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Airborne Mycoflora of Zanjan-Iran

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ABSTRACT

Air pollution is one of the most serious problems to human health. Fungi are among the most important natural pollutants, which can be pathogenic under special circumstance. Otomycosis, Chronic Bronchitis, Emphysema, Asthma, Allergy and Systemic mycosis are among the fungal diseases. In view of the high prevalence of allergy in Zanjan, Iran, this study was conducted to determine the airborne fungal flora in the Zanjan city. In general, the incidence of fungi was higher in the winter (79.07%) and lower (4.4%) in summer. Moreover, the incidence was high at noon in all seasons. Overall, results showed that *Cladosporium* was the most filamentous fungus (45.8%) in autumn and yeast fungi were common in winter. Large number of colonies was obtained from overcrowded places. Colony count and temperature had negative but moisture showed positive correlation. The importance of different factors affecting frequency and seasonal variations of these fungi are discussed.

Key Works: Airborne fungi; Aerobiology; Asthma; Allergy; Systemic mycosis

INTRODUCTION

Air pollution an important environmental issue, which seriously affects the human health, and airborne fungi are most significant natural pollutants and pathogenic under specific circumstance (Abdel Hafez, 1984; Shelton *et al.*, 2002). Elevated levels of particle air pollution are associated with a decreased lung function, increased respiratory symptoms as coughing, shortness of breath, wheezing and asthma attacks as well as chronic obstructive pulmonary disease, cardiovascular disease, systemic mycosis in predisposed persons and lung cancer (Halonen *et al.*, 1997; Hargreaves *et al.*, 2003).

Researcher believed that, more than 80 genera of fungi are associated with symptoms of respiratory tract allergies (Horner *et al.*, 1995). Over 100 species of fungi were involved with serious human and animal infections, whereas many other species caused serious plant diseases (Cvetnic *et al.*, 1997). *Alternaria*, *Cladosporium*, *Aspergillus*, *Penicillium* and *Fusarium* were among the most common allergenic genera, for instance, elevated concentration of Cladosporium were usually associated with respiratory symptom (Su *et al.*, 1992). Higher concentrations of *Cladosporium* and *Penicillium* indoor may cause allergy (Li *et al.*, 1995). Reponen *et al.* (1996) reported that the deposition of fungal spores in lungs and their effects on human healthy not only depended on their composition and concentration but also size. First finding by Charlz blacky showed that fungi spores have related asthma and allergic disease that was caused by *Chaetomium* and *Penicillium* (Rippon, 1988) Larger spores (> 10 µm) were deposited in the upper airway like nose, pharynx and might result in high fever symptoms, while smaller spores (< 5 µm) could penetrate the lower airways and might lead to asthma and allergens (Horner *et al.*, 1995).

In view of their adverse effects on the human health, many studies were carried out on the airborne fungi. Hariri *et al*. (1978) reported that the most prevalent airborne fungi in Ahvaz-Iran were *Penicillium*, *Alternaria* and *Aspergillus*. Abdel-Hafez *et al.* (1987) showed that the maximum airborne fungi were in winter and the minimum in the summer. Many authors indicated that the dominant fungi were *Cladosporium*, *Alternaria*, *Penicillium* and *Aspergillus* in the atmosphere and their concentration differed from place to place, because of local environmental variables, fungal substrates and human activities.

Recent studies by Fung *et al.* (2005) showed that dominant fungi in Beijing was *Cladosporium* and the highest fungal concentration was noted in summer, because of the suitable conditions for fungal growth such as air temperature and moisture. Since fungal air spores can play a significant role in several allergic manifestations, the identification of geographic areas of mold distribution could be helpful to the clinician, especially if associated with fungal air spore recording in homes or working environments of sensitized subjects, in determining the real clinical importance of sensitization to fungi. As a result, regarding the high prevalence of allergy and lung disease in Iran as well as in Zanjan and little information about the species and number of airborne fungi we designed this survey to determine the airborne fungi flora in Zanjan.

MATERIALS AND METHODS

Present study was carried out during one year from 2003 to 2004 on outdoor Zanjan. Zanjan is located in North-west of Iran and it has a territorially mountainous climate. It has a cold season from October to December and warm

seasons from May to September. Seven sites were selected for this study including six from crowded areas that have traffic jam in rush hour and near the city center with a little green area, one site for comparison without any human activity and vehicle. It was 10 km far from city center at each sampling site. Samples from these sites were taken in the middle of the each month of each season, except holiday and weekend from June 2003 to May 2004. The samples were collected using the air sampler (MSA 100, Merck, Germany), which was positioned 1.5 m above the ground and oriented vertically towards the sky. Samples were collected for three times; 7:00 to 9:00 a.m. in the morning, 12:00 to 13:00 at noon and 17:00 to 20:00 in the evening the culture plate exposure method. For collection we use 8 cm petridish containing Sabouraud Dextrose Agar with chloramphenicol (SC) to inhibit bacterial growth on 1.5 m above ground for 15 min. The petridishes were taken to the laboratory and incubated for three days at 27ºC. Results were expressed as number of colony per petridish. On incubation fungal colonies growing on each dish were counted and identified to the genus level based on colony morphology, hyphae, conidia and sporangia. Slide cultures were used for all samples and cultured on enrichment media for producing spores such as potato dextrose agar (PDA) for better identification.

RESULTS

Considering all samples sites, 5782 colonies were separated from 252 plates during one year. There were 1500 fungal, 4253 yeast and 29 sterile mycelia colonies. The most prevalent airborne fungi species were: yeast 70.13%, *Cladosporium* 11.9%, *Penicillium* 6.2%, *Aspergillus* 2.4%, *Alternaria* 1.9% (Fig. 1). Six sampling sites including Amirkabir, Khayam, Engelab, Honarestan, Aslamabad & Esteghlal where are crowded and full of vehicle and human activity. There was no significant different in the number of colonies. At Ghavazang without any vehicle and human activity, the fungi were well spread. Significantly higher (34.5%) fungal isolations were found in Honarestan area and lower (2.5%) fungal isolations were in Ghavazang area (Fig. 2). There was great difference in fungal isolations among seasons. A highest frequency of fungi (79.07%) was obtained in winter and a lowest (4.4%) in summer.

Metrological data showed that moisture in the air was higher during winter (65.7%), precipitation in spring, while mean temperature and wind variability in summer. Highest frequency of fungi was recorded at noon in summer, at evening in spring and at noon in winter. These isolations were mainly from Honarestan area.

Finding in this research and information from meteorological office showed that fungal distribution had a close relationship ($P < 0.0005$) with wind, while temperature, rain and moisture variable had no relationship ($P > 0.05$). In general, the fungal isolation in Zanjan was different compared to the studies conducted in other cities.

Fig. 1. Frequency of the fungal isolation in all sites of the Zanjan

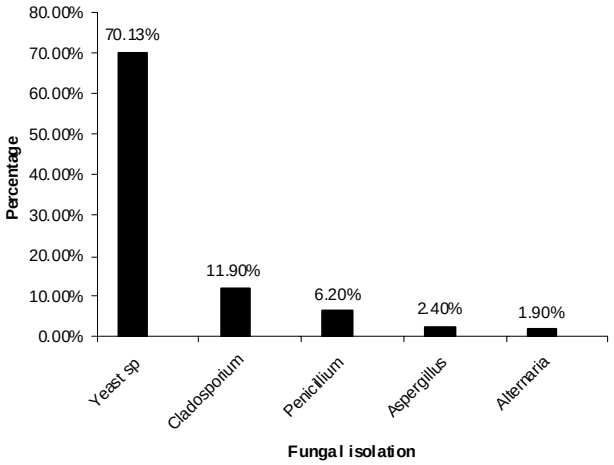
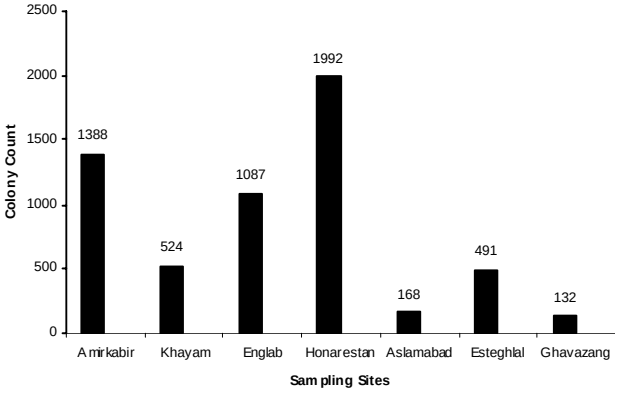


Fig. 2. Frequency and fungal distribution in each site in Zanjan



DISCUSSION

The fungal concentration in the atmosphere varied greatly in different sampling sites during one year in Zanjan. Adhikari *et al.* (2004) attributed such variations to the micro-environmental conditions, sampling time of a day and year and climatic conditions. In other studies, there were large difference in total fungal concentrations and the great variations were also found in the cities of other countries (Giorgio *et al.*, 1996; Takahashi, 1997). For examples, Rosas *et al.* (1997) recorded a geometric mean of concentration of 143 CFU m^{-3} in Mexico City. Shelton *et al.* (2002) reported the medium concentration (500 CFU m^{-3}) in the United State. Takahashi (1997) showed that a concentration range of 13 - 2750 CFU/m³ from the city of Yokohama, Japan. These great differences could be due to geographic location, fungal growth substrates in different countries as well as different sampling methods used (Adhikari *et al.*, 2004). Moreover, the human activities might also increase the fungal frequency in the atmosphere.

Fang *et al.* (2005) reported a high frequency of airborne fungi in regions with high vegetation coverage in

summer in Beijing and also mentioned that, most of the airborne fungal spores came from vegetation rather than soil (Fang *et al.,* 2005). Picco and Rodolfi (2000) reported that, in the vigorous growth of plants in summer, the phylloplanes can allow for the growth of several saprophytic and parasitic fungi. On the other hand, air temperature and moisture in the environment in summer could be adapted for germination, growth and propagation of airborne fungi. So it might be attributed to a lack of vegetation and many other environment factors inhibiting the growth of fungi in summer, such as serious pollution, high wind velocity and low moisture near the ground (Picoo & Rodolfi 2000). As mentioned above, Zanjan is surrounded by mountains and has little vegetation, and greater vegetation has a close relationship with fungal distribution (Fang *et al.,* 2005).

Huang *et al.* (2002) reported that airborne fungi were higher in winter than other seasons in municipal landfill sites in southern Taiwan and ascribed it to the geographic characteristics of the sampling area. It is very similar to our study, which means that the highest fungal airborne was in winter seasons rather than others (Fig. 1 & 2). The prevalent fungal groups from all the sampling sites were yeast species, *Cladosporium*, *Penicillum*, *Aspergillus* and *Alternaria*; former two being the most dominant. Similar results were found by Wu *et al.* (1982) and Fu *et al.* (2000) in Beijing. However, studies in China and south of United State reported the presence of few *Cladosporium* in the atmosphere (Zhang *et al.,* 1987; Zhai *et al.,* 2000). It might be caused by the sampling methods, sampling environments and culture medium. Furthermore, different culture medium could permit the isolation of some species of airborne fungi (Morring *et al.,* 1983). Studies showed that some of the airborne fungi were observed seasonality, which corresponded to their seasonal occurrence; *Cladosporium* was predominant during winter, *Alternaria* during summer and *Penicillium* species during autumn (Singh *et al.,* 1990). In the present study yeast species and *Cladosporium* were predominant in all seasons (Fig. 1 & 2).

In crux *Cladosporium* was the most abundant in autumn and yeast in winter. Large number of colonies was obtained from overcrowded areas. Colony count had a reverse correlation with temperature but a positive one with moisture. Fungal spores are a serious problem for human health in Zanjan. This information can be useful for studies on clinically important fungal diseases in Iran.

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