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

2. Abundance and diversity of soil fungi

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Abundance of fungi and their species diversity in soil of seven deciduous forests situated at a distance of 0.7, 3, 4, 5, 8, 9 and 15 km from a chemical factory in Lithuania were investigated. Fungal abundance and species composition were determined by the colony count technique and identification by macro- and micro-morphological observations of fungal cultures after subculture on potato dextrose agar, malt extract agar, cornmeal agar, Czapek's agar and Sabouroud agar media. The number of fungi viable on the isolation media negatively correlated (r = –0.58) with the distance from the pollution source and with the content of some heavy metals in soil (r = – 0.46, –0.44 and –0.35, As, Cr and Zn, respectively). A positive correlation between fungal abundance and the content of nutrients, organic mater and water in soil (r = 0.77, 0.88, 0.74 and 0.46, for N, P, OMC and WC, respectively) was observed. In contrast to the abundance, the number of isolated fungal genera (and species) positively correlated with the distance from the factory and heavy metal content in soil. A lower fungus diversity in more polluted soils provided evidence that pollution may reduce the suitability of forest soil as a habitat for specific groups of fungi, such as the genera *Geomyces* and *Acremonium* and some species of the *Penicillium* and *Trichoderma* genera. Other fungi (*Paecilomyces*, *Talaromyces, Beauveria* genera and sterile mycelia forms) were abundant in the soil of more polluted forest plots.

Key words: deciduous forest, fungal communities, abundance, diversity, pollution effects, heavy metals, resistance

INTRODUCTION

Different microbial groups inhabit various niches within an ecosystem and therefore differ in their sensitivity to nutritional and environmental change (Giller et al., 1998). In both field and laboratory experiments, structural changes in different microbial communities under heavy metal stress were observed (Frostegård et al., 1993b; Pennanen et al., 1996; 1998; Moffet et al., 2003; Abaye et al., 2005; Frey et al., 2006; Macdonald et al., 2007). Community structure is an important aspect of the microbial biomass as the microbial community structure is the parameter controlling microbial activity (Ramsey et al., 2005) and influences ecosystem functioning (Gadd, 2008). Because the microbial community regulates decomposition processes and nutrient cycling, it is of keen interest to understand how its structure is affected by industrial pollution stress (Kiikkila, 2003; Gadd, 2008). Soils under pollution stress can maintain microbial activity because sensitive microbial communities can be replaced by more tolerant ones (Pennanen et al., 1996; Bååth et al., 2005). Changes in microbial community structure were observed in a long-term studies (Sandaa et al., 2001; Macdonalds et al., 2007), however, most studies of the impact of heavy metal concentrations on the diversity and structure of soil microbial communities have largely been concerned with analysis of bacterial community (Sandaa et al., 2001; Moffet et al., 2003; Ton-Petersen et al., 2003), total microbial biomass, total microbial activity in the soil or with the microbial community structure analyzed by the phospholipid fatty acid method (Frostegård et al., 1993a; Åkerblom et al., 2007).

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The aim of this study was to investigate the impact of long-term industrial pollution, characterized by elevated concentrations and co-varying levels of a complex mixture of various metals and enlarged content of nutrients (nitrogen and phosphorus) on the abundance and diversity of fungi in deciduous forests situated at a different distance from a chemical factory in Lithuania. The first product of this chemical enterprise was obtained in 1963. At present, the major product of the company is nitrogen phosphorous fertilizer. In the study period, hard pollutants emitted from the chimneys made up about 140 t per year (data provided by Ministry of the Environment of Lithuania).

MATERIALS AND METHODS

Study area. The chemical factory near the city of Kėdainiai in Central Lithuania was founded in 1963. At present, the major product of the company is nitrogen phosphorous fertilizer. The main emission products are NO_x, CO, sulfur anhydride, apatite and fluorine. Heavy metals accumulate in the organic topsoil close to the factory.

The present study was done along a heavy metal gradient west-east and northwest-southeast of the factory. Four different samplings were made at seven sites of differently polluted coniferous forests. The sites were selected with the aim to minimize differences due to environmental factors other than heavy metal pollution. The nearest plots were located at a distance of about 0.7–3 km in the eastern and southeastern directions; other investigated forests were located about 4 km to the northeast, 5 km to the southwest, 8 km to the southeast, 9 km and about 15 km to the east from the emission source. The sites were mature coniferous forest stands. Dominant tree species were *Fraxinus excelsior* L., *Populus tremula* L., *Padus avium* Mill., and *Corylus avellana* L. A more detailed description of the study area is found in Stankevičienė and Pečiulytė (2004).

Analytical methods. The sites were sampled in spring and early summer (May and June) and in autumn (September and October) of 2001 and 2002. All seven sites were common to the four samplings. 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 size) prior to further use in the experiment. Fungi were isolated by the serial dilution method, the level of dilution being selected to give 30–50 colonies on medium plate, and incubated at 25 ± 2 °C. Fungi were isolated and grown on five media: Rose Bengal agar (RBA), potato dextrose agar (PDA), Czapek's agar (CA), corn meal agar (CMA), and malt extract agar (MEA). To avoid bacterial contamination, 50 µl of streptomycin (33 mg ml^{-1} stock solution) was added to 50 ml of the media, except RBA medium. Abundance of viable fungi in soil was determined on MEA medium after 7 days of incubation at 25 ± 2 °C in the dark and was expressed as colony-forming units (CFU) in one g of dry weight soil.

Standard procedures based on colony, spore and structural morphology were followed for identification at the generic and species level (Ellis, 1971; Watanabe, 1994; Kiffer and Morelet, 1999; Domsch and Gams, 1988). Colonies that obviously belonged to different types but could not be conclusively identified at the generic level were denoted by serial numbers and by letters.

Soil chemical analysis. The concentration of nitrogen and phosphorus was determined with the SPECOL11 photometer, potassium with the FLAPHO41 flame photometer, and the content of humus was established calorimetrically (Минеев, 1989). After digestion of a sample with a mixture of HNO_3 and HCl ((1 : 3, v/v) (aqua regia), the total concentration of heavy metals (As, Cd, Cr, Cu, Ni, Pb and Zn) in the soil was measured by atomic absorption spectrophotometry (EAAS) using a Perkin–Elmer–Zeeman 3030 spectrophotometer. Soil pH_{KCl} was measured with a glass electrode using a mixture of soil with a 1.0 M KCl suspension. Moisture content in soil was determined by overnight drying at 105 °C.

Statistical analysis. For data analysis, the standard errors of fungal abundance, number of fungus genera and soil parameters were estimated, and the significance level of the 0.05 was used throughout employing the recommended methods (Zar, 1999).

RESULTS AND DISCUSSION

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 of potassium in the 4th and 5th plots, which were distanced 8 km, 9 km and 15 km, respectively. A significant negative correlation (r = –0.65) was observed between potassium concentration in the forest soil and distance from the pollution source (Table 2). Potassium can affect heavy metal concentration level in soil (Nordgren et al., 1985). Concentration of nitrogen and phosphorus have strong positive correlation with concentration of humus content in soil (r = 0.98 and r = 0.94, respectively), especially with humus content in plots located at a distance of 0.7 and 5 km from the factory. Mobility of heavy metals in soil depends on soil pH as well as on the organic compound (humus) amount in it (Gadd, 2008). Soil pH in forest plots investigated varied from 4.0 to 6.0 (Table 1) and in some plots can be characterized as moderate acid (pH 4.6–5.0). The stronger acidity of soil (pH 4.0 ± 0.2) during investigation period was determined in the forest plot, situated next to the factory (0.7 km). Medium negative correlation (r = –0.33) between pH and the distance from the factory was observed. Small positive correlation (r = 0.18) between pH and the total content (sum) of heavy metals in soil were observed, however, medium positive correlation between As

and Zn content in soil and soil pH (r = 0.69 and 0.52, respectively) were determined (Table 2). The highest concentration of heavy metals was determined in soil of the 7th plot situated at a distance of 5 km from the factory and in soil of the 1st and 3rd (3 and 4 km, respectively) plots, and the lowest – in soil of the 6th plot (8 km). 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 forests revealed a negative correlation between the distance and the concentration of Pb and Cu (r = –0.52 and r = –0.44, respectively). Thus, forests situated at a distance of about 3–5 km from the factory were most heavily polluted with heavy metals. The highest concentrations of Zn, As, Cr and Ni distinguished these forests. Moisture content in soil positively (r = 0.78) correlated with the humus content in soil, as well as the content of some heavy metals (Cd and Ni) positively correlated with water content in soil. A more detailed description of the content of heavy metals in soils of these forest plots is found in Stankevičienė and Pečiulytė (2004).

Abundance and diversity of fungi. Fungal communities in soils are an important component, because they participate in regulating microbial activity in polluted soils (Ramsey et al., 2005; Frey et al., 2006). To increase our ability to optimize the management of soil fungi in field situations, there is a need for more information on how industrial practices influence the variation in fungal community development in different soils. The first step is to fully characterize the fungal community abundance. Despite the fact that the soil plots studied cannot be attributed to highly polluted areas, the abundance of fungi correlated with the soil parameters, suggesting an impact of long-term heavy metal pollution (Table 2). Our results can be used to estimate the potential of heavy metal pollution to exert a negative effect on soil fungus populations in contaminated deciduous forest soils. It is, however, always difficult to compare different soils in relation to what portion of the total metal loading is available. Soil pollution with heavy metals in our investigation as well as in other studies can be characterized by elevated concentrations and co-varying levels of a complex mixture of metal species (Åkerblom et al., 2007). Therefore, the observation of negative effects on soil microorganisms with high levels of metal pollution can rarely be attributed to a single metal. Despite the different media used in the investigation to improve the data obtained, the fungal abundance and their

Table 1. **Main soil properties of seven deciduous forest plots (mean ± standard error) (n = 36)**

Distance from the factory (plot)	N (% ds)	P (% ds)	K (mg/kg)	Humus (% ds)	Heavy metals*	Water content, %	pH (KCl)
0.7 km (2nd)	0.86 ± 0.45	0.106 ± 0.024	133.02 ± 68.2	9.89 ± 3.46	52.94 ± 0.2	30.6 ± 4.2	4.0 ± 0.2
3 km (1st)	0.39 ± 0.05	0.052 ± 0.015	143.3 ± 35.6	6.23 ± 1.06	64.34 ± 0.5	11.0 ± 3.6	4.6 ± 0.2
4 km (3rd)	0.42 ± 0.08	0.049 ± 0.01	117.7 ± 31.1	7.03 ± 0.98	64.06 ± 1.2	16.7 ± 2.7	6.1 ± 0.8
5 km (7th)	0.74 ± 0.09	0.076 ± 0.019	146.07 ± 52.4	9.06 ± 1.72	78.74 ± 1.6	32.5 ± 5.3	5.2 ± 0.1
8 km (6th)	0.25 ± 0.03	0.033 ± 0.012	129.05 ± 42.7	4.57 ± 1.02	46.28 ± 0.7	23.1 ± 4.8	4.8 ± 0.3
9 km (4th)	0.43 ± 0.06	0.051 ± 0.014	105.47 ± 22.7	6.76 ± 0.67	52.17 ± 0.4	23.1 ± 6.1	6.1 ± 0.1
15 km (5th)	0.41 ± 0.08	0.051 ± 0.022	109.7 ± 39.8	6.75 ± 0.93	54.14 ± 1.3	18.2 ± 3.5	5.0 ± 0.8

ds – dry weight soil; * – sum (mg of Pb, Cd, Ni, Cr, Cu, Zn, and As) kg–1 of dry weight soil.

Table 2. **Coefficients of correlation between fungal characteristics and soil chemical parameters (n = 24)**

	Total number	Number of genera	As	Cd	Cr	Cu	Ni	Pb	Zn	N	P	K
Distance from the factory	–0.58	**0.93**	0.14	–0.03	–0.04	–0.44	–0.09	–0.52	0.18	–0.52	–0.54	**–0.65**
pH	**–0.71**	0.36	0.69	0.16	0.31	–0.09	0.12	–0.30	0.52	–0.39	–0.52	**–0.52**
WC	**0.46**	–0.45	–0.24	**0.67**	0.44	–0.43	**0.63**	–0.26	–0.18	**0.90**	**0.82**	0.16
OMC	**0.74**	**–0.68**	–0.17	**0.69**	0.33	**0.84**	0.51	0.08	0.28	**0.98**	**0.94**	0.29
As	–0.46	0.06										
Cd	–0.01	–0.34										
Cr	–0.44	–0.24										
Cu	0.16	–0.67										
Ni	–0.31	–0.31										
Pb	0.36	**–0.58**										
Zn	–0.35	–0.34										
Sum*	–0.11	**–0.55**										
N	**0.77**	**–0.70**										
P	**0.88**	**–0.71**										
K	0.42	**–0.70**										

* Sum – the total content of heavy metals.

species diversity did not show a very strong relationship with the pollution levels (only some statistically significant data were obtained). The biomass of the total soil microbial communities is usually negatively correlated with metal stress (Chander et al., 2001; Bååth et al., 2005; Wilke et al., 2005) but is less affected than community structure. Our results obtained in a two-year period agree with this statement. The resistance of bacteria and fungi in soils polluted with heavy metals has been studied in the field (Pannanen et al., 1998; Ramsey et al., 2005) and in laboratory studies (Rajapaksha et al., 2004), indicating that fungi are favoured compared to bacteria in metal-stressed soils. Our results obtained during a complex investigation of forest plots situated along a pollution gradient next to a fertilizer factory in central Lithuania also suggest an increase in the relative fungal / bacterial ratio (in press).

Despite the limitations of the method used in this investigation, significant differences in the abundance of fungi as well as in species composition were found. The abundance of fungi was evidently different in separate plots ($P < 0.05$) and depended on the sampling period (Fig. 1).

The average amount fungi determined during the investigation period in different plots varied from 25.3 to $162.4 \cdot 10^5$ CFU g^{-1} of dry weight soil (Fig. 1). Mean fungal counts determined in soil samples in May were the highest in the 2nd plot and statistically significantly differed ($P < 0.05$) from their abundance in soil samples collected from the other plots. Analysis of soil chemical composition revealed the lowest concentrations of nutrients and heavy metals in the forests situated at a larger distance from the factory (8–15 km), however, the abundance of fungi in soils was higher in the plots located closer to the factory (at a distance of 0.7–4 km). A relative abundance ($14.02 \cdot 10^5$ CFU g^{-1} of soil) was determined in soil of the 4th and 5 forest plots, while the counts determined in the 1st, 2nd and 3rd plot soil were highest and decreased with the distance from the factory. The lowest number of fungi was observed in the 6th plot located at a distance of 8 km from the pollution source and perhaps was due to the low organic matter and nitrogen content in the soil of that forest plot (Table 1). Some seasonal variations in fungal counts were also observed. The counts of fungi were significantly higher ($P < 0.05$) in soil samples collected from some plots in May than in September. No differences in fungus abundance were determined in May, June and September of the 5th and 6th plot soils, and these differences were not statistically significant in other plots. The number of fungi in forest soils was lower in June and in October than in May and September, especially in soil of the 1st, 3rd and 4th forest plots. A medium-negative correlation between fungus abundance in soil of forest plots and the distance from the pollution source ($r = -0.58$) was observed (Table 3). A significant negative correlation was found 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 plot location and the abundance of total fungal counts. A positive correlation between fungal abundance and nitrogen, phosphorus and humus ($r = 0.74$) content in soil was determined ($r = 0.77$, 0.88 and 0.74, respectively). Fungus abundance negatively correlated with soil pH ($r = -0.71$). This is worth noting because the mobility of heavy metals in soil depends on soil pH. The soil plots studied cannot be attributed to highly polluted areas, however, a medium-negative correlation between fungal abundance and the content of separate metals in soil was observed (Table 3). A positive correlation between the fungal counts and Cu ($r = 0.16$) as well as Pb ($r = 0.36$) concentrations were noted, but, in contrast to abundance, the species diversity of fungi was negatively correlated with the content of these two metals.

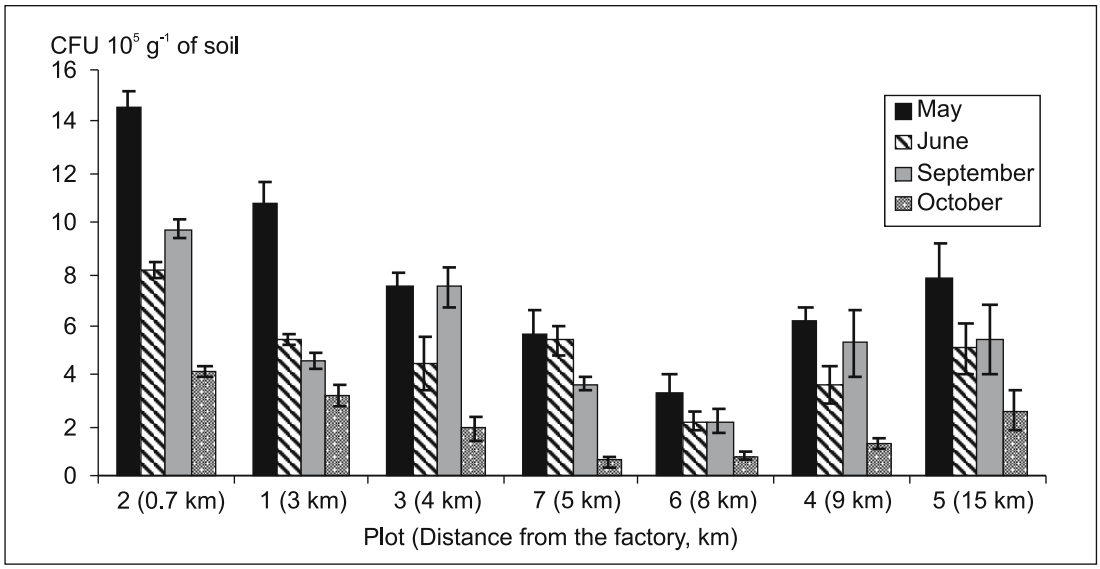

Fig. 1. Abundance of fungi (CFU $\cdot 10^5$ g^{-1} of dry weight soil) of soil fungi in seven deciduous forest plots located at a different distance from a fertilizer factory. Mean of two years (2001–2002) ± standard deviation (n = 24)

Table 3. **The number of fungal species in genus (SN) and relative frequency (RF, %) of genus isolates in soil of seven forest plots [* – site (distance from a fertilizer factory)]**

Genus	Total number of species in the genus	2nd (0.7 km)*		1st (3 km)		3rd (4 km)		7th (5 km)		6th (8 km)		4th (9 km)		5th (15 km)	
		SN	RF	SN	RF	SN	RF	SN	RF	SN	RF	SN	RF	SN	RF
Phylum Zygomycota Class Zygomycetes Order Mucorales															
Absidia Tiegh.	2	2	24.2							1	14.3	2	2.3	1	3.4
Cunninghamella Matr.	2			2	6.9	2	3.9	2	2.9	1	3.4			1	1.1
Mucor Fresen.	3			1	1.3	1	0.9	1	1.3	2	1.3	2	0.9	2	0.6
Rhizopus Ehrenb.	2			2	3.4	2	0.6	2	0.9	2	2.2	1	0.6	1	0.4
Zygorhinchus Vuill.	2			2	2.6	2	1.8	2	2.3	1	1.20	1	0.4	1	0.7
Umbelopsis Amos & H. L. Barnett	2			2	5.3	1	5.6	2	6.5	1	2.4			1	1.3
Order Mortierellales															
Mortierella Coem.	3									1	1.8	2	3.1	2	2.6
Phylum Ascomycota Class Eurotiomycetes Order Chaetothyriales															
Phialophora Medlar	1									1	2.4				
Order Eurotiales															
Aspergillus P. Micheli ex Link	6	1	6.3							1	2.9	2	4.8	2	3.3
Eurotium Link	1			1	2.7					1	2.2			1	0.5
Paecilomyces Bainier	4	1	2.8	3	6.1	3	7.9	4	8.4	1	2.1	2	4.4	4	3.4
Penicillium Link	18	3	11.8	5	10.4	4	10.4	6	7.3	10	14.7	12	33.1	15	36.3
Talaromyces C. R. Benj.	2			1	10.9	1	3.8	1	5.6	1	0.8	1	0.8		
Order Onygenales															
Chrysosporium Corda	2			2	4.9	2	3.2	2	3.9						
Class Dothideomycetes Order Capnodiales															
Cladosporium Link	2	1	7.6	2	4.5	2	5.2	2	6.7	1	0.4	1	2.8	2	2.2
Order Dothideales															
Aureobasidium Viala & G. Boyer	1			1	6.4	1	5.3	1	6.2	1	1.1				
Order Pleosporales															
Alternaria Nees	2	2	2.3							1	2.5	1	1.7	1	0.8
Curvularia Boedijn	1											1	0.1	1	0.4
Epicoccum Link	1	1	3.7							1	1.8	2	0.9	1	0.2
Leptosphaeria Ces. & De Not.	1	1	2.3							1	1,4				
Oidiodendron Robak	1									1	0.8	2	1.3	1	1.9
Phoma Sacc.	2	2	4.5			1	0.7			1	0.8	1	0.9	1	2.3
Stemphylium Wallr.	1			1	1.3	1	0.4					1	0.8	1	0.3
Ulocladium Preuss	1			1	1.8	1	0.3	1	0.7			1	0.4	1	0.8
Incertae sedis															
Geomyces Traaen	1					1	0.2			1	0.8	1	1.2	1	0.4
Class Leotiomycetes Order Helotiales															
Scytalidium Pesante	1									1	1.8	1	2.1		
Botrytis P. Micheli & Pers.	1			1	0.3										
Class Saccharomycetes Order Saccharomycetales															
Geotrichum Link.	1									1	1.4			1	0.2
Class Sordariomycetes Order Hypocreales															
Acremonium Link	2									1	2.6	2	3.9	2	3.1
Beauveria Vuill.	2			1	3.1	2	5.5	1	4.5			1	0.9	2	0.3

Table 3 **(continued)**

Genus	Total number of species in the genus	2nd (0.7 km)*		1st (3 km)		3rd (4 km)		7th (5 km)		6th (8 km)		4th (9 km)		5th (15 km)	
		SN	RF	SN	RF	SN	RF	SN	RF	SN	RF	SN	RF	SN	RF
Clonostachys Corda	2	1	2.5							1	3.8	2	3.1	2	4.7
Cylindrocarpon Wollenw.	1	1	2.2							1	2.4	1	2.8	1	3.3
Cylindrocladium Morgan	1	1	8.9											1	0.8
Fusarium Link	4			2	1.5	1	1.6	2	2.4	1	3.6	2	2.1	2	1.9
Gliocladium Corda	1	1	6.7	1	3.5	1	4.2	1	5.2	1	3.6	1	4.1	1	3.4
Gliomastix Guég.	2					2	1.6	2	2,1						
Lecanicillium W. Gams & Zare	2			2	5.7	2	4.1	1	5.4	1	0.9	1	1.3	1	0.4
Metarhizium Sorokin	1			1	5.9	1	4.6	1	6.7	1	2.3	1	0.4	1	0.2
Myrothecium Tode	1									1	1.5	1	2.2	1	0.3
Stachybotrys Corda	2					1	0.3							1	0.8
Trichoderma Pers.	4	1	6.8	2	2.7	2	0.8	1	1.9	4	5.7	3	4.1	4	3.8
Trichothecium Link	1	1	0.4												
Incertae sedis															
Arthrinium Kunze	1			1	2.2	1	1.9	1	2.1					1	1.2
Verticillium Nees	2					1	11.4	1	8.7	1	2.2	2	3.1	2	2.4
Order Melanosporales															
Gonadobotrys Corda	1	1	2.8												
Order Microascales															
Cephalotrichum Link.	2	1	1.4									1	0.4	1	0.8
Scopulariopsis Bainier	2	2	4.6							1	0.08	1	0.5	1	0.6
Wardomyces F. T. brooks & Hansf.	1									1	1.4	1	1.1	1	0.8
Order Sordariales															
Botryotrichum Sacc. & Narchal	1					1	0.2	1	0.7	1	0.8			1	0.6
Chaetomium Kunze	2			2	5.7	2	4.2	2	2.9			1	2.3	1	1.1
Humicola Traaen	1			1	0.7					1	0.6	1	1.3	1	2.7
Trichocladium Harz.	2					1	2.8	2	2.4	1	1.4				
Class Insertae sedis Order Insertae sedis															
Gilmaniella G. L. Barron	1											1	0.6	1	0.4
Mitosporic fungi															
Order Agonomycetales															
Mycelia sterilia (white)	4									1	0.03	2	0.2	4	0.6
Mycelia sterilia (dark)	3							3	1.3	1	1.2	1	1.4	3	1.2
Mycelia sterilia (yellow)	2									1	0.2	1	0.1	2	0.2
Phyllum Basidiomycota Class Agaricomycetes Order Atheliales															
Athelia Pers.	1									1	0.2	1	0.4	1	0.7
Order Polyporales															
Sporotrichum Link	1									1	0.4			1	0.2
Order Cantharellales															
Rhizoctonia DC	1									1	0.5	1	0.2	1	0.4
Total number of genera (species):	59 (121)	18 (22)		24 (40)		28 (42)		24 (44)		43 (58)		41 (65)		48 (83)	

Diversity of fungi. Despite the fact that fungal abundance negatively correlated with the distance from the pollution source, their species diversity was positively correlated with this parameter. The mean values of data obtained during the study period are presented in Fig. 2. Contrasting, changes of the total abundance of fungi and the number of fungal genera along the pollution gradient were pronounced (Fig. 2). A decrease of fungal diversity is a basic tendency in polluted soils (Chander et al., 2001; Dighton, 2004; Невская и др., 2006; Bååth et al., 2005). Spore germination is known to be more sensitive to metals than mycelia growth (Amie, Pineau, 1998). Perhaps spores (or conidia) of more resistant fungi kept their

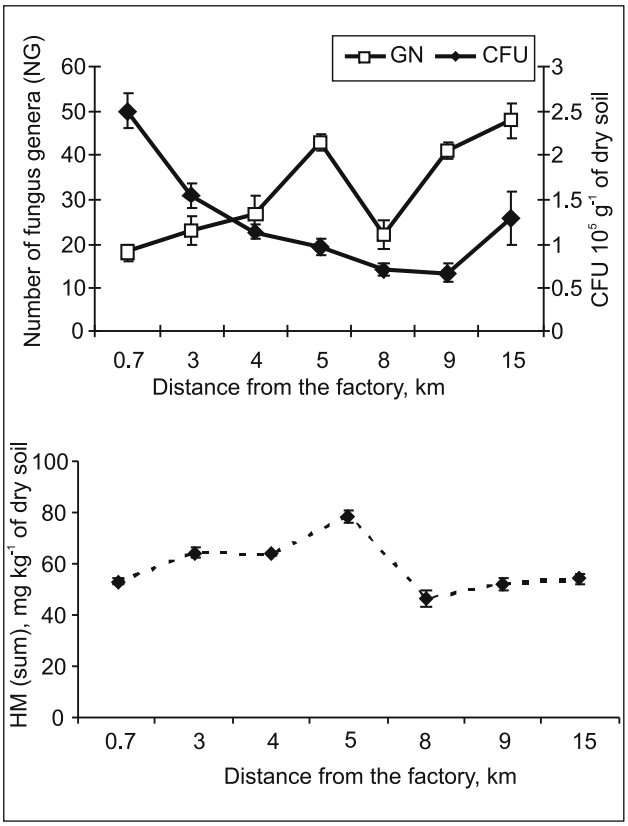

Fig. 2. Abundance of fungi (CFU · 10^5 g^{-1} of dry weight soil), number of genera (NG) of isolated fungi and heavy metal (HM) concentration (sum of Pb, Cd, Ni, Cr, Cu, Zn, and As), mg kg^{-1} of dry weight soil of forest plots. Mean of eight samplings (in May, June, September, October, 2001–2002) ± standard deviation (n = 24)

viability in the polluted soils in our study and were abundant on the isolation media. One hundred twenty one species belonging to 59 genera, presented in Table 3, were identified during this investigation of the differently polluted forest plots. Different numbers of fungal species and their genera were observed. In the soil of separate forest plots, the number of genera varied from 22 to 83 and from 18 to 48, respectively (Table 3). A positive correlation between the number of fungal genera and the distance from the factory (r = 0.93) and a negative correlation with the content of heavy metals in soil, especially of Pb (r = –0.58) and Cu (r = –0.67), was found (Table 2).

The fungal community composition at the level of genera was affected by heavy metal pollution (Table 3). The abundance of the genus *Penicillium* Link ex Fr. (18 identified species) decreased from 36.3% and 33.1% in the total number of fungal genera isolated from the soil of the 5th and 4th sites (at a distance of 15 km and 9 km, respectively) to 11.8%, 10.4.5% and 8.4% isolated from the soil of the 2nd, 1st and 7th sites (at a distance of 0.7, 3 and 5 km from the pollution source, respectively). A similar decrease of the number was found for the genera *Acremonium* (2 species), *Oidiodendron* (4 species), and *Trichoderma* (5 species). These results are in agreement with data reported by Nordgren et al. (1983) on the fungal community composition along the heavy metal gradient in a coniferous forest in Sweden. The abundance of other genera, such as *Paecilomyces* (4 species), *Geomyces* (1 species), *Torulomyces* (2 species), *Trichocladium* (2 species), *Gliomastix* (2 species), *Mortierella* (3 species), *Umbelopsis* (2 species), and *Mycelia sterilia* increased in the soil of plots located close to the factory and in the soil of more polluted plots. A similar effect of heavy metal pollution was found for the genera *Beauveria*, *Metarhizium*, and *Lecanicillium*: the number of species was higher in polluted soils. Abundance of some fungi belonging to the genera *Paecillomyces*, *Beauveria*, *Metarhizium*, *Lecanizillium* and *Fusarium* increased in the soil of the 1st, 3rd and 7th plots where the content of Cu, Zn or both was higher. Fungi from the genera *Curvularia* and *Fusarium*, known as common in agricultural soils, were more sensitive to metals (Ni, Co, Fe, Mn, Mg) than the other fungi. Similar results had been obtained by Amie and Pineau (1998). The copper-adaptation is referred to as a result of physiological mechanisms, and the activated biochemical processes explain resistance to copper ions and to other heavy metals (Romero et al., 2006). It is possible that some common soil fungi were not isolated from the polluted soil samples in our study because of their elimination by competitors that are more tolerant to heavy metals. Fungi belonging to the genera *Monilia* and *Geoterichum* showed a relatively low tolerance to all metals in comparison to other ones. The genera *Aspergillus*, *Rhizopus*, *Penicillium*, *Fusarium*, *Chaetomium*, *Geomyces* and *Paecilomyces* were also isolated from the soil of the forest plots polluted with heavy metals (Cu, Cd, Pb, As, and Zn). Most *Penicillium* species are considered as ubiquitous, opportunistic saprophytes able to grow in almost any environment with mineral salts, a wide range of pH and the redox potential (Gadd, 2008). *Talaromyces* isolates were observed only in forest plots located closer to the factory (at a distance of 0.7, 3 and 4 km). The number of *Talaromyces* colonies contains 5.6% of all fungi isolated from the soil of the 7th plot where Cu content was the highest. Romero et al. (2006) have found that *Talaeromyces helicus* is able to survive in soil polluted with copper.

Analysis of the study results suggests an interesting negative correlation between fungal community structure and heavy metal pollution level and, in contrast, a positive correlation between fungal abundance and heavy metal pollution level. That observation is in agreement with data of other investigators. The biomass of soil microbial communities was negatively correlated with metal stress, but usually it is less affected than community structure (Chander et al., 2001; Bååth et al., 2005; Wilke et al., 2005).

CONCLUSIONS

Soil fungi are an important component because they participate in the regulation of soil microbial activity in polluted areas. Although heavy metal pollution in the study area around the chemical factory is relatively low (compared with foreign

smelters and great factories), it influences the community of soil fungi. Strong or medium correlations of fungus abundance and their diversity with some soil parameters observed in the investigation suggests a tangible impact of the long-term industrial pollution on fungal populations in soil.

Abundance of soil fungi negatively correlated with the distance from the pollution source as well as with the heavy metal content in soil. The diversity of fungal genera positively correlated with the distance from the pollution source; the number of genera in differently polluted forest plots varied by the pollution level, but it was more dependent on organic mater, phosphorus and nitrogen content in soil.

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ILGALAIKĖS PRAMONINĖS TARŠOS ĮTAKA SKIRTINGU ATSTUMU NUO GAMYKLOS ESANČIO LAPUOČIŲ MIŠKO DIRVOŽEMIO MIKROMICETAMS 2. MIKROMICETŲ GAUSA IR ĮVAIROVĖ

S a n t r a u k a

Ištirta mikromicetų gausa ir jų populiacijų gentinė struktūra septyniuose lapuočių miško plotuose, esančiuose 0,7, 3, 4, 5, 8, 9 ir 15 km atstumu nuo centrinėje Lietuvos dalyje esančios trąšų gamyklos. Mikromicetų gausa tirtuose dirvožemiuose įvertinta kolonijas sudarančių vienetų skaičiumi (ksv g^{-1} sauso dirvožemio), o jų bendrijų struktūra pagal skirtingose (bulvių ekstrakto su gliukoze, salyklo ekstrakto, kukurūzų ekstrakto, Čapeko ir sabūro) terpėse išaugintų augalų kolonijų ir mikrostruktūrų charakteristikas. Nustatyta neigiama koreliacija tarp išskirtų mikromicetų gausos ir atstumo nuo taršos šaltinio (koreliacijos koeficientas –0,58) bei tarp jų gausos ir kai kurių sunkiųjų metalų koncentracijos dirvožemyje (koreliacijos koeficientai As, Cr ir Zn buvo atitinkamai lygūs –0,46, –0,44 ir –0,35). Teigiamai koreliuoja mikromicetų koncentracija ir mitybinių medžiagų, humuso bei drėgmės kiekiai dirvožemyje (mikromicetų gausos ir azoto, fosforo, humuso bei drėgmės kiekio koreliacijos koeficientai buvo atitinkamai 0,77, 0,88, 0,74 ir 0,46). Kitaip negu mikromicetų gausa dirvožemyje, jų genčių skaičius buvo didesnis didesniu atstumu nuo gamyklos esančių miško plotų dirvožemyje. Nustatyta skurdesnė sunkiaisiais metalais užteršto dirvožemio mikromicetų bendrijų įvairovė leidžia teigti, kad tarša neigiamai veikia kai kurių mikromicetų, ypač *Geomyces* ir *Acremonium* genčių bei kai kurių *Penicillium* ir *Trichoderma* genčių vystymąsi dirvožemyje. Kiti mikromicetai (*Paecilomyces*, *Talaromyces* ir *Beauveria* gentys bei sterilų micelį formuojantys grybai) gausesni labiau užterštame dirvožemyje.

Raktažodžiai: miško dirvožemis, mikromicetų bendrijos, gausa, įvairovė, taršos poveikis, sunkieji metalai, atsparumas