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RADIATION AND BIOSPHERE

Atmospheric Aerosol Fungi Concentration and Diversity in the South of Western Siberia

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Abstract—The results of the two-year observation of the atmospheric aerosol fungi concentration and diversity in the south of Western Siberia are presented. It is found that the fungi concentration in samples of atmospheric air can change dramatically: from less than 10 up to several thousands of viable fungi per cubic meter. A total of 18 genera of fungi referring to 3 subdivisions (*Zygomycotina, Ascomycotina,* and *Deuteromycotina*) were identified in the samples under study. Among them are representatives of those genera that are potentially pathogenic for human health (e.g., *Aspergillus, Cladosporium, Penicillium, Alternaria, Trichoderma,* etc.) and those that may be useful in modern biotechnology (e.g., *Aspergillus, Aureobasidium, Ganoderma,* etc.).

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INTRODUCTION

Many of the viable microorganisms in atmospheric aerosol are fungi. Propagating with the aerosol, they are liable to cause different human, animal, and plant diseases. In particular, data on the intercontinental transport of plant diseases caused by various fungi are given in [1]. There are large volumes of published data for other regions regarding the presence of one or another genera of fungi in atmospheric air (see, e.g., [2–11]); however, they are cited mainly for seasonal measurements, not systematic ones, and do not include altitude measurements. This work describes the two-year detailed observation of the atmospheric aerosol fungi concentration and diversity in the atmospheric air in the south of Western Siberia.

MATERIALS AND METHODS

In this work, we present the data obtained from the analysis of atmospheric aerosol sampled over 2 years (from June 2006 to May 2007 and from June 2007 to May 2008) during the course of aircraft sounding of the atmosphere and ground-based measurements in the south of Western Siberia.

Sampling of Atmospheric Air

The altitude sampling was carried out 50-km south of Novosibirsk once during the last 10 days of every month with the help of the Optik-E AN-30D aircraft laboratory. The aircraft flew over forested area during the daytime at altitudes of 7000, 5500, 4000, 3000, 2000, 1500, 1000, and 500 m. Air samples were collected in sterile impingers for 8–10 min at each altitude. Ground air was sampled in the same impingers for 30 min with a 50 l/min flow rate at sites located on the territory of the Vector State Research Center of Virology and Biotechnology and in the village of Kluchi near Novosibirsk Akademgorodok. In Kluchi they were taken over the course of 7 days a month in different seasons. To reveal the diurnal variations of the measured parameters, air sampling was carried out four times per day at the site of the Vector State Research Center of Virology and Biotechnology during 1 day in the middle of the month.

Analysis of the Viable Bacteria Concentration in the Samples

The viable microorganism concentration was detected on Petri dishes in Sabouraud's medium [12]. If needed, the samples were serially diluted. The culture and morphological characters of the germinating fungus colonies were considered and described twice (5th and 14th days) for a more complete accounting of the fungi, including those that were in dormant stages and needed a longer time for germination in the medium. The qualitative consideration included a description of the colony morphologies of the entire variety of germinating fungi and the microscopy of isolated strains.

Using an AXIOSCOP ZEISS microscope, images of the conidiophores, conidium, and mycelium of some strains were obtained. To identify the fungi, standard instructions and identifiers were used [13–19]. After determining the genera of the identified isolators, some fungi cultures were reseeded in the medium

Table 1. Concentrations of fungi revealed in the high-altitude samples, CFU/m^3

Month of sampling	Altitude, m							
	500	1000	1500	2000	3000	4000	5500	7000
June, 2006	250	0	0	0	0	0	0	83
July, 2006	–	125	83	208	0	250	42	42
August, 2006	583	333	0	167	42	417	208	83
September, 2006	333	250	542	83	42	125	83	42
October, 2006	542	208	0	0	0	42	0	125
November, 2006	0	0	0	0	0	0	83	83
December, 2006	42	0	0	0	42	0	0	0
January, 2007	0	0	0	0	0	0	0	0
February, 2007	167	458	0	0	0	0	0	0
March, 2007	0	0	125	0	0	0	42	208
April, 2007	0	0	83	83	42	83	0	0
May, 2007	0	42	1042	0	0	0	0	0
June, 2007	292	0	42	42	0	42	0	0
July, 2007	–	–	–	–	–	–	–	–
August, 2007	83	0	42	0	42	42	42	0
September, 2007	208	42	0	0	0	0	0	0
October, 2007	83	0	0	0	0	0	0	125
November, 2007	83	0	0	0	0	0	0	0
December, 2007	0	0	0	0	0	0	42	0
January, 2008	0	0	0	0	83	0	0	0
February, 2008	83	125	0	0	0	0	0	83
March, 2008	0	0	0	0	0	83	0	0
April, 2008	–	–	–	–	–	–	–	–
May, 2008	125	42	0	42	0	125	0	42

Note: (–) lack of data.

to obtain pure culture collections and then study their properties.

The viable count (in colony-forming units CFU) in the samples was determined by common techniques; the amount of microorganisms was averaged over 3–4 parallels of samples inoculated on medium. The rms error of the microorganism concentration did not exceed $\pm 0.2 \log_{10}$ in these conditions. Taking into account the volume of atmospheric air samples, the minimum detection threshold of the viable bacteria concentration in the atmosphere was 40 CFU/m^3 for high-altitude samples and 11 CFU/m^3 for ground ones.

RESULTS AND DISCUSSION

The following results were obtained from the study of atmospheric air samples.

No definite monthly trends were revealed in the distribution of the quantity and frequency of individual taxons within a fungi complex (Tables 1–3), similar to those for viable microorganisms in the south of Western Siberia [20]. The percentage of viable bacteria in the investigated atmospheric air samples is larger than that for fungi; hence, the cyclic annual variations of the fungi concentration should reliably appear in long-term observations, like for the total concentration of viable microorganisms [20]. Probably, the daily variations of the fungi concentration could be also revealed.

Note that the weather can noticeably influence the obtained data on the fungi concentration: depending on the region from which the air masses have come to the sampling site, they are more-or-less enriched with bioaerosols; precipitations or dead calm can decrease the atmospheric concentration of viable microorganisms. However, as shown in [21], such variations are much smaller than the data spread given in the present work. The high diversity of the concentration of viable microorganisms was noted earlier in [22].

A total of 18 genera of fungi referring to 3 subdivisions (*Zygomycotina, Ascomycotina,* and *Deuteromyco-*

Table 2. Atmospheric concentrations of fungi revealed at the sampling site on the territory of the Vector State Research Center of Virology and Biotechnology, CFU/m^3

Month of sampling	Time of sampling			
	10:00–10:30	16:00–16:30	22:00–22:30	04:00–04:30
June, 2006	167	11	211	822
July, 2006	178	322	611	622
August, 2006	278	344	689	800
September, 2006	633	711	444	333
October, 2006	89	11	33	33
November, 2006	22	33	11	311
December, 2006	100	100	22	144
January, 2007	78	78	156	122
February, 2007	100	11	33	22
March, 2007	0	11	11	33
April, 2007	44	11	89	167
May, 2007	711	833	444	544
June, 2007	178	178	300	489
July, 2007	444	156	300	267
August, 2007	356	467	0	533
September, 2007	67	56	67	111
October, 2007	222	222	144	89
November, 2007	22	78	56	33
December, 2007	500	478	100	78
January, 2008	0	11	0	44
February, 2008	0	0	333	0
March, 2008	11	11	22	0
April, 2008	156	22	33	0
May, 2008	33	0	11	111

Table 3. Atmospheric concentrations of fungi revealed at the sampling site in the village of Kluchi, CFU/m^3

Season of sampling	The date of sampling beginning (the 1st day)	Day of sampling						
		1	2	3	4	5	6	7
Summer, 2006	July 4, 2006	247	153	117	142	117	244	197
Autumn, 2006	October 10, 2006	39	33	36	42	67	19	28
Winter, 2007	February 1, 2007	25	28	3	0	169	11	8
Spring, 2007	May 4, 2007	203	22	11	17	22	53	36
Summer, 2007	July 5, 2007	78	47	33	61	92	308	–
Autumn, 2007	September 3, 2007	22	42	75	53	22	64	11
Winter, 2008	January 29, 2008	31	3	6	0	0	0	3
Spring, 2008	April 29, 2008	50	17	19	0	17	6	25

Note: (–) lack of data.

Table 4. The number of fungi genera identified in atmospheric air samples during the study

Sampling site	The number of identified fungi genera	
	June, 2006–May, 2007	June, 2007–May, 2008
Vector State Research Center of Virology and Biotechnology	13	15
High-altitude samples	15	7
Kluchi village	10	10

tina) were identified in the samples under study. Changes in the ratio between the identified genera have been noted during the period of the study, depending on sampling site (Table 4).

The maximum fungi diversity was recorded in high-altitude samples during the first year of the study. A sharp decrease (more than twofold) in the biodiversity of the fungi was observed during the second year. The amount of fungi slightly increased in the atmospheric air samples taken at the site of the Vector State Research Center of Virology and Biotechnology. The amount of fungi at the sampling site in the village of Kluchi virtually did not change during the period of the study. Qualitative changes in the content of fungi genera isolated from the air aerosol samples took place during this period (Table 5).

The obtained data do not allow for the revelation of the altitude dependence of the total concentration of fungi and their separate genera, despite the well-known fact that the calculation of the aerosol concentration is reduced by more than one order of magnitude with an increasing altitude of up to 7 km [23]. In addition, the obtained long-term data for viable microorganisms in the south of Western Siberia revealed the unique dependence or, more precisely, the independence of their concentration from an altitude of up to 7 km [24]. Bacteria constitute the main part of the total amount of viable microorganisms, and fungi make up a slightly smaller part; hence, such a dependence does not appear to be unique.

The study of the biodiversity of fungi in the atmospheric aerosol in the south of Western Siberia shows that these microorganisms are usually structural and functional components of land ecosystems. Among the fungi isolated from atmospheric air samples (see

Table 5. Fungi isolated from atmospheric air samples during the study

Fungi genera	Sampling Site					
	Vector State Research Center of Virology and Biotechnology		High-altitude samples		Kluchi village	
	1st year of the study	2nd year of the study	1st year of the study	2nd year of the study	1st year of the study	2nd year of the study
Alternaria	+	+	+	+	+	+
Aspergillus	+	+	+	+	+	+
Aureobasidium	+	+	–	+	+	+
Basidiomycetes	+	+	–	+	–	+
Botrytis	–	+	–	–	–	+
Cephalosporium	+	+	+	–	–	+
Cladosporium	+	+	+	+	+	–
Drechslera	–	–	+	–	–	–
Fusarium	+	+	+	–	+	+
Ganoderma	+	–	–	–	–	–
Geotrichum	–	+	–	–	–	+
Mucor	–	–	+	+	–	–
Mycelia sterilia	–	–	–	–	–	+
Penicillium	+	+	+	+	+	+
Rhizopus	–	–	+	–	–	–
Stereum	+	–	–	–	–	–
Trichoderma	–	+	+	–	–	–
Verticillium	+	+	+	+	–	–
Unidentified	+	+	+	+	+	+

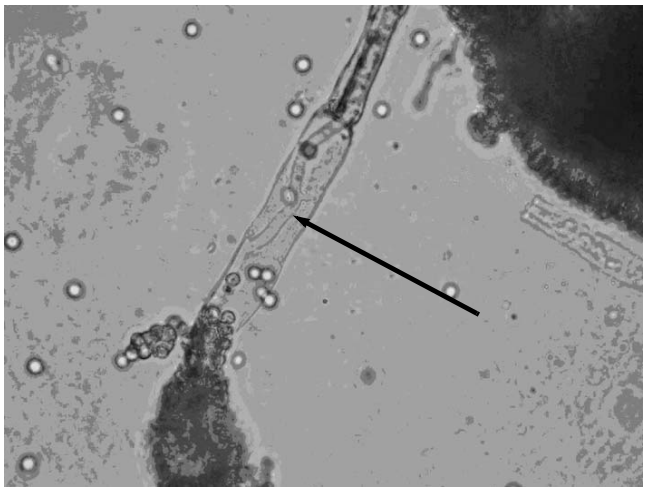

Fig. 1. Mycophile fungus (pointed by the arrow) inside the conidiophores of the *Aspergillus* fungus. 1000-power magnification.

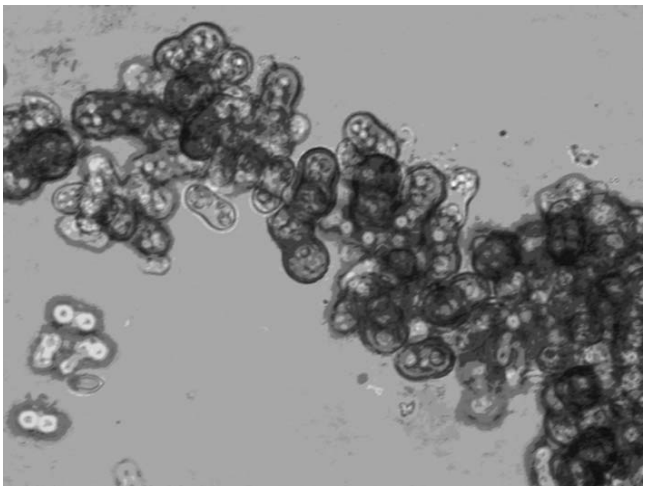

Fig. 2. Conidium of *Aerobasidium* fungus from the collection of the samples taken on March 15, 2007 at 10:00–10:30 at the site of the Vector State Research Center of Virology and Biotechnology. 1000-power magnification.

Table 5), there are genera liable to cause different plant, animal, and human diseases. The presence of spores of *Aspergillus*, *Cladosporium*, and *Penicillium* fungi in the air and inhabited areas is usually considered to be a human health risk, since representatives of these fungi are allergen and mycotic disease producers [10, 25]. It is known that environmental fungi dangerous to human health enter into the composition of mycobiota from the surrounding media. *Alternaria, Aspergillus, Trichoderma,* etc., fungi potentially liable to cause mycoses, have been revealed in museums [26].

The diversity of *Aspergillus* fungi types differing in colony color and micromorphology is striking. Among them there are types developing ascospores in the cleistothecium formed in culture media. This state of fungi development supports the fact that fungi of this genus belong to ascomycetes, but more often they occur in an imperfect state, only with conidiospores.

The toxigenic ability of aspergillic fungi has been known for a long time; the pollution of plant production by toxins of these fungi is the subject of investigation in many countries [11, 27]. Among plant pathogenic fungi, representatives of the *Fusarium*, *Cladosporium, Botrytis, Drechslera,* and *Cephalosporium* genera were isolated from the samples. Fungi of the *Aspergillus, Penicillium, Cladosporium,* and *Alternaria* genera predominated in the atmospheric air samples; they were also recorded in other regions of the Commonwealth of Independent States, such as Adzhariya and St. Petersburg [10, 11].

Having a powerful fermentative apparatus, fungi can destroy many man-made materials, i.e., wooden constructions, building materials, and even jet fuel [28–30]. Considering the danger of biodeterioration, the term "biologically active media" has entered into construction regulations since 1997; scientists suggest implementing an obligatory follow-up analysis of fuel and fuel systems during scheduled aircraft technical inspections using common mycological and microbiological methods and including the analysis schemes in the corresponding regulatory documents [29].

Among the fungi revealed in the collection of the atmospheric air samples, there are those that can be used as producers of various bioactive substances after the corresponding investigations. Germinating colonies were sometimes mixtures of two or more fungi types differing in color; mycoparasites in the mycelium of other types were visible in a microscope (Fig. 1).

Producers of fibrinolytic ferments could be selected among these mycoparasites [31]; this is topical in regards to medicine. In this context, wood-destroying fungi are of interest. They are widespread in nature, since they participate in cellulose and xylogen destruction. The question is not only about basidium fungi well-known by their conks, but also about such basidiomycetes as stereum fungi that play a significant role in wood destruction. These fungi were identified in the samples by the presence of "buckles," particular structures on floccus.

There is an upsurge in interest in basidium fungi all over the world because of their immunomodulatory, antitumoral, and antiviral effects; therefore, representatives of stereum fungi could be of interest for studying these properties. Among germinating colonies, phaeic fungi containing pigment melanin were present in all of the samples. This pigment is of growing interest due to its acting as a universal protector during the attack of physical and chemical factors of a mutagenic and carcinogenic nature in addition to its function of a regulator cell metabolism [32]. Some types of *Aspergillus, Clàdosporium, Alternaria, Aureobasidium,* etc., genera are related to melaniferous fungi. High-quality water-soluble melanin has been obtained from *Aspergillus* carbonarium fungus at the Scientific

Research Institute of Biology at Irkutsk State University [33]; it can be used in farm animal production to increase milking capacity and broiler chicken production and to strength human immunity. Fungus melanin is used in Belarus to produce dermatological creams [34]. *Aureobasidium* fungi, frequently appearing in collections of the samples, are of interest (Fig. 2).

Based on one of its types, the production of a new plasmalike substance has begun [34] in Belarus. The further development of the medical biotechnology of new pharmaceutical drugs depends on the search of effective producers among the natural varieties.

CONCLUSIONS

The conducted observations have shown that the fungi concentration in atmospheric air samples is highly variable. The obtained results did not allow us to reveal the dependences of the seasonal variations of their concentration and diversity in ground-based and high-altitude samples of atmospheric air in the south of Western Siberia.

It is shown that fungi of multiple genera are present in samples of atmospheric air. Representatives of those genera that contain potentially human pathogenic fungi have been noted among them (such as *Aspergillus, Cladosporium, Penicillium, Alternaria, Trichoderma,* etc.), as well as those properties which are usable in modern biotechnology (such as *Aspergillus, Aureobasidium,* and *Ganoderma*).

In addition, we emphasize once more that both the fungi concentration and diversity in different samples of atmospheric air in the south of Western Siberia are highly variable. Therefore, long-term investigations are required to obtain reliable annual, altitudinal, and other dependences of their concentration and the diversity of the samples.

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