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SEASONAL DISTRIBUTION OF AEROMYCOSPORA OF KARACHI: A MULTIVARIATE APPROACH

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ABSTRACT

This study focuses on the seasonal distribution of aerospora of Karachi city. Sampling of aeromycospora was performed at five localities within the city and multivariate techniques were employed for data analysis. The climate of Karachi is of subtropical maritime desert with three main seasons, *i.e*., winter, summer and monsoon. Generally, the temperature and humidity are high providing conducive environment for the growth of fungi. Pollution of various kinds, particularly unattended refuse dumping sites in city also provide favorable sites for fungal growth. A total of 10 fungal species were recorded including *Aspergillus candidus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *A. wentii*, *Alternaria solani*, *Curvulari aclavata*, *Drechslera dematioidea* and *Penicillium notatum*. Aerospora was dominated by *Aspergillus* species. Differences among localities were disclosed by cluster analysis and principal component analysis (PCA).

Key- words: Aeromycospora, cluster analysis, PCA, Karachi.

INTRODUCTION

Karachi is an ideal city for determination of seasonal distribution of aeromycospora of the atmosphere. The weather of Karachi is divided into three main seasons *i.e.* winter, summer and monsoon, while the temperature fluctuations occur in all seasons in contrast to other inland areas of the country (Gadiwala, 2006). Some other studies, categorized the climate of Karachi as subtropical maritime deserted climate (Naqvi, 1956; Chaudhri, 1961).

High humidity prevailing during May to September results in increased fungal growth in the atmosphere (Afzal*et al.,* 2005).In addition, the various pollution types *i.e.*air, water and soil are also favorable for the fungal growth and spread of the many types of diseases such as those of plants, animal as well as human beings (Gregory, 1973). Fungal spores are the important particles of air and their wide distribution and rapid growth has attracted many studies, they constitute a permanent component of bioaerosol of polluted air (Adams, 1964; Larsen, 1981; Nikkels *et al*., 1996; Diaz *et al.,* 1998; Mitakakis and Guest, 2001).

In aerobiological studies morphological identification of many fungal spores or conidia are often difficult (Haines *et al.,* 2000). Many researchers concentrated on the airborne plant pathogenic fungal taxa and their parasitisation of field crops (Diaz *et al.,* 1998; Frenguelli, 1998; Hirst, 1991), while some fungi produced carcinogenic and teratogenicmycotoxins as well as being responsible for creating the hypersensitivity reactions in the human body, whereby these fungi produce adverse effects on human health (Bush and Portnoy, 2001; Dutkiewicz, 1997; Corden and Millington, 2001; Gravesen, 1979; Myszkowska *et al*.,2002;Stepalska *et al.,* 1999). Further studies on air revealed that 20 to 30% population of the world becomes infected by some aeromycospora and that were responsible for causing allergic rhinitis, atopic dermatitis and bronchial asthma (Schafer and Ring, 1997; Smith and Salim, 1988).

In addition, airborne conidia of *Aspergillus* spp. are the dominant allergens of the air to create allergic disorders (Austwick, 1965; Bisht *et al.,* 2003; Blackley, 1873; Millins *et al.,* 1976; Tewary and Mishra, 1996; Sarma, 2002). The present study is based on three years extensive survey of aeromycospora of five different areas of Karachi and reveals the seasonal distribution of the airborne fungal spores and conidia of the atmosphere of Karachi. This study is part of an ongoing investigation of collection, identification and seasonal distribution of aeromycospora of Karachi city. The present study determines the seasonal distribution of airborne fungal flora of Karachi employing advanced multivariate techniques including cluster analysis and principal component to identify group, structure and trends in aeromycospora assemblages with the ultimate aim to providing help to public health concerning bodies as well as common people about the health disorders related to these organisms.

MATERIALS AND METHODS

Five different areas were selected in Karachi. Criteria of area selection were: 1) sufficiently large and homogeneous area. 2) the area should have poor sanitary conditions, with the exception of Gulshan-e-Maymar, 3) with low per capita income (see Rao *et al*.,2009; 2011a). In each area sampling of aerospora was performed by a locally made spore trapper for qualitative analysis of spores. The spores were trapped on glass slides having a layer of Vaseline. Quantitative analysis was carried out by exposing Petri plates with PDA (potato dextrose agar) and Czapek-Dox agar. The exposed Petri plates were incubated at 27º C in an incubator and the colony forming units (CFU's) were counted. For the identification of fungal species a number of manuals were consulted (Thom and Raper, 1945; Domsch *et al*., 1980; Ellis and Ellis, 1997; Barnett and Hunter, 1998). To unravel the group structure and trends in aerospora, multivariate analysis including cluster analyses and principal component analysiswere performed with the mean values of the three years aeromycological data (Orloci and Kenkel, 1985; Kenkel and Booth, 1992).These method permit parsimony of representation of the recorded information (Wold *et al.,* 1987; Jolliffe, 2002).

Cluster analysis and principal component analysis were carried out using the software program PC-ORD Version 5.10 (McCune and Mefford, 2005).

RESULTS

A total of 10 fungal species were recorded on an overall three years basis acrossthe aeromycological survey of five different areas of Karachi. Five different areas showed considerable variation in the composition of fungal species except some *Aspergillus* species.

Malir Area

From Malir area a total of five fungal species were found during the study period. They were *Aspergillus flavus, Aspergillus niger, Aspergillus terreus, Alternaria solani* and *Curvularia clavata*. *Aspergillus niger* showed the highest abundance in the area over different seasons as well as in 36 months (Table 1). This species showed highest mean CFU's with standard error value of the coloniesof 936 ± 253 and 935 ± 252 in the months of March and August, respectively. Abundance of *Aspergillus flavus* was 679± 169 and 659 ± 149 in the months of August and March, respectively. *Alternaria solani* also gained third place in order of abundance in this area with 315 ± 103 during the month of August, while *Aspergillus terreus* and *Curvularia clavata* showed similar magnitude in their distributions as presented in Table1.

Korangi Area

In Korangi area a total of seven fungal species were recorded. The most dominant species was *Aspergillus niger* with 1165 ± 295 and 1112 ± 249 mean CFU's ± standard error values during the month of September and July respectively. *Aspergillus flavus* also showed marked abundance and attained second position in this location with mean ± SE 855 ± 254 and 776 ± 164 in September and July respectively. Another species *Alternaria solani* reached at third position with mean ±SE of 374 ± 83 and 351 ± 110 in July and September respectively. In addition, the other four species showed their presence in a similar manner (Table2).

Metroville S.I.T.E. Area

Metroville SITE area only showed low species diversity with only five different fungal species found from the area. These species were *Aspergillus flavus*, *Aspergillus niger*, *Alternaria solani*, *Drechslera dematioidea* and *Penicillium notatum*. *Aspergillus niger* and *Aspergillus flavus* were recorded with their highest mean and standard error values. In the month of July these two species were distributed with mean CFU's ±SE of 977 ± 203 and 616 ± 185, respectively, while in the month of September they showed 923 ± 225 and 585 ± 197, respectively. Other three species of the area demonstrated more or less similar distribution (Table 3).

Gulshan-e-Maymar

At the location of Gulshan-e-Maymar areaa total of five different fungal species were found. *Aspergillus niger* was again the dominant fungal species with 595 ± 152 mean ± SE during the month of August. Another highly distributed species was *Alternaria solani* with mean ± SE of 207 ± 36 attained in August. Other three species were recorded in a similar pattern from this area (Table 4).

Table 1. Mean with ± standard error of 3 years aeromycospora from Malir Area.

Months	*A. flavus*	*A. niger*	*A. terreus*	*Alternaria*	*Curvularia*
Mar. 07-09	679.33 ± 169.3	935.67 ± 252.78	170 ± 44	315.33 ± 103.71	47 ± 24.27
Apr. 07-09	443 ± 137.04	618 ± 206.51	131.33 ± 38.25	222.67 ± 75.84	146.67 ± 54.03
May.07- 09	586.67 ± 139.32	753.67 ± 188.49	146.67 ± 31.8	249 ± 62.85	143.67 ± 35.83
June. 07- 09	621 ± 107.51	798.33 ± 138.3	153.67 ± 32.41	265 ± 36.47	82.33 ± 35.59
Jul. 07- 09	568.67 ± 130.34	802.33 ± 174.22	153 ± 44.54	277 ± 71.63	58 ± 29.96
Aug. 07- 09	679.33 ± 169.3	935.67 ± 252.78	170 ± 44	315.33 ± 103.71	47 ± 24.27
Sept.07- 09	577.33 ± 83.68	848.67 ± 92.21	148 ± 24.68	245 ± 39.28	48.67 ± 24.36
Oct. 07- 09	390.67 ± 67.19	628.33 ± 122.4	111 ± 10.6	177.67 ± 13.96	46 ± 23.26
Nov.07- 09	385.33 ± 120.76	687.33 ± 240.72	134 ± 30.57	177 ± 29.82	69.33 ± 30.51
Dec. 07-09	277.67 ± 79.83	440.33 ± 134.64	97 ± 20.23	118.33 ± 13.78	54.67 ± 5.24
Jan. 08-10	329 ± 100.62	467.67 ± 142.87	118 ± 22.74	143.33 ± 39.07	60.33 ± 13.37
Feb. 08-10	318.67 ± 76.23	442.33 ± 105.02	123.67 ± 27.91	144 ± 24.95	53.67 ± 0.88

Table 2. Mean with standard error of 3 years aeromycospora from Korangi Area.

Months	*A. candidus*	*A. flavus*	*A. fumigatus*	*A. niger*	*Alternaria*	*Penicillium*	*A. terreus*
Mar. 07-09	68.33 ± 14.17	191.33 ± 48.27	76.33 ± 20.67	228.67 ± 46.49	90 ± 17.47	36.33 ± 3.28	76 ± 1
Apr. 07-09	129.33 ± 35.71	347 ± 113.81	125.67 ± 27.34	412.33 ± 123.72	208 ± 63.04	92.67 ± 15.6	147 ± 4
May.07- 09	138.33 ± 28.88	397.67 ± 102.84	118 ± 34.6	560 ± 67.27	271.67 ± 64.43	65 ± 21.5	135 ± 1
June. 07- 09	132 ± 44.06	506.33 ± 124.68	130.33 ± 30.69	783 ± 160.52	299.67 ± 66.91	70 ± 23.03	117.5 ± 2.5
Jul. 07- 09	161.67 ± 42.4	775.67 ± 164.44	127 ± 32.72	1112.33 ± 249.11	374.33 ± 83.31	83 ± 4.04	136.5 ± 0.5
Aug. 07- 09	114 ± 33.08	688.33 ± 163.96	107.33 ± 36.77	989 ± 172.46	310.67 ± 82.89	97.67 ± 27.55	140.5 ± 4.5
Sept.07- 09	156 ± 42.78	854.67 ± 253.88	143.67 ± 33.93	1165.33 ± 295.08	351 ± 110.39	113.33 ± 27.28	166.5 ± 8.5
Oct. 07- 09	98 ± 21.17	704.67 ± 98.34	90 ± 8.5	875.67 ± 145.84	265.33 ± 35.88	51 ± 3.79	69 ± 1.22
Nov.07- 09	76 ± 1	483 ± 116.5	72 ± 3.51	640.33 ± 166.35	157.33 ± 23.54	29.33 ± 7.45	55.5 ± 2.5
Dec. 07-09	60.33 ± 9.74	387 ± 115.44	71 ± 4.36	551.67 ± 192.17	146.67 ± 38.75	46.67 ± 2.91	55 ± 5
Jan. 08-10	54 ± 11.02	295.67 ± 50.85	57 ± 17.62	409.33 ± 97.66	108 ± 31.01	48.33 ± 9.35	44.5 ± 2.5
Feb. 08-10	46.67 ± 1.2	299.33 ± 67.44	48 ± 2.08	447 ± 138.9	123 ± 27.32	46 ± 1.15	49 ± 1

Table 3. Mean with ± standard error of 3 years aeromycospora from Metroville SITE Area.

Months	*A. flavus*	*A. niger*	*Alternaria*	*Drechslera*	*Penicillium*
Mar. 07-09	202.33 ± 46.11	324 ± 92.74	122 ± 6.43	61.67 ± 8.82	98.33 ± 7.26
Apr. 07-09	334 ± 122.03	514 ± 207.23	145.67 ± 25.75	101 ± 28.75	126 ± 30.02
May. 07- 09	430 ± 12.77	709.33 ± 99.35	150 ± 2.89	53.67 ± 3.33	114 ± 10.6
June. 07- 09	435.33 ± 120.94	665.33 ± 226.16	157.67 ± 22.51	42.33 ± 7.84	115 ± 26.1
Jul. 07- 09	616 ± 185.39	976.67 ± 202.84	219 ± 39	32.67 ± 16.83	162 ± 42.52
Aug. 07- 09	508.33 ± 128.19	836.33 ± 143.18	177 ± 33.05	27.67 ± 14.19	119.67 ± 32.84
Sept. 07- 09	585.33 ± 196.72	923.33 ± 225.37	187.67 ± 45.34	36 ± 18.58	125.33 ± 55.69
Oct. 07- 09	440 ± 89.89	700.33 ± 70.2	140.33 ± 20.2	32.67 ± 16.42	122.33 ± 29.69
Nov. 07- 09	357.33 ± 63.73	557.33 ± 77.62	104.33 ± 10.2	46.67 ± 14.97	89.33 ± 21.17
Dec. 07-09	344 ± 73.55	515 ± 111.81	90.67 ± 4.33	57.67 ± 18.34	88 ± 13.58
Jan. 08-10	263.67 ± 46.69	364 ± 48.77	77.33 ± 15.43	39.67 ± 20.09	70.67 ± 9.87
Feb. 08-10	286.33 ± 48.17	370 ± 66.11	73 ± 5.57	33.67 ± 16.84	72 ± 16.62

Table 4. Mean with standard error of 3 years aeromycospora from Gulshan-e-Maymar.

Months	*A. niger*	*A. wentii*	*Alternaria*	*Drechslera*	*Penicillium*
Mar. 07-09	303.67 ± 50.9	82.67 ± 8.17	91 ± 13.05	64.33 ± 5.67	79.33 ± 14.1
Apr. 07-09	312 ± 29.1	71 ± 5.51	104.33 ± 0.33	55.33 ± 2.19	76.67 ± 2.85
May. 07- 09	447.67 ± 68.69	98.67 ± 8.74	161.33 ± 2.4	58 ± 8.66	113 ± 11.59
June. 07- 09	459.67 ± 26.91	80.67 ± 25.3	152 ± 8.54	44 ± 16.29	91 ± 4.36
Jul. 07- 09	453.67 ± 90.74	74.33 ± 4.98	145.67 ± 24.39	35.67 ± 7.42	78 ± 15.72
Aug. 07- 09	595 ± 152.15	94 ± 9.5	207.33 ± 36.19	42.67 ± 10.67	115.67 ± 19.94
Sept. 07- 09	484.67 ± 46.84	90.33 ± 21.88	170 ± 3.51	49.33 ± 7.54	111 ± 13.01
Oct. 07- 09	455 ± 72.06	109.67 ± 30.82	127.67 ± 3.18	49 ± 3.21	94 ± 5.77
Nov. 07- 09	414.33 ± 105.95	78 ± 18.18	102.67 ± 6.36	60 ± 5.29	85 ± 12.5
Dec. 07-09	287 ± 57.66	59 ± 17.06	60 ± 6.24	45 ± 2.52	68.33 ± 2.91
Jan. 08-10	305 ± 67.57	60.33 ± 12.67	80 ± 10.54	54.33 ± 11.29	88 ± 2
Feb. 08-10	250.67 ± 13.91	61.67 ± 13.86	66 ± 1.15	48 ± 3.51	88.67 ± 10.68

Table 5. Mean with standard error of 3 years aeromycospora from Lines Area.

Months	*A. candidus*	*A. flavus*	*A. fumigatus*	*A. niger*	*A. terreus*	*A. wentii*	*Alternaria*	*Curvularia*
Mar. 07-09	50.33 ± 2.85	214 ± 26.84	73.67 ± 3.33	397.33 ± 103.2	69.33 ± 3.76	53.67 ± 4.48	104.67 ± 22.34	51.67 ± 8.88
Apr. 07-09	86.33 ± 19.43	364 ± 104.15	123.67 ± 35.83	628.33 ± 215.84	63 ± 6.43	52 ± 8.5	172.33 ± 47.84	59 ± 12.06
May. 07- 09	106.33 ± 29.72	482 ± 46.69	143 ± 21.5	761.33 ± 103.83	75.67 ± 3.67	51.67 ± 9.28	193.33 ± 23.31	51 ± 2.52
June. 07- 09	115.33 ± 36.42	524 ± 101.52	159 ± 46.52	818 ± 180.45	82.67 ± 13.09	59 ± 3.61	200 ± 44.3	51 ± 5.57
Jul. 07- 09	141.67 ± 44.33	729.67 ± 138.28	163.33 ± 30.72	1038.67 ± 242	99.33 ± 14.71	67.33 ± 5.78	266 ± 35.38	64.67 ± 7.22
Aug. 07- 09	122.67 ± 36.34	638.67 ± 119.69	117 ± 18.88	831.67 ± 191.17	79.67 ± 15.96	50.33 ± 3.93	205.67 ± 30.85	45 ± 4
Sept. 07- 09	325.67 ± 231.33	685.67 ± 143.68	143.67 ± 26.96	974 ± 256.59	95.67 ± 21.87	60.33 ± 9.91	225.67 ± 32.51	69.33 ± 6.69
Oct. 07- 09	101.67 ± 21.71	511 ± 24.11	112.33 ± 16.05	762.67 ± 97.4	71.67 ± 6.69	52.33 ± 7.06	148.33 ± 12.45	78.67 ± 9.33
Nov. 07- 09	67 ± 9.54	384 ± 25.24	70.67 ± 3.33	560 ± 48.5	49.67 ± 3.76	40.33 ± 4.98	96.33 ± 11.89	64.67 ± 2.03
Dec. 07-09	52 ± 4.36	283.33 ± 58.81	66.67 ± 9.74	439 ± 113.06	46.33 ± 3.28	41.67 ± 7.13	74.33 ± 8.09	67 ± 9.29
Jan. 08-10	49.67 ± 4.06	244.67 ± 30.38	60.33 ± 18.41	368.67 ± 77.95	51 ± 11.06	47 ± 12.66	82 ± 5.86	61.67 ± 7.88
Feb. 08-10	52.33 ± 7.31	297.67 ± 73.21	48 ± 4.16	437.67 ± 132.49	46 ± 3.51	44.67 ± 4.81	77.67 ± 8.19	49 ± 7.23

Lines Area

Lines Area showed the highest number of fungal speciesand a total of eight different species were found. These species were *Aspergillus candidus*, *Aspergillu sflavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus wentii*, *Alternaria solani* and *Curvularia clavata*. Highly distributed species was *Aspergillus niger* with the mean ± SE of 1038 ± 242 in July. *Aspergillus flavus* was obtained with 729 ± 138 mean ± SE and prevailed as second highly distributed fungal species of the area. Another species *Aspergillus candidus* was recorded in high a value which was 326 ± 231 mean ± SE in the month of September. Other remaining five species were distributed in a similar manner (Table 5).

Multivariate Analysis

Cluster analysis (Ward's method with Euclidean distance measure) was applied on the data set. This analysis clearly divided the species in distinct groupings on the basis of their distributions.

Malir Area

In Malir area, the recorded fungal species showed two significantly different groups in their distribution pattern at $478x10^4$ level of Euclidean distance and 87.5% of total information at which the groups were recognized. *Aspergillus flavus* and *Aspergillus niger* were recognized as group 1 and *Aspergillus terreus,Curvularia clavata* and *Alternaria solani* were placed in group 2 (Fig. 1.A).

Table 6. Results of Principal component analysis (PCA) of five different areas of Karachi in terms of eigenvalues and eigenvectors.

Sites	Eigen value	% of Variance	Cumulative % of Variance	Eigenvector Coefficient	Associated Variables
Malir Component 1	3.422	68.446	68.446	0.5338	As
				0.4840	Af
				0.4753	An
				0.4621	At
Component 2	1.269	25.376	93.823	0.7841	Cc
				0.3889	An
				0.3445	At
Component 3	0.240	4.801	98.624	0.3353	Af
				0.6897	At
				0.5676	Cc
				0.3910	Af
Component 4	0.046	0.926	99.549	0.1782	An
				0.7686	An
				0.5698	Af
				0.2531	As
				0.1381	Cc
Korangi Component 1	5.731	81.871	81.871	0.4046	As
				0.3961	Ac
				0.3876	Afu
				0.3785	At
Component 2	0.916	13.087	94.959	0.5511	Af
				0.5441	An
				0.4161	At
				0.3173	Afu
Component 3	0.262	3.750	98.708	0.7871	Pn
				0.4401	Ac
				0.3177	Afu
				0.2391	As
Component 4	0.041	0.590	99.298	0.6899	As
				0.5509	Afu
				0.4524	Af
				0.0784	At
Metroville Component 1	3.753	75.066	75.066	0.5064	An
				0.5004	Af
				0.4922	As
				0.4672	Pn
Component 2	1.067	21.345	96.412	0.8952	Dd
				0.3575	Pn
				0.2134	As
				0.1341	Af
Component 3	0.137	2.743	99.155	0.5252	Af
				0.4543	Pn
				0.4387	An
				0.4076	As
Component 4	0.036	0.727	99.82	0.7254	As
				0.6668	Pn
				0.1334	Af
				0.0801	Dd

Continued……..

				0.5281	As
Maymar Component 1	3.343	66.854	66.854	0.5232	An
				0.4685	Pn
				0.4538	Aw
				0.8936	Dd
Component 2	1.124	22.475	89.328	0.3454	Aw
				0.2249	Pn
				0.1474	An
				0.7449	Pn
Component 3	0.329	6.581	95.910	0.6428	Aw
				0.1621	An
				0.0636	As
				0.5023	Aw
Component 4	0.164	3.279	99.188	0.4624	As
				0.4452	An
				0.4197	Dd
				0.3977	As
Lines Area Component 1	6.012	75.149	75.149	0.3969	An
				0.3951	At
				0.3817	Af
				0.9302	Cc
Component 2	1.085	13.561	88.711	0.2734	Ac
				0.1505	As
				0.1189	Af
				0.6396	Ac
Component 3	0.416	5.198	93.909	0.5459	Aw
				0.3240	Cc
				0.3048	Af
				0.5697	Ac
Component 4	0.320	4.003	97.912	0.4815	Aw
				0.4041	Af
				0.3488	An
				0.5023	Aw
Component 4	0.164	3.279	99.188	0.4624	As
				0.4452	An
				0.4197	Dd

Note: Ac = *Aspergillus candidus*, Af = *A. flavus*, Afu = *A. fumigatus*, An = *A. niger*, At = *A. terreus*, Aw = *A. wentii* As = *Alternaria solani*, Cc = *Curvularia clavata*, Dd = *Drechslera dematioidea* and Pn = *Penicillium notatum*

Korangi Area

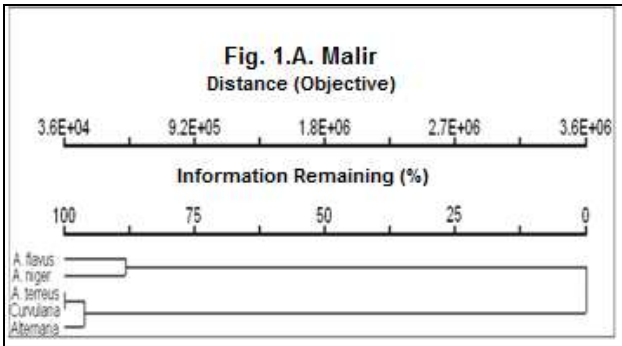
From the area of Korangi, seven fungal species disclosed their groupings with species clearly delineated in 2 main groups at $6505x10^2$ Euclidean distance (Fig. 1.B). Both groups were divided further into four sub groups such as *Aspergilluscandidus*, *A. fumigatus*, *A. terreus* and *Penicilliumnotatum* belonged to 1a group, while *Alternariasolani* to group 1b. The group 2 also showed two sub groups which were 2a *Aspergillusflavus* and 2b *Aspergillusniger*.

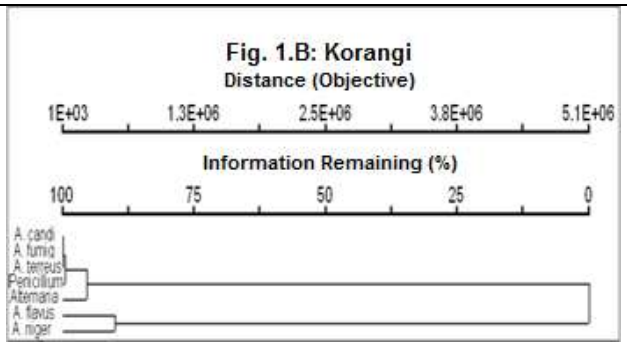
Metroville S.I.T.E. Area

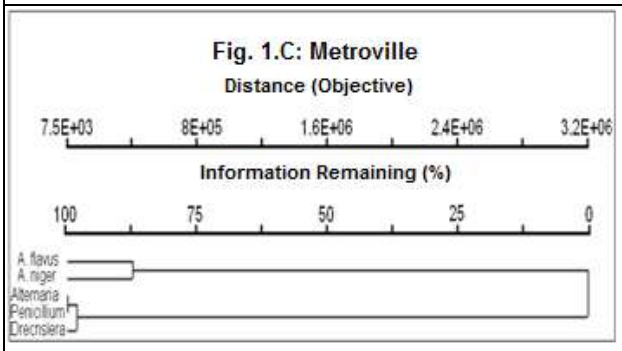
The dendrogram forMetroville S.I.T.E area showed two main groups of fungal species and four subgroups. Main groups formed at the level of $4375x10^2$ Euclidean distance. *Aspergillus flavus*and *Aspergillus niger* were placed together, while *Alternaria solani*, *Penicillium notatum* and *Drechslera dematioidea* represented another group (Fig. 1.C).

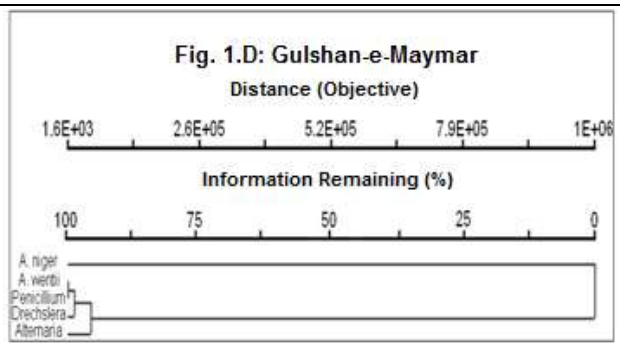
Gulshan-e-Maymar

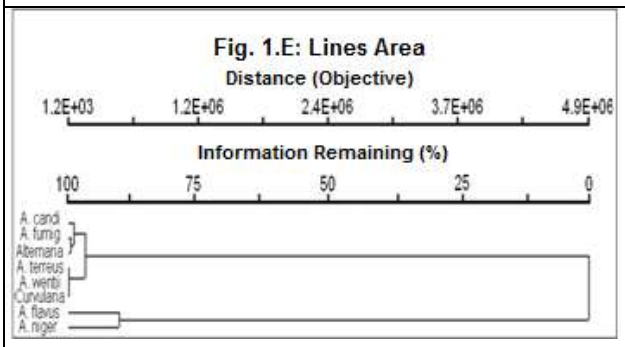
For Gulshan-e-Maymararea four fungal species together formed the main group and *Aspergillus niger* showed an isolated group. The main group was further divided into two sub-groups *i.e.* 1a sub-group included *Aspergillus wentii*, *Penicillium notatum* and *Drechslera dematioidea*, while the sub-group 1b consisted only of *Alternaria solani*. These species formed the cluster at 96 % of information and the Euclidean distance was $1.6x10^3$ (Fig. 1.D).

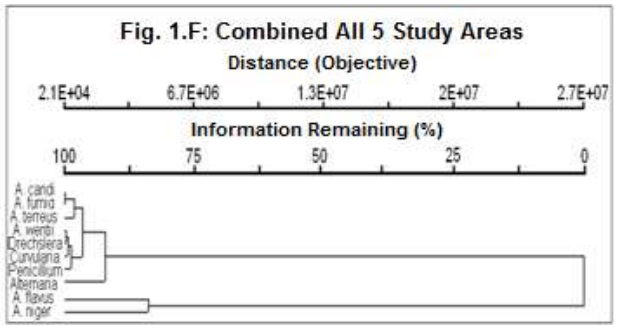












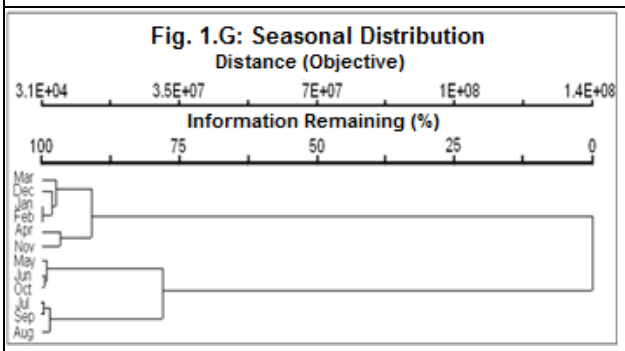


Fig. 1. (A to G) Dendrograms of Cluster Analysis of airborne Aeromycospora with monthly distribution from five different locations (areas) of Karachi.
Note: Ac = *Aspergillus candidus*, Af= *A. flavus*, Afu = *A. fumigatus*, An = *A. niger*, At = *A.terreus*, Aw = *A. wentii*, As = *Alternaria solani*, Cc = *Curvularia clavata*, Dd = *Drechslera dematioidea* and Pn = *Penicillium notatum*.

Lines Area

In Lines Area two main groups emerged at $3003x10^3$ Euclidean distance by the eight fungal species. These two groups were further separated into four sub-groups. 1a sub-group consisted of*Aspergillus candidus*, *A. fumigatus* and *Alternaria solani*, whereas 1b sub-group was based on *Aspergillus terreus*, *A. wentii* and *Curvularia clavata* (Fig.

1.E). Group 2 was also divided into two sub-groups. These two sub-groups 2a and 2b comprised of*Aspergillus flavus* and *Aspergillus niger*, respectively.

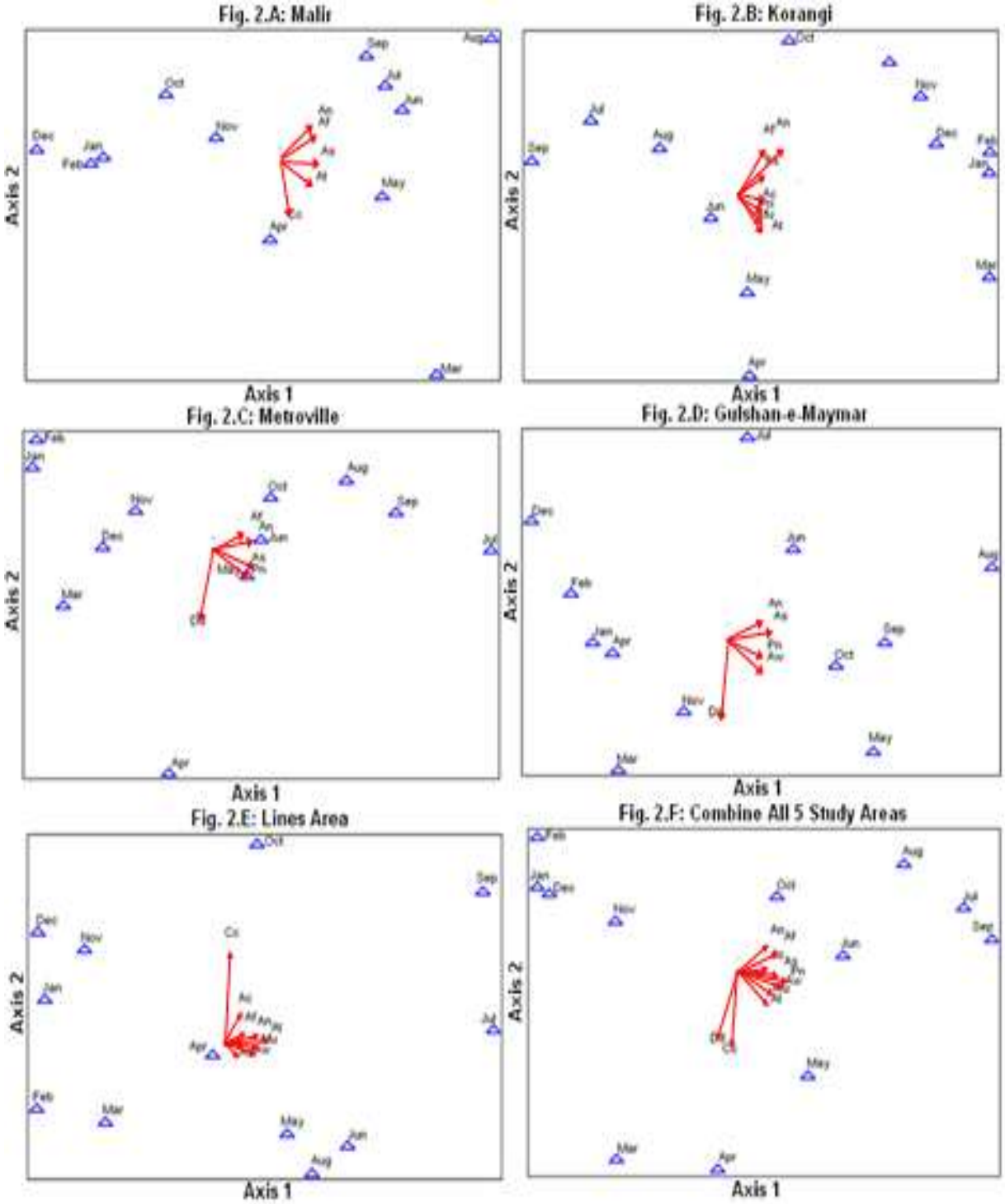


Fig. 2. (A to F) Principal component analysis of Aeromycospora from five different Areas of Karachi.
Note: Ac = *Aspergillus candidus*, Af = *A. flavus*, Afu = *A. fumigatus*, An = *A. niger*, At = *A. terreus*, Aw = *A. wentii*, As = *Alternaria solani*, Cc = *Curvularia clavata*, Dd = *Drechslera dematioidea* and Pn = *Penicillium notatum*.

Combined data of Five Areas

All five areas were subjected to combine clustering to confirm significant grouping in the overall fungal species of Karachi. Fungal species showed two main groups with regard to their distribution. *Aspergillus candidus*, *A. fumigatus*, *A. terreus*, *A. wentii*, *Drechslera dematioidea*, *Curvularia clavata*, *Penicillium notatum* and *Alternaria solani* constituted group 1(Fig. 1.F). *Aspergillus flavus* and *Aspergillus niger* were found placed together in group 2.

Agglomerative clustering with respect to months (Fig. 1.G) showed that May to October (summer months) grouped together while winter and spring (November to April) together formed a separate group. This larger group contained two sub-groups. Sub-group 1a comprised of true winter months, December, January, February while 1b included April and November which have higher average temperature.

Fig.2.A-E shows the PCA ordination of each locality. In general, 2-D ordination of each locality shows separation of summer and winter months. With respect to species vectors, in general, *Aspergillus* species depict correlation with the first PCA component while *Drechslera* and *Curvularia* seem to be associated with the second PCA component. The result of PCA are summarized in Table 6 which shows that the first three components together for Malir, Korangi, Metroville, Gulshan-e-Maymar and Lines Area explained 98.3, 98.70, 99.15, 95.91 and 93.90 percent of total variance respectively. The first principal component, in general, was regulated by *Aspergillus niger*, *Aspergillus flavus* and *Alternaria solani*. The second component, generally, was the function of *Curvularia clavata* and *Drechslera dematioidea*.

DISCUSSION

Karachi is a thickly populated city of Pakistan with an estimated current population of 18 million. Abundance of population of this city creates large number of turbulations for residents of Karachi. Of the most common and alarming aspect is pollution including air, water and soil pollution which affects all types of life existing in the city (Rao *et al.,* 2011a). In addition, due to coastal region the humid and moist climate provides favorable growth conditions for different types of microorganism present in the atmospheric air of the city. Fungi (spores and conidia), bacteria, actinomycetes and dust mites are the major component of airborne communities in this area (Rao *et al.,* 2009). The study disclosed the seasonal distribution of airborne fungi in 5 different areas (localities) of Karachi, based on 3 years survey of atmospheric air of the city. Multivariate analytical techniques including cluster analysis and principal component analysis were employed. Both the techniques proved reasonably effective in exposing the underlying group structure and trends inherent in the data structure.

The first four axes of PCA of individual and combined localities explained greater than 99 percent variability in the data sets providing testimony to the effectiveness of this technique. The results showed significant grouping in aeromycological data sets for the five selected areas. These two analyses supported and complemented each other's results on the basis of monthly and seasonal distribution of fungal species in the aerospora. Furthermore, mean values and the grouping obtained from multivariate methods also confirmed the seasonal distribution pattern of fungal species present in the atmospheric air of Karachi.

The results at hand showed that *Aspergillus niger*, *A. flavus* and *Alternaria solani* were abundantly distributed fungal species of the atmosphere of all 5 sites except Gulshan-e-Maymar where *Aspergillus flavus* was not found in any season. Climatic conditions *i.e.* high relative humidity of air with high temperature are known to increase the growth of airborne microorganisms especially that of fungal species (Nunes *et al.,* 2005).

The results of the current study correspond with the findings of the previous workers (Garcia-Pentaleon, 1992; Meriggi *et al*., 1996; Singh and Singh, 1999; Peterman, 2002) who found *Aspergillus* spp. as dominants. Our results show high abundance of airborne fungal species in June to September during which both the temperature and humidity remain high in the city. Cluster analysis performed on combined data of five localities to assess monthly distribution pattern also confirmed the influence of temperature and humidity on the distribution of fungal species prevailing in the atmosphere of Karachi. The summer months May to October, during which temperature and humidity remain high formed one group while Nov., Dec., Jan., Feb., Mar. and April (winter) in which temperature and humidity are lower formed the other group in the dendrogram derived from agglomerative clustering. Likewise, the PCA of individual localities and that of combined data of all 5 localities exhibited well-defined demarcation between the same two groups corresponding to the two main seasons, *i.e*., summer and winter. With respect to species grouping depicted by the dendrograms and species vectors in PCA an important observation is that the species of *Aspergillus* that are dominant in the aerospora tend to be collected as a group of their own, while other species including *Drechslera* and *Curvularia* tend to remain as a separate cluster.

Due to pollution in the city mainly owing to unmanaged garbage, open dumping sites and insanitary conditions create favorable environment for fungal growth in soil, water, leaf surfaces (phylloplanes) as well as in atmospheric

air of the city (Rao *et al.,* 2011a). A recent study (Shaukat *et al.,* 2013) has demonstrated that a number of fungal species found on the phylloplane are also abundantly represented in the aerospora of the city.

Overall ten airborne fungal species namely *Aspergillus candidus, A. flavus, A. fumigatus, A. niger, A. terreus, A. wentii, Alternaria solani, Curvularia clavata, Drechslera dematioidea* and *Penicillium notatum* are recorded from five study sites of Karachi. Species of *Aspergillus* appear as the dominant species of the aerospora of localities which corresponds with the results of previous studies (Afzal *et al*., 2005; Rao *et al.,* 2009). In addition, fungal species recorded from five locations are mostly saprophytic except for some, such as *Aspergillus niger*, *Aspergillus flavus* and *Alternaria solani* that are epidemiologically parasitic in nature. *Alternaria solani* causes early blight in potato and tomato (Weir *et al*., 1998).Several studies have reported that *Alternaria solani* produces hypersensitivity reactions in humans and that *Aspergillus niger* and *A. flavus* also cause bronchopulmonary aspergillosis and asthma in human beings (Hasnain *et al.,* 1998; Haq, 2004).

In general, Metroville and Korangi area are both residential and industrial areas, these sites retain residential and industrial pollution caused by different types of industries especially dyeing mills and tanneries (Rao *et al.,* 2009). Malir locality comprises of agricultural fields as well as thickly populated areas and because of huge population and poor sanitation unhygienic conditions prevail that create health threatening environment for the residents of the area. Lines Area is also congested with high traffic density coupled with insanitary conditions. The vehicular emissions and high atmospheric fungal load together are responsible for greater intensity of air pollution of the area.

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