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AIRBORNE FUNGAL FLORA OF KARACHI, PAKISTAN

TOQEER AHMED RAO, ABDUL HAKEEM SHAIKH AND MOINUDDIN AHMED

Department of Botany, Federal Urdu University of Arts, Science and Technology, Gulshan-e-Iqbal Campus, Karachi-75300, Pakistan.

Abstract

Investigations in the airborne mycoflora of Karachi was carried out using Spore Trapper technique along with culture media Petri plate technique. In order to collect different types of airborne fungal spores, five sites were selected for installing the spore trappers. In the course of collected spores, different types of fungal spores were identified. The study revealed that the fungal spores were the major components of the air micro flora of the environment of Karachi. These microorganisms were present throughout the year with different mean values in different seasons. Normally fungi were recorded more in summer than in winter season. During the study, different types of spores viz., *Alternaria solani, Aspergillus candidus, A. flavus, A. fumigatus, A. niger, A. terreus, A. wentii, Curvularia clavata, Drechslera dematioidea* and *Penicillium notatum* were found from five selected sites. These fungi are the major spora of the atmospheric air of Karachi in all seasons.

Introduction

Karachi is one of the biggest cities of Pakistan. The city is inhabited by over 15 million people and there has been a breakdown of conservancy services which has made it one of the dirtiest cities of the world. The soil, water and air of Karachi have become highly polluted and contaminated resulting in unhygienic condition. This appalling situation has increased the incidence of water and air borne diseases in the city (Afzal *et al*., 2005). Fungi and bacteria contaminate water and air, causing many human, animal and plant diseases (Gregory, 1973). Fungal spores present in atmosphere may cause a number of allergic reactions (Sanches *et al*., 1999). The contaminated air and water are believed to be responsible for gradual increase in the incidence of dermal and respiratory diseases in the city of Karachi (Mughal, 2002). The occurrence of species of *Aspergillus*, *Alternaria*, *Cladosporium*, *Penicillium* and *Saccharomyces cerevisae* from the atmosphere of Karachi has been reported by Afzal *et al*., (2004). It was noted that temperature and relative humidity play an important role in increasing fungal population in the environment of Karachi (Afzal *et al*., 2004).

The city of Karachi is a typically ideal city for the study of microbial pollution due to its thickly populated areas, poor sanitary conditions, highest traffic in the country, large industrial areas, agricultural fields and dry humid climate. The city is located between latitudes 24° 42' N to 25° 42' N and longitudes 66° 30' E, which is a sub-tropical location in character. The average temperature in coastal area is 30.1°C and inland it is 31.5°C (Gadiwala, 2006). With every change in weather, thousands of people of varying ages visit hospitals and doctors indicate different type of respiratory problems and diseases. Intensity of fungal conidia, fungal spores, pollen grains and other microorganisms are extremely high in atmosphere of Karachi, responsible for the Bronco-respiratory and skin disorders. Some of the isolated fungi from Karachi have been reported to cause allergic diseases in countries where atmospheric fungi present indoors have been studied in detail in relation to such diseases (Nasir & Jalaluddin, 2005). There is an increase in dermal and respiratory diseases among the residents of Karachi City. There have been several studies of the relationships between environmental factors, particularly air pollution and

*Corresponding author e-mail: toqeer81@yahoo.com

attacks of asthma. Most of these studies have ignored the potential confounding effects of aeroallergens such as fungal spores. The interaction of fungal spores with lung structures may be important for the development of respiratory allergies induced by air borne fungal allergens (Geiser *et al*., 2000). Apart from their direct allergenic effect, fungi may carry mycotoxins in their spores or produce volatile metabolites (Miller, 1998). Inhalation of mycotoxins such as aflatoxins, secalonic acid, zearalenone and tricothecenes produced by *Aspergillus, Penicillium* and *Fusarium* spp, may affect the immunological response of the lung tissues or cause other hazards to human health (Gerberick, 1984). Fungi grow on structural materials where humidity is high or on food and stored products where there is condensation. Humidifiers, air-conditioning systems, carpeting and damp walls are the potent sources of indoor fungal allergens. Outdoor fungi such as species of *Alternaria, Aspergillus, Botrytis, Cladosporium, Curvularia, Epicoccum, Fusarium, Penicillium, Phoma, Trichoderma* etc., have been reported regularly from damp indoors. *Aspergillus, Eurotium and Penicillium* have been observed to colonize food scraps and other organic materials. No attempts have been made to record seasonal distribution of fungal spores in Karachi atmosphere to explore potential of these microorganisms. Therefore this paper is a part of a research in which allergic microorganisms would be identified.

Materials and Methods

This work was started in March 2007. Five different locations were selected in Karachi City to install "Spore Trapper" in order to trap the fungal spores from the atmospheric air. Map of study area with GPS locations are shown in Figure 1. Five locations selected in Karachi are Malir Extension Colony, Korangi Area, Metroville S.I.T.E Area, Gulshan-e-Maymar and Lines Area (Fig. 1). The local version of Spore Trapper was designed and constructed indigenously to save foreign exchange (Fig. 2).

At each location a Spore Trapper was installed for sampling spores. From above mentioned sites, each week samples were collected and new slides for next week were placed in Spore Trapper. Six plates of culture media (3 Czapek- Dox Agar and 3 Potato Dextrose Agar) were also exposed for 5-10 minutes at each visit to sampling site, the time of Petri plates exposure was mid of the day. Samples on slides and media containing Petri plates were brought to the laboratory for identification purpose, and microscopic examination. The Petri plates were incubated in incubator for 24 to 48 hours at 37^{o}C.

Seven sterilized slides for seven days immersed with Vaseline grease were filled in the chamber of the spore trapper. A system was established in spore trapper to suck the atmospheric air of the area at 5 liters per minute airflow in the chamber of the spore trapper. Each slide was rotated after 24 hours automatically. After one week, these slides were removed and new slides were inserted in the Spore Trapper and same procedure was followed regularly at all above-mentioned sites. All the removed slides were stained with lectophenol cotton blue. The slides were individually examined under an electric microscope (model-ys-100) with magnifying range of 40-X and 100-X; eyepiece power is 15-X. The fungal spores were seen on the slides through microscope. The numbers of different types of fungal conidia were recorded in the data sheet for one year. The Cluster Analysis (Orloci & Kenkel.1985) was performed to see grouping of the fungal species from different sites.

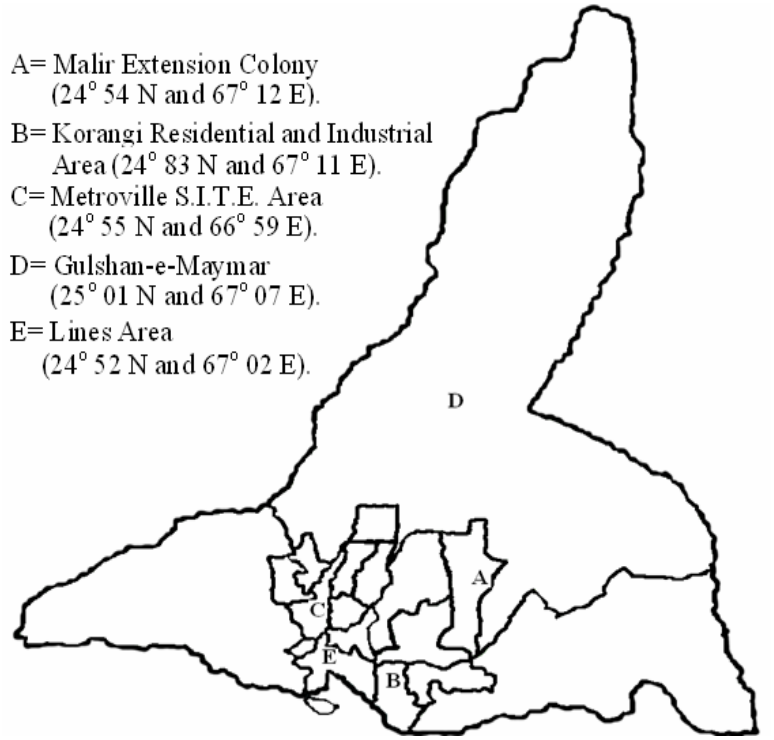

Fig. 1. Map of Karachi, locations of study area are shown by A, B, C, D and E.

Fig. 2. Local Version Spore Trapper Designed by Prof. Dr. Abdul Hakeem Shaikh, Prof. Dr. Moinuddin Ahmed and Prof. Dr. Qaisar Abbas
Department of Botany, Federal Urdu University of Arts, Science and Technology, Gulshan-e-Iqbal, Karachi-75300, Pakistan.

Results and Discussion

The present research work was started on March, 2007 upto the end in February, 2008. This study is based on the collection of fungal spores from the atmospheric air by spore trapping technique along with culture media in Petri plates. This work was carried out at five different locations of Karachi City. The fungal populations were very high in the atmospheric air of Karachi. During this research study 10 fungal species were recorded viz., *Alternaria solani*, *Aspergillus candidus*, *A. flavu*s, *A. fumigatus*, *A. nige*r, *A. terreus*, *A. wentii*, *Curvularia clavata*, *Drechslera dematioidea* and *Penicillium notatum*. Seasonal distributions of air mycoflora in different locations are described below.

Malir extension colony: This location is open but thickly populated area close to the Malir agriculture fields. The population of this location is middle class with low income. The environmental conditions are poor. Open garbage dumps, poor sanitary conditions, open man-holes and scattered residential waste made conditions unhygienic and polluted in the environment of this area. This situation is favorable for diseases and their causal agents. The fungi are present in the atmospheric air of the location and this pollution is helpful for the growth of fungi. A total of 5 different fungal species viz., *Aspergillus flavus*, *A. niger*, *A. terreus*, *Alternaria solani* and *Curvularia clavata* were recorded from this location. The distribution of all five fungal species of the locality was similar during December to April (Fig. 3A). However, after the month of April, *Aspergillus niger* and *Aspergillus flavus* gained highest mean values due to the favorable climatic conditions. The 1st dominant fungal species was *Aspergillus niger* while the 2nd dominant species of the locality was *Aspergillus flavus.* These two species obtained highest mean values in the months of September (22.17) and August (14.21) respectively, but the other three fungal species were distributed in similar way as in December to April. The change in climatic and environmental conditions did not affect the numbers of colonies of these three fungal species in this location.

Korangi residential and industrial area: Korangi is closed to coastal area, with various types of industries such as tanneries, oil, food, garments, textiles and pharmaceuticals etc. In addition, the location has residential population area. The population is comprised of poor and lower middle class. Due to huge population and different type of industries especially tanneries, this area remained polluted throughout the year. A tanneries waste water treatment plant is also operated in this area but the pollution is still increasing. A total of 6 fungal species were recorded in this area including *Aspergillus candidus, A. flavus, A. fumigatus, A. niger, Alternaria solani and Penicillium notatum.* The unhygienic conditions of the area may have promoted the growth of some hazardous fungal species especially *Aspergillus niger* and *Aspergillus flavus*. Distributions of the fungal species were same in December to April. The distribution of *Aspergillus niger, Aspergillus flavus* and *Alternaria solani* gradually increased after April (Fig. 3B). Due to the highest mean value (26.92) of *Aspergillus niger* during the month of August this species was considered the first dominant species of the locality, while the second highest mean value 15.04 was recorded for *Aspergillus flavus* in the month of October. The third dominant species of the area was *Alternaria solani* which obtained 6.93 mean values in the month of July. The distribution of other three fungal species was almost constant throughout the year.

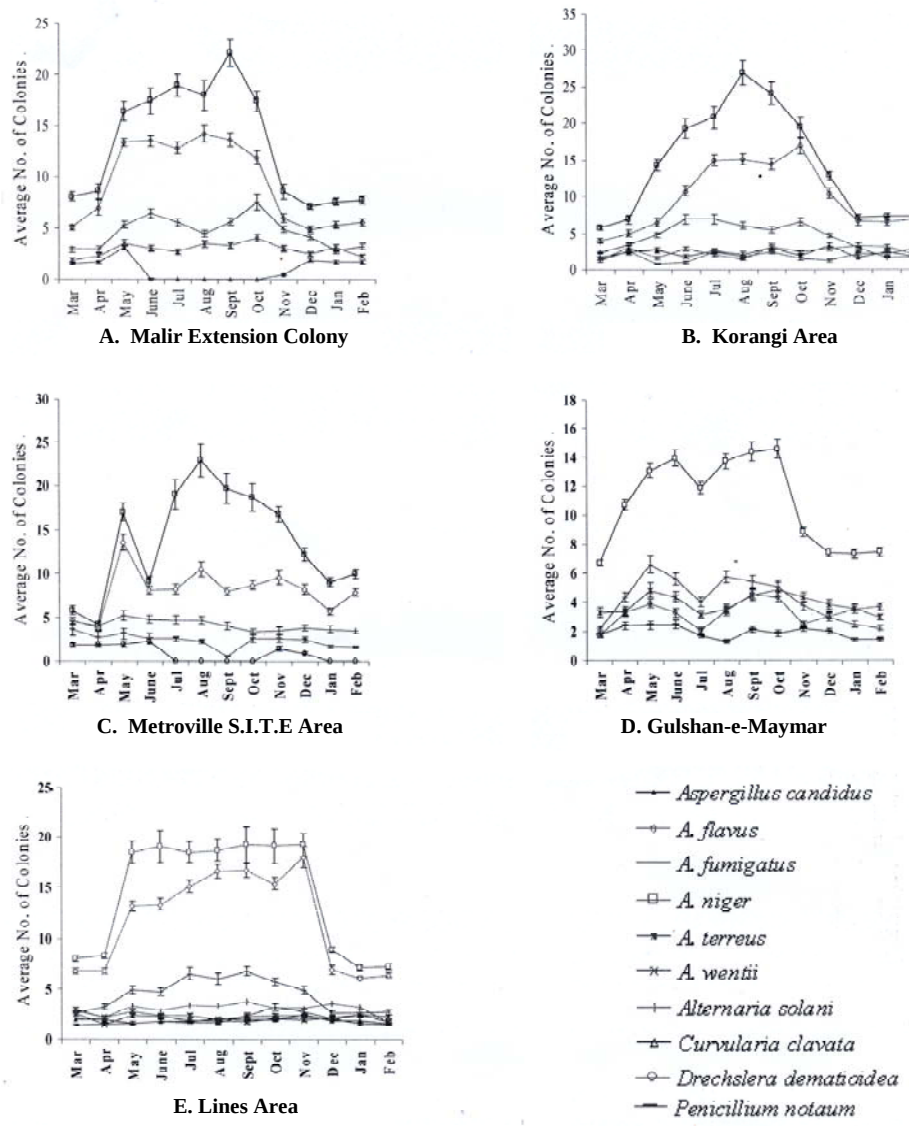

Fig. 3. Distribution of fungal colonies in various months of the year from five different locations of Karachi.

Metroville S.I.T.E. area: Metroville residential area is close to the Sindh Industrial Estate. This Estate also has different types of industries, such as pharmaceutical, textile, dyeing, chemical, flour and rubber industries. This area is open but thickly populated and the population of the locality comprises of middle class and lower middle class. Due to the presence of huge population and industries, the pollution level is also very high in this locality. The waste of residential area and industries made the open land into a garbage land. These wastages and polluted matters are favorable ground for the growth of airborne microorganisms including fungal spores.

A total of 5 different fungal species was found from this location. These were *Aspergillus flavus*, *Aspergillus niger*, *Alternaria solani*, *Drechslera dematioidea* and *Penicillium notatum*. The distribution of *A. solani*, *D. dematioidea* and *P. notatum* remained constant throughout the year, while *A. niger* and *A. flavus* showed different distributions in different months (Fig. 3C). The *A. niger* gained highest distribution in the month of August while *A. flavus* obtained highest distribution during the month of May. These two species were the first and second dominant species of the locality due to their highest mean values of 22.92 and 13.5 respectively. The climatic factors (humidity and temperature) were responsible for the distributions rate of above mentioned two dominant fungal species, but did not affect the other three fungal species of the location.

Gulshan-e-Maymar: This suburb of the city is located near the Super highway. This is an open residential area with low population, less traffic emissions and no industries. The population of the locality is middle class and upper middle class. The sanitary and garbage dumping conditions were better in contrast to the other four locations of the city. However the location is dusty due to loose sandy soil, high wind speed and comparatively less number of high rise buildings. Some time the velocity of the dusty wind is very high and the clusters of dust cover all possessions.

Five different fungal species viz., *Aspergillus niger*, *Aspergillus wentii, Alternaria solani*, *Drechslera dematioidea* and *Penicillium notatum* were recorded from this locality. The distribution of the species was different in different months of the year (Fig. 3D). *A. niger* was the most dominant species of the locality due to its high mean values throughout the year, it obtained highest mean value of 14.58 during the month of October. The fluctuation in distribution throughout the year showed the seasonal variation. The seasonal variation may play an important role in the distribution of the fungal species.

Lines area: This sampling site is congested, thickly populated and high traffic area due to the center of the city. Large population, high traffic emissions residential waste, inadequate sewerage system and garbage dumps made the locality highly polluted. In addition, the perilous gases and metallic particles of the exhaust from the automobiles also affect the natural environmental conditions of the location. The population of the location comprises of lower middle class and low income creates dangerous problems for hygienic environment. These grimy situations produced collectively favorable environment for the growth of airborne microorganisms especially fungal species. During this study a total of 8 fungal species *Aspergillus candidus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *A. wentii*, *Alternaria solani* and *Curvularia clavata* were found from this location. The presence of highest fungal species in contrast to other four localities of the city showed that this location was highly polluted. Airborne *Alternaria solani* can be a potential allergic sensitizer in susceptible individuals and can be a risk factor in sensitized individuals with symptoms of bronchial asthma and allergic rhinitis (Hasnain *et al.*, 1998). Some airborne *Aspergillu*s conidia were responsible for the prevalence of bronco- pulmonary aspergilloses (Haque, 2004).

Karachi may divide into three seasons namely winter, summer (including pre- and post-monsoon) and monsoon. Pre-monsoon hot season is from April to mid July and post-monsoon hot period from mid-September to October. Mean minimum temperature in coastal area is higher than that of inland and mean maximum temperature in coastal area is lesser than that of inland area (Gadiwala, 2006). The humidity was high throughout the year, while July to September were most humid months. The reason for the variation lies in the interplay of arid and marine influences. These climatic variations may affect the growth and distribution of some above-mentioned fungal species.

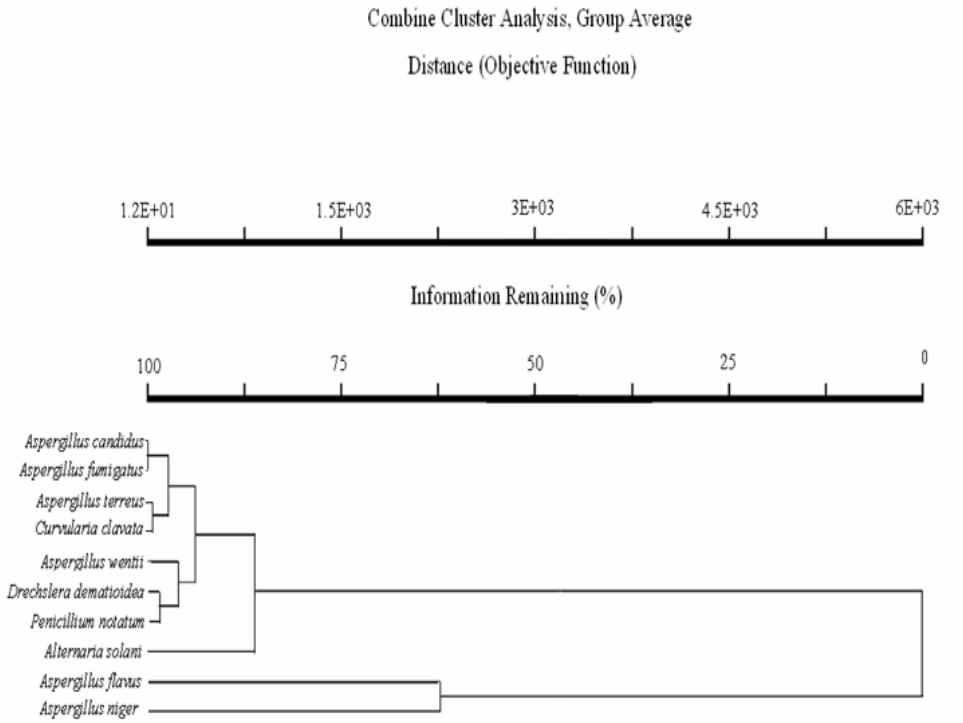

Fig. 4. Dendrogram of Cluster Analysis (Group Average) of five different locations of Karachi.

Combine cluster analysis (Fig. 4) showed significant grouping between fungal species. The results indicate highly significant differences in the distribution of fungal species in 5 different locations. The high mean values of *Aspergillus niger* and *Aspergillus flavus* clearly show that these two fungal species were dominant fungal species of the aeromycoflora of the atmospheric air of Karachi. The distribution of the fungal species showed same pattern during the months of December to April, but after the month of April fluctuation occurs in distribution of the fungal species especially in *A. niger* and *A. flavus* (Fig. 3E). *A. niger* showed high distribution and gained highest mean value which was 19.29, while *A. flavus* attained mean value 18.04. The other fungal species do not possess significant result in this area. Distribution of different fungi in different months of the year and different sites are shown in Fig. 1A to Fig. 3E. Despite the distances, traffic, pollution, population status, people's way of life, hygienic condition, industrial or domestic waste, *A. niger* and *A. flavus* are the widely distributed fungi in the study area, except Gulshan-e-Maymar, where marked absence of *A. flavus* was observed (Fig. 3D) in contrast to other areas. *A. solani* was the other fungus commonly found in all 5 study areas. Since no logical explanation can be presented in the same species restricted to two sampling sites. For example, *A. terreus* and *Curvularia clavata* were recorded from Malir Extension and Lines area, while *Aspergillus candidus* and *A. fumigatus* were found in Korangi and lines area. Similarly *D. dematioidea* was distributed in Metroville S.I.T.E and Gulshan-e-Maymar study area. *P. notatum* was observed in Korangi, Metroville S.I.T.E and Gulshan-e-Maymar sampling sites.

A. wentii was restricted to Gulshan-e-Maymar and Lines Area only. It is evident; that there was a considerable amount of overlaps in fungal spores found in different sampling sites, which suggested that specific fungi may be distributed in the atmosphere regardless of location. *A. niger* and *A. flavus* seem to be sensitive to the changing weather, *i.e.* temperature and humidity (Fig. 3A-E), while spores of other species seem to have a relatively constant distribution throughout the year. However at this stage, it is hard to identify the process responsible for the variability of the microorganism. According to Jones & Harrison (2004), a wide variety of biotic and abiotic processes may interact to enhance microbial distribution in atmosphere. Therefore more extensive sampling and monitoring is required to determine the factors responsible for airborne microbial distribution in an area.

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