ISSN 0026-2617, Microbiology, 2011, Vol. 80, No. 6, pp. 870–876. © Pleiades Publishing, Ltd., 2011.
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ARTICLES FROM THE RUSSIAN JOURNAL
MIKOLOGIYA I FITOPATOLOGIYA
(MYCOLOGY AND PHYTOPATHOLOGY)

Opportunistic Fungi in Soils and Surface Air of a Megalopolis (for the Tushino Region, Moscow)

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Received December 21, 2010

Abstract—The number and composition of opportunistic microscopic fungi was studied in soils and surface air (0.2 and 1.5 m above the surface) in the megalopolis districts (Tushino, Moscow) of different age of construction (6 and 40 years) and in urban recreational forests. The highest number (up to 1500 CFU/m^3) determined by plating from air was found in the summer in new-built quarters. Direct count of fungal diaspores in airborne dust yielded significantly higher values (up to $4 \times 10^5/m^3$). The composition of the soil fungal population differed significantly from that of the air. In soil, the diversity of potentially pathogenic fungi was higher, while in air, their abundance was greater. The highest content of opportunistic fungi in soil and air was observed in spring and late summer–autumn, respectively. The fungi known as allergenic (mostly *Cladosporium* spp.) predominate in the air in autumn, especially in the new-built quarters.

Keywords: microscopic fungi, potential pathogens, urban environment, soil, air.

DOI: 10.1134/S0026261711060142

Accumulation of potentially pathogenic (opportunistic) fungi in inhabited environments may increase the risk of secondary mycoses and allergies. Assessment of their presence in the surface air layers is especially important at the sites with high population density. The presence of opportunistic fungi in urban environments has been previously investigated for living spaces [1–5]. Assessment of potentially dangerous fungi outside the buildings is no less important. The effect of urban soils on the composition of fungi in the surface air has not been previously investigated.

The goal of the present work was to assess the presence of microscopic fungi in the surface air layers and soils of neighborhoods of different ages and of the recreational territories of a megalopolis.

MATERIALS AND METHODS

The composition of fungi in the aeroplankton and soil was studied in the Tushino district (Moscow, Russia) at the sites where the composition of soils and the functional diversity of the soil mycobiota have been already investigated [6, 7]: urban sites of different age of construction (6 and 40 years) and urban forest with different recreational load. The urban neighborhoods contained panel 5- and 9-storey buildings. The vegetation cover of the new neighborhood was a cereal lawn mixture, while in the older neighborhood, rarefied herbaceous vegetation (bluegrass, dandelion, clover, etc.) and planted birch, linden, rowan, and maple. The soils were fine clay loam technozem and urbanozem (Urbic Technosoils) on the covering loams with up to 2.75% humus content in the upper horizon and pH 6.4. A nearby site of an 18th century estate park, presently under intense recreational load, was treated as the oldest urban territory. Its arboreal cover consisted of elm, maple, and linden, and its herbaceous cover, of bluegrass, buttercup, and glague. The soil was mid-density sandy-clay loam urbanozem on the cultural layer, underlayered by the horizons of sod–podzolic soil. A slightly disturbed parcel of mixed forest at the territory of the regional woodland park was used as the control. Its vegetation consisted of pine with a complex underbrush of oak, birch, and linden. The soil was sod–podzolic, deeply gleyed sandy-clay loam on clay loams, with up to 4.9% humus in the upper horizon and pH 4.2 [7].

The samples were collected in 2007–2009. Air samples (250 ml) were taken from the surface layer (0.2 m) and the level of respiration of a medium-sized human (1.5 m) using a PU-1B aspirator and plated in triplicate on Czapek medium with 2% sucrose. In the samples of aerial dust collected in 2009, the concentration of fungal propagules was determined by epifluorescence microscopy of calcofluor-stained samples [8].

At the time of air sampling, soil samples were collected from the upper soil horizon. The soil dilutions (1 : 100) were analyzed by plating in five repeats on Czapek medium. Occurrence of the microscopic fungi was determined by spatial frequency of occurrence and relative abundance of the species. In spring and autumn 2008, microscopic fungi from the surfaces of

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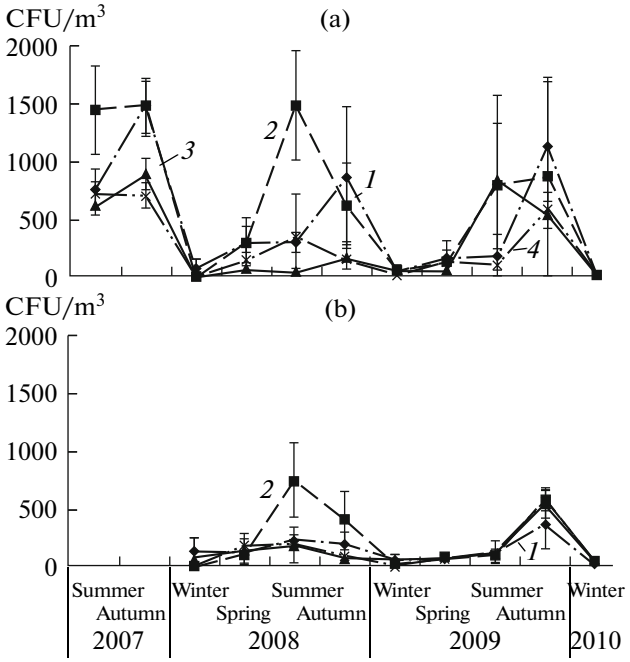

Fig. 1. Dynamics of the total number of cultured microscopic fungi (CFU/m^3) in the surface air layers at urban territories of different ages of construction (Tushino, Moscow): surface layer (0.2 m above the soil) (a), 1.5 m above the soil (b), woodland park (control) (*1*), 6-year neighborhood (*2*), 40-year neighborhood (*3*), and estate (300 years) (*4*).

the panel and concrete buildings were analyzed. For this purpose, RidaCount Yeast and Mold test plates (R-Biopharm AG) were used. The samples were taken by imprints in five repeats from the walls of the buildings. For quantitative determination, the imprints were developed with the physiological saline in a sterile box. For the isolation of pure cultures, the films were placed on Czapek agar with 2% sucrose. The species composition of the aeroplankton and soil biota was determined based on the cultural and morphological characteristics using the relevant identification guides. For a number of dominant species, which were isolated as a sterile mycelium, identification by the ITS1 and ITS2 rDNA sites was carried out by Syntol (Russia). Statistical treatment of the results was carried out using the Microsoft Office Excel 2003 and Statistika 8.0 software packages.

Table 1. Number of fungal spores (10^3/m^3) in the air of an urban territory (2009)

Site	Spring	Summer	Autumn
6-year neighborhood	305 ± 120	405 ± 55	235 ± 10
40-year neighborhood	109 ± 150	310 ± 80	305 ± 20
Estate (300 years)	170 ± 25	195 ± 125	227 ± 25

Note: Spore numbers were determined by fluorescence microscopy.

RESULTS AND DISCUSSION

The number of fungal propagules in the urban air varied significantly in different seasons (Fig. 1). In 2009, the highest number of microscopic fungi was observed in summer (~1500 CFU/m^3) and autumn (up to 1100 CFU/m^3), while the lowest (30–50 CFU/m^3) was in winter. Throughout the study, the distribution of fungal spores in the air was stratified, with higher abundance in the surface air (Fig. 1a) and a 1.5–2 times lower numbers at the man's height (Fig. 1b). Among different urban sites, the highest number of fungi in the surface air layer (up to 1500 CFU/m^3) was observed in summer at the 6-year neighborhood. This is three times the permissible concentration recommended for living spaces [9].

The total number of microfungi (from 20000 to 80000 CFU/g) was typical of the zonal soils. The highest number of fungi (8×10^4 CFU/g) was observed in autumn, unlike the aerial environment, where the lowest number of fungi in soil (2×10^4 CFU/g) was revealed in summer, similar to the air.

Direct count by fluorescence microscopy revealed, however, significantly higher number of fungi in the air than plating. For instance, the number of diaspores in the air could be 4×10^5/m^3 (Table 1). Although some of these spores probably do not grow on the media used in this study or are not viable, our data indicate that the amount of fungal biomass aspirated by humans may significantly exceed the estimates obtained by traditional techniques.

Apart from the seasonal variation in the number of fungi in the air of the urban and woodland park sites, changes in the species composition were observed. A total of 82 fungal species were isolated from the surface layer. Their highest diversity was observed in spring at the control woodland park site. *Cladosporium cladosporioides* (Fresen.) G.A. de Vries, which was practically invariably isolated from the air, dominated in the new neighborhood in summer and at almost all sites in autumn (up to 1000 CFU/m^3). *Fusarium verticilloides* (Sacc.) Nirenberg was also present in the air, its numbers peaking in summer. Other species had more pronounced seasonal dynamics. In autumn, the content of dark-colored species increased drastically in the surface air of all sites: *Alternaria alternata* (Fr.) Keissl., *Cladosporium cladosporioides, Stachybotrys chartarum* (Ehrenb.) S. Huges, *Ulocladium atrum* Preuss, and *Phoma leveillei* Boerema et. G.J.Bollen. In spring, members of the genera *Acremonium, Fusarium*, and *Aspergillus* were common in air, as well as *Epicoccum nigrum* Link. *Penicillium* was more often isolated in winter and spring, mainly the species *P. rubulosum* Thom, *P. brevicompactum* Dierckx, *P. corylophilum* Dierckx, *P. canescens* Sopp, *P. citrinum* Sopp, *P. crustosum* Thom, *P. duclauxii* Delacr.,

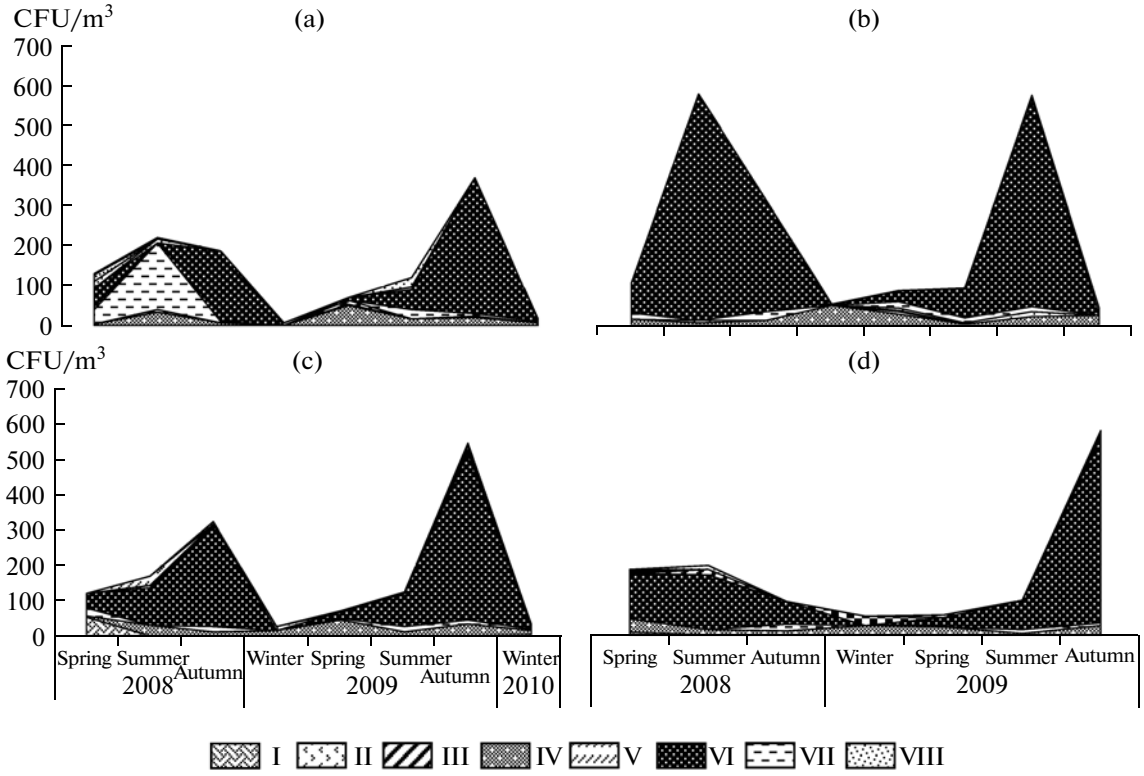

Fig. 2. Dynamics of the numbers of different groups of microscopic fungi in air at 1.5 m in 2008–2009: woodland park (control) (a), 6-year neighborhood (b), 40-year neighborhood (c), and estate (300 years) (d), *Aspergillus* spp (I), *Botrytis* spp. (II), *Fusarium* spp. (III), *Penicillium* spp. (IV), *Trichoderma* spp. (V), dark-colored (VI), sterile mycelium (VII), and others (VIII).

P. glabrum (Wehmer) Westling, *P. hirsutum* Dierckx, *P. implicatum* Biourge, *P. lividum* Westling, *P. miczynskii* K.M. Zalessky, *P. simplicissimum* (Oudem.) Thom, *P. verruculosum* Peyronel, *P. vulpinum* (Cooke et Massee) Seifert et Samson, and *P. waksmanii* K.M. Zalessky.

The highest species diversity was observed in the woodland park air. Unlike the urban territories, three *Trichoderma* species, *T. harzianum* Rifai, *T. atroviride* P. Karst, and *T. viride* Pers., which are known cellulose degraders, were present from spring to autumn. In spring, *Aspergillus niger* Tiegh., *A. flavus* Link, *A. wentii* Wehmer, *A. ustus* (Bainier) Thom et Church, and *A. fumigatus* Fresen were found in the woodland park and the 40-year neighborhood (Figs. 2a, 2c). The reasons for this increase in the abundance of *Aspergillus* are presently unclear.

Differences existed between urban sites of different age in the species composition of fungal aeroplankton, which was more stable in the new neighborhood. The share of dark-colored fungi was highest there. Unlike other territories where a peak of dark-colored fungi was recorded only in autumn, in the new neighborhood high content of this group was sometimes observed both in summer and in autumn (Fig. 2b). Their dynamics in the surface air layer was similar. The share of dark-colored species, was, however, even higher (up to 1500 CFU/m^3), possibly due to their growth on the surfaces of lawn grasses.

Determining the sources of formation of the urban aeroplankton is important for assessment of the formation of the urban mycobiota. These sources may include wind transfer of soil dust from the surfaces of plants and urban constructions. The mykobiota of urban soils is known to differ significantly from the zonal one [10]. Our results treated by cluster analysis show the differences between the fungal complexes in soil and air (Fig. 3). For different seasons, the similarity of the fungal aeroplankton from different urban neighborhoods was shown to be higher than between the soil populations of the same territories, indicating the insignificant role of transfer of soil dust under urban conditions.

Importantly, the urban territories of different age of construction and exploitation differed in the character of their aeroplankton. For example, two years of investigation clearly showed the differences between the structure of the fungal complexes in the surface air of the 6-year neighborhood and of other urban territories (Fig. 4). The new neighborhood also exhibited the poor seasonal dynamics of aeroplankton, while the composition and structure of aerial microfungi at other urban territories varied strongly in different years of investigation.

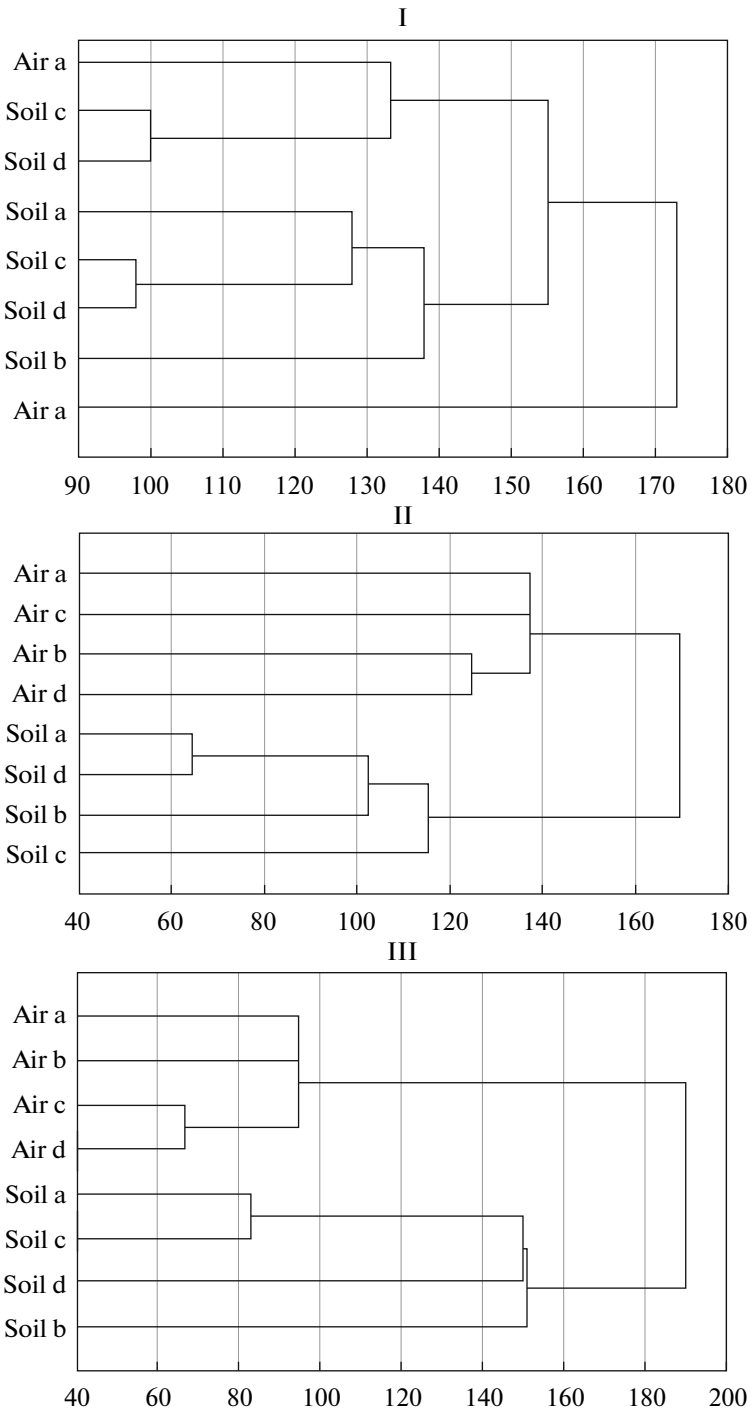

Fig. 3. Cluster analysis of the spatial frequency of occurrence (2009) for the fungal complexes in soil and air (at 1.5 m): spring (I), summer (II), and autumn (III), woodland park (control) (a), 6-year neighborhood (b), 40-year neighborhood (c), and estate (300 years) (d).

The composition of airborne fungi may be possibly affected by the fungal complexes colonizing the surfaces of urban constructions. We analyzed the composition of fungi from the outer walls of domestic buildings at the investigated urban sites. *Cladosporium cladosporioides* dominated on the house surfaces and in the air in autumn, while the *Penicillium* fungi *P. simplicissimum* and *P. vulpinum* prevailed during winter. No clear relation was found between the mycobiota of the air and of the house surfaces.

Potentially pathogenic fungi were always present at the urban territories investigated. Their composition

and the dynamics of their presence in the surface air and in soils differed significantly. In soil, potentially pathogenic species predominated in spring (up to 60%). Their content was highest at the site of the new neighborhood and at the control woodland park. The species belonging to the most potentially dangerous fungi, Scopulariopis *brevicaulis* (Sacc.) Bainier and *Aspergillus fumigatus* [11] were isolated only from soils.

In the air, only the potentially pathogenic species of the BSL-1 group were found, which is the one least dangerous to humans among the species known as opportunistic ones [11]. Their presence had a pronounced seasonal dynamic: it was low in winter, somewhat higher in spring, and high during summer and autumn (Table 2). The highest number of potentially pathogenic fungi was revealed during the dry summer of 2009, when the average monthly temperatures were within the climatic norm, while precipitation was below 80% of the seasonal norm (http://seakc.meteoinfo.ru). The number of potentially pathogenic species was significantly higher in the near-earth surface layer (up to 360 ± 45 CFU/m^3) and decreased three- to fourfold (to 80 ± 45 CFU/m^3) at the man's height level. This resulted from the elevated concentration of *Cladosporium cladosporioides* in the surface layer, since this species is primarily considered an allergenic for humans [12].

The studies on the quality and concentration of the fungal propagules in urban environments were mostly carried out in living spaces, since people spend most of their time there. However, many urban dwellers, especially children, spend much time outdoors, and the data on the environmental aeromycota are therefore required for more complete assessment of occurrence of opportunistic fungi in urban air.

Clearly established criteria for the safe level of fungal propagules in the air are absent at present. These recommendations exist only for living quarters, where 500 CFU/m^3 was suggested as a safe level [13]. This value may be much higher outdoors, both in natural and urban environments. In the cities, such situations may occur at the storage sites for industrial and household garbage, at waste composting areas, etc. [14, 15]. According to our data, significant variations in the numbers of fungal aeroplankton revealed in one of the nonindustrial living districts of Moscow depended primarily on the season. The highest values (up to

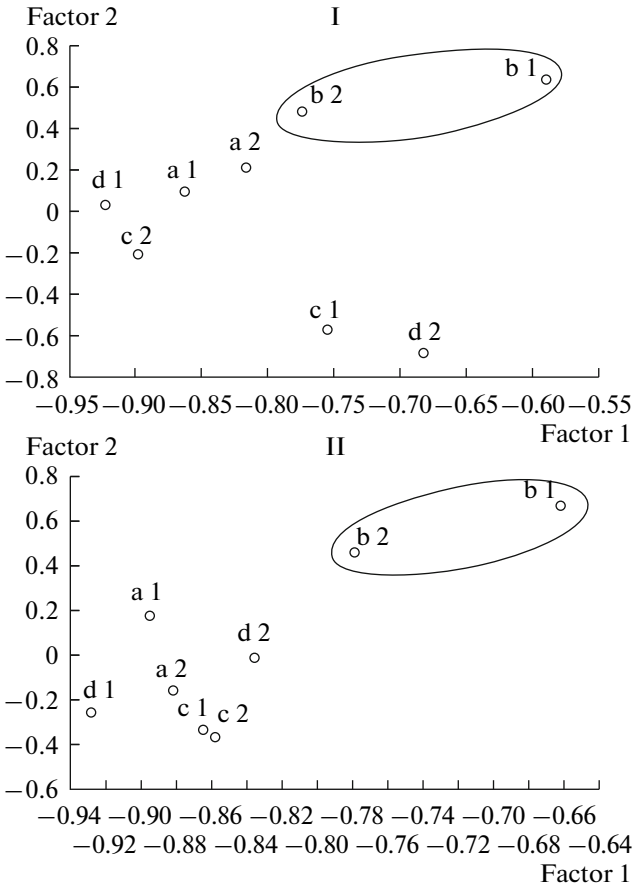

Fig. 4. Factor analysis of the composition of microscopic fungi in air at different urban territories (summer 2008 and 2009). Designations: 2008 (I) and 2009 (II), woodland park (control) (a), 6-year neighborhood (b), 40-year neighborhood (c), and estate (300 years) (d). Sampling level: surface layer 0.2 m (1) and 1.5 m (2).

Table 2. Ratios of potentially dangerous fungi (BSL1 group) in the air of urban neighborhoods and the woodland park, %

Object	Sampling level, m	2008			2009				2010
		Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
Control (woodland park)	0.2	11	55	99	16	45	85	88	21
	1.5	7	78	98	44	17	72	89	33
6-year neighborhood	0.2	25	99	98	14	43	91	86	11
	1.5	61	96	92	27	31	80	88	12
40-year neighborhood	0.2	98	54	77	40	20	94	78	10
	1.5	53	67	69	27	37	83	85	48
Estate (300 years)	0.2	56	12	86	50	48	92	78	18
	1.5	41	86	74	25	50	85	88	5

1500 CFU/m^3) occurred in summer and autumn. Similar patterns of fungal dynamics in the air (increased abundance in autumn) are known for natural environments [16, 17].

It should be kept in mind that the content of fungal diaspores in the aerial medium may be significantly higher than the values obtained by traditional plating techniques. Our results obtained by fluorescence microscopy show that the content of fungi in surface air may be several times higher than the numbers determined by plating. These differences may result from the limitations in the action of the impactors, the impossibility of isolation of many fungal groups on solid media, and the presence of nongerminating spores. Further investigation is required to determine the ratio of viable and dead diaspores under different seasonal and ecological conditions of urban environments and to establish their number directly contacting with humans. The latter may be determined using individual nasal impactors [4].

Comparison of our data on the aeromycota of a Moscow district to the data obtained in other cities is of interest. For example, compared to St.-Petersburg [3], the content of fungal diaspores in the air was higher, including the content of dark-colored fungi, which was more pronounced during summer and autumn. The complex of the dominant species isolated from the air of St.-Petersburg green areas included *Fusarium oxysporum* and *Penicillium chrysogenum*. During the moist autumn season, the most pronounced increase of the ratio of phytopathogenic microfungi of the genera *Botrytis, Fusarium, Monilia*, etc. was recorded [3]. We also observed *Botrytis* and *Monilia* in the air of Moscow urban territories in autumn, although *Cladosporium cladosporioides* was predominant.

Considering the low immune status of the urban population, assessment of the spores and fragments of potentially dangerous (potentially pathogenic and allergenic) microscopic fungi capable of causing unfavorable health consequences is important. Accumulation of potentially pathogenic fungi in urban soils is known [6, 18]. In the present work, the highest content of opportunistic fungi in soil fungal communities was observed in spring, while in the air their content increased in late summer and autumn.

Dark-colored fungi with large spores, primarily *Alternaria* and *Cladosporium* species, are most often considered allergenic for humans [12]. Comparison of the urban and recreational park territories revealed the highest content of these fungi in the air of the new neighborhood. This tendency was observed for two years during different seasons. Elevated levels of such spores in aerial environments were most often detected in autumn.

In general, the content of potentially dangerous fungal species was higher above the surface (20 cm) than at the respiration height of a middle-sized human (1.5 m). Accumulation of dangerous species in the surface layer is possibly caused not only by their release from soil, but also by their development in the litter (in the recreational park zone, while they were practically absent on the lawn grass of the urban zone). The elevated level of potentially dangerous molds in the urban air at low height is especially important due to its potentially unfavorable effect on the health of children.

Thus, determination of the sources of formation of the urban aerial mycobiota is an important issue. The possible release of dangerous fungi into the air during repair and reconstruction of the buildings was reported [19]. We did not observe any clear correlation between the aerial mycobiota and the fungal complexes on the surface of the outer walls of the buildings, although some common tendencies were found, such as development of *Cladosporium* in autumn and the presence of *Penicillium* species in winter. According to our results, soil dust was not the main source of formation of the mycobiota of the surface air layers, since the composition and structure of the fungal complexes in soil and in the air differed significantly. Introduction from external sources and from plant surfaces is possibly of greater importance. This conclusion is supported by significant increase in the content of dark-colored fungi in the air in autumn, when they develop actively on plant surfaces and in the litter. Further investigation is required in order to elucidate all the possible sources and scale of the introduction of fungal diaspores for formation of the urban mycobiotas.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research, project no. 08-04-0359a.

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