

Influence of dust storms on concentration and content of fungi in the atmosphere of Haifa, Israel

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Abstract Dust storms originating primarily in the Saharan desert heavily affect the Eastern Mediterranean, mostly during the winter and spring season. The main goal of our study addresses the qualitative and quantitative aspects of dust-associated fungal communities sampled during dust events in the years 2004–2005 in Haifa, Israel, and their comparison with the communities sampled on adjacent clear days. The effect of particulate matter concentrations and elemental composition of the atmospheric particles on fungal communities was also estimated. Airborne fungi were collected with the Six Stage Andersen Viable Impactor. Their taxonomic identification was based mainly on morphological characteristics; one repeatedly isolated type of non-sporulated colonies was identified employing the polymerase chain reaction. During six dust events and the adjacent clear days, 98 species were collected—79 and 32 species in dusty and clear days, respectively. The dust-associated fungal communities were significantly richer than the communities of clear days (Wilcoxon signed-rank test, $p = 0.03$). Remarkable increases in the concentration of airborne fungi during the dust events compared to

the adjacent clear days have been also revealed. The canonical correspondence analysis showed that the concentration of fine atmospheric particles was the dominant environmental factor influencing the distribution of most common fungal species followed by the concentration of geological elements and coarse particles. Overall, the study revealed a distinct pattern of distribution of fungi in the atmosphere of Haifa that was strongly related to Saharan dust storms.

Keywords Airborne fungi · Eastern Mediterranean · Particulate matter · Saharan dust storms

1 Introduction

Dust storms moving from large deserts on the planet are phenomena that affect air quality in vast areas all over the world. An annual amount of desert dust subjected to regional or global airborne transport has been estimated within the range of 0.5–5.0 billion tons (Perkins 2001). The biogenic component of airborne dust contains numerous fungal fragments, mostly spores, which are extremely mobile and can be transferred by wind for very long distances (e.g., Goudie and Middleton 2001; Griffin et al. 2003; Griffin 2007). A diverse viable dust-associated fungal community has been detected, for example, at a mid-Atlantic ocean research site located a long distance

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from the North African dust source (Griffin et al. 2006).

Israel is exposed every year to dust storms originating primarily in the Saharan desert in North Africa, but also from Arabian deserts, mainly during autumn, winter, and spring. The amounts of Saharan dust transported eastward have been estimated to be near 70 million tons per year, of which 20–30 million tons are deposited in the Eastern Mediterranean (Mamane and Ganor 1981). The physical and chemical nature of these dust storms, their origins, and the meteorological conditions leading to their generation have already been documented (e.g., Alpert and Ganor 2001; Ganor and Foner 1996, 2001; Ganor and Mamane 1982; Nickovic et al. 1997). In contrast, the microbial content of dust storms affecting the Israeli region remained almost unknown, even though in Israel, the presence of biological particles in the atmosphere containing pollen grains and fungal spores has been studied in different areas (Barkai-Golan 1958; Barkai-Golan and Galzer 1962; Barkai-Golan et al. 1977; Keynan et al. 1991; Waisel et al. 1997). Our previous study described the concentration of airborne bacteria and fungi and species composition of fungi during four dust events and adjacent clear days in the city of Haifa (Schlesinger et al. 2006). This study revealed remarkable increases in the concentration of airborne microorganisms during the dust events compared to the adjacent clear days accompanied by variations in the composition of microscopic fungi. It suggested that Saharan and other desert dust events in the Eastern Mediterranean had a significant effect on the airborne microbial communities, which might have a great impact on human health, agriculture, and natural ecology.

Our current investigation continues the study of microbial content of dust particles in the atmosphere of Haifa. The main goal of the present study address the qualitative and quantitative aspects of dust-associated fungal communities samples during dust events in the years 2004–2005 and their comparison with fungal communities samples during adjacent clear days. The following characteristics of the communities were examined: species composition, species diversity (richness), and concentration of colony-forming units (CFU per cubic meter). The effect of particulate matter concentrations and elemental composition of the atmospheric particles on the above characteristics was also estimated.

2 Materials and methods

2.1 Collection of airborne fungi

Days of sampling were selected based on meteorological and dust forecasts of the Tel-Aviv University Dust Forecasting System (Alpert et al. 2002). Continuous air quality data of the coarse fraction of particulate matter (PM₁₀) were used to confirm actual sampling times. Airborne fungi were sampled on the roof of a three-story building at the campus of the Technion—The Israeli Institute of Technology, Haifa, Israel, during seven dust storm events and adjacent clear days (before or after the dust events, depending on the meteorological conditions), during the winter and spring seasons of 2004–2005. During one of the dust storms (April 2004), a series of five samplings was conducted in order to obtain information about the dynamics of airborne fungi during the dust event. Additionally, for the characterization of the atmospheric fungal background in the summer and winter seasons, air samples were collected on clear days twice in August 2004 and seven times during January and February 2005. Airborne fungi were sampled with the Six Stage Andersen Viable Impactor (Andersen 1958). The concentration of fungi was expressed as a number of colony-forming units (CFU) per cubic meter. Details of sampling, incubation, and storage of fungal isolates are described in a previous publication (Schlesinger et al. 2006).

2.2 Taxonomic identification of fungal isolates

Cryogenically stored fungal clones were inoculated on Potato Dextrose Agar (PDA) plates and incubated at 30 °C for 2–7 days. Taxonomic identification was based mainly on the morphological characteristics of fungal isolates: microscopic details of sporulation and cultural features of colonies. One repeatedly isolated type of non-sporulated colonies was identified employing the polymerase chain reaction (PCR) technique. For DNA extraction, frozen fungal isolates were grown on PDA during 7 days at 30 °C. The mycelia were transferred by means of a sterilized microbiological needle to a 1.5-ml Eppendorf tube containing 200 µl of sterile distilled water and then suspended repetitively with a sterile pipette tip. The suspension was placed at 95 °C for 10 min and then transferred into ice for 3 min in order to enhance the lysis of fungal cells. The

obtained lysate was centrifuged (13,000 rpm, 3 min, 4 °C), and the supernatant was stored at −20 °C until PCR analysis. The extracted DNA was used as a template for the amplification of a segment of the 18S rRNA gene. Universal 18S rRNA fungal primers EF3 and EF4 were utilized for amplification (Smit et al. 1999). The primers were synthesized by SIGMA-Israel (Rehovot, Israel) (EF3—5′-TCC TCT AAA TGA CCA AGT TTG-3′; EF4—5′-GGA AGG G[G/A]T GTA TTT ATT AG-3′). The reaction mix included 2 µl of fungal DNA template, 1× PCR buffer, 0.2 mM dNTPS (deoxynucleotide triphosphates), 1× Q-Solution, 20 pmol of each primer, and 1.25 units Hot-Star Taq DNA Polymerase (Qiagen Inc., Hilden, Germany), in a total volume of 50 µl. The PCR amplification was carried out in the DNA Engine PTC-200 (MJ-Research) thermocycler as follows: one step of 15-min incubation at 95 °C, followed by 40 cycles of 1-min denaturation at 94 °C, 1-min annealing at 48 °C, and 1-min elongation at 72 °C, followed by an additional 10-min elongation step at 72 °C. Two microliters of 6× running buffer (30 % glycerol, 0.15 % Alizarin Red) was added to 10 µl of the PCR mix. The 1.5-kilobase pairs amplicons were visualized using electrophoresis in 2 % agarose gel with Tris–Acetate–EDTA buffer (Sambrook et al. 1989). The PCR products were sequenced by the division of molecular biology of Daniel-Biotech Inc. (Rehovot, Israel) using the EF3 primer. The DNA sequence, of the length of 882 base pairs, was used for sequence/isolate identification by GenBank Blast Search (<http://www.ncbi.nlm.nih.gov/BLAST/>).

2.3 Collection and analysis of dust particles

Along with the sampling of biological particles, fine (PM_{2.5}, aerodynamic diameter of 0–2.5 µm) and coarse (PM₁₀, 2.5–10 µm) particles were also collected using a dichotomous sampler (Loo and Cork 1988). The sampler filters were analyzed for elemental content using the X-ray fluorescence technique (Dzubay and Stevens 1975).

2.4 Analysis of fungal data

Statistical analysis was conducted using XLSTAT (<http://www.xlstat.com>). We employed the nonparametric Wilcoxon signed-rank test to compare data on the concentration of CFU and species diversity of fungal communities collected during dust events and

adjacent clear days. The relationship of these data with particulate matter concentrations and elemental composition of the atmospheric particles was estimated by the linear regression analysis. Canonical correspondence analysis (CCA) was performed with the XLSTAT software in order to reveal the association between the distribution of most common fungal species and the measured atmospheric variables. To evaluate the similarity between dust-associated fungal communities and fungal communities of the adjacent clear days, clustering of the communities based on the abundance of species was conducted by the unweighted pair-group average method with Euclidian distance as the distance coefficient.

3 Results

3.1 Species composition and species diversity

During the 2004–2005 sampling period, a total of 202 identified fungal species were collected. They belong to Mucoromycotina (3 species), teleomorphic (sexual) Ascomycota (27 species), anamorphic (asexual) Ascomycota (169 species), and Basidiomycota (3 species). The species belonged to 71 genera; the most prominent were *Penicillium* (42 species), *Aspergillus* (21), *Chaetomium* (11), and *Acremonium* (10). Based on their origin, 132 species can be considered as typical soil fungi (e.g., Domsch et al. 2007), while the other 70 species are known mainly or only as plant inhabiting fungi (Ellis 1971, 1976; Ellis and Ellis 1997; Sutton 1980).

The following species were most frequently isolated: in >70 % of the samples—*Alternaria alternata* (19.2 and 2.8 % of the total number of fungal isolates collected in dusty and clear days, respectively), *Phlebia* sp. (the basidiomycete species identified by PCR) (15.4 and 65.6 %), *Cladosporium cladosporioides* (4.3 and 2.7 %), *Aspergillus niger* (2.7 and 0.2 %), *Sporotrichum aurantiacum* (4 and 2.8 %), *Penicillium chrysogenum* (5.2 and 0.1 %), *P. glabrum* (1.3 and 1.9 %), and *A. versicolor* (0.1 and 0.8 %); in >40 % of samplings—*A. fumigatus* (2 and 0.04 %), *C. sphaerospermum* (0.4 and 0.5 %), *P. aurantiogriseum* (0.4 and 0.8 %), *P. griseoroseum* (1 and 0.7 %), *P. purpurogenum* (0.1 and 0.2 %), and *Pleospora tarda* (0.1 and 0.2 %). Most of these species, except *P. glabrum*, *P. griseoroseum*, *P. tarda*, *S. aurantiacum*,

and *Phlebia* sp., are known as opportunistic fungi of BSL-1 (bio-safety level 1) non-consistently causing different health problems in immuno-compromised persons (de Hoog et al. 2000).

During six dust events and the adjacent clear days, 98 species were collected—79 and 32 species in dusty and clear days, respectively, with 27 species sampled in either day types. Species diversity (richness) of the samplings varied within the range of 16–47 species for dust events and 6–16 species for clear days (Fig. 1). The dust-associated fungal communities were significantly richer than the communities of the background days (Wilcoxon signed-rank test, $p = 0.03$).

3.2 Concentration of fungi

During dust events, concentration of fungal isolates varied from 113 to 890 CFU/m³ (Fig. 2). During clear days, this variation was less pronounced—31 to 263 CFU/m³. In five out of seven dust samples, the highest amounts of fungal isolates were collected in the small-size stages of the Six Stage Andersen Viable Impactor (aerodynamic diameter ranges of 1.1–2.2 and 2.1–3.3 μ m). The dust-associated fungal communities significantly differed on the concentration of CFU from the communities of clear days (Wilcoxon test, $p = 0.017$). Concentration of airborne fungi peaked on the dust event of March 31, 2005, and it was accompanied by the highest number of species collected (47 species—see Fig. 1).

3.3 Similarity

Cluster analysis (Fig. 3) showed that the dust-associated fungal communities consist of four separate groups while the communities found in clear days

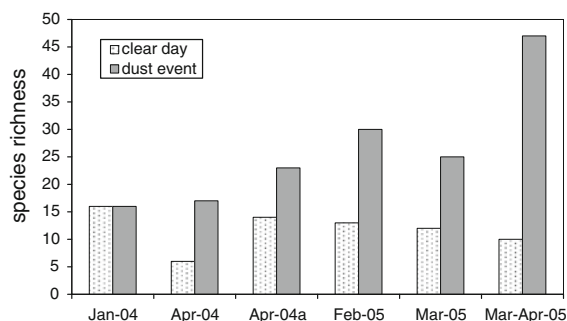


Fig. 1 Species richness of airborne fungi collected in the atmosphere of Haifa during dust events and adjacent clear days

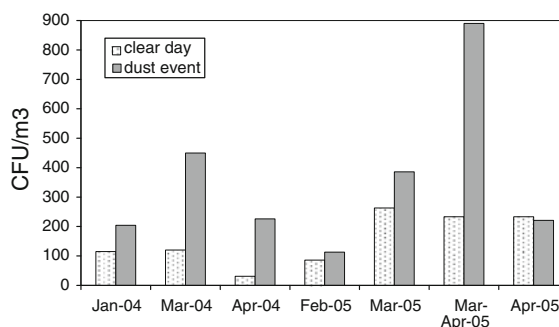


Fig. 2 Concentration of airborne fungi (CFU/m³) in the atmosphere of Haifa during dust events and adjacent clear days

were more homogenous and formed two distinct groups. The communities of dusty days and their respective background days did not cluster together although in these pairs of communities sampled at the end of January 2004, and at the beginning of April 2004, similarity was comparatively high. The fungal community of the dust event occurred at the end of March 2005 was most dissimilar to other communities. As was already mentioned, this community was characterized by the highest concentration of fungi and highest species diversity.

3.4 Analysis of atmospheric particles

The concentrations and elemental contents of the particles collected during the dust events and clear days are given in Table 1. In most cases, coarse particle concentrations were higher than the fine

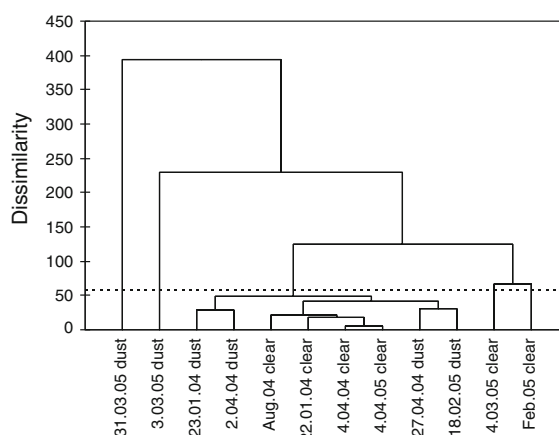


Fig. 3 Clustering the airborne fungal communities collected in the atmosphere of Haifa during dust events and adjacent clear days based on species relative abundance

particle concentrations (1.9- to 4.5-fold), and, in all cases, concentrations of atmospheric particles during the dust events were remarkably higher than in their adjacent clear days (5.3- to 7.8-fold for PM₁₀ and 2.1- to 14-fold for PM_{2.5}).

During dust events, a considerable increase in the concentration of chemical elements of geological (terrestrial) origin was also observed (Table 1). The concentration of Cl linked to sea water spray increased when the sampled air masses passed previously over the Mediterranean Sea, irrespective of the movement of desert dust plumes. Variation in the concentrations of elements from anthropogenic sources—vehicle emission and heavy fuel combustion—was not related to the transport of dust plumes as well. Nevertheless, there were cases in which the concentration of anthropogenic pollutants increased during dust events in the coarse particle fraction.

3.5 Effect of concentration of atmospheric particles and elements on airborne fungal communities

Although concentration of fungi and their respective species diversity significantly increased in the atmosphere during dust events as compared to the adjacent clear days, neither of these two parameters displayed a significant linear relationship with the concentration of coarse or fine atmospheric particles as well as with concentrations of different chemical elements composing the particles.

CCA revealed that the sampling/species data were not linearly related to the variables' data (the permutation test, $p = 0.067$) although the p value was just above the chosen threshold (0.05 against 0.067). The inertia (variation) test showed that all explanatory variables explained 93.8 % of the variation in the data. Within the CCA eigen analysis, most of the variation was carried by the first (horizontal) axis (48.38 %). With the second (vertical) axis, it resulted in 76.27 % of the variation. These results mean that the first two axes explain 71.5 % of the variation in the data, which is considered a high value for ecological datasets (Lira-Noriega et al. 2007).

According to the arrow length on the CCA map (Fig. 4), the dominant environmental factors were the concentration of fine atmospheric particles followed by the concentration of geological elements and coarse particles; it should be taken into account, however,

Table 1 Concentration of particulate matter and elements ($\mu\text{g}/\text{m}^3$) in atmospheric particles collected at Haifa, Israel, and sources of the sampled air masses (according to HYSPLIT—Hybrid Single-Particle Lagrangian Integrated Trajectory, http://www.arl.noaa.gov/HYSPLIT_data2ar1.php)

Date of sampling	Source of air masses	Particulate matter		Elements						Marine		Fuel burning	
				Geological		Al	Si	Ca	Fe	K	Cl	S	
		PM ₁₀	PM _{2.5}										
January 23, 2004, dust	Libya/Egypt	700	280	18	47.7	76.7	31.3	11	8.8	2			
January 22, 2004, clear	Egypt/Sinai	90	20	1.4	4.6	8.3	3.2	1.3	2.7	0.25			
April 2, 2004, dust	Mediterranean sea/Egypt	130	40	4.3	12.7	16.4	4.7	2.6	2.2	1.6			
April 4, 2004, clear	North of Europe	20	10	0.3	1	1.9	0.45	0.3	1.4	0.8			
August 10, 2004, clear	Eastern Mediterranean	20	10	0.14	0.45	0.8	0.23	0.24	1.2	2.2			
August 12, 2004, clear	Eastern Mediterranean	40	40	0.15	0.4	1.3	0.25	0.33	3.3	3.8			
March 3, 2005, dust	Libya/Egypt	80	40	4.3	12.5	7.9	4.6	2.4	3.2	1.2			
March 4, 2005, clear	Sardinia/Mediterranean sea	15	8	0.07	0.13	0.3	0.09	0.2	4.1	1			
March 31, 2005, dust	Egypt	80	25	1.7	5.4	7.8	2.6	1.2	4.6	1.9			
April 4, 2005, clear	Turkey/Mediterranean sea	11	12	0.08	0.15	0.4	0.13	0.14	2	1.7			

that these variables are strongly collinear. The position of each species in relation to the environmental arrows on the map provides the information about the species-variable relationships. Abundance of *Aspergillus versicolor*, *P. chrysogenum*, and especially *P. griseoroseum* increased with the increased concentration of atmospheric particles and their elements of geological origin. Three other species—*A. alternata*, *P. tarda*, and *C. cladosporioides*—form the group that is unrelated in its distribution to the above parameters and sensitive to the atmospheric pollutants. In contrast, the basidiomycete species—*Phlebia* sp. and *Sporotrichum aureum*—seem to be the least sensitive to the elements from anthropogenic sources (sulfur). *A. niger*, *A. fumigatus*, and *C. sphaerospermum* were more abundant in the air masses passed above the

Mediterranean Sea and enriched by Cl. The species found near the center of the axes—*P. aurantiogriseum*—can be considered as a generalist (ter Braak 1986).

4 Discussion

Our present study, as a follow-up of our previous investigation (Schlesinger et al. 2006), showed that dust storms substantially influenced both the quantitative and qualitative characteristics of fungal communities in the atmosphere of Haifa. During dust events, concentrations of airborne fungi significantly increased and were accompanied by the increase in numbers of collected species, as well as by variation in species composition. Such an enriching effect of dust storms has been well established for the regions periodically subjected to these events (e.g., Kellogg and Griffin 2006; Griffin 2007, and references therein). Cluster analysis revealed low similarity between most dust-associated fungal communities and their respective background communities (see Fig. 3). This is likely because the air masses carrying fungal propagules during dust events and clear days originate from different geographic regions (see Table 1). Neither concentration of fungi nor their species diversity is significantly linearly related to the concentration of coarse and fine atmospheric particles or concentrations of different chemical elements composing the particles. Based on these atmospheric variables, we cannot explain, for example, the highest number of species and their highest concentration during the dust event occurred at the end of March 2005; this phenomenon may be caused by other, probably meteorological, factors. Nevertheless, the CCA analysis gave a possibility to separate different groups among the most frequently collected fungal species associated in their distribution with concentration and composition of air particulate matter (see Fig. 4).

Nearly half of the species (94) sampled in the atmosphere of Haifa during the course of our study contained melanin. Moreover, conidia of some light-colored fungi from the *Penicillium* and *Aspergillus* genera frequently recorded in the air are known to contain greenish pigments of a melanin nature (e.g., Coelho et al. 1997; Cuadros et al. 1999; Youngchim et al. 2004). Dominance or frequent occurrence of dematiaceous airborne fungal species has been well

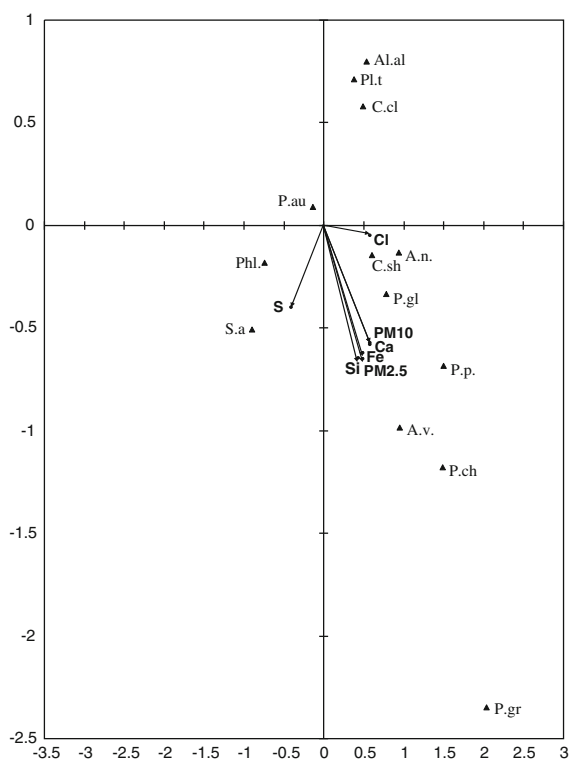


Fig. 4 CCA ordination biplot showing concentration of coarse (PM10) and fine (PM2.5) atmospheric particles, and geological elements (Ca, Cl, Fe, S, and S) (arrows) and fungal species distribution (triangles). Al.al. = *Alternaria alternata*, A.n. = *Aspergillus niger*, A.v. = *A. versicolor*, C.c. = *Cladosporium cladosporioides*, C.sh. = *C. sphaerospermum*, Phl. = *Phlebia* sp., P.au. = *Penicillium aurantiogriseum*, P.ch. = *P. chrysogenum*, P.gl. = *P. glabrum*, P.gr. = *P. griseoroseum*, P.p. = *P. purpurogenum*, Pl.t. = *Pleospora tarda*, S.a. = *Sporotrichum aureum*

documented all over the world (e.g., Kellogg and Griffin 2006) including Israel (Waisel et al. 1997). Protective pigmentation of cell walls with melanin and melanin-like pigments makes airborne fungal propagules less susceptible to UV radiation damage (e.g., Ulevicius et al. 2004).

Both typical soil-borne fungi and phyllosphere fungi (in an approximate proportion of 2:1) combined the collected airborne mycobiota. Leaf surface fungi, mainly from the genera *Alternaria*, *Cladosporium*, *Ulocladium*, and *Phoma*, were found to be considerable contributors to atmospheric fungi (Hameed and Awad 2005; Levetin and Dorsey 2006). All species of phyllosphere origin found in the air of Haifa are melanin-containing, and a great majority of them produce comparatively large, thick-walled and multi-cellular spores. Such morphological adaptations protect these fungi, not only from the harmful influence of UV radiation but also from desiccation, which is crucial for their long distance dispersal.

Notably, a polyporous lignicolous species, *Phlebia* sp., is one of the most abundantly isolated fungi during the dust events and overwhelmingly prevailed in the clear days' samplings of winter-spring 2005; while in 2004, *Phlebia* sp. was detected only once and in very low amounts. Such sharp increases in abundance of this species could be associated with massive maturation and release of its basidiospores in the regions where air masses originated or passed through. On the whole, remarkable concentrations of basidiospores belonging to the class Agaricomycetes have been found in the atmosphere all over the world (e.g., Hasnain et al. 2005; Morales et al. 2006; De Antoni Zoppas et al. 2006; Levetin and Dorsey 2006). A high amount of this kind of basidiospores has been detected in air particulate matter also by the culture-independent DNA-based technique (Frohlich-Nowoisky et al. 2009). In this study, fungi from Basidiomycota (mainly from Agaricomycetes) were found even in higher proportion than ascomycetous fungi, and the authors supposed that basidiomycetous fungi might enrich the atmosphere due to their abundant presence on vegetation and soil surface or because of more effective mechanisms of spore dispersing.

In conclusion, our present study reveals a distinct pattern of distribution of fungi in the atmosphere of Haifa, strongly affected by dust storms originating mainly from the Saharan desert. The study should be continued in order to search for other parameters,

probably meteorological, which can significantly influence the content and concentration of atmospheric fungi in our region. International monitoring studies conducted in parallel in the countries where the dust clouds pass through would substantially enrich and enhance our knowledge in this important field of aeromycology.

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