ORIGINAL ARTICLES

CONCENTRATION AND SPECIES DIVERSITY OF AIRBORNE FUNGI NEAR BUSY STREETS IN LITHUANIAN URBAN AREAS

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Lugauskas A, Šveistytė L, Ulevičius V: Concentration and species diversity of airborne fungi near busy streets in Lithuanian urban areas. *Ann Agric Environ Med* 2003, **10**, 233-239.

Abstract: The investigations on air pollution in industrial cities of Lithuania: Vilnius, Alytus, Kaunas, Marijampole and Elektrenai were aimed at detecting the presence of fungi and aerosol particles during different seasons of the year. Sampling of fungal spores was carried out at 20 sampling sites. Active air sampling was performed simultaneously with the use of passive sedimentation plates. Data on the spread of various micromycete species in the air of cities contaminated with various pollutants are presented. Micromycetes of 430 species belonging to 165 genera, 19 families, 13 orders, 4 classes, and 3 phylla were isolated and identified. We found 21 species, 11 genera, 7 families from *Ascomycota*, 6 species, 1 genus, 1 family from *Oomycota*, 45 species, 15 genera, 8 families from *Zygomycota*. Mitosporic fungi comprised 138 genera, 358 species, and comprised the vast majority of identified species: 358 out of 430 (83.25%). Conditionally pathogenic species were also isolated. It was concluded that the abundance of such fungi as *Aspergillus niger*, *A. fumigatus*, *Cladosporium herbarum*, *Alternaria alternata*, *Aureobasidium pullulans* in the air can be a significant criterion for the evaluation of air pollution.

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Key words: aerosols, fungi, species, diversity, urban areas.

INTRODUCTION

Recently, attention to air pollution, not just by chemical and physical pollutants but also by micromycete propagules, has increased. The literature [29] suggests that air is the primary way for microorganisms of many species to spread. Investigations on the atmosphere aerosols, their composition, and impact of their components upon certain groups of microorganisms and their viability, as well as possibilities of microorganisms' adaptation to atmospheric conditions, were performed. Knowledge about the survival of microorganisms under extreme conditions of ambient air when their cells are affected by a profusion of chemical and physical factors [25, 30, 40] is being accumulated.

Atmospheric aerosols consist of biological and nonbiological components. Bioaerosols of ambient air consist of various particles of organic origin: bacteria, their endospores, micromycete spores, conidia, and hyphal fragments, metabolites excreted by microorganisms, viruses, hair and skin particles of mammals, as well as many other organic wastes and decaying products. Toxines, excreted by microorganisms and other live organisms, are important elements of bioaerosols. Components of bioaerosols get into the air from various sources: soil, living plants and animals, surface of organic

Received: 30 September 2003

Accepted: 4 December 2003

litter, damaged wooden and stone buildings, and many other places. Winds and brief air movements contaminate the air with dust of mechanic origin, together with a lot of organic particles which rise into the air. Plenty of viable microorganism propagules are carried long distances by airflow from the places and substratum they have formed [15, 19, 30].

All around the world the propagules of various microscopic fungi constitute the largest part of biological particles in the air [6]. Propagules of micromycetes can travel far and on their way contaminate various substrata of the environment. Therefore, micromycetes can affect the activities of people and can cause diseases of plants, animals and people [1, 13, 17, 21, 22, 23, 31].

Airborne microorganisms may cause ill effects in humans ranging from mild irritation to disease. Examples of infections which may result from the presence of microorganisms in the air, are tuberculosis, measles, legionellosis, histoplasmosis, various mycoses, and toxicosis [21].

There is considerable concern in environmental health circles about chemical and physical contaminants being emitted by road transport. Epidemiological studies are consistently reporting an association between fine particulate pollution and health [3]. Motor vehicle emissions are considered to be the main source of fine particles in ambient air of cities that are not directly influenced by industrial emissions.

Vehicle movement creates local turbulence which promotes aerosolization of fungal spores from surrounding buildings, trees, and soil. Therefore, concentrations of fungal spores can be considerably increased near busy streets. In the construction field, demolition of old buildings may release concentrations of fungi high into the surrounding air. These particles, in fact, play a very important role in triggering allergies, asthmatic symptoms, skin and systemic mycoses, and may at times be fatal, as in the case of invasive aspergillosis which easily develops in an immunodepressed host. Ambient particulate matter (PM) and co-pollutants deposited in the respiratory tract cause cardiovascular and systemic effects, especially to persons with pre-existing conditions, such as allergic hyperresponsiveness, pulmonary, cardiac, and vascular diseases [7, 41]. Therefore, recently the evaluation of airborne microorganisms has gained increasing importance. The aim of this paper was to estimate seasonal variation of composition and concentrations of airborne fungi and aerosol particles in the ambient air of industrial Lithuanian cities.

MATERIALS AND METHODS

Geographical Location and Climate. Lithuania is situated on the Baltic Sea. Lithuania's climate is transitional between maritime and continental with a mean air temperature of about 6°C. Average for January is about -5°C and in July about 17°C. Annual precipitation levels vary from an average of 540 mm (21.3") in the

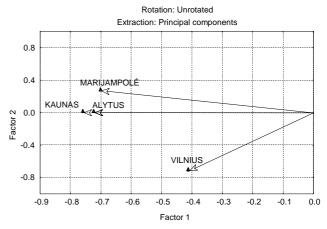


Figure 1. Diversity of micromycetes in the designated Lithuanian cities.

Central Lowlands to 930 mm (36.6") on the south-eastern slopes of the Samogitian Highlands. Inland, precipitation is heaviest in August, and on the coast in October. The vegetation season lasts from 169–202 days (Eastern Lithuania and on the coast, respectively).

Sampling and identification of fungi. Measurements of atmospheric bioaerosols were performed in the course of different seasons during 1996–1999 in the ambient air of several industrial cities (Vilnius, Kaunas, Marijampolė, Alytus, and Elektrėnai). In the cities, the sampling sites were located a few meters from driveways, in areas with different traffic intensity.

The intensity of traffic was calculated by dividing the vehicles into passenger cars, light duty vehicles and heavy duty vehicles. The average number of vehicles was between 300-800 per hour. In the period of sampling the temperature averages were: in winter -2°C, in spring +10°C, in summer +20°C, in autumn +6°C. Air humidity varied from 60 to 80%.

The samples at each sampling site were taken three times during daytime. Measurements of fungal spores were carried out at 20 sampling sites and 882 samples were analysed. Aerosol particle concentration was measured with optical spectrometer LAS-15A (Institute of Physics, Lithuania).

Active air sampling was performed simultaneously with the use of passive sedimentation plates. Samples of fungal propagules were taken into water with an AGI-30 impinger [18] and onto an open-faced Whatman-40 filter (Whatman International Ltd.). The period of sampling was set to 15 min. Both samplers were operated at flow rates of 10 lpm. The reported cut-off size of the impinger was below 0.4 μ m; therefore, it can be expected that collection efficiency for fungal propagules could be near 100%. The impinger was filled with 20 ml of saline. Before each sampling procedure the equipment was sterilised with ethyl alcohol and washed with sterile water.

The data from the gravitational method is suitable for the qualitative analysis of micromycetes, but this method is less precise in the calculation of quantitative parameters

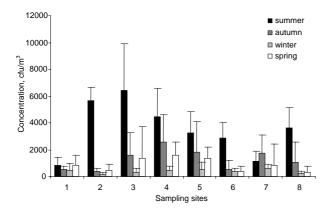


Figure 2. Seasonal concentrations of airborne fungal propagules in Vilnius city (means, standard deviations)

of the airborne micromycetes. The micromycete concentrations are presented by gathering micromycetes from the air into AGI-30.

A standard spread plate technique was used for enumeration and identification of viable fungal propagules collected into the impinger. Three aliquots (0.1, 0.5, and 1.0 ml) of impinger fluid were spread on agar plates and about 16 ml of fluid were filtered through Millipore filters of 0.4 mm pore size. Filter samples were analysed making imprints from each filter on 3 agar plates and further covering the filters with a thin layer of agar on the fourth plate. A malt extract agar (Difco), steep agar, and Czapek agar were used as culture media. Samples were incubated for 5 days at a temperature of $26 \pm 2^{\circ}$ C. After incubation, the colonies were counted and identified to species level according to different manuals [8, 9, 10, 33, 34, 38, 42]. The concentrations of fungal propagules were expressed as colony forming units (cfu) per cubic meter of air.

Statistical analysis. The variety of species was studied using factor analysis and principal components analysis (PCA). The analysis was performed using factor extraction turn for maximal dispersion run, minimum eigenvalue 1. Heterogeneity or Interaction G test [36] was used.

Table 1. The most common airborne fungal species in the air of Lithuania.

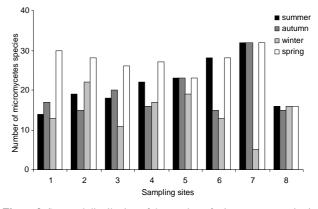


Figure 3. Seasonal distribution of the number of micromycete species in the air of Vilnius.

RESULTS

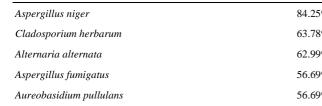
Investigations of the micromycete species diversity in the ambient air of industrial cities in Lithuania (Vilnius, Alytus, Kaunas, Marijampolė, and Elektrėnai) were performed. We found 21 species, 11 genera, 10 families from Ascomycota, 6 species, 1 genus, 1 family from Oomycota, 45 species, 15 genera, 8 families from Zygomycota. Mitosporic fungi comprised 138 genera, 358 species comprised the vast majority of the identified species: 358 out of 430 species (83.25%).

The dominant family (in terms of the number of species) was Mucoraceae - 24 micromycete species belong to this family, which constitutes 6.7% of the total number of species. This family can also be characterised by a high number of genera - 4 genera belong to the aforementioned family. Ascomycota division has the highest number of orders - 8 (61.54%) as well as families - 10 (52.63%). The highest number of genera 138 (83.00%) and species 358 (83.25%) were isolated from the division Mitosporic fungi.

The diversity of airborne micromycetes species in industrial cities of Kaunas, Alytus, and Marijampolė is similar. It differs from the species composition revealed

Table 2. Percentile expression of the most common airborne micromycete species by seasons (n = 882)

on frequency%	Species	Percent of samples			
84.25%		winter	spring	summer	autumn
63.78%	Alternaria alternata	2.67	36.00	21.33	40.00
62.99%	Aspergillus niger	21.36	32.04	19.42	27.18
56.69%	Cladosporium herbarum	15.00	30.00	30.00	45.00
56.69%	Aspergillus fumigatus	35.21	33.80	9.86	21.33
56.69% 43.31%	Aureobasidium pullulans	17.65	27.94	16.18	38.24
45.52%	Botrytis cinerea	1.08	41.67	27.08	29.17
33.07%	Cladosporium cladosporioides	5.48	42.47	19.18	32.88
27.56%	Penicillium funiculosum	28.13	15.63	12.50	43.75



Species

Cladosporium cladosporiodes

Cladosporium sphaerospermum

Penicillium funiculosum

Geotrichum candidum

Botrytis cinerea

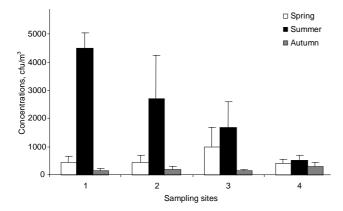


Figure 4. Seasonal concentrations of micromycetes in the air in Lithuanian urban areas: 1. Elektrėnai; 2. Kaunas; 3. Marijampolė; 4. Alytus (means, standard deviations).

in the air of Vilnius (Fig. 1). Diversity of micromycete species in the ambient air depended upon environmental factors, intensity of traffic, infrastructure of the city, meteorological conditions at the moment of sampling, proximity of pollution sources, and other factors.

Concentrations and composition of airborne micromycetes in Vilnius city. The concentrations of micromycete propagules in the air of Vilnius city are shown in Figure 2. The figure shows clearly that the lowest concentrations of micromycete propagules in the air of Vilnius city were measured in winter. They ranged from 178-522 cfu/m³. The average concentration was 379 micromycetes cfu/m³. The concentrations of micromycetes increased in spring ranging from 333-1,600 cfu/ m³; average concentration was 898 cfu/m³. The same tendency occurred in autumn when concentrations ranged from 516–2,657 propagules/m³, and the average concentration was 1,275 cfu/m³. The highest concentrations of micromycetes in Vilnius city were observed in summer; average concentration was 3,625 cfu/m³, and ranged from 800–6,400 cfu/m³. Figure 2 shows the spatial differences in concentrations measured during different seasons. The concentrations measured in summer and autumn in the centre of Vilnius city (sampling site numbers 3, 4, 5) were higher than those measured in the suburbs (sampling site numbers 1, 2, 8). Such rises in the concentrations in summer and autumn could be explained by the increase of the sources of micromycetes, as well as meteorological factors forming favourable conditions for the accumulation of pollutants. Pollutants accumulate in the centre of the city while weak winds from south, south-west, and south-east are blowing.

The composition of airborne fungi varied during different seasons of the year. After performing mycological analysis, 402 species of micromycetes were isolated. They were identified and classified as *Ascomycota*, *Zygomycota*, and Mitosporic fungi. The most common micromycete species in Vilnius city during various seasons of the year were the following: *Alternaria alternata* (96.87%), *Aspergillus fumigatus* (85.94%), *Aspergillus niger* (87.50%), *Aureobasidium pullulans*

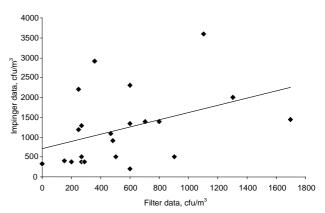


Figure 5. Comparison of filter sampler and impinger data.

(78.13%), Botrytis cinerea (79.69%), Cladosporium herbarum (93.75%), Geotrichum candidum (57.8%), Paecilomyces puntonii (34.75%), Penicillium funiculosum (31.25%).

The composition of the micromycete species in the air of Vilnius city differed during various seasons of the year. The highest number of species from the air in Vilnius were isolated in spring (196 species), and the smallest number in winter (102). Figure 3 shows the total number of micromycete species isolated from the air during different seasons. The highest species diversity in each sampling site was noted in spring and autumn. The following micromycete species dominated in the air of Vilnius city in spring: C. cladosporioides, C. herbarum, A. alternata, Aureobasidium pullulans, B. cinerea, Sclerotinia sclerotiorum. In autumn Alternaria alternata, Aureobasidium pullulans, Cladosporium sphaerospermum, C. cladosporioides, C. herbarum dominated. Together with the above-mentioned species, in the zone of heavily polluted air (3 site), rather noteworthy from the ecological and sanitary point of view are also Atrhroderma insingulare, Microsporum ferrugineum, Torula herbarum, Absidia glauca, Zygosporium gibbum, Hyalodendron lignicola, Mortierella lignicolis, Tieghemiomyces parasiticus. The majority of these species are rare in Lithuania or recorded for the first time, so there is no data about their ecological peculiarities and the impact they could produce upon people. Some of them are described as damaging nails, skin, other organs.

In summer, during the whole period of investigations, together with the above-mentioned species, Aspergilus carbonarius, Monilia brunnea, M. implicata, M. koningii, Periconia laminella, Olpitrichum macrosporum, Alternaria sonchi, Gliocladium viride, Cristulariella pyramidalis, Pseudorotium zonatum, Oidiodendron griseum, Choanephora trispora, Gymnoascus reesii, Fusarium moniliforme, Geomyces pannorum, etc. were isolated from the polluted air samples in Vilnius city.

Although the concentrations of airborne fungal propagules were relatively low in winter, there was still a great variety of fungal species. *Oidiodendron echinulatum, A. fumigatus, Rhizopus stolonifer, Penicillium oxalicum, P.*

Table 3. Micromycete species influencing human health.

Micromycete species	Characteristics of the species
Rhizomucor pusillus (Lindt) Schipper	4
Mucor hiemalis Wehmer	4
Chaetomium globosum Kunze	1, 2
Eurotium amstelodami (Mangin) Thom and Church	4
Aspergillus clavatus Desm.	1
Aspergllus flavus Link	1, 2
Aspergillus fumigatus Fresen.	1, 3
Aspergillus niger Tiegh.	1,3
Aspergillus ochraceus Wilhelm	1
Aspergillus sydowi (Bainier et Sartory) Thom et Church	4
Aspergillus versicolor (Vuill.) Tirab.	4
Paecilomyces variotii Bain.	3
Penicillium chrysogenum Thom	1, 2
Penicillium citrinum Thom	4
Penicillium expansum Link ex Gray	4
Penicillium frequentans Westling	1
Penicillium purpurogenum Stoll	1
Alternaria alternata (Fr.) Keissl.	1,3
Arthrinium phaeospermum (Corda) M.B. Ellis	1
Aureobasidium pullulans (De Bary) Arn.	4
Botrytis cinerea Pers. ex Fr.	1
Cladosporium cladosporioides (Fres.) de Vries	1, 2
Cladosporium herbarum (Pers.) Link ex Gray	1
Cladosporium sphaerospermum Penz.	4
Rhinocladiella mansonii (Castell.) Schol-Schwarz	3
Scopulariopsis brevicaulis (Sacc.) Bain	3
Phoma eupyrena Sacc.	4

1 - micromycetes able to cause allergies, 2 - micromycetes causing diseases, 3 - opportunistic micromycetes, often causing diseases, 4 - micromycetes, very seldom, occasionally causing diseases.

lividum, and *C. herbarum* dominated in samples. It should be mentioned that in winter the species composition of the *Penicillium* genus changed. *Penicillium lanoso-coerulleum* and *P. paxilli* became dominant; the abundance of *Aspergilus penicillioides* increased. In the air samples from the city centre, especially close to places with intensive movement of people, *Trichopyton rubrum* and *Microsporum canis* appeared; they are considered pathogenic to people and animals.

Concentrations and compositions of airborne fungi in other Lithuanian cities. The air of the areas in Kaunas, Marijampolė, and Elektrėnai, situated close to highways, was heavily contaminated with micromycete propagules, especially during dry weather in summer $(2,727 \pm 160; 1,636 \pm 489; 4,545 \pm 2,627 \text{ cfu/m}^3 \text{ were}$ detected respectively).

The richest species diversity of micromycetes have been revealed in the investigated cities during summer time. In Elektrenai, species diversity was much poorer, despite high numbers of micromycetes. Fungi distributions analysis revealed that *C. herbarum*, *C. cladosporioides*, A. alternata, A. niger, A. fumigatus, Penicillium paxilli, P. decumbens, P. funiculosum, P. chrysogenum, P. viridicatum, P. expansum species dominated in samples. These fungi in general are characterised as non-pathogenic and non-toxigenic, though some of the strains may possess the ability of producing toxins or causing diseases. These peculiarities are characteristics of A. fumigatus, A. niger, Penicillium expansum, P. verrucosum, P. viridicatum, A. alternata, and occasionally of other fungi.

In the air of some areas in these cities, during certain seasons, a lot of yeast-like fungi (*Geotrichum candidum*) were detected. Which could be related with additional pollution of the environment with micromycete propagules from specific environmental sources. This possibly occurred due to high concentration of people, pet waste, under the influence of butchery enterprise and a soft drink factory. Micromycete species most frequently recorded in the air of Lithuanian cities all through the year are listed in Table 1.

Simultaneously with air sampling for the investigations on microorganisms, the concentration of physical solid particles (1.5–10 µm diameter) in the air was measured. The concentration of these particles in the air of Vilnius city ranged from 15×10^6 – 14×10^7 particles in 1 m³ of air, in Alytus - from 20×10^6 – 40×10^6 , in Marijampolė from 25×10^6 – 30×10^6 , in Kaunas - from 20×10^6 – 23×10^6 , in Elektrėnai from 20×10^6 – 50×10^6 . The concentration of solid particles strongly depended upon the season, meteorological conditions, especially strength of wind, intensity of traffic, and other environmental factors. Still, no evident correlation between the abundance of solid particles and micromycete propagules was determined.

Micromycete species isolated from the air of Lithuanian cities that negatively affect the human health are listed in Table 3.

Eight species, most frequently isolated from the air and known to be allergens and agents of plant diseases, were selected for statistical analysis (Tab. 2). Analysis of interactions or heterogeneity by G-test considering the season (winter, spring, summer, autumn) revealed that winter reliably differs from other seasons (p < 0.05), while spring, summer, and autumn are similar regarding the most frequently detected species.

The least number of micromycete species were detected in winter (13.83%), while in spring - 33.67%, in summer - 19.33%, in autumn - 33.17%. Analysis of interspecific differences showed that the season influenced *Alternaria alternata* and *Botrytis cinerea*, which were detected most seldom, as well as *Aspergillus fumigatus*, which was most frequent in winter (p < 0.05).

Therefore, spring and autumn in Lithuania can be characterised by increasing numbers of the most frequently isolated species that may produce a negative impact upon the health of people, plants, and animals.

Comparison of filter sampler and impinger data. The comparison of experimental data on the concentrations of airborne fungal propagules obtained by impinger and filter sampler showed that impinger's yields were considerably higher than those of the filter sampler.

It has been stated that many results obtained by different authors contradict each other because the samplers used in the studies differ considerably in their physical and biological collection aspects. Furthermore, different methods are used for microbial analysis, and airborne microorganisms were sampled from a variety of air environments.

Taking into account the above-discussed structural differences of various fungal species, we can explain the differences in concentrations of fungal propagules (Fig. 5) measured, by applying different bioaerosol sampling methods. The cultural assay yielded one colony-forming unit when the agglomerate of fungal conidia was sampled onto a Whatman 40 filter. A similar agglomerate of fungal conidia taken into the impinger's liquid was possibly broken into a number of single conidia by the highly developed turbulence of the impinger's liquid, and few colony-forming units developed after the cultural assay [14]. Therefore, the differences in concentrations of fungal propagules (Fig. 5) show that many airborne fungal propagules found in air were possibly agglomerated fungal propagules.

DISCUSSION

In Lithuania, the lowest concentration of micromycetes was registered in winter; in Vilnius the average concentration of airborne micromycete propagules was 333 ± 15 cfu/m³. Finnish scientists [35] have presented similar data. In regions of subarctic and temperate climate the concentrations of propagules in the air are the lowest in winter when the main sources of fungal propagules are frozen or covered by snow. In Finland, the average concentrations of fungal propagules in winter (during months with snow cover) was 20 cfu/m³, while in summer (during months without snow cover) it reached 950 cfu/m³, being highest in August and September.

In Lithuania, the highest concentrations of micromycetes were observed in summer and autumn. It is noted in the literature that in regions of subarctic climate the number of airborne fungal propagules reaches from 10^2-10^4 cfu/m³ in summer [35]. Another Finnish study reported an average concentration of 190–410 cfu/m³ for airborne fungal propagules during October-December [16]. In the course of investigations performed at the foot of the Tatra Mountains, in southern Poland, the maximum concentrations of micromycete propagules were detected in July and August. In Warsaw, the highest concentrations were in June and July [37].

However, it is rather complicated to compare the data on the concentrations of micromycetes and species composition obtained in our research with that of other authors because investigators use different devices for collecting fungal propagules, different media, and cultivating conditions.

In the works of other researchers the following results have been presented: in the ambient air of the city of Turin (Italy), an area of temperate, suboceanic climate, the majority of detected fungi species belonged to Mitosporic fungi; micromycetes of the *Penicillium* (62.8%) and Aspergillus (23.3%) genera dominated [25, 26]; investigations performed in Milan revealed the prevalence of the Cladosporium genus micromycetes (55%). C. cladosporioides, C. herbarum, C. sphaerospermum, as well as micromycetes of the Penicilium and Alternaria genera were constantly isolated [32]. The literature claims that Penicillium [2, 16, 27], Aspergillus [2, 16, 28, 39], Alternaria [2, 12, 19, 21, 24], Botrytis [6, 31], and Aureobasidium [31] are frequently detected in the air. Comparison of our research results with those presented in the literature shows that the micromycete species most frequently isolated from the air in Lithuania are cosmopolitan, spread in different climatic zones.

In Europe and America [31], micromycetes of the *Aspergillus* genus dominate in winter, though they do not show strict seasonal prevalence.

Therefore, spring and autumn in Lithuania can be characterised by increasing numbers of most frequently isolated species that can produce a negative impact upon the health of people, plants, and animals.

The species diversity of micromycetes was unequal in the tested air samples. Micromycetes found in the air in Vilnius, especially in industrial sites, differed from the micromycetes isolated from the samples taken in smaller cities (Kaunas, Alytus, Marijampolė). It is probably predetermined not just by the size of the city, traffic intensity, but also by the geographical situation of the city. As Vilnius is situated among hills in the valley of the Neris river, the samples were taken there. In other cities the geographical impact was slight or completely absent.

However, it was a noticeable tendency that in the air of Vilnius city the frequency of conditionally pathogenic micromycete species was high. It caused anxiety, as people living in cities are often immunodepressed [28], and those species can have a significant impact on the human environment.

CONCLUSIONS

1. The abundance of micromycete propagules in the air of the investigated cities varied, causet by different seasons. Higher amounts of fungal propagules are recorded in industrial areas of Vilnius and areas close to the highways with heavy traffic; they reach 6,400 cfu/m³ (in summer). Meanwhile the air pollution in smaller cities is: Kaunas - 2,700, Marijampolė - 1,636, Alytus - 484, Elektrėnai - 4,545.

2. The highest species diversity of micromycetes in the air of Lithuanian cities is recorded in summer and autumn when conditions for the spread of microorganisms are most favourable (humidity and temperature), and the contamination sources in the environment are most abundant (rotting leaves, branches, various remnants).

3. Most frequent in the polluted air of all investigated cities are *Aspergillus fumigatus* (detection frequency 56.9%), *A. niger* (84.25%), *Cladosporium herbarum* (63.78%), *C. cladosporioides* (56.69%), *C. sphaerospermum* (56.69%), *Penicillium funiculosum* (45.52%), *Geotrichum candidum* (33.07%). Separate strains of the dominant micromycete species can be conditionally pathogenic to the health of people, cause allergies and disorders of respiratory organs.

4. The comparison of experimental data on airborne fungal propagule concentrations obtained by impinger and filter sampler showed that impinger's yields were considerably higher than that of the filter sampler.

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