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

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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]
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 $\leq 2.5$ $\mu$m) and Bauer et al., found that fungal spores accounted for up to 21% of PM10 ($\leq 10$ $\mu$m) 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.

An analysis of data was carried out by Mann-Whitney test calculating by SPSS 20. Data reported as mean $\pm$ 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.
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].

### 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     | 4466.020           |         |
| *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           | 407      | 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             |         |
| *Stemphyllum* 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*.
The mean of PSI, the amount of 24 h PM2.5 and PM10 in μg/m³ 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 PM10 concentrations than rural areas; the coarse size fraction (PM10-2.5) 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. flavus 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 were 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**
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.

ACKNOWLEDGMENTS

The authors would like to acknowledge Isfahan University of Medical Sciences for its financial support to carry out the current research. The authors declare that there are no conflicts of interest.

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Source of Support: Nil, Conflict of Interest: None declared.