plots ($P < 0.05$).

Table 4. **Correlation coefficients between microbial characteristics, nutrients content and heavy metal content in soil of the investigated forest plots (n = 12), distance from the pollution source and between the amounts of different microorganisms in soil samples**

	Total number of fungi	Total number of bacteria	Total number of actinomycetes
Pb	0.3590	–0.4516	–0.0501
Cd	–0.0044	0.8855	–0.1082
Ni	–0.3082	0.8446	–0.0658
Cr	–0.4360	0.8803	–0.0332
Cu	0.1668	0.6618	–0.2021
Zn	–0.3524	0.7512	0.0141
As	–0.4643	0.1448	–0.1241
sum	–0.1084	0.7901	–0.0704
N	0.7720	0.4814	–0.1158
P	0.8764	0.3326	–0.1566
K	0.4197	0.2443	–0.3393
Humus	0.7345	0.5514	–0.0965
pH	–0.7073	0.1873	0.5151
Distance from the pollution source	–0.5628	0.2589	0.1586
Water content	0.4644	0.7828	0.1281
Total number of fungi	X	–0.3284	–0.4262
Total number of bacteria	–0.3284	X	–0.3402
Total number of actinomycetes	–0.4262	–0.3402	X

the soil of other forest plots. In Central Lithuania where the fertilizer factory is located, prevailing wind direction is from north-west (NW) (Katinas and Markevičius, 2001). Perhaps, industrial pollutants (the main emission products, such as NO_x, CO, sulphur anhydride, apatite and fluorine) could affect the populations of actinomycetes in the forest soil. Positive correlation was found between the total amount of actonomycetes and soil pH (r = 0.52), and only slight negative correlation (r = –0.07) was determined between the concentration of actonomycetes and the total content of the heavy metals in the soil (Table 4). Increase in the abundance of actinomycetes was determined in the plots where the content of Cd, Cu, Ni and Zn in the soil was highest.

Each biogeocenosis is characterized by the specific soil actinomycetes complex as well as their abundance. Actinomycetes belong to the order Actinomycetales (Super kingdom: *Bacteria*, Phylum: *Firmicutes*, Class: *Actinobacteria*, Subclass: *Actinobacteridae*). The total number of actinomycetes on the primary isolation plates was 2534. An average of 22–28 mycelium-forming actinomycete colonies per plate were observed with numbers increasing to 38 when only the plates that yielded actinomycetes were considered. 1897 colonies were picked based on actinomycete-like morphology; about 637 of them formed powdery colonies with well-developed aerial hyphae fragmented into spore chains. These isolates were tentatively termed as Streptomyces-like actinomycetes. The main part of the remaining isolates formed orange to red or black pigmented colonies with solid colony texture. Morphological examination of dominating in plates isolates clearly indicates that they belong to the following genera: *Streptomyces*, family *Streptomycetaceae* (spore chain with coiling and branching); *Micromonospora*, family *Micromonosporaceae* (clusters of spore chain, single conidia on substrate mycelia). The isolates were diverse in color of their conidia (white 43.2%, yellow 11.4%, brown 11%, grey 6.4%, creamy 16%, and black 12%). Actinomycetes with black conidia dominated in the 7th plot (5 km from the pollution source) soil where they comprised 86.45% of all the actinomycetes isolated from the soil samples collected from this forest plot. Soil of this forest was characterized by the highest amount of pollutants. The number of morphotypes in different plots positively correlated with the distance from the pollution source (r = 0.86) and negatively correlated with the heavy-metal pollution (r = –0.76). The largest diversity (all 6 morphotypes by color) of the actinomycetes was determined in the 4th forest plot where their total counts were also the largest and was followed by the diversity of the actinomycetes in the 3rd plot which was located at a distance of 4 km from the pollution source (Fig. 2). Both these forests are located in northeastern direction from the fertilizer factory which is situated in the central part of Lithuania (Fig. 1). Perhaps, the major emission products from the chimneys deposit at the locations which are situated on the leeward side (the 1st, 2nd, and 6th sites). The 7th plot is located in the forest which is situated in the southwestern direction from the factory and also does not fit the wind direction. The total counts of the actinomycetes in that plot soil statistically do not differ from the counts determined in the soil from the 3rd and 4th plots ($P > 0.05$) (Fig. 2), however, they are characterized by poverty of morphotypes (3 from the 6 viable on the appropriate medium).

Fungi. The standard plate count, direct enumeration method used for fungus population abundance analysis in current investigation is of fairly limited value. The number and diversity of the fungi in soil can be extremely large, but often only a small proportion (generally < 10%) of them is available or form colonies on used medium plates. Many fungi from natural environment do not grow on synthetic media, or they form colonies that have very similar morphology, therefore can be confused with the other members of the community. The fungal population analysis in that investigation was performed on malt extract agar (MEA) medium. We tried to compare abundance of fungi in soil samples collected from differently polluted forest soil. Despite the limitations of the method used in that investigation, significant differences in abundance of fungi as well as in species composition was found out. Abundance of the fungi was evidently different in separate investigation plots ($P < 0.05$). Average amount of viable on MEA fungi during the investigation period in different forests varied from 23.3 to 387.9 thousand CFU g^{-1} of dry weight soil (Fig. 2). Mean counts of the fungi, determined in soil samples in May and September, both in 2001 and 2002, were the highest in the 1st and 2nd plots and statistically significantly differed ($P < 0.05$) from their concentrations in the soil samples collected from other plots. In spring 2001, the lowest number of fungi was observed in the 6th plot, located 8 km from the pollution source, which was due to low organic matter (presented as humus content) and least water content (not presented) in soil of that forest plot. Their number negatively correlated with the distance from the pollution source (r = –0.56) (Table 4). Significant negative correlation was observed between the total content of heavy metals in soil and the distance from the pollution source (r = –0.78). This is in accordance with the relationship between the distance and abundance of total fungal counts. Statistically significant and high positive correlation between the fungal concentration and nitrogen (r = 0.77), phosphorus (r = 0.88) and humus (r = 0.74) content in soil was determined. The lowest number of fungal genera was determined in the forest plots where content of the heavy metals (especially Pb, Cr, Zn and As), nutrients (nitrogen, phosphorus), and humus in soil was the highest. Their abundance in the soils investigated depended on the location of the plot. The higher plate counts were in the plots (1st, 2nd, 5th and 6th) located on the leeward side of the the fertilizer factory (Figs. 1, 2).

As expected, there was a strong relationship ($P < 0.05$) between humus content in soil and fungal abundance (r = 0.73) (Table 4). The lowest fungal concentrations were observed in the soil of the 4th plot in 2001 and in soil of the 6th plot in 2002 (Figs. 2 and 3), however, differences were not statistically

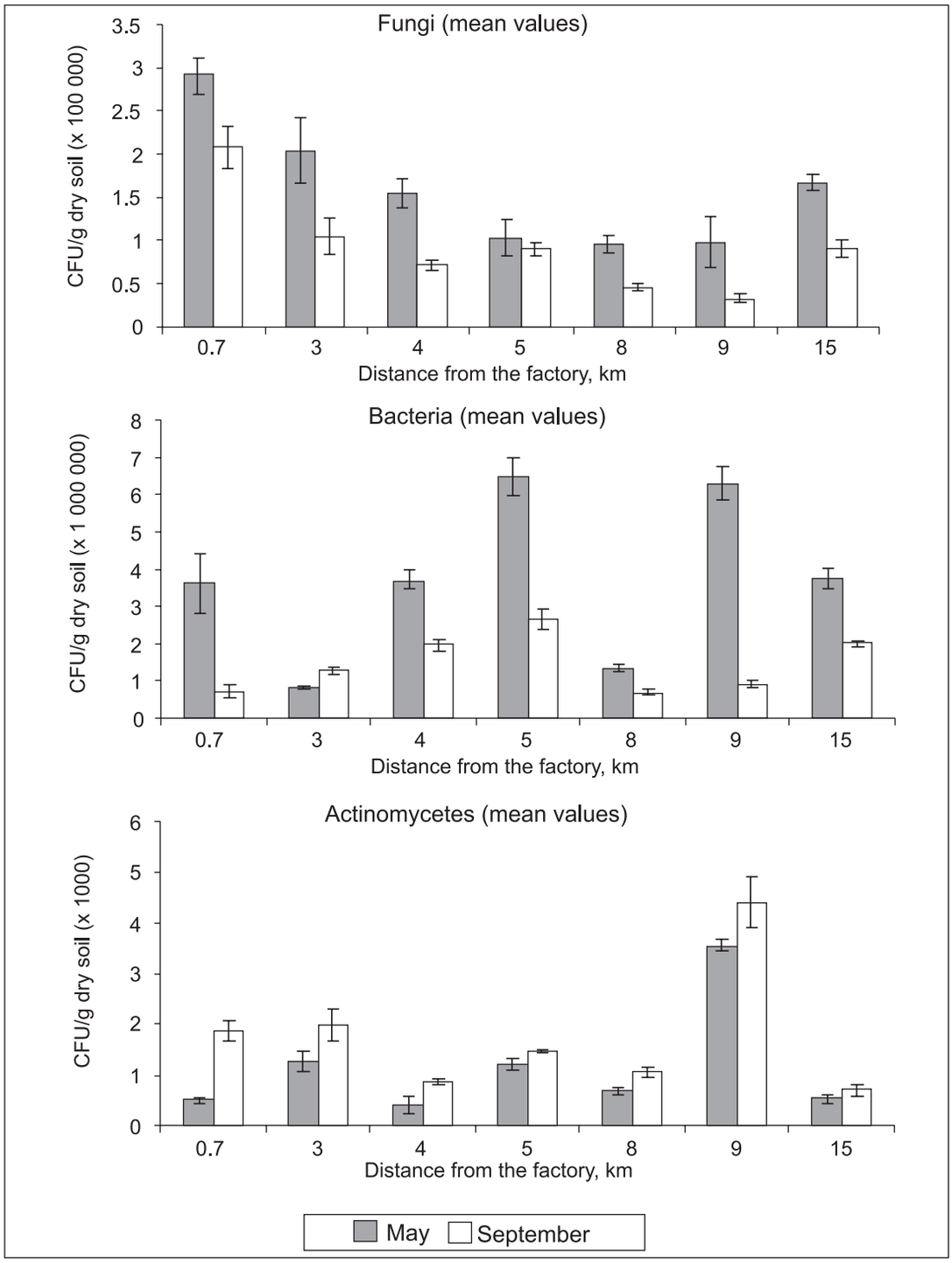

Fig. 3. Total counts (mean values of two years of investigation) of the microorganisms in soil (CFU g^{-1} of dry weight soil) in the soil of seven forest plots located at a different distance from the fertilizer factory. Error bars in figures denote standard error (n = 3)

significant ($P > 0.05$). The lowest fungal counts, determined in the soil of the 6th plot, perhaps, were due to low organic matter content and the least water content in the soil of that forest plot. Average number of filamentous fungi was distinctly higher in the plots located closer to the factory (Fig. 2 and 3, and Table 3) and, in contrast to the bacterial counts, – negatively correlated with the heavy metal concentrations in the soil of the investigated plots (Table 4). However, variability of some results was rather high and the differences were not statistically significant.

Loss of microbial diversity is evident as we move towards higher concentration of heavy metal in soil (Ahmad et al., 2005). In that investigation, statistical negative relationship between the number of isolated fungal genera and the

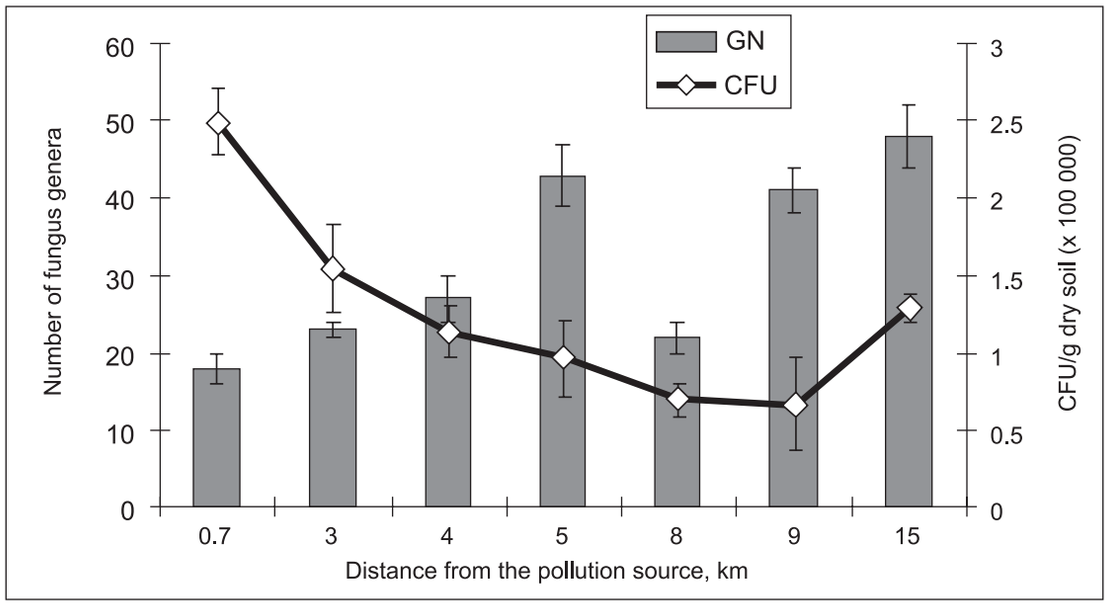

Fig. 4. Total number of the fungal genera (GN) and average numbers of the colony forming units (CFU g^{-1} of dry weight soil) on MEA medium in the soil of seven forest plots located at a different distance from the fertilizer factory

average total counts of the viable on MEA fungi was observed (Fig. 4). Total number of fungal genera isolated from the soil of the seven forest plots investigated varied from 18 to 46. The highest diversity of fungi was determined in soil samples collected from the 5th and 7th plots, and the lowest in the soil samples collected from the 2nd and 6th plots. Fungi belonging to the genus *Penicillium* Link. were the most common among the isolates on MEA medium in all samples except soil samples from the 2nd and 6th plots, where their proportions were similar to those of the *Gliocladium* Corda, *Acremonium* Link and *Cladosporium* Link genera. It must be noted that *Cladosporium* spp. dominated in the fungal community of the 2nd plot, located next (at a distance of 0.7 km) to the pollution source. Fungal community in the 4th plot during that investigation was dominated by *Absidia* Tiegh., *Cladosporium*, *Mucor* Fresen, and *Trichoderma* Pers. genera, and the community of the 5th plot soil were dominated by *Mortierella* Coem. and *Gliocladium* genera. With the increasing distance from the pollution source, fungal diversity slightly increased (Fig. 4), but fungal community composition depended in the highest degree on the soil characteristics and especially on the total concentration of nutrients and heavy metal ions.

DISCUSSION

The effect of long-term industrial contamination on soil microbial communities was assessed with a view to determining whether analysis of these communities could be used for ecological assessment of contaminated sites. Toxic effects of heavy metals on soil microorganisms have been extensively studied in the past (Bååth, 1989; Giller et al., 1998; Williamson et al., 1998), and almost every group of organisms has been studied in terms of resistance. Fungi and bacteria constitute the main components of the soil microbial biomass. It has often been stated that fungal populations are more tolerant to heavy metals than bacterial populations (Hiroki, 1992; Doelman et al., 1994; Brokes, 1995; Rajapaksha et al., 2004; Dirginčiutė, Pečiulytė, 2007; Stefanowicz et al., 2008). Similar conclusions were made when phospholipid fatty acid (PLFA) analysis was used to differentiate between the fungal and bacterial components of the soil microbial biomass (Frostegård et al., 1993, 1996; Pennanen et al., 1996; Kelly et al., 1999) and after the investigation of soils treated with a waste sludge (Pennanen et al., 1996; Bååth, 1998). The precision in the community tolerance measurements differed between metals, being highest for Cu and lowest for Ni (Bååth et al., 1998). This might indicate that community tolerance can be used to compare actual metal toxicity between soil types. Different biomass measurements or plate counting techniques and activity measurements have also indicated that heavy metals affect bacteria and fungi in soil differently (Müller et al., 2001; Rajapaksha et al., 2004). Our study provides the first direct evidence on a differential response of fungi and bacteria to the pollution with heavy metals in deciduous forest situated along pollution gradient next to a fertilizer factory in Lithuania. The plate count data obtained indicated that pollution differently affect fungi, bacteria and actinomycetes. Because of the limitation of the plate technique for determining populations of soil fungi, bacteria or actinobacteria, the results can be taken only as an indication of what is likely to occur in the field. As distinct from the investigations of the authors listed above, fungal abundance in the soil during the investigation was found to be negatively correlated,

while bacterial concentration manifested a positive correlation with the distance from the pollution source. The number of investigated plots was limited; therefore, our experiment does not provide sufficient evidence to state that fungi or bacteria are more resistant to heavy metals pollution. From the results obtained it is evident that relative fungal / bacterial ratio rose alongside the increasing metal pollution. Those results agree with the data obtained by Rajapaksha et al. (2004) during short-term treatment of soils with Cu and Zn. Increase was the largest for the Cu-amended soils, where the ratio increased from about 0.075 in the control to almost 0.14 in the most-polluted soils (Rajapaksha et al., 2004). In our investigation bacterial concentration in soils investigated was less correlated with the Cu content than with the content of other metals in soil, however, the significant positive correlation ($r = 0.66$) was determined. The data of relative fungi/bacteria ratio relationship with the Cu content in soil in our investigation is comparable with that obtained by Rajapaksha et al. (2004). The negative correlation between the abundance of actinomycetes and abundance of other two groups of microorganisms was also negatively correlated. Hence we may conclude that the antagonistic relationship between different microorganisms is important for the formation of the communities of soil microorganisms, as was established by Rajapaksha et al. (2004). Recent studies in different ecosystems have indicated that fungi and bacteria can have antagonistic relationships: increase in activity of one organism group results in a decrease in the activity of the other. In general it can be stated that in our investigation actinomycetes were less tolerant than bacterial population. These observations are comparable with other findings (Ahmad et al., 2005). Increase of the amount of actinomycetes was determined in some soil samples in which content of Cd, Cu, Ni and Zn was the highest. Increase in amount of the actinomycetes in soil artificially polluted with Cd, Ni and Cu was observed in calcareous loamy chernozem soil (Szili-Kovács, 2008). The ratio between the number of actinomycetes and filamentous fungi was recommended for the estimation of environmental stress on soil microflora (Wang et al., 2007). Analysis of the results obtained during the investigation can show that the higher the stress, the lower the ratio.

Significant negative correlation between the total counts of the fungi and soil pH ($r = -0.707$) and the positive correlation of the total counts of the actionomycetes and soil pH ($r = 0.52$) was determined. Bacteria took intermediate position with slight positive correlation. Lower water content in the soil of all the investigated forest plots in 2002 (not presented) was obviously one of the reasons for the lower level of the basal abundance of fungi and bacteria in the soil samples. All three groups of the microorganisms positively correlated with the water content in soil ($r = 0.46$, 0.78 and 0.12 for bacteria, fungi and actinomycetes, respectively) (Table 4). Litter decomposition rate in the 2nd plot (0.7 km from the pollution source) was slower than that in other sites. Total numbers of fungi and bacteria in that plot were high enough and even outnumbered those in other sites (Fig. 2 and Table 3). Abundance of the ectomycorrhizal (ECM) fungi determined in parallel with micromycetes which are referred to above (Stankevičienė, Pečiulytė, 2004) was also high in the 2nd plot (number of ECM root tips per 100 cm^{-3} of soil in that plot was twice higher than in the 1st, 3rd and 7th plots). Water content in the soil of the 2nd plot was about 30.64% during investigation period and it was 2–3 times higher than water content in the 1st and 3rd plots. The content of nitrogen (N, Table 1) was also two times higher in the 2nd plot than in the other two. The only explanation for the negative correlation between the rate of plant remnants destruction process and the abundance of the below-ground community of microorganisms could be possibly higher levels of pollutants emitted from the factory chimney, characterised by fast sedimentation period. The 2nd plot is located next to the factory and leeward. Average wind rage at that district of central Lithuania is low – 4 m/s (Katinas, Markevičius, 2001). Industrial pollution decrease lignin and cellulose degrading microorganisms. That is a possible way of reduction of the microbial variety of soil, which requires further research.

CONCLUSIONS

Significant negative correlation was observed between the total content of heavy metals in soil and the distance from the pollution source ($r = -0.78$).

Statistically significant differences in abundance of fungi, bacteria and actinomycetes in the soil of the investigated forests plots were detected. Total numbers of fungi, bacteria and actinomycetes correlated with the content of nutrients and pollutants in the investigated soils in a different way.

Fungal populations were the most abundant in the plots, situated near the fertilizer factory and decreased with the increasing distance from the pollution source. In contrast, the number of the fungal genera grew with the increase of the distance from the pollution source. Significant and high positive correlation between the fungal concentration and nitrogen ($r = 0.77$) and phosphorus ($r = 0.88$) content in soil was determined, however, the lowest number fungal genera was determined in the forest plots where the content of heavy metals (especially Pb, Cr, Zn and As), nutrients (nitrogen, phosphorus), and humus in soil was the highest.

As expected, there was a strong relationship ($P < 0.05$) between humus content in soil and fungal abundance ($r = 0.73$), followed by abundance of bacterial populations ($r = 0.55$).

Unexpected positive correlation between the amount of bacteria determined by plate count method and amount of metals Cd, Ni, Cr, Cu and Zn was determined ($r = 0.88$, 0.84, 0.88, 0.66 and 0.75, respectively).

Bacteria positively correlated with the distance from the pollution source. No correlation was determined between the abundance of actinomycetes and the distance from the pollution source.

The lower pH in contaminated soil enhanced the negative effect on bacteria, especially on actinomycetes, and the positive effect on fungi.

Changes of the relative fungi / bacteria and fungi / actinomycetes ratio were observed in forest plots situated at a different distance from the fertilizer factory and characterized by different chemical composition, water content, humus content in soil and soil pH. Their abundance and distribution can explain possible effects of industrial pollution on microorganisms inhabiting the soil, their resistance and activity.

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ILGALAIKĖS PRAMONINĖS TARŠOS ĮTAKA LAPUOČIŲ MIŠKŲ DIRVOŽEMIO MIKROORGANIZMAMS. 1. BAKTERIJŲ, AKTINOMICETŲ IR MIKROMICETŲ GAUSA

S a n t r a u k a

Ištirtos 7 lapuočių miško teritorijos, esančios skirtingu atstumu nuo trąšų gamyklos Lietuvoje. Gyvybingų mikroorganizmų koncentracija vertinant pagal kolonijas sudarančius vienetus grame sauso dirvožemio (ksv g^{-1}) tirta salyklo agaro (mikromicetai), mitybinio agaro (bakterijos) ir krakmolo-kazeino agaro (aktinomicetai) terpėse. Tyrimo metu (2001–2002 m.) gyvybingų mikromicetų koncentracija buvo nuo 23,3 iki 387,9 tūkstančio, bakterijų – nuo 380,2 tūkstančio iki 9,02 milijono, aktinomicetų – nuo 0,11 iki 7,58 tūkstančio ksv g^{-1}. Mikromicetų ir aktinomicetų koncentracija dirvožemio mėginiuose didėjant atstumui nuo gamyklos mažėjo (koreliacijos koeficientai atitinkamai r = –0,56 ir r = –0,05), o bakterijų koncentracija didėjo. Teigiama koreliacija nustatyta tarp sunkiųjų metalų (Cu, Cr, Cd, Ni ir Zn) koncentracijos ir humuso kiekio dirvožemyje, tačiau koreliacijos tarp sunkiųjų metalų koncentracijos ir atstumo nuo taršos šaltinio nebuvo. Didžiausia sunkiųjų metalų koncentracija nustatyta 5 km atstumu nuo taršos šaltinio. Stipri teigiama koreliacija nustatyta tarp mikromicetų koncentracijos ir azoto (r = 0,77), fosforo (r = 0,88) bei humuso (r = 0,74) koncentracijos dirvožemyje. Mikromicetų įvairovė buvo skurdžiausia plotuose, kuriuose aptiktas didžiausias kiekis sunkiųjų metalų (ypač Pb, Cr, Zn ir As), azoto, fosforo ir humuso. Gauti duomenys parodė, kad bakterijų ir mikromicetų populiacijos gausios didelės taršos zonose, tačiau rūšinė įvairovė mažesnė.

Raktažodžiai: dirvožemio mikroorganizmai, pramoninė tarša, sunkieji metalai, mikroorganizmų bendrijos, bakterijos, aktinomicetai, mikromicetai