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SEASONAL AND DIURNAL VARIATION OF ATMOSPHERIC FUNGAL SPORE CONCENTRATIONS IN HYDERABAD; TANDOJAM-SINDH AND THE EFFECTS OF CLIMATIC CONDITIONS

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Abstract

Airborne biological particles are present in every type of environment. Different types of geographical localities have different type of airspora, which affect human health. The current study is conducted for the first time to identify the airborne fungal spores from Hyderabad: Tando-Jam, Sindh. For this purpose, Burkard's 7-Days recording volumetric spore trap was used for a period of one year. A total of 68,183 spores/m^3 were recorded throughout the study period, belonging to 41 fungal spores types. The presented data revealed that Deuteromycetes spore type was predominant. *Cladosporium* sp. spores were detected in the highest concentration i.e., 50.83%, which was followed by *Aspergillus* sp. (18.63%) and *Alternaria* sp. (11.04%). The highest spore count was captured in the month of September-2008 (17,294 spores/m^3), while lowest spore count was found in the month of June-2009. Diurnal patterns of individual fungal spore types was observed to be mid-day to evening maxima for various species. Spearman rank correlation coefficient "r" was determined for correlation of fungal spore counts with climatic factors by using IBM software SPSS ver. 20. Results of the current study revealed that fungal spore concentration was increased in high humid weather while low count was found in hot and windy climate that was also confirmed by statistical analysis. The presented work demonstrated that various types of allergenic and phytopathogenic fungal spores were present in the atmosphere of Hyderabad: Tando-Jam. It was also observed that meteorological conditions had a significant impact on dispersal and concentration of fungal spores.

Key words: Airborne spores, Spore count, Sindh, Allergy, Asthma, Fungal spores, Aeromycology, Aerobiology.

Introduction

Aerobiology is an important filed of palynology. It is a well-known fact that the pollen and spores are present in the air that affect human beings (Nair, 1964). These biological particles cause bronchial asthma and allergies in human. Air current also carries pathogens, which can harm human, plants, and animals (Waqar *et al*., 2010).

Fungal spores varies in shape, size, and color. These are released by a variety of ways (Ingold, 1971). This variation in dispersal of fungal spores is necessary for them to flee into the turbulent wind (Gregory, 1973). The quantity, and types of fungal spores changes with respect to area, day timings, month of the year, and geographical location. For this reason, the sampling period must be carefully decided.

Various airborne fungal spores, Actinomycetes, and bacteria are able to cause several diseases in human beings and animals by infecting the living tissues, by toxicoses, or by allergies and asthma. Respiratory allergy in human can show symptoms immediately as in the case of hay fever or allergic rhinitis, or its delayed symptoms may appear as in Farmer's Lung. Airborne spores are found in different materials either in stored products that include straw, hay, food grains, wood chips, organic compost, and many others (Lacey & Jonathan, 2006).

In addition to airway irritations, few aerial microorganisms (excluding microbes causing colds, pneumonia, and influenza) may cause respirational contagions (Samson *et al*., 2001). *Aspergillus fumigatus*, a saprophytic fungus, can harm human respiratory tract by causing *Bronchopulmonary aspergillosis*. It sometimes produces colony showing infection in lungs. Serious problems may appear in people having hypersensitive immune systems (Campbell *et al*., 1996).

Introduction to the study area

Tando-Jam is a town of Hyderabad city, Sindh, Pakistan. It is situated at 25°25'60N 68°31'60E. Hyderabad is the second largest city of the Sindh province. According to demographic reports, it has a population of over 2 million people. 60.07% of the total population is urban which makes it the second most urbanized city of Sindh after Karachi. Hyderabad is situated on the bank of the river Indus.

Material and Methods

Collection of airborne biological particles

Fungal spores were captured by using Seven Day Burkard volumetric spore trap. The trap was installed on the roof department of plant protection, Agriculture University, Tando-Jam. The trap was placed 10 meters above the ground level. It sucked sample of 10 liters of air per minute, through a narrow horizontal orifice which was present on the front side of trap. Behind the slit, rotating drum was present on which a sticky tape was wrapped, so that the particles present in the air adhere to the tape. The drum was rotating with a speed of 2mm per hour. Every week the exposed drum was replaced by new drum. Then the tape, having impacts of aerobiological data, was removed from the drum and divided into seven sections of 48 mm length each section represented 24 hour/day.

On each day, hourly data wasrecorded for 24 hours during the study period of one year. Quantification of spore count was done according to the guidelines of British Aerobiology Federation (BAF, 1995). Fungal spores were identified by using reference slides as well as literature survey (Smith, 1990).

Meteorological Data

Meteorological data from August-2008 to July-2009 were received from the meteorological department, Hyderabad: Tando-Jam, Sindh, which included daily temperature, humidity, and wind speed. Spearman rank correlation coefficient "r" was determined for the correlation between spore counts and the environmental parameters by using IBM software SPSS ver. 20.

Results

Quantification and identification of airborne fungal data from Tando-Jam was carried out by using Burkard's seven day recording spore trap. The data was collected for the year of August-2008 to July-2009. During the study period a total of 68,183 spores/m3 were captured belonging to 41 fungal genera (Table 1). Spores were mostly identified at generic level. Out of 41 types about 17 belonged to Ascomycetes (A), 13 belonged to Deuteromycetes / imperfect fungi (I), 10 belonged to Basidiomycets (B), and 01 belonged to Oomycetes (O). Current study revealed that the highest concentration of fungal spores was contributed by fungi species belonging to Deutromycete. *Cladosporium* sp. (33598 conidia/m^3), *Aspergillus* sp. (12312 spores/m^3), Alternaria sp. (7294 conidia/m^3), *Periconia* sp. (5208 spores/m^3), *Bipolaris* sp. (2065 conidia/m^3) were predominant that constituted almost 91.5% of total airspora.

During the study period of one year, the maximum count of fungal spores was recorded in the month of September-2008 with a fungal spore count of 17,294 spores/m^3. Major contributors in the month of September-2008 were *Cladosporium* sp. (10022 spores/m^3), *Aspergillus* (920 spores/m^3), *Alternaria* sp. (1627 spores/m^3), *Periconia* sp. (937 spores/m^3), *Bipolaris* sp. (765 spores/m^3), *Coprinus* sp. (535 spores/m^3), Myxomycetes spp. (336 spores/m^3), *Agaricus* sp. (354 spores/m^3), *Curvularia* sp. (279 spores/m^3), and *Ganoderma* sp. (283 spores/m^3). The second highest concentration of fungal spores was detected in the month of December-2008 i.e. 9850spores/m^3. This count was contributed by *Cladosporium* sp. (6768 spores/m^3), *Aspergillus* (1039 spores/m^3), *Alternaria* sp. (172 spores/m^3), *Periconia*sp. (973s pores/m^3), *Bipolaris* sp. (239 spores/m^3), and *Coprinus* sp. (186 spores/m^3), *Sporomiella* sp. (155 spores/m^3). Lowest fungal spores count was observed in the month of June-2009 (Fig. 1).

Captured fungal spores were divided into two groups i.e. major group (fungal spore concentration more than 3%) and minor group (fungal spore concentration less than 3%). The data revealed that in major group highest concentration of fungal spores was contributed by the 05 members of Deuteromycota or imperfect fungi viz., *Cladosporium* sp. (50.83%), *Aspergillus* (18.63%), *Alternaria* sp. (11.04%), *Periconia* sp. (7.88%), and *Bipolaris* sp. (3.12%). Minor group included all of the 17 members of Ascomycota, 09 members of Deuteromycota fungi, 08 members of Basidiomycota, and 01 member of Oomycota (Table 2).

Seasonal periodicity of fungal spores deals with the appearance pattern of different fungal spores in various months of the year. September-2008 was found to be the most important month as the highest concentration of spores (17,294 spores/m^3) was recorded during this month. *Cladosporium* sp. having a 10022 spores/m^3 was most the dominant fungal spore type of this month. Furthermore, data also revealed that the highest number of fungal spore types was also recorded in the month of September-2008. 33 types, out of 41 fungal spore types, were present in that month. Second highest peak (9850 spore/m^3) of fungal spores was detected in December-2008, contributed by 17 fungal spore types. This highest count was due to 6768 spore/m^3 of *Cladosporium* sp. Other important fungal spores were *Aspergillus* sp., *Periconia* sp., *Bipolaris* sp., *Alternaria* sp., and *Coprinus* sp. Higher concentration of *Alternaria* sp. and *Cladosporium* sp. is detected in autumn season due to decay of organic material. The month of September is also suggested to be an important month for the fungal spore catch. Major fungal spore types are prevalent throughout the year *viz*. *Alternaria*sp., *Aspergillus* sp., and *Cladosporium* sp., while *Bipolaris* sp. and *Periconia* sp. spores that appeared for 11 and 10 months. Second highest concentration of fungal spores from Tando-Jam was recorded in the month of December-2008.

Diurnal pattern of fungal spores

Mean yearly concentration of fungal spore types were calculated for 24 hours. Data revealed some fungal spores possessed very sharp peak concentration at specific time of a day while other fungal spore types had homogenous pattern of spores distribution throughout the 24-hours' time scale. *Agaricus* sp. showed peak count at 6 pm (48 spores/m^3; Fig. 2). *Alternaria* sp. did not show any sharp peak of fungal spores (Fig. 3). *Arthrinium* sp. showed an early morning peak count at 7 am (33 spores/m^3; Fig. 4). *Aspergillus* sp. showed a very sharp maximum concentration at 8 pm (1764 spores/m^3; Fig. 5). *Bipolaris* sp. data also showed higher concentration in afternoon at 4 pm (81 spores/m^3; Fig. 6). Lower concentration of *Cladosporium* sp. fungal spores were detected in from 01 am to 12 pm and then there was gradual increase in fungal spore count after 12 pm that reaches to maximum level at 11 pm (1398 spores/m^3; Fig. 7). *Coprinus* sp. fungal spores showed late evening maxima at 7 pm (79 spores/m^3; Fig. 8). In case of *Curvularia* sp., a higher count was observed during mid-day timings (Fig. 9). *Ganoderma* sp. showed an interesting diurnal pattern of fungal spore count. It showed three peaks of higher concentrations first at early morning timings at 7 am (93 spores/m^3), second at 5 pm (36 spores/m^3) and third at 7 pm (36 spores/m^3; Fig. 10).

Statistical analysis of spore count with meteorological conditions

Spearman rank correlation coefficient "r" was calculated to determine the correlation between important fungal spores count and climatic factors by using statistics software SPSS, ver. 20. Mean monthly fungal spores concentrations were correlated with mean monthly values of temperature, humidity, and wind velocity.

Our data revealed that *Alternaria* sp. spores count was significantly negatively correlated with humidity. *Arthrinium* sp. spore count were negatively correlated with wind speed (Table 3). Over all most of the fungal spore types were negatively correlated with the temperature and wind speed while positive correlation with humidity was observed.

Table 1: Mean monthly airborne fungal spores concentration (spores/m^3) from Hyderabad: Tando-Jam, Sindh (August 2008-July 2009).

Types	Months												Total
	Aug-08	Sep-08	Oct-08	Nov-08	Dec-08	Jan-09	Feb-09	March-09	April-09	May-09	June-09	July-09	
Agaricus sp. (B)	0	354	66	93	13	0	0	106	53	9	4	9	707
Agrocybe sp. (B)	0	4	0	0	9	0	0	0	0	0	0	0	13
Alternaria sp. (I)	31	1627	588	561	172	508	822	1388	1454	75	35	31	7294
Amphisphaeria sp. (A)	0	35	0	13	13	0	0	57	0	0	0	0	119
Arthrinium sp. (I)	0	168	0	18	18	4	9	40	4	0	0	0	261
Ascobolus sp. (A)	0	53	9	44	0	0	0	0	0	0	0	0	106
Aspergillus (I)	22	920	942	738	1039	3342	234	203	4819	22	13	18	12312
Bipolaris sp. (I)	0	765	305	407	239	57	44	115	102	13	13	4	2065
Cerebella sp. (A)	0	137	4	0	0	0	0	0	0	0	0	0	141
Chaetomium sp. (A)	0	4	0	0	22	0	0	0	0	4	0	0	31
Chlorophyllum molybdites (B)	0	53	0	0	0	0	0	0	0	0	0	0	53
Cladosporium sp. (I)	66	10022	5252	3289	6768	4032	1185	1950	964	27	13	31	33598
Comoclathris sp. (A)	0	0	0	4	0		0	0	0	0	0	0	4
Coprinus sp. (B)	9	535	146	119	186	40	31	66	137	0	0	0	1269
Curvularia sp. (I)	0	279	62	35	18	4	9	9	0	0	0	0	416
Ganoderma sp. (B)	0	283	57	18	13	9	4	9	0	0	0	0	393
Inocybe sp. (B)	0	0	0	9	0	0	0	0	0	0	0	0	9
Leptosphaeria sp. (A)	0	13	4	0	0	0	4	0	0	0	0	0	22
Lophiostoma sp. (A)	0	9	4	4	0	0	0	9	0	0	0	0	27
Massaria spp. (A)	0	4	0	0	0	0	0	0	0	0	0	0	4
Monodictys sp. (A)	0	35	0	0	0	0	0	0	0	0	0	0	35
Myxomycetes spp. (I)	0	336	159	195	88	4	44	13	0	9	0	0	849
Panaeolus sp. (B)	0	119	22	9	4	0	0	0	0	0	0	0	155
Periconia sp. (I)	0	937	1331	420	973	234	447	522	221	97	0	27	5208
Peronospora sp. (O)	0	71	0	0	0	0	0	0	0	0	0	0	71
Phaeosphaeria sp. (A)	0	4	0	0	0	0	0	0	0	0	0	0	4
Pithomyces sp. (I)	0	18	13	0	0	4	0	0	0	0	0	0	35
Pleospora sp. (A)	0	0	0	9	0		0	0	0	0	0	0	9
Pseudocercospora sp. (A)	0	4	0	9	0	0	0	0	0	0	0	0	13
Puccinia sp. (B)	0	0	4	0	0	0	4	22	44	0	0	0	75
Rust (B)	4	62	57	0	0	9	9	0	27	0	4	0	172
Saccobolus sp. (A)	0	44	31	18	0	0	0	9	0	0	0	0	102
Sordaria sp. (A)	0	0	27	0	0	0	0	0	0	0	0	0	27
Spegazzinia sp. (I)	22	0	0	0	0		0	0	0	0	0	0	22
Spondylocladiella botrytiodes (A)	0	9	0	0	0	0	0	0	0	0	0	0	9
Sporomiella sp. (A)	0	0	0	0	155	0	0	0	0	0	0	0	155
Stemphylium sp. (I)	0	9	4	9	40	40	31	0	88	4	4	9	239
Tetraploa sp. (I)	0	13	0	4	0	4	0	0	0	0	0	0	22
Tilletia sp. (B)	0	0	0	4	0		0	0	0	0	0	0	4
Torula sp. (I)	0	18	0	0	0	0	0	0	0	0	0	0	18
Xylariaceae (A)	0	4	0	0	0	0	0	0	0	0	0	0	4
Damaged/Unidentified	0	345	310	75	80	22	35	44	1184	13	0	0	2108
Total	155	17294	9399	6105	9850	8316	2913	4562	9098	274	88	128	68183

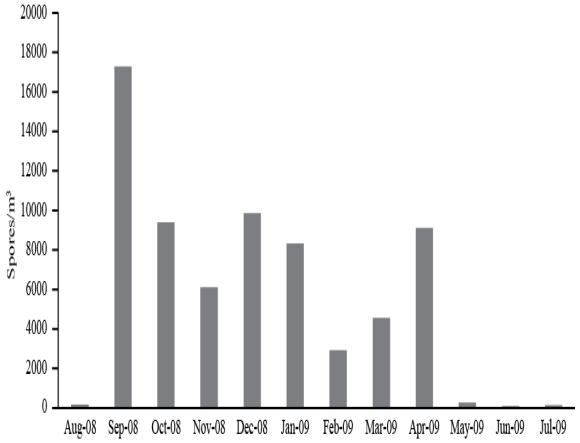

Fig. 1. Total monthly fungal spores count.

Table 2. Percentage of the captured fungal spore types from Hyderabad: Tando-Jam, Sindh (August 2008-July 2009).

Types	Spores/m^3	Percentage
Major group		
Cladosporium sp. (I)	33598	50.83
*Aspergillus*sp. (I)	12312	18.63
Alternaria sp. (I)	7294	11.04
Periconia sp. (I)	5208	7.88
Bipolaris sp. (I)	2065	3.12
Minor group		
Myxomycetes spp. (I)	849	1.28
Coprinus sp. (B)	1269	1.92
Agaricus sp. (B)	707	1.07
Arthrinium sp. (I)	261	<1%
Stemphylium sp. (I)	239	<1%
Rust (B)	172	<1%
Panaeolus sp. (B)	155	<1%
Sporomiella sp. (A)	155	<1%
Cerebella sp. (A)	141	<1%
Amphisphaeria sp. (A)	119	<1%
Ascobolus sp. (A)	106	<1%
Saccobolus sp. (A)	102	<1%
Puccinia sp. (B)	75	<1%
Peronospora sp. (O)	71	<1%
Chlorophyllum molybdites (B)	53	<1%
Monodictys sp. (A)	35	<1%
Pithomyces sp. (I)	35	<1%
Chaetomium sp. (A)	31	<1%
*Sordaria*sp. (A)	27	<1%
Lophiostoma sp. (A)	27	<1%
Spegazzinia sp. (I)	22	<1%
Leptosphaeria sp. (A)	22	<1%
Tetraploa sp. (I)	22	<1%
Torula sp. (I)	18	<1%
Agrocybe sp. (B)	13	<1%
Pseudocercospora sp. (A)	13	<1%
Inocybe sp. (B)	9	<1%
Pleospora sp. (A)	9	<1%
Spondylocladiellabotrytiodes (A)	9	<1%
Comoclathris sp. (A)	4	<1%
Massaria spp. (A)	4	<1%
Phaeosphaeria sp. (A)	4	<1%
Tilletia sp. (B)	4	<1%
Xylariaceae (A)	4	<1%

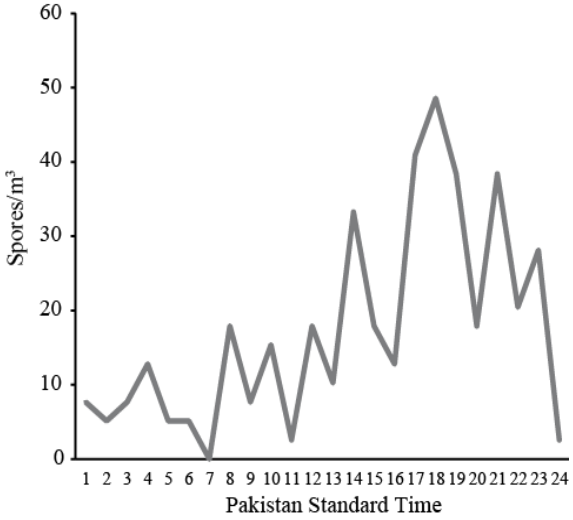

Fig. 2. Diurnal pattern of *Agaricus* sp. spores/m^3.

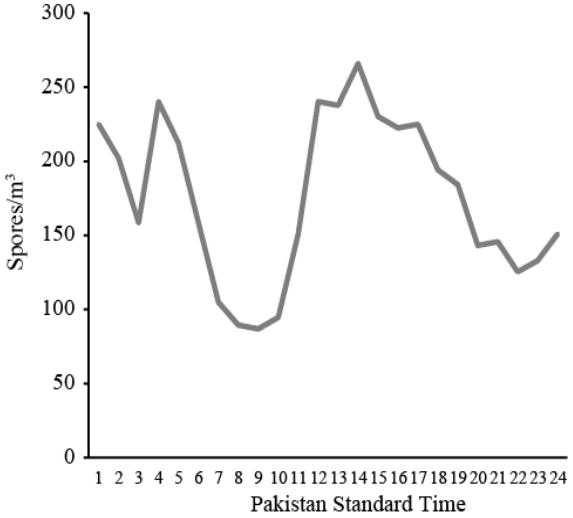

Fig. 3. Diurnal pattern of *Alternaria* sp. spores/m^3.

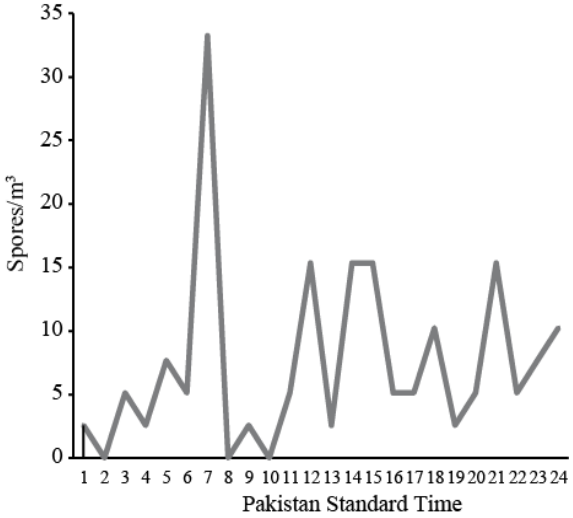

Fig. 4. Diurnal pattern of *Arthrinium* sp. spores/m^3.

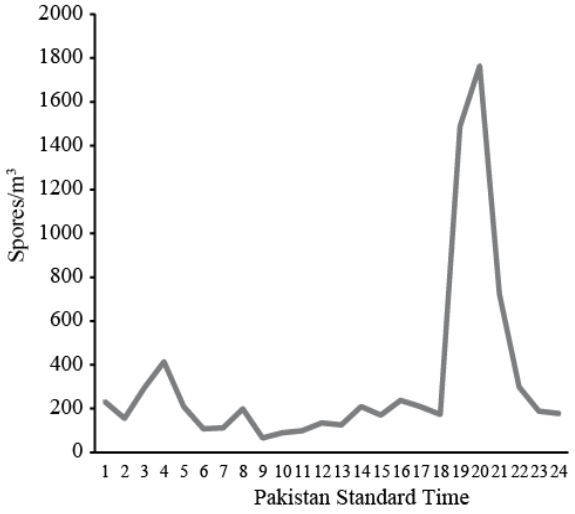

Fig. 5. Diurnal pattern of *Aspergillus* sp. spores/m^3.

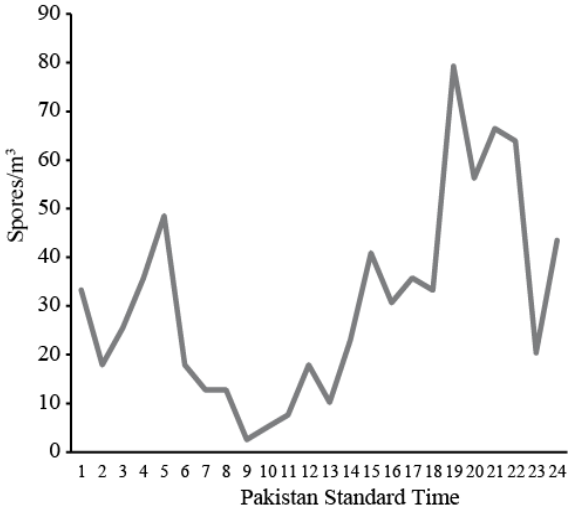

Fig. 8. Diurnal pattern of *Coprinus* sp. spores/m^3.

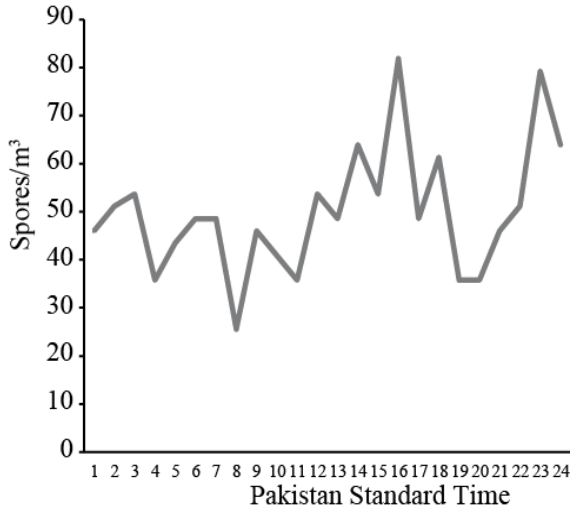

Fig. 6. Diurnal pattern of *Bipolaris* sp. spores/m^3.

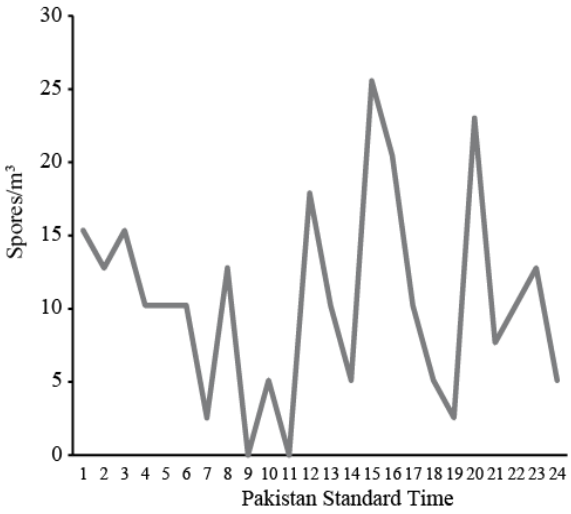

Fig. 9. Diurnal pattern of *Curvularia* sp. spores/m^3.

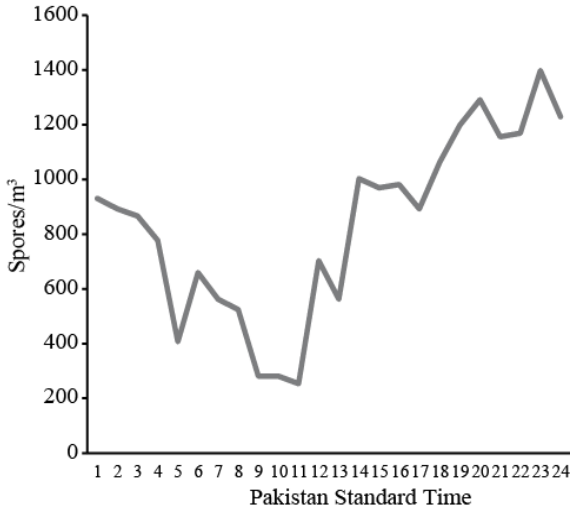

Fig. 7. Diurnal pattern of *Cladosporium* sp. spores/m^3.

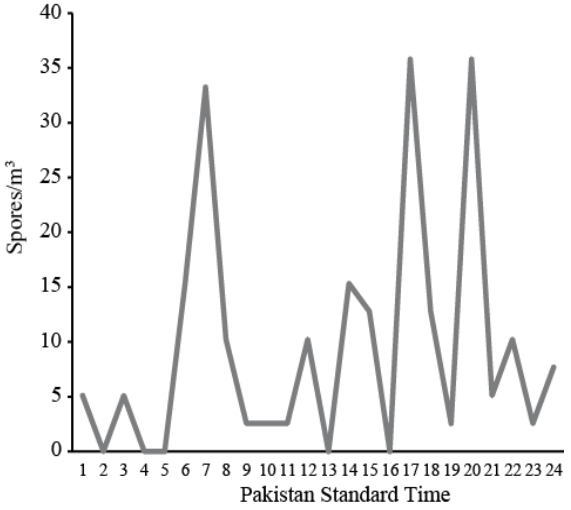

Fig. 10. Diurnal pattern of *Ganoderma* sp. spores/m.

Table 3. Correlation of various fungal spores count to weather conditions.

Name of Species	Temperature (°C)	Relative Humidity (%)	Wind Speed (Km/Hr)
Cladosporium sp.	-0.348	0.212	-0.233
Aspergillus sp.	-0.21	-0.496	-0.451
Alternaria sp.	-0.043	-0.557[a]	-0.37
Periconia sp.	-0.0238	0.072	-0.335
Bipolaris sp.	-0.067	0.007	-0.037
Coprinus sp.	-0.024	0	-0.024
Myxomycetes spp.	-0.071	0.084	0.024
Agaricus sp.	0.122	-0.096	0.112
Curvularia sp.	0.062	0.12	0.209
Ganoderma sp.	0.072	0.128	0.106
Arthrinium sp.	0.02	0.058	-0.566[a]
Stemphylium sp.	-0.224	-0.479	0.16
Rust	0.159	-0.076	0.25
Panaeolus sp.	0.097	0.124	-0.229
Amphisphaeria sp.	-0.055	-0.142	-0.342

Note: Correlation is significant at 0.05 level (Two tailed test)

Discussion

The aerobiological survey of fungal flora at Tando-Jam was conducted for the first time. During the present study various pathogenic airborne fungal spores were recognized. Current study revealed that *Cladosporium* sp., *Aspergillus* sp., and *Alternaria* sp. constituted about 80% of total fungal spore count from Tando-Jam. The highest fungal spore concentration was contributed by *Cladosporium* sp. (50.83%). *Cladosporium* sp. was also the most common spore type that was reported in other aerobiological surveys (Al-Suwaine *et al*., 1999; Kalyoncu, 2010; Hussain *et al*., 2013). It is also recognized as an important aeroallergen (Denning *et al*., 2006; Waqar *et al*., 2009). *Aspergillus* sp. spores were recorded in second highest concentration followed by *Alternaria* sp. fungal spores. Both *Aspergillus* sp. and *Alternaria* sp. are characterized as bronchial allergy causing fungi (Hasnain *et al*., 1998; Peat *et al*., 2004; Hasnain *et al*., 2012). More than 100 types of fungi species have been characterized as aeroallergens throughout the world (Green *et al*., 2005). Severity of asthma attack is significantly correlated with the concentration of fungal spores in the air. According to the results of research conducted in Saudi Arabia, 31.3% of asthmatic individuals were sensitized with *Cladosporium* sp. (Hasnain *et al*., 1994). Fungal spores of *Aspergillus* sp. were also reported to elicit bronchial allergies and asthma (Leenders *et al*., 1999). Other important fungal spores types that have been recognized and reported as aeroallergens are *Agaricus* sp., *Coprinus* sp. (Santilli *et al*., 1985), *Agrocybe* sp. (Gioulekas *et al*., 2004), *Bipolaris* sp. (Schubert & Geotz, 1998), *Chaetomium* sp., *Curvularia* sp. (Smith, 1990), *Ganoderma* sp. (Tarlo *et al*., 1979; Cutten, 1988), *Leptosphaeria* sp., *Puccinia* sp., *Stemphylium* sp., *Tetraploa* sp., and *Torula* sp. (Gioulekas *et al*., 2004).

Our study also reported low fungal spores concentration in the months of May, June and July (Fig. 1) similar was the case in Kuwait, in which the lowest spore count were encountered in mid-summer month of July (Moustafa & Kamel, 1976).

Diurnal periodicities of fungal spores were analyzed in order to detect that at what time of the day concentration of suspended fungal spores were maximum. This knowledge would help the allergy suffering individuals to plan their daily outdoor activities. The data of Tando-Jam, various peaks of the fungal spore concentration were analyzed. *Agaricus* sp. showed peak count at 6 pm which is a late evening maxima. *Alternaria* sp. did not show any sharp peak of fungal spores which is similar to a study performed in Karachi, Pakistan (Hasnain *et al.*, 2012). *Arthrinium* sp. showed an early morning peak count at 7 am. *Aspergillus* sp. showed a very sharp maximum concentration at 8 pm. *Bipolaris* sp. data also showed higher concentration in afternoon at 4 pm from Tando-Jam. An increase in the concentration of *Bipolaris* sp. in the afternoon time is reported in a similar kind of study from Karachi (Hasnain *et al.*, 2012). *Cladosporium* sp., also showed a post morning maxima of spore concentrations which is similar to a study in which late evening peak of fungal spores was declared due to *Cladosporium* sp.(Carinanos *et al.*, 1999). According to the aerobiological survey, high count of *Curvularia* sp. spores was observed during mid-day timings to late evening. Current study also showed higher mid-day *Curvularia* sp. fungal spores count. *Periconia* sp., *Stemphylium* sp., and Myxomycetes sp. did not show any clear pattern of highest spore count at specific hour of the day which was also similar to the results obtained by Hasnain *et al.* (2012).

The fungal spore counts were correlated with meteorological conditions by using statistical software SPSS and Spearman rank correlation coefficient "r". Over all most of the fungal spore types are negatively correlated with the temperature and wind speed while

positive correlation with humidity is observed. Humidity plays a key role in the fungal growth and spore production. Thus, the relation of humidity and fungal spore count has always been positively correlated. Other studies also demonstrated that increase in the humidity level will increases the spore count (Afzal *et al.*, 2004; Hasnain *et al.*, 2012).

Conclusion

Epidemiological surveys suggest that the prevalence of allergies and asthma is about 5 to 30% in school going children and affect 2 to 30% of adult persons. Unfortunately, there are no records of aerobiological data of fungal spores of Tando-Jam, Sindh. For the first time this study has provided a comprehensive data base comprising details of airborne biological data, and effect of meteorological conditions on spore counts. This data would aid in the treatment of allergy suffering individuals of the study area.

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