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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

3. Species diversity and community structure of soil fungi

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The present study was conducted to establish whether a long-term (57 years) chemical industry pollution could influence fungal community structure in deciduous forest soil. This paper presents results of two-year investigations of soil fungi in seven deciduous forest plots located at a different distance (0.7–15 km) from the emission source (ES) – a fertilizer factory in Central Lithuania. Fungal abundance as colony forming units (CFUs) was determined by the plate dilution technique, and the identification of fungi to a species was based of their micro- and macromorphology. Their abundance varied from 0.88 to 2.59 thousand CFU g^{-1} dry weight soil and was significantly negatively correlated with the distance from the ES (Spearman's correlation $r_s = -0.78$). Phosphorus and humus content was the major factor to influence the fungal abundance ($r_s = 0.88$ and 0.79, respectively). Copper and lead were the two pollutants (out of Cu, Cd, Ni, Pb, Zn and As investigated), which exerted a significant impact on the community structure. In total, 158 species out of 11 394 isolates were identified. Most of the isolates belonged to Anamorphic (Mitosporic) fungi (74.87% of the total CFU), followed by Zygomycetes (15.56%), Ascomycetes (7.78%), with only 3 isolates from Basidiomycetes. The majority of fungi (33.22%) were from the genus *Penicillium*, followed by *Trichoderma* (6.98%) and *Paecilomyces* (6.58%).

Key words: forest soil, fungi, fungal communities, abundance, species diversity, pollution impact, heavy metals

INTRODUCTION

Owing to a rapid increase of fertilizer industries, there has been a substantial increase of pollutants discharged into surroundings. This causes varying degrees of air, water and soil pollution. Soil has the greatest capacity for accepting and decomposing pollutants of different kinds. However, if the input of pollutants is higher than the purifying capacity of soil, the pollution decreases the effectiveness of soil microorganisms considerably; thus, the physical-chemical properties of the soil are also adversely affected. Soil fungi take a very important position in the structure and function of an ecosystem, but they are sensitive to a wide variety of organic and inorganic pollutants and environmental changes (Francland et al., 1996; Gadd, 1993, 2005, 2007; Dighton, 2003; Kelly et al., 2003). The impairment of fungal activity could have important consequences for ecosystem function since fungi play a major role in carbon, nitrogen, phosphorus and other biogeochemical cycles (Gadd, 2005, 2008). It is obviously desirable to know as much as possible about the impact of pollutants on these organisms. However, there are very few data on a community structure of soil fungi in industry-specific areas, such as land and forests located next to fertilizer factories. Lithuania is not an exception. The first investigation of soil fungi next to a phosphorus fertilizer factory was conducted by L. Lebedeva and A. Lugauskas (Lebedeva, Lugauskas, 1985). The values of fungus abundance, determined by the dilution plate count method, were higher in the plots next to the factory; however, no correlation between the abundance of fungal populations and the soil pollution level was determined. In 2001–2003, complex investigations

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on lichens (Motiejūnaitė, 2007), fungal endophytes, ectomycorrhizae (Stankevičienė, Pečiulytė, 2004) and soil fungi from eight deciduous forest plots were performed.

In this paper, we report on the effects of long-term pollution from a fertilizer factory on the abundance and community composition of soil fungi. The effect of pollution on fungal population / community size and composition is particularly difficult to assess. Furthermore, the dilution plate count method used to assess changes in fungal community composition has been criticised (Gadd, 2007). According to Mueller et al. (2004), only investigations coupled with appropriate manipulations of the isolation methods could give some idea of the relative effects of environmental factors on the abundance and structure of a soil fungus community. During this investigation, we attempted to approach the problem in different ways, monitoring the soil fungi in seven differently polluted locations during two years (four times a year), analyzing soil samples immediately after they had been collected to stop temporal changes, with minimal effects on species composition and abundance, balancing the number of soils and the number of isolation media. The main aims were: (*i*) to isolate and identify soil fungi from unequally polluted plots located at a different distance from a fertilizer factory in Central Lithuania, and (*ii*) to compare species diversity and community structure of soil fungi in differently polluted deciduous forest plots.

MATERIALS AND METHODS

Sampling sites

The present study was conducted near a chemical factory located in the district of Kėdainiai, situated in Central Lithuania, where the climate is subcontinental with average temperatures of 17.5 °C in July and 6.0 °C in January, annual precipitation being 500–550 mm and the prevailing winds W, SW and NW (Perkauskas et al., 1996); the study plots are situated downwind. The phosphorus fertilizer factory, founded in 1963, is situated in the south-eastern part of the Kėdainiai city. At present, the major products of the company ("Kemira-Lifosa") are aluminium fluoride, sulphur and phosphoric acids and phosphates. The emission consists mainly of nitrogen oxides, sulphur dioxide, carbon monoxide, volatile organic compounds, ammonia, fluorine compounds and dust particles. Heavy metals also accumulate in the organic topsoil close to the factory (Stoškus, 2005). Seven areas chosen for the present study (30 × 30 m) were located at a different distance (from 0.7 to 15 km) from the main emission source (ES: "Lifosa" phosphorus fertilizer factory) located at a distance increasing from the pollution source. Care was taken to choose plots with similar vegetation. The study area is occupied by deciduous forest stands. The prevailing tree species were *Fraxinus excelsior* L., *Populus tremula* L., *Padus avium* Mill., and *Corylus avellana* L. (Stankevičienė, Pečiulytė, 2004). The nearest plots Juodkiškis (**S1** – 55°16' N, 24°01' E) and Zabieliškis-Šilainėliai (**S2** – 55°14' N, 24°01' E) were at a distance of about 0.7–3 km in the eastern and south-eastern directions, respectively, Vilainiai (**S3** – 55°18' N, 24°01' E) and Pašiliai (**S4** – 55°03' N, 23°58' E) are about 4 km in the north-eastern and 5 km in the south-western directions, Berunkiškis (*S5* – 55°13' N, 24°08' E) and Stebuliai (*S6* – 55°19' N, 24°06' E) being at about 8 km in the south-eastern and 9 km in the north-eastern directions, respectively. The farthest forest plot – Lančiūnava (*S7* – 55°20' N, 24°12' E) – is located about 15 km in the eastern direction from the emission source.

Soil sampling and chemical analysis

Soil samples were collected from seven sites (S1, S2, S3, S4, S5, S6 and S7, according to a distance from the ES) four times (in the spring and autumn period of 2001 and 2002: in May, June, September and October). A sample taken from each site for microbiological analysis was placed separately in a clean, sterile plastic bag and stored at 4 °C. Three replicate samples were taken randomly from each sampling site; they consisted of 18–20 sub-samples collected from the topsoil layer (0–10 cm horizon, after removal of litter). The composite soil samples were sieved through a 2-mm screen and homogenized, air-dried, ground to pass a 1-mm sieve, adjusted to 40% of water holding capacity and stored in a plastic bag at 4 °C prior to analysing soil microbial parameters. Samples collected for chemical analysis were air-dried. Some physical and chemical properties of the soil were measured by routine analytical methods and are listed in Tables 1 and 2.

Table 1. **Chemical properties of soil of seven forest plots located at a different distance from the emission source (ES) – chemical factory; correlation coefficients (Spearman's r_s) and significance level (p) for linear regressions of chemical property variables versus the distance from the ES**

Plots (distance from the factory, km)	N (g kg^{-1})	P (g kg^{-1})	K (g kg^{-1})	Humus (%)	pH_{KCl}
S1 (0.7 km)	8.6 ± 4.5	1.06 ± 0.24	0.133 ± 0.07	9.89 ± 3.46	4.01 ± 0.2
S2 (3 km)	3.9 ± 0.5	0.52 ± 0.15	0.143 ± 0.04	6.23 ± 1.06	4.66 ± 0.23
S3 (4 km)	4.2 ± 0.8	0.49 ± 0.11	0.118 ± 0.03	7.03 ± 0.98	6.02 ± 0.83
S4 (5 km)	7.4 ± 0.9	0.76 ± 0.19	0.146 ± 0.05	9.06 ± 1.72	5.27 ± 0.11
S5 (8 km)	2.5 ± 0.3	0.33 ± 0.12	0.129 ± 0.04	4.57 ± 1.02	4.85 ± 0.35
S6 (9 km)	4.3 ± 0.6	0.51 ± 0.14	0.105 ± 0.02	6.76 ± 0.67	6.09 ± 0.15
S7 (15 km)	4.1 ± 0.8	0.51 ± 0.22	0.109 ± 0.04	6.75 ± 0.93	5.03 ± 0.82
r_s	–0.32	–0.52	–0.64	–0.43	–0.33
p	0.43	0.21	0.11	0.29	0.21

Table 2. **Mean concentration of heavy metals in soil (mg kg^{-1} dry weight soil) of seven forest plots; correlation coefficients (Spearman's r_s) and significance level (p) for linear regressions of the total metal values versus the distance from the emission source (ES)**

Plots	Pb*	Cd	Ni	Cr	Cu	Zn	As
S1	13	0.14	6	10.5	6.5	16	0.8
S2	20.5	0.12	5.7	12.5	4.82	19.5	1.2
S3	12.5	0.16	6.5	14.5	5.8	23	1.6
S4	11.2	0.24	11.5	21	8.5	25	1.3
S5	8.5	0.06	5.6	11.5	3.6	16	1.02
S6	10.4	0.12	5.7	12	4.35	18.5	1.1
S7	10.5	0.17	6.4	13	4.82	18	1.25
r_s	**–0.82**	0.70	–0.07	0.21	–0.52	–0.07	0.18
p	**0.04**	0.84	0.87	0.59	0.21	0.87	0.66

* Analysis of total metals (Pb, Cd, Zn, Cu, Cr, Ni and As) was made after digestion with a mixture of concentrated HNO_3 : HCl (1 : 3, v/v).

The soil humus content was determined colorimetrically (Mineev, 1989), total N and P in soil extracts were determined photometrically with a SPECOL 11 photometer and potassium with a FLAPHO 41 flame photometer. Soil pH was measured using a pH meter with a glass electrode in a 1.0 KCl suspension. Analysis of the total content of each metal (Pb, Cd, Zn, Cu, Cr, Ni and As) in soil samples was made using the Perkin-Elmer Zeemer Zeeman 3030 atomic adsorption spectrophotometer after digestion with a mixture of concentrated HNO_3–HCl (1 : 3, v/v) (aqua regia) (Soon, Abbout, 1993). Organic matter content (OMC) was calculated as the percentage of loss-on-ignition (at 550 °C for a minimum of 3 h) from soil dry matter (Howard, Howard, 1990). Moisture content was determined by overnight drying at 105 °C.

Soil fungus community analysis

The soil dilution technique was used to determine the number of fungus colony forming units (CFUs) in soil samples and isolate pure cultures. Ten grams (on dry weight basis) of the soil subsample was added to a 250 ml sterilized conical flask containing 90 ml of sterilized NaCl solution (0.5%). Flasks were shaken vigorously for 10 minutes to detach soil-surface fungus propagules. Suspensions of 1 : 100 and 1 : 1 000 dilutions were obtained aseptically transferring 10 ml of the previous dilution to the 250 ml conical flasks containing 90 ml of sterilized distilled water. The prepared dilutions (0.5 ml each) were plated aseptically into sterilized Petri dishes and flooded with either of the media: 2% malt extract agar (MEA, Liofilchem), Czapek's agar (CA, Liofilchem, Italy) and potato dextrose agar (PDA, Liofilchem) supplemented with 0.06 g/l of streptomycin sulphate. Three replicates were maintained for each dilution and medium. Plates with the medium inoculated with soil dilutions were incubated at 25 ± 1 °C for 5–7 days, and then the number of colonies was counted.

Each type of colony originating from each soil sample was recorded, counted and isolated on a slope of MEA medium for species identification. The percentage of occurrence of each taxon in the soil of each forest plot was calculated for three media (MEA, CDA, and PDA) used for 24 samples (3 replicates × 8 samplings). Thus, the isolation frequency (*F*) of each taxon per site soil was calculated as the percentage of the total CFU numbers isolated from a sample. The dilution factor and moisture content of a soil sample were taken into account. The relative abundance or isolation frequency (*F*) of fungi in soil samples was calculated by the following equation:

$$F = \frac{CFUs\ of\ a\ species}{CFUs\ of\ all\ species} \times 100\%.$$

Pure cultures of isolated fungi were identified on the basis of their micro- and macro-morphology according to Gilman (1966), Barnet (1967), Ellis (1971), Charmichael et al. (1980), Domsch et al. (1980), Kiffer, Morelet (1999), Watanabe (2000). The system of classification was made following handbooks (Clements, Shear, 1954; Kendrick, 1981; Kiffer, Morollet, 2000; Hawksworth et al., 2001). The colonies that obviously belonged to different types but could not be conclusively identified at the generic (or order) level were left as morphotypes and were denoted by a serial No.

The following indices of diversity, with a species as the operational taxonomic unit (CFU), were computed (Krebs, 1989). A colony obtained on the primary growth plate was identified as a single viable propagule (spore, conidium, arthroconidium, etc.) in a soil sample. The Shannon–Wiener index (*H'*) was applied to evaluate the diversity of fungi in the soil of seven plots (Magurran, 1988; Krebs, 1989):

$$H' = -\sum_{i=1}^{S} P_i \log_e P_i,$$

where *S* is the number of CFUs and $P_i = \frac{N_i}{N}$ is the proportion of total samples belonging to the *i*th CFU. The *H'* value varies between 0 and $\log_2 S$ and is a information content of a relevant sample. The value of *H'* close to 0 indicates a low diversity, whereas a value close to $\log_2 S$ indicates a high diversity.

Sørensen's index of similarity (*S'*) was plotted to evaluate different fungal communities and expressed with the values between 0 (no similarity) and 1 (absolute similarity) (Krebs, 1989):

$$S' = \frac{2c}{a+b},$$

where *S* is Sørensen's index of similarity; *a*, *b* are numbers of species in community *a*, *b*, respectively, and *c* is the number of species found in both communities *a* and *b*.

The non-parametric measurement of statistical dependence between two variables (X and Y) was made by calculating Spearman's rank correlation coefficient r_s (Lehmann, Abrera, 1996). The calculations were made using free statistics software (Wessa, 2010):

$$r_s = 1 - \frac{6\sum d^2}{n(n^2-1)},$$

where *n* is the number of raw cores, *d* is a difference between the ranks in two columns (the first column is the rank of the *i*th value of one variable (X) and the second column is the rank of the *i*th value of the other variable (Y). The analysis of correlation is designed to test the significance.

RESULTS

Chemical properties of forest soil

The highest content of nutrients (N, P, K) was determined in sites S1 and S4 distanced 0.7 and 5 km from the ES, respectively (Table 1). The lowest content of nitrogen and phosphorus was found in the soil of site S5 and of potassium in the soil of sites S6 and S7 located at a distance of about 8 km, 9 km and 15 km, respectively. The total content of N, P and K did not correlate ($p > 0.05$, Table 3) with the distance from the ES. The total content of nitrogen significantly correlated ($r_s = 0.96$ at $p = 0.02$) with the humus content, especially in the soil of forest plots situated at a distance of 0.7 and 5 km from the ES. The highest content of all heavy metals (sum) was also determined in site S4 (5 km from the ES), except Pb, the content of which was the highest in the soil of site S2 situated closer to the factory (3 km) (Table 2). A relatively high content of heavy metals was determined in the soil of sites S2 and S3 (distanced 3 and 4 km, respectively) and the lowest in the soil of site S5 (8 km), in which humus content was the lowest (Tables 1, 2). Forest sites situated at a distance of 0.7, 8 and 15 km (S1, S6 and S7, respectively) took an intermediate position. Evaluation of the distribution of various heavy metals in different forests revealed a negative correlation between Pb content and the distance from the ES ($r_s = -0.82$ at $p = 0.04$) (Table 3). Correlation trends of Cd content in soil and the distance from the ES were observed, but this correlation was insignificant ($r_s = 0.70$ at $p > 0.05$).

Fungal communities, species composition and richness

The community structure and the number of species in the soil of the forest plots varied significantly; 77 genera and 158 species of fungi were isolated from 11 394 fungal colony forming units (CFU). Over 0.3% of the isolates did not sporulate and were grouped into a sterile category (separate morphotypes) and remained unclassified. The average number of viable fungal propagules in the soil of different forests varied from 0.88 to 2.69 thousand CFU · g^{-1} dw soil (Figure). A statistically significant negative correlation between the number of CFUs and the distance from the ES was determined ($r_s = -0.78$ at $p = 0.05$). Fungal abundance positively correlated with the *P* content in the plot soil ($r_s = 0.88$ at $p = 0.03$) (Table 3). Surprisingly, a positive correlation was determined between the number of CFU and the copper content in soil ($r_s = 0.84$; $p = 0.04$). A low, if any, correlation was determined between the total values of N, K and content of most of the heavy metals and the abundance (number of CFU) of fungi in soil (Table 3). The number of CFU positively correlated with humus content ($r_s = 0.79$, $p = 0.05$). The lowest number of fungal CFU was determined in the forest spaced at a distance of about 5 km from the ES, in which the number of species was the highest (Figure). Fungal abundance in the soil of the forest located at a distance of about 15 km from the ES was also low (1.13 thousand CFU g^{-1} soil). The highest number (2.69 thousand CFU g^{-1} soil) of viable propagules was determined in the soil of sites S1 and S4, followed by the soil of site S2. The soil of sites S1 and S4 was characterized by the highest values of N, P and humus and the lowest soil pH (pH 4.01) (site S1). The highest metal content (except Pb) was also determined in the soil of site S4 (Table 1). The soil of site S2 was characterized by the highest Pb and K levels. The lowest content of nutrients and heavy metals was registered in the forests furthermost from the ES (8–15 km), where the abundance of soil fungi was low.

The number of species in fungus community varied from 29 in site S1 to 81 in site S5 (Figure). Species richness increased with the decreasing distance from the pollution source, developing a significant negative correlation ($r_s = 0.78$ at $p = 0.05$). Evaluation of the nutrients (N, P) and K distribution in different forests revealed a negative correlation between the number of fungal species and the content of chemicals in the soil; however, only the value for linear regressions of the species number variables versus the phosphorus content in the soil was significant ($r_s = -0.88$ at $p = 0.03$) (Table 3). A significant negative impact of total Cu and Pb on

Table 3. **Correlation coefficients (Spearman's r_s) and significance values (p) for linear regressions of fungal community response variables (colony forming units (CFU) and species number) versus the main soil chemical parameters and heavy metal concentration in soil of seven forest plots**

		Cd	Cr	Cu	Ni	Pb	Zn	N	P	K	Hum	pH
CFU	r_s	0.39	0.07	**0.84***	0.47	0.51	0.32	0.75	**0.88**	0.64	0.79	–0.32
	p	*0.32*	*0.86*	***0.04***	*0.25*	***0.05***	*0.44*	*0.06*	***0.03***	*0.11*	***0.05***	*0.42*
Species number	r_s	–0.39	–0.07	**–0.84**	–0.47	–0.78	–0.32	–0.75	**–0.88**	–0.64	–0.79	0.32
	p	*0.34*	*0.86*	***0.04***	*0.44*	***0.05***	*0.44*	*0.07*	***0.03***	*0.11*	***0.05***	*0.43*

* The most significant ($p < 0.05$) correlations are highlighted.

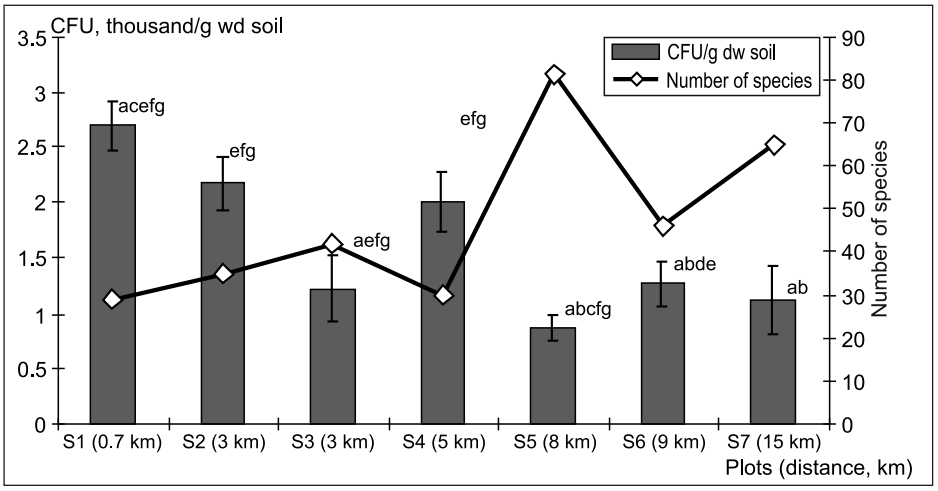

Figure. Abundance (thousand CFU · g^{-1} of dry weight soil) and species diversity (number of recorded species) of fungi in soil of seven deciduous forest plots. Mean values ± standard deviation (N = 24)

the fungus community structure was determined (r_s = – 0.75 at p = 0.04 and r_s = – 0.78 at p = 0.05, respectively). However, the N, K and Cd variables that were not significantly related to the fungal community data contributed to the variations of species number (Table 3).

Totally, 158 fungal species and 9 morphotypes belonging to 77 genera were identified from 168 soil samples (3 replicates × 8 samplings) collected at seven forest sites. All fungi isolated by using different growth media were classified into four phyla according to their morphology (Table 4). Anamorphic fungi (mitosporic fungi) dominated among the isolated fungal taxa; 125 species of anamorphic fungi belonging to 43 genera, were attributed to 5 orders and comprised 74.87% of the total number of isolated fungal CFU. The fungal species belonging to the Zygomycota and Ascomycota phyla comprised 15.56% and 7.78% of the total number of isolated fungi, respectively. Only three morphotypes of Basidiomycota according to the mycelium morphology were determined. The structure of the fungal communities in the seven forest plots differed significantly. Anamorphic fungi dominated in all seven sites; however, their percentage in the total number of isolated CFU varied from 89.71% to 67.26% (Table 5).

Fungal species arranged in appropriate taxonomic groups are presented in Table 6. The fungi included in the Table were those recorded in the soil of two or more plots or their frequency was >2.0%. Thus, 76 fungi (out of 158) were included in this table. This reduced set of 76 species accounted for about 87.4% of all isolated CFUs. The Shannon–Wiener index (H'), presented in Table 6, was calculated for all isolates (158 fungus species + 9 different morphotypes). A wider spectrum of fungal species (all and common) was present in the soil of sites S4 and S7, while the lower number of fungal species and the lower species richness were determined in sites S1 and S2 located at a distance of 0.7 km and 3 km from the ES, respectively. The highest fungal diversity was found in site S3 (H' = 9.67), followed by site S6 (H' = 9.49), site S7 (H' = 3.45) and site S4 (H' = 3.27) (Table 6).

Table 4. **Distribution of all fungi (167 taxa) isolated from seven forest sites among different taxonomic groups**

	No. of classes	No. of orders	No. of genera	No. of species or morphotypes	Percentage in the total number of colony forming units (CFU)
Zygomycota	1	5	12	26	15.56
Basidiomycota	–	–	–	3	1.79
Ascomycota	3	4	9	13	7.78
Anamorphic fungi*	3	5	43	125	74.87
Total number				167	100

* Anamorphic fungi (mitosporic fungi) – heterogeneous group of fungi whose common characteristic is absence of the sexual state.

Table 5. **Distribution of soil fungi from each separate forest plot (total numbers and species lists in Table 6) among different taxonomic groups: number of species (mean percentage of CFUs in the total number of fungi isolated from the site soil)**

	Site (distance from the factory)						
	S1 (0.7 km)	S2 (3 km)	S3 (4 km)	S4 (5 km)	S5 (8 km)	S6 (9 km)	S7 (15 km)
Zygomycota	9 (13.01)	6 (11.19)	4 (5.19)	5 (6.80)	12 (14.78)	7 (12.66)	11 (9.02)
Ascomycota	2 (4.72)	5 (21.37)	4 (4.94)	2 (7.09)	8 (4.90)	5 (9.59)	5 (6.64)
Anamorphic fungi (including *Mycelia sterilia*)	18 (82.28)	23 (67.26)	32 (89.71)	23 (86.16)	59 (79.53)	34 (77.75)	48 (84.34)
Unidentified fungi*	–	1 (0.18)	1 (0.16)	–	2 (0.79)	–	2 (0.16)

* *Mycelia sterilia* was attributed to the order Agonomycetales (class Hyphomycetes).

Table 6. **Mean frequency (%) of occurrence of most common fungal taxa, the total number of identified taxa and the number of taxa unidentified in different forests' soil. Each value is a mean of eight collections and three replicate sub-samples (n = 24)**

Fungal taxa	Site No. (distance from the chemical factory)						
	S1 (0.7 km)	S2 (3 km)	S3 (4 km)	S4 (5 km)	S5 (8 km)	S6 (9 km)	S6 (15 km)
Phylum ZYGOMYCOTA Class Zygomycetes Order *Mortierellales*							
Mortierella alpina Peyronel				1.79			3.73
Mortierella polycephala Coem.	0.78				1.71		
Mortierella verrucosa Linnem.	0.26				0.45		
Order *Mucorales*							
Absidia cylindrospora Hagem var. *cylindrospora*	1.56			1.34		4.4	0.89
Absidia glauca Hagem				1.04	2.95		
Absidia spinosa Lendn. var. *spinosa*	3.89						0.70
Cunninghamella elegans Lendner		1.75	0.81	1.29	1.82		0.35
Mucor circinelloides Tiegh. f. *circinelloides*			0.32			2.67	
Mucor hiemalis Wehmer f. *hiemalis*		2.95					0.70
Umbelopsis ramanniana (A. Möller) W. Gams		1.84			0.68		
Umbelopsis issabellina (Oudem.) W. Gams	0.26				1.59		1.42
Umbelopsis vinacea (Dixton-Stew.) Arx	0.26		2.11				0.89
Zygorrhynchus moeleri Vuill.	5.86	4.24		1.34		2.83	
Phylum ASCOMYCOTA Class Eurotiomycetes Order *Eurotiales*							
Eupenicillium euglaucum (J. F. H. Beyma) Stolk & Samson					0.68		0.35
Talaromyces flavus (Klöcker) Stolk & Samson var. *flavus*	2.0	4.42	1.70		1.93		
Talaromyces luteus (Zukal) C. R. Benj.		9.77		5.35		2.20	
Talaromyces stipitatua (Thom) Benjamin			0.32		0.11		
Class Pyrenomycetes Order *Shaeriales*							
Apiospora montagnei Sacc.					0.45		0.08
Chaetomium globosum Kunze			1.78			0.63	
Chaetomium murorum Corda		0.37				2.52	
Eurotium herbariorum (F. H. Wigg.) Link		0.18					0.53
Geomyces pannorum (Link) Sigler & J. W. Carmich.	2.72	6.63	1.14			3.77	4.26
Class Sordariomycetes Order *Opiostomales*							
Sporothrix schenckii Hektoen & C. F. Perkins var. *schenckii*				1.74	0.94		1.42
*ANAMORPHIC FUNGI Class Hyphomycetes Order *Moniliales*							
Acremonium killiense Grütz				2.88	0.45		
Acremonium strictum W. Gams					1.82		1.42
Alternaria alternate (Fr.) Keissl.	6.74	0.61			1.59	2.67	
Arthrinium phaeospermum Fuckel		4.42	1.46				3.19
Aureobasidium pullulans (de Bary) G. Arnoud var. *pullulans*		0.74	3.08	4.77	0.68		2.13
Aspergillus niger Tiegh. var. *niger*	3.64				1.59		
Aspergillus terreus Thom					2.39		0.7
Aspergillus ustus (Bainier) Thom & Church						4.4	
Beauveria bassiana (Bals.-Criv.) Vuill.			4.22		0.91	2.91	
Botryotrichum piluliferum Sacc. & Marchal					0.45	1.89	
Chrysosporium merdarium (Ehrenb.) J. W. Carmich.		8.29	5.52	4.87	3.30		
Cladosporium cladosporioides (Fres.) de Vries	7.30	1.66	2.92	4.32	1.82		0.89
Cladosporium herbarum (Pers.) Link ex S. F. Gray			2.77		0.57	0.94	
Clonostachys rosea f. *catenulata* (J. C. Gilman & E. V. Abbott) Schroers	1.30	0.78					

Table 6 **(continued)**

Fungal taxa	Site No. (distance from the chemical factory)						
	S1 (0.7 km)	S2 (3 km)	S3 (4 km)	S4 (5 km)	S5 (8 km)	S6 (9 km)	S6 (15 km)
Clonostachys rosea f. *rosea* (Link) Schroers, Samuels, Seifert & W. Gams	3.11				2.16	4.4	0.44
Cylindrocarpon destructans (Zinssm.) Scholten var. *destructans*	8.82						3.55
Epicoccum nigrum Link	0.93				0.68		
Geotrichum candidum Link.		1.10			0.71		0.70
Gliomastics murorum (Corda) S. Huges var. murorum			5.19	3.08			
Humicola fuscoatra Traaen		3.87			0.34		0.53
Humicola grisea Traaen			2.27		1.02	1.65	
Lecanicillium fungicola (Preuss) Zare & W. Gams var. *fungicola*			2.27				1.77
Lecanicillium lecanii (Zimm.) Zare & W. Gams							2.13
Paecilomyces farinosus (Holmsk.) A. H. S. Br. & G. Sm.		2.76	2.92	2.09			
Paecilomyces lilacinus (Thom) Samson	8.82	2.21	5.10	4.07	2.16		2.48
Penicillium aurantiogriseum Dierckx							2.84
Penicillium brevicompactum Dierckx							3.37
Penicillium chrysogenum Thom var. *chrysogenum*			2.61				
Penicillium citrinum Thom						2.52	
Penicillium commune Thom						5.03	
Penicillium corylophilum Dierckx				2.78			
Penicillium decumbens Thom			4.06				1.86
Penicillium expansum Link	3.37				1.59		
Penicillium fellutanum Biourge var. *fellutanum*					6.60		1.42
Penicillium funiculosum Thom			5.84	6.16			
Penicillium glabrum (Wehmer) Westling	9.42	4.24		7.15	3.31		
Penicillium janczewskii K. M. Zalessky			5.19		3.98		
Penicillium jantinellum Biourge					2.95		
Penicillium jensenii K. M. Zalessky		8.84	6.16				7.28
Penicillium lividum Westling				4.67	1.02		
Penicillium melinii Thom		2.21					1.06
Penicillium oxalicum Currie & Thom				2.38			
Penicillium purpurogenum Stol				2.68			
Penicillium restrictum J. G. Gilman & E. V. Abbott				3.38			3.02
Penicillium rubrum Stoll					0.68		
Penicillium simplicissimum (Oudem.) Thom					2.73		3.02
Penicillium spinulosum Thom				4.17		2.52	
Penicillium steckii Zaleski						4.40	
Penicillium thomii Maire	0.89	6.82		6.55	3.52		3.73
Penicillium waksmani K. M. Zalessky				1.79	2.39		2.84
Scopularipsis brevicaulis (Sacc.) Bainier	4.15				1.59	1.18	2.13
Scytalidium aurantiacum Klingström & L. Beyer			0.81		1.14	3.46	
Stachybotrys parvispora S. Hughes					0.11		0.35
Stemphylium botryosum Sacc.		1.66	2.27			1.26	
Tolypocladium geodes W. Gams					0.34	0.71	
Trichocladium asperum Harz				0.74	0.45		
Trichoderma hamatum (Bonord.) Bainier					0.68		1.60
Trichoderma harzianum Rifai			4.54			6.29	3.19
Trichoderma koningii Oudem.		2.21					
Trichoderma polysporum (Link) Fifai	3.86			3.18			
Trichoderma virens (J. H. Mill., Giddens & A. A. Foster) Arx		4.79		0.79	2.73		
Trichoderma viride Pers.				5.11	2.95		2.31
Trichoderma spirale Bissett					1.82		

Table 6 **(continued)**

Fungal taxa	Site No. (distance from the chemical factory)						
	S1 (0.7 km)	S2 (3 km)	S3 (4 km)	S4 (5 km)	S5 (8 km)	S6 (9 km)	S6 (15 km)
Verticillium albo-atrum Reinke & Berthold			1.95				1.42
Verticillium chlamydosporum Goddard var. *chlamydosporum*					0.91	1.89	
Volutella ciliata Alb. & Schw. ex fr.			1.46		1.14		
Ulocladium oudemansii E. G. Simmons		1.47	0.97				0.70
Wallemia sebi (Fr.) Arx					0.68		
Wardomyces inflatus (Marchal) Hennebert					1.14	1.57	0.70
Order *Tuberculariales*							
Fusarium oxysporum Schltdl.	5.12	3.88	3.73	4.37	9.82		2.13
Fusarium sambucinum Fuckel					0.34		
Fusarium solani (Mart.) Sacc			3.57	4.07	2.95	2.99	2.48
Metarhizium anisopliae Metschn.			5.19		2.04	2.04	1.24
Myrothecium roridum Tode					0.23		1.86
Myrothecium verrucaria (Alb. & Schwein.) Ditmar					0.45	1.42	0.79
Class Coelomycetes Order *Sphaeropsidales*							
Phoma glomerata (Corda) Wollenw. & Hochapfel			0.97		1.36	1.26	
Phoma exigua Sacc. var. *exigua*					0.23	2.75	1.06
Number of most common genera	15	23	24	16	36	25	31
Number of all determined genera	22	28	29	16	50	37	40
Number of most common species	21	29	33	29	58	30	47
N (individual number of all determined species)	29	35	42	30	81	46	65
H' (Shanon–Weiner index)	1.085	3.070	9.669	3.269	2.442	9.487	3.447

* Anamorphic fungi are a heterogenous group of fungi whose common characteristic is absence of sexual state.

The lowest diversity of fungi was recorded in site S1 (*H'* = 1.08). *Penicillium* isolates were abundant in soil samples of all eight collections and constituted 33.22% of all isolates. A detailed study showed that this genus was represented by 27 species. The genus *Penicillium* was also characterized as a genus with the highest number of more common species (24 species), followed by the genus *Trichoderma* (8 common species; 6.98%), the genera *Absidia*, *Mortierella*, *Umbelopsis*, *Talaromyces*, *Fusarium* (3 common species each and 3.27, 1.13, 4.84 and 5.64%, respectively), *Acremonium*, *Cladosporium*, *Clonostachys*, *Chaetomium*, *Humicola*, *Verticillium* and *Phoma* (2 species each and 1.57, 3.82, 1.93, 0.63, 1.35, 0.64 and 0.81%, respectively).

The *Cladosporium cladosporioides*, *Paecilomyces lilacinus* and *Fusarium oxysporum* species were isolated from the soil of six forest areas in both years. The species *Aureobasidium pullulans*, *Cunninghamella elegans*, *Fusarium solani*, *Geomyces pannorum*, *Penicillium glabrum* were isolated from the soil of four forest areas and the species *Absidia cylindrospora*, *Alternaria alternata*, *Chrysosporium merdarium*, *Clonostachys rosea* var. *rosea*, *Metarhizium anisopliae*, *Scopulariopsis brevicaulis*, *Talaromyces lutea* and *Zygorhynchus moelleri* from four out of seven forest areas. Most of these species were isolated more frequently as well. *Talaromyces luteus* was more frequent (*F* = 9.77%) in the soil of site S2, *Paecilomyces lilacinus* (8.82%) – of site S1, *Fusarium oxysporum* (9.82% and 5.12%) – of sites S5 and S1, *Cladosporium cladosporioides* (7.3%) – of site S1, *Chrysosporium merdarium* (8.29% and 5.52%) – of sites S2 and S3, *Penicillium thomii* (6.82% and 6.55%) – of sites S2 and S4, respectively. The fungal species recorded in two or three forest plots but characterized by a higher frequency of occurrence were *Cylindrocarpon*

Table 7. **Sørensen's similarity indices of fungal communities among different forest sites**

Sites	Sørensen's index (*S'*)					
	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
Site 1 (S1)	0.31	0.16	0.27	0.24	0.16	0.23
Site 2 (S2)		0.34	0.37	0.24	0.09	0.34
Site 3 (S3)			0.28	0.26	0.25	0.30
Site 4 (S4)				0.31	0.13	0.27
Site 5 (S5)					0.28	0.32
Site 6 (S6)						0.18

destructans (site S1 8.2%), *Penicillium jensenii* (S2 8.84% and S3 6.16%), *Penicillium funiculosum* (S3 5.84%, S4 6.16%), *Alternaria alternata* (S1 6.74%), *Zygorhynchus moelleri* (S1 5.86%), *Gliomastix murorum* (S3 5.19%) and *Trichoderma harzianum* (S3 4.54%, S6 6.29%).

The varying number of the total and common fungal species in a site determined a very low similarity indices (*S*' values from 0.13–0.34) among the fungal communities (Table 7). The lowest similarity (*S*' = 0.13) was found between the communities of sites S4 and S6 and the highest (*S*' = 0.37) between the communities of sites S2 and S4.

DISCUSSION

Chemical industry activities in the regions of Central Lithuania over the last five decades have resulted in a serious damage to the surrounding terrestrial ecosystem (Stoškus, 2005). The Kėdainiai district where the forest plots of the present investigation are located is under a strong anthropogenic pressure: only 26.2% of land is occupied by forests and water basins, the rest being industrial, urban or agricultural areas (Motiejūnaitė, 2007). No precise air quality data in every plot could be obtained as these are measured in the Kėdainiai city and the Dotnuva meteorological station 10 km NW of Kėdainiai. However, according to available air monitoring data, compared with earlier evaluations, it can be assumed that the pollution level is not very high in the area. SO_2 concentrations currently range from 5 to 15 μg m^{-3} (Lietuvos valstybinio..., 2004), reaching 60 μg m^{-3} in the most polluted area (Petrauskas et al., 1996). NO_2 levels have increased, ranging from 5 to 30 μg m^{-3} in the most polluted area. The location of the most polluted area, determined by Petrauskas et al. (1996) coincides with sites S1 and S2 of the present study, where the lowest number of fungal species and the highest number of CFU was recorded. The acidity of precipitation in the region (pH 5) is close to the average for Lithuania (Juknys et al., 2002). Besides the chemical factory, there are several smaller industrial enterprises which, together with agriculture and transport, add to environmental pollution in the region. Human-induced disturbances in this region led also to the contamination by heavy metals (Cu, Pb, Ni, Cd, Zn and As) (Mažvila, 2001). The total content of lead in the soil of the plots located at a distance of 0.7–15 km from the ES varied within 8.5–20.5 g kg^{-1} dry weight soil, total Cu 3.6–8.5, Zn 16.0–25.0, Cr 12.0–21.0, Ni 5.6–11.5, Zn 16.0–25.0, and As 0.8–1.6 g kg^{-1} dry soil. These values are low; according to B. J. Alloway (1995), they could be equated with a classical soil background. Nevertheless, Renella et al. (2002) performed a short-term laboratory experiment to evaluate the impact on soil microorganisms of different metals in combination and found that, in all cases, poly-metal contamination had a greater impact on microbial biomass than did single-metal contamination, leading the authors to conclude that there were additive effects of metal toxicity (Renella et al., 2002). Among the heavy metals, cadmium, mercury and copper are known to have deleterious effects on the abundance, activity and diversity of soil microorganisms (Almås et al., 2004; Ranjard et al., 2006). The cumulative effect of Cd and Cu on soil microorganisms was determined under laboratory conditions (Ranjard et al., 2006). In field, poly-metal effects on soil microorganisms are generally associated with inorganic nutrients and organic matter. Therefore, it is difficult to distinguish the effect of one metal from the effect of another (Bååth et al., 1998; Sandaa et al., 2001; Turpeinen et al., 2004; Frey et al., 2006). Despite the relatively low heavy metal pollution level of the study sites, the impact of copper and lead on the number of fugal CFUs and species diversity was noticeable. Fungal abundance significantly positively and species number significantly negatively correlated with the Cu and Pb content in the soil. An insignificant correlation among fungus abundance, diversity and the total amount of other heavy metals in the soil was determined.

Mycological analysis of the soil enabled to ascertain a total of 11 394 isolates of fungi representing 158 species. The number of fungal species decreased with an increase of the total content of N and K in soil (r_s = –0.75 and –0.64, respectively), although the correlation coefficients were statistically unreliable (p = 0.07 and 0.11, respectively). The humus and P content in the soil were the factors significantly influencing fungus species diversity. Surprisingly, a statistically reliable negative correlation of the fungus species diversity with the humus and phosphorus content in the soil was determined (r_s = –0.79 at p = 0.05 and r_s = –0.88 at p = 0.03 for humus and P, respectively). Firstly, the negative correlation with P content in soil may be related to phosphorus availability. Humus-bound P could be indigestive to most fungi. Moreover, P limitation decrease the microbial decomposition of the litter material (Joergensen, Schen, 1999). Therefore, the development of the fungi unable to decompose organic mater and assimilate P bound with humus could be restricted. A positive correlation between *P* and humus content in the soil of the study plots (r_s = 0.94 at p = 0.04) was determined (Pečiulytė, Dirginčiutė-Volodkienė, 2009b) and can confirm such a possibility. A positive correlation between P and humus content as well as a correlation between metal and humus content in the soil of Central Lithuania had been determined also by other investigations (Mažvila, 2001). Seasonal changes of P content in soil samples of seven sites were ascertained (data not published). Naturally, phosphorus is mineralized by increasing microbial activity in spring, consumed by trees in spring and summer, but it is accumulated as a result of increased organic inputs, slower plant growth and low microbial activity in late autumn (Chen et al., 2007). Another possible explanation of the negative correlation between the diversity of fungi and P content in soil, and herewith an opposite correlation between fungal abundance, species number and humus content in soil can be caused by an elevated Cd and Cu content in more humous soils. A significant correlation of Cd and Cu content with humus content in soil (r_s = 0.69 and 0.84, respectively at p < 0.05) was determined (Pečiulytė,

Dirginčiutė-Volodkienė, 2009b). In our study, it was apparent that the fungal community was particularly responsive to Cu content in soil. Comparable results were obtained by Yamamoto et al. (1981). They noticed an increase in fungal population with an increase of Cu content in soil, and this increase was attributed to the populations of Cu-tolerant fungi. Effects of Cd and Cu on microbial biomass, activity and community diversity was determined by other investigations (Fostergård et al., Ranjard et al., 2006; Shentu et al., 2008). Dar, Mishra (1996) studied the effect of Cd and found that increased Cd concentrations caused a sizeable reduction in the growth of *Penicillium*, *Rhizopus*, *Fusarium* and *Geomyces*, but stimulated the growth of *Aspergillus*, *Trichoderma* and *Paecilomyces*. They concluded that certain species were tolerant to the Cd levels used and, in the absence of competition, proliferated rapidly.

Most of the isolated fungi belonged to the Anamorfic (74.87%) fungi, followed by Zygomycetes (15.56%), Ascomycetes (7.78%), with only three fungi isolated from Basidiomycetes. The majority (33.22%) were from the genus *Penicillium*; the next two in the order of dominance were *Trichoderma* (6.98%), *Paecilomyces* (6.58%) and *Fusarium* (5.64%). Fungi of the genera *Actinomucor*, *Coemansia*, *Dimargaris* and *Piptocephalis* were found just once. *Penicillium* was the most abundant genus represented by 27 species. The species *Penicillium thomii* (5.8%), *P. glabrum* (4.64%) and *P. jensenii* (3.13%) dominated in soil of all sites. Most *Penicillium* species are considered as ubiquitous, opportunistic saprophytes able to grow in almost any environment with mineral salts and a wide range of pH (Gadd, 2008). The genus *Penicillium* dominated in sites S4, S5 and S7 located at a distance of about 5–8 km and 15 km from the ES; however, the species distribution did not correlate with the chemical parameters of the soil. *Paecilomyces lilacinus* (4.26%) was also an abundant fungus in the soil of seven forest plots. The other species followed them in descending order: *Cladosporium cladosporioides* (3.82%), *Talaromyces lutes* (3.69%), *Fusarium oxysporum* (3.54%), *Geomyces pannorum* (2.92%) and *Zygorhynchus moellerii* (2.81%). The species *Actinomucor elegans* (Eidam) C. R. Benj. & Hesselt., *Coemansia aciculifera* Linder, *Dimargaris bacilispora* R. K. Benj., *Mortierella bisporus* (Thaxt.) Björl., and *Piptocephalis cylindrospora* Bainier were new for Lithuania and were recorded only in complexes with other fungi.

The diversity indices (*H'*) calculated from all the isolated taxa (not from those listed in Table 6) confirmed clear differences in soil fungus community structure of seven forest plots. Soil communities in sites S3 and S6 were most numerous (*H'* = 9.66 and 9.48, respectively); the community of site S1 was the poorest, although it was characterized by the highest number of CFUs. Sørensen's indices confirmed differences of the community structure in the soil of seven sites. The highest similarity was determined between the communities of sites S2 and S4 (*S'* = 0.37) and the lowest between sites S4 and S6 (*S'* = 0.13). Site S4 was characterized by soil with the highest content of heavy metals, whereas the total content of Pb, Ni, Cu and Zn was the lowest in soil of site S6.

Reported values of soil fungal diversity and population density are often a reflection of the methods used to recover the fungi. The technique of direct isolation from soil particles can yield more counts, but the faster growing fungi can be favoured (Satish et al., 2007). We used the dilution plate count technique. An advantage of using this method is that all the developing colonies can be picked off from the plates for further the sub-culturing. Fungi are so nutritionally diverse that there is no one medium that can isolate all of them. Therefore, Czapeks' salts medium was modified by different carbon sources (glucose, starch, cellulose, lignin and chitin). These modifications allowed determining some seasonal changes in the fungus community composition as well as diversity differences depending on the humus, P and N content in the soil (data not presented). It is very difficult to compare the results obtained in the present investigation with those presented in other articles. Most of them report changes in the community structure as measured by phospholipid fatty acid (PLFA) analysis. Moreover, it is always difficult to compare different soils as to what portion of the total metal loading is available. Results of the present investigation suggest that the community structure can be a sensitive measure of detecting environmental perturbation and assessing the long-term impacts after such perturbation. Long-term industrial pollutants in Central Lithuania have influenced other organisms too. Changes of the abundance and diversity of bacteria (and actinomycetes) (Pečiulytė, Dirginčiutė-Volodkienė, 2009 a, b) and ectomycorrhizae (Stankevičienė, Pečiulytė, 2004) as well as changes in the community structure of endophytic lichens (Motiejūnaitė, 2007) were also observed.

CONCLUSIONS

The fungus community structure and abundance conversely correlated with the distance from the pollution sources and with the content of main pollutants in soil. Abundance of fungi significantly negatively correlated with their species number. Fungal abundance in soil decreased, whereas species diversity increased with increasing the distance from the factory. Similarly, a positive correlation between fungus abundance and heavy metal (especially Cu and Pb) content in soil and, conversely, a negative correlation between species diversity and heavy metal content in soil was determined. Species diversity negatively correlated also with the humus and phosphorus content in soil. Fungi were more abundant in soil with a higher humus and phosphorus content. These correlations suggest that shifts in the fungus community structure under long-term pollution impact depend on the ability of fungi to develop resistance to pollution and on the competitiveness among the dominating populations.

Out of the 158 species recorded in the soil of seven forest plots, four species – *Actinomucor elegans* (Eidam) C. R. Benj. & Hesselt., *Coemansia aciculifera* Linder,

Dimargaris bacilispora R. K. Benj., *Mortierella bisporus* (Thaxt.) Björl., and *Piptocephalis cylindrospora* Bainier – were new for Lithuania and recorded only in complexes with other fungi.

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ILGALAIKĖS PRAMONINĖS TARŠOS ĮTAKA SKIRTINGU ATSTUMU NUO GAMYKLOS ESANČIO LAPUOČIŲ MIŠKO DIRVOŽEMIO MIKROORGANIZMAMS

3. MIKROMICETŲ GAUSA IR RŪŠINĖ DIRVOŽEMIO BENDRIJŲ STRUKTŪRA

S a n t r a u k a

Tirta ilgalaikės (57 metų) pramoninės taršos įtaka dirvožemio mikromicetų įvairovei ir jų bendrijų struktūrai. Analizuojami septynių lapuočių miško plotų, parinktų skirtingu atstumu (nuo 0,7 iki 15 km) nuo taršos šaltino (TŠ) – trąšų gamyklos Vidurio Lietuvoje, dirvožemio mikromicetų bendrijų tyrimo rezultatai. Mikromicetų gausą – kolonijas sudarančių vienetų (KSV) skaičius nustatytas dirvožemio suspensijos skiedimų pasėlių metodu. Mikromicetų kiekis kito nuo 0,88 iki 2,69 tūkst. KSV g^{-1} dirvožemio ir mažėjo didėjant atstumui nuo gamyklos (r_s = – 0,78). Mikromicetų gausa priklausė nuo fosforo ir humuso kiekio dirvožemyje (atitinkamai r_s = 0,88 ir 0,79). Iš tirtų sunkiųjų metalų (Cu, Cd, Ni, Pb, Zn, As) tik Cu ir Pb turėjo įtakos mikromicetų bendrijų gausai ir rūšinei sudėčiai. Bendra 11 394 padermių mikologinė analizė leido identifikuoti 158 mikromicetų rūšis. Dauguma rūšių priklauso anamorfiniams (mitosporiniams) mikromicetams (sudarė 74,87 % visų išskirtųjų mikromicetų), mažiau užfiksuota zigomicetų (Zygomycetes – 15,56 %), askomicetų (Ascomycetes – 7,78 %) ir tik trys morfotipai bazidiomicetų (Basidiomycetes). Vyravo *Penicillium*, *Trichoderma* ir *Paecilomyces* gentys (atitinkamai 33,22, 6,98 ir 6,58 %).

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