Original Article

Enumeration and identification of dust fungal elements from the weather inversion phenomenon in Isfahan, Iran

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Abstract

Background: Fungi are the major pathogens or allergens for which the air is the natural medium of their dispersal. Since the air pollution is associated with a wide range of adverse health outcomes, then identification of the type and population of fungi in these conditions will help the management of hygienic and control of fungal disease.

Materials and Methods: A total of 103 dust samples were collected from glass surfaces of different places by sedimentation method. Pollution standard indexes were provided by Environmental Protection Agency in Isfahan. All dust samples were mixed and homogenized in distilled water containing antibacterial agents. Serial cultures were done in 5 times experiments on two standard culture media. Isolated fungal colonies were identified by their standard morphologic and physiologic criteria. The analysis was performed by Mann-Whitney test calculating by SPSS version 20 (SPSS Inc., Chicago, IL, USA).

Results: The real mean of total culture-able fungi in 1 g of sedimentation dust were account about 44800 colonies of different fungi. More than half of the viable fungi (62.8%) could grow out of 1 g of dust on Mycosel agar were the genera of *Aspergillus*, *Penicillium* and *Cladosporium* with 28.8%, 23.4% and 10.6% respectively. The dominant genus could grow on Sabouraud dextrose agar with chloramphenicol medium were the genera of *Aspergillus, Cladosporium* and *Penicillium* with 23.7%, 21.1% and 14.5% respectively.

Conclusions: Our data show the amount and variety of viable colony-forming fungi, which we are faced with in Isfahan during the air pollution condition. The real abundance of fungal particles and non-cultivable fungi in dust are still poorly understood and remain for further study in the future.

Key Words: Air pollution, fungi, identification, Iran, particulate matter

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INTRODUCTION

Today, air pollution is one of the major health problems in big cities. In addition to the air pollution derived from the dust storms of deserts, a number of big cities in Iran, like Tehran and Isfahan are confronted with the weather inversion phenomenon in cold seasons. Dust-borne microorganisms, such as fungi are the major pathogens or allergens for humans, animals and plants; for which the air is the natural medium of their dispersal.[1,2]

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The number of fungi typically found in 1 g of top soil is approximately 10^6. However the concentration of spores and their diversity in soil or outdoor airborne are not completely known and is depended on the amount of humidity, temperature and the composition of nutritional elements and bioenvironmental factors.[3]

Once the pollutants are released into the atmosphere they are moved by wind, rain or snow, pushing the particles back down to the earth, which they contaminate the air and the surface water.

There are the air quality indexes and pollution standard indexes (PSI) which are concerned in human health by environment protection agency.[4,5]

Dust-borne microorganisms, in particular, can directly affect the human health through pathogenesis, or through the exposure of sensitive individuals to cellular components.[6]

The harmful effects of air pollution on the cardiovascular system and also respiratory and allergic diseases are well-documented.[7]

The air particulate matter (PM) with the biological origin includes viable cells such as pollen of plants, bacteria, fungal spores and dead microorganisms as well as the other non-viable materials such as plant, animal and fungal fragments, allergenic compounds, mycotoxins and endotoxins.[8-10]

The climate change such as inversion phenomenon can have a strong impact on the concentration and composition of airborne spores, which in turn may influence the effects of fungi on plants, animals and human health, the biosphere, and climate and result in negative effects. The study by Womiloju *et al*., reported that the material of fungi contributed 4-11% of the mass of fine (PM2.5, aerodynamic diameter ≤2.5 μm) and Bauer *et al*., found that fungal spores accounted for up to 21% of PM10 (≤10 μm) mass.[11,12]

On December 2010 in Isfahan, the air quality descriptor was very unhealthy during some days and the government recommended people with respiratory heart disease, the elderly and children should avoid outdoor activity as well as everyone should avoid prolonged exertion.

This study was designed to discern the types of fungi we are faced with in air pollution resulted from the inversion phenomenon in Isfahan area. For this end, the traditional cultivation/microscopy and physiological techniques were applied to identify viable fungi in PM.

MATERIALS AND METHODS

A total of 103 dust samples were randomly collected from the glass surfaces of different places by sedimentation method on December 2010.

From 103 dust samples, a 7.25 g dust was totally gathered and then a 1 g of mixed dust was solved in 5 ml of distilled water.

Before doing colony count, a pilot study was done to determine how much dust should be cultured to find the separate colonies on culture media. So for preparing appropriate suspension, 25 ml of distilled water containing 2 g/L penicillin, 1 g/L streptomycin and 0.2 g of mixed dust were used.

Suspension was mixed well and 50 λ of it was spread on Sabouraud dextrose agar containing 50 mg/L chloramphenicol (Sc) plates by L shape spreaders. As, some slow growing molds could masked by fast growing fungi, so a series of dust samples were cultured on Sabouraud dextrose agar with chloramphenicol and cycloheximide (Scc). Doing serial cultures were repeated in 5 times experiment on both culture media.

The plates were incubated at 25°C for 2 weeks and the accounts of growing colonies were recorded every 3 days on two series culture media (Sc and Scc) in all 5 times experiments.

Colonies were identified by preparing tease mount or slide culture technique and other standard methods.[13]

An analysis of data was carried out by Mann-Whitney test calculating by SPSS 20. Data reported as mean ± standard deviation or median interquartile range.

The pollution weather row data were provided by Environmental Protection Agency in four stations of Isfahan during 21 days. The analysis of the data to PM2.5, PM10 and PSI were done by environmental health center of Isfahan.

RESULTS

The results showed from a total of 103 dust samples, 7.25 g of dust were gathered. The real mean of total culture-able fungi in 1 g of sedimentation dust were about 44800 colonies of hyaline, pheohyphomycete molds and also yeasts in 5 times experiment.

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Table 1: Mean of fungal colonies by culture media (Sc and Scc)

Name of fungus	Culture media	Mean	Standard deviation	*P* value
Aspergillus sp. (total)	Sc	10621	2948.953	0.614
	Scc	9370	4446.029	
A. flavus	Sc	4644	1695.275	0.841
	Scc	4200	4468.020	
A. niger	Sc	2552	2552.443	0.426
	Scc	2477	2477.249	
Penicillium sp.	Sc	6502	1365.097	0.430
	Scc	7633	2721.606	
Cladosporium sp.	Sc	9490	2529.935	0.002
	Scc	3271	1733.742	
Rhizopus sp.	Sc	2262	1630.299	0.079
	Scc	606	856.601	
Alternaria sp.	Sc	1656	873.160	0.009
	Scc	283	180.674	
Yeast	Sc	2261	1573.112	0.032
	Scc	404	319.390	
Chatumium sp.	Sc	40	90.337	1.000
	Scc	40	90.337	
Acremonium sp.	Sc	0.00	0.000	0.207
	Scc	162	263.376	
Scopulariopsis sp.	Sc	40	90.337	0.347
	Scc	0.00	0.000	
Epicoccum sp.	Sc	81	110.640	0.545
	Scc	40	90.337	
Drechslera sp.	Sc	0.00	0.000	0.347
	Scc	40	90.337	
Stemphylum sp.	Sc	0.00	0.000	0.347
	Scc	81	180.674	
Unknown	Sc	283	338.011	0.025
	Scc	1050	522.323	
Mycelium sterile	Sc	848.00	1896.186	0.394
	Scc	1896.186	180.674	
Phoma sp.	Sc	81	180.674	0.667
	Scc	40	90.337	

Sc: Sabouraud dextrose agar with chloramphenicol, Scc: Sabouraud dextrose agar with chloramphenicol and cycloheximide. *P* values calculated by Mann-Whitney-test. *A. flavus: Aspergillus flavus, A. niger: Aspergillus niger*

Table 2: The PSIs and the average amount of PM10 and PM2.5 in 4 stations, during 21 days in Isfashan (22 November to 12 December 2010)

Date	Station 1		Station 2		Station 3		Station 4	
	Ave24h PM2.5 μg/m³	PSI	Ave24h PM10 μg/m³	PSI	Ave24h PM2.5 μg/m³	PSI	Ave24h PM10 μg/m³	PSI
2010/11/22	86.1	163	170.6	109	79.7	159	189.9	119
2010/11/23	93.4	167	190.1	119	71.9	155	199.7	124
2010/11/24	94.1	168	180.4	114	78.5	158	190.6	119
2010/11/25	90.6	165	187.8	118	86.2	163	201.1	124
2010/11/26	88.1	164	189.9	119	82.1	161	191.4	120
2010/11/27	92.5	167	187.1	117	70.5	154	199.6	124
2010/11/28	98.3	170	210.9	129	100.5	171	209.7	129
2010/11/29	124.1	185	261.5	154	109.7	177	239.1	143
2010/11/30	207.3	257	391.5	254	190.9	241	311.4	179
2010/12/1	171.7	222	362.8	213	166.3	216	359.8	209
2010/12/2	163.9	214	350.3	198	147.3	198	362.0	212
2010/12/3	147.6	198	340.1	193	122.4	184	329.2	188
2010/12/4	120.1	183	289.9	168	110.7	177	302.6	174
2010/12/5	109.3	176	240.5	144	96.8	169	201.3	124
2010/12/6	111.7	178	240.1	144	94.7	168	229.4	138
2010/12/7	116.9	181	250.0	148	100.4	171	240.1	144
2010/12/8	120.1	183	249.9	148	112.7	178	270.0	158
2010/12/9	228.6	278	259.9	153	167.7	218	271.7	159
2010/12/10	229.4	279	277.0	162	229.4	279	270.4	158
2010/12/11	226.0	276	275.4	161	226.0	276	281.9	164
2010/12/12	189.3	239	200.0	124	189.3	239	198.8	123

The indexes value of PSI; 201-300 describe the quality of the air is very unhealthy according to the National Environment Agency. PSI: Pollution standard index

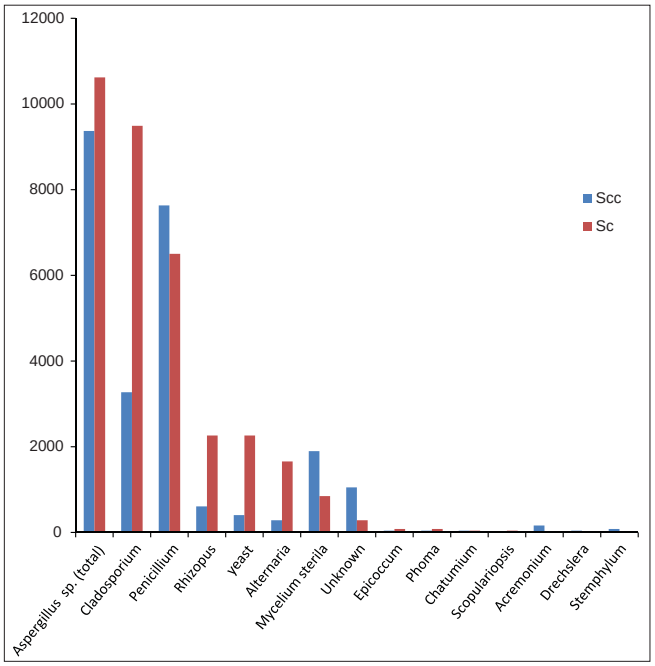

Figure 1: Comparing mean of colonies growing on Sc and Scc culture media done by colony count

The results showed more than half of viable fungi (62.8%) could grow in 1 g of dust on Scc medium were the genera of *Aspergillus, Penicillium* and *Cladosporium* with 28.8%, 23.4% and 10.6 respectively. The dominant genus could grow on Sc medium were the genera of *Aspergillus, Cladosporium* and *Penicillium* with 23.7%, 21.1% and 14.5% respectively. Among the *Aspergillus* species, *Aspergillus flavus* were dominant on Sc (43.7%) and Scc (44.8%) culture media [Table 1 and Figure 1].

As it has shown in Table 1, the mean of colonies number of *Cladosporium*, *Alternaria,* yeasts and unknown species are different on Sc and Scc culture media and their *P* value indexes significantly indicate the differences of the number of colonies on both culture media [Table 1].

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The mean of PSI, the amount of 24 h PM2.5 and PM10 in μg/m^3 during 21 days (from 22 of November to 12 of December in 2010) is outlined in Table 2.

DISCUSSION

In air pollution, PMs can stay in the air for minutes, hours and weeks and can travel many hundred miles.[14] Urban areas have higher PM_{10} concentrations than rural areas; the coarse size fraction ($PM_{10-2.5}$) has been identified as the cause of these differences.[9]

Spores of fungi enhance survival during transport and prolonged environmental stress such as ultra violet exposure stress and desiccation.[15,16]

As several allergens and pathogens are frequently found in both fine and coarse particle samples (e.g. *Cladosporium* sp., *Alternaria* sp., *Penicillium* sp., *Aspergillus* sp.) so, the exposure to fungal spores can cause a wide spectrum of allergenic reactions such as asthma, hypersensitivity of pneumonitis and so on.[2]

In susceptible or immune-compromised individuals some severe diseases such as allergic and invasive aspergillosis, fungal sinusitis and invasive fungal infections may be also found.[17-19]

Fungal spores are typically 2-10 μm in size. Species the genera of *Aspergillus*, *Cladosporium*, *Alternaria* and *Penicillium* are more often involved in allergenic fungal disease.[6]

It has shown in Figure 1 that, the dominant genus could grow on Scc medium was *Aspergillus* with 28.8%. The results show that more than half of viable fungi on Scc (62.8%) are present in dust from the inversion phenomenon are the genera of *Aspergillus*, *Cladosporium* and *Penicillium* [Figure 1].

Penicillium and *Aspergillus* spp. are both well-known soil fungi and are commonly considered indoor fungi in aerobiology, although they are also prevalent in outdoor air environment.[20]

The smaller spore types of fungi like *Aspergillus* and *Penicillium* may reach the alveoli, whereas the larger spore types, may deposit to a greater extent in the lower and upper airways rather than in the alveoli.[21] *A. flav*us spores are larger than the spores of *A. fumigatus* and tend to infect paranasal sinuses.[19,22] The results showed, among the *Aspergillus* species, *A. flavus* were dominant on Sc (43.7%) and Scc (44.8%) culture media [Table 1].

It is believed in conventional culture-based method, however, only 1% ~17% of environmental microorganisms is cultivable on any given medium but non-cultivable cells, dead ones, or cell debris are not detected by cultivation at all. Fungal fragments like cell walls or cytoplasmatic material are easily suspended and inhaled as fine air particulate matter.[6,23-25]

Our results showed there are 44,800 viable particles of fungi in 1 g of dust due to inversion weather phenomenon under our laboratory condition. You can suppose the amount of travelling organisms such as fungi in the dust storm events which are not also rare in Iran, when many tones of dust are transferred from deserts of neighbor desert countries. These arid regions could be an important source for the long-range transport of viable microorganisms to our country.

In a study in Qatar, *Alternaria* and *Cladosporium*, were the most common genera in air (40.1% and 21%, of the total) whereas they accounted for only 4.06% and 2.8% of the total soil fungi in that country.[26]

Second predominance genus mold at the present investigation was *Cladosporium* (21.1%) on Sc medium which is in agreement the results of Al-Subai. This mold can also interact with airborne pollen and increasing allergic problems.[27]

A. flavus has been reported to be the etiologic agent of rhinosinusitis, in healthy and immunocompromised individuals in Iran.[19] In the present study, the results showed a predominance growth of *A. flavus* and *Aspergillus niger* colonies on both culture media. Similarly, the study of fungus allergens inside and outside the residences of atopic and control children, showed that, *A. flavus* and *A. niger* were predominant species in *Aspergillus* composition.[20]

Fungi are found in almost every environment.[24] During weather pollution, dust was settled not only, everywhere in outdoors area such as streets, farms, soils, waters, vegetables, plants and fruits surfaces but also on the floors, tables and mirrors, dishes of food and everywhere in indoor environments.

Although the concentration of cultivable fungi is low in our samples, allergic reactions can be participated by dead fungal material as well. *In vitro* studies have shown that submicron particles of several fungal species are aerosolized in much higher concentrations (300-500 times) than spores.[6]

CONCLUSIONS

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This study shows the significant concentrations of viable colony-forming fungi which we are faced with or inhale at polluted days from inversion phenomenon in big cities. Air pollution conditions, which are not rare in Isfahan and Tehran, cause many health problems particularly for children and elderly population. Every breath in polluted air causes to inhale many spores. The actual abundance of particles and components are, however, still poorly understood and quantified. Especially, the information about the dead and non-cultivable fungi of dust is extremely inadequate due to the lack of some sampling equipment in our laboratory condition. To gain the accurate and adequate information further studies are necessary to identify all species of fungal elements in dust. It is therefore important to investigate and evaluate the type and population of microorganisms for the management of hygienic and control of fungal disease in the future.

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