ATMOSPHERIC MOLD SPORE COUNT IN CEBU CITY, PHILIPPINES†

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Many common soil fungi living on dead or dying vegetation have long been known to cause inhalant allergies. Their spores and mycelial fragments blown by the wind, make up a significant part of airborne matter and they maybe allergenic by inhalation in the same way as are other allergens, such as pollens. Numerous investigations have amply confirmed the fact that hypersensitibity to fungi not infrequently occurs giving rise to asthma, rhinitis and dermatitis (Feinberg, 1946).

In studying mold allergy in a country, it is essential first of all to gather information regarding atmospheric mold spores in different part of the country, inorder to provide information on geographic and seasonal variation (if any) and the kind of fungus predominating in a locality. The knowledge of the predominating fungus genera in the atmosphere constitutes an invaluable aid to allergist in evaluating mold allergies in that locality.

Studies on airborne fungus spores have been reported from many localities throughout the world. Bocobo and Suguitan (1959) have compiled the results of studies showing the predominating atmospheric molds in different parts of the world. In the Philippines, 2 atmospheric mold surveys have been reported, both involving the Manila area (Bocobo and Suguitan, 1959; Agbayani *et al*, 1968). In both studies, the predominating airborne fungi in the area were: *Hormodendrum*, *Aspergillus*, and *Penicillium*. This investigation was undertaken inorder to obtain information regarding the kind and quantity of airborne fungus spores in Cebu City area. The work presented below covers a period of one year, from October, 1967 to September, 1968.

MATERIALS AND METHODS

The culture plate technic was employed in this survey using Sabouraud's glucose agar as growth medium. The site selected for exposure of plates was the roof of the M. H. Aznar Building on the campus of the Southwestern University in Cebu City. This building composed of 5 storeys (now has 7 storeys), is the tallest on campus and its roof is exposed to wind coming from all directions.

A Sabouraud's glucose agar plate was exposed to the air at approximately 9:30 A.M. and another plate at approximately 2:30 P.M. for a period of 10 minutes each. The time of exposure was chosen for convenience. After exposure, the plates were wrapped in clean paper and sent by air to Manila. All plates were processed at the Institute of Hygiene. The plates were kept at room temperature and examined on the beginning of the fourth day for fungus colonies which were identified and counted. Those which could not be identified in situ were subcultured onto Sabouraud's glucose agar salants for further studies. Czapek medium, potato dextrose agar and corn meal agar were used in the identification when

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necessary. Identification was made to the level of the genus only.

RESULTS AND DISCUSSION

A total of 6223 colonies representing 41 genera of fungi were isolated from 472 plates exposed during the survey. The number of colonies of each genus counted with their corresponding percentages and the number of times each group was observed during the 236 exposure days are given in Table 1. The average daily spore counts for all genera combined and for the genera *Aspergillus*, *Hormodendrum*, and *Penicillium* are shown in Table 2 and Fig. 1 The meteorological data for Cebu City corresponding to the period of the survey is given in Table 3.

Table 1
Fungi Isolated from the Air, Cebu City
in 236 Exposure Days from October, 1967 to September, 1968.

Genera	Colonies Isolated		Number of Appearance
	Number	% of Total	
1. *Aspergillus*	1,643	26.40	196
2. *Hormodendrum*	1,299	20.87	211
3. *Penicillium*	980	15.75	162
4. *Curvularia*	357	5.74	109
5. *Helminthosporium*	262	4.21	127
6. *Fusarium*	242	3.89	109
7. *Nigrospora*	202	3.25	120
8. *Mycelia sterila*	197	3.17	91
9. *Pullularia*	194	3.12	85
10. *Yeast*	173	2.78	41
11. *Streptomyces*	160	2.57	64
12. *Paecylomyces*	65	1.04	29
13. *Phoma*	62	1.00	48
14. *Rhizopus*	57	0.91	40
15. *Monilia*	40	0.64	30
16. *Trichoderma*	34	0.55	27
17. Others*	256	4.11	–
Total	6,223	100.00	

* Others, less than 0.5% each (in the order of descending frequency).

Unidentified; *Mucor; Hormiscium; Syncephlastrum; Pestalotia; Stemphylium; Geotrichum; Ustilago; Heterosporium; Alternaria; Scopulariopsis; Cephalosporium; Oospora; Monochaetia; Sporotrichum; Rhodotorula; Chaetomium; Verticillum; Thielaviopsis; Cunninghamella; Leptodiscus; Epicoccum; Sarcinella; Gliocladium; Diplocladium and Papularia.*

Table 2
Average Daily Spore Count by Months, Cebu City, October, 1967 to September, 1968.

Month	Average Daily Spore Count			
	All Genera (Total)	*Aspergillus*	*Hormodendrum*	*Penicillium*
October, 1967	16.1	3.5	3.8	0.9
November	22.4	2.7	4.9	2.2
December	25.5	2.0	8.6	4.3
January, 1968	24.9	2.9	5.6	2.5
February	41.5	3.3	12.6	12.2
March	39.0	8.8	13.3	4.0
April	38.0	12.2	5.5	4.1
May	27.9	15.9	2.4	4.2
June	17.3	8.3	1.0	4.0
July	28.0	12.8	3.4	3.4
August	26.0	9.1	2.2	6.9
September	13.4	8.4	1.0	1.0

Table 3
Meteorological Data, Cebu City, October, 1967 to September, 1968.

Month	Mean Wind Velocity (Knots)	Mean Rainfall (mm)	Mean Relative Humidity (%)	Mean Monthly Temperature (°C)	Mean Prevaling Direction (16 points)
October, 1967	4	107.1	80	27.07	S
November	5	139.3	80	26.76	NE
December	5	59.6	79	25.56	NE
January, 1968	6	57.1	79	26.43	NE
February	6	56.4	76	25.95	NE
March	7	58.1	75	27.35	NE
April	7	32.6	70	28.25	NE
May	6	5.1	69	29.32	NE
June	4	144.6	77	28.65	NE & S*
July	5	115.7	80	27.83	S
August	5	134.9	82	27.76	SW
September	4	177.3	79	27.85	SW

* Two prevailing winds.

Apsergillus appeared to be the most predominant fungus in Cebu City atmosphere constituting 26.4% of all the colonies counted (Table 1). This was followed in descending order of abundance by *Hormodendrum*, 20.8%; *Penicillium*, 15.75%; *Curvularia*, 5.74%; *Helminthosporium*, 4.21%; *Fusarium*, 3.8%; *Nigrospora*, 3.2%; *Mycelia sterila*,

3.17%; *Pullularia*, 3.12%; and *Yeast*, 2.78%. These 10 most common genera constituted 89.17% of the total number of colonies counted.

It was noted that although *Aspergillus* spores were the most abundant fungus in the air, it came out second only to *Hormodendrum* in terms of frequency of appearance irrespective of number. The latter genus appeared in the air 211 times in 236 exposure days or 89%. *Aspergillus* on the other hand, appeared 196 times or 83% only (Table 1). In general, however, the fungus groups dominant in total number were also dominant in number of appearances.

The fungal spores of each genus were present in the atmosphere throughout the year. The total fungus colony count tended to be higher during the dry months—February to April—with peak count obtained in February (Table 2 and Fig. 1). However, no seasonal trend was noted for the individual genus. *Aspergillus* spores predominated in the air from April through September; *Hormodendrum* spores from November through March (Fig. 1).

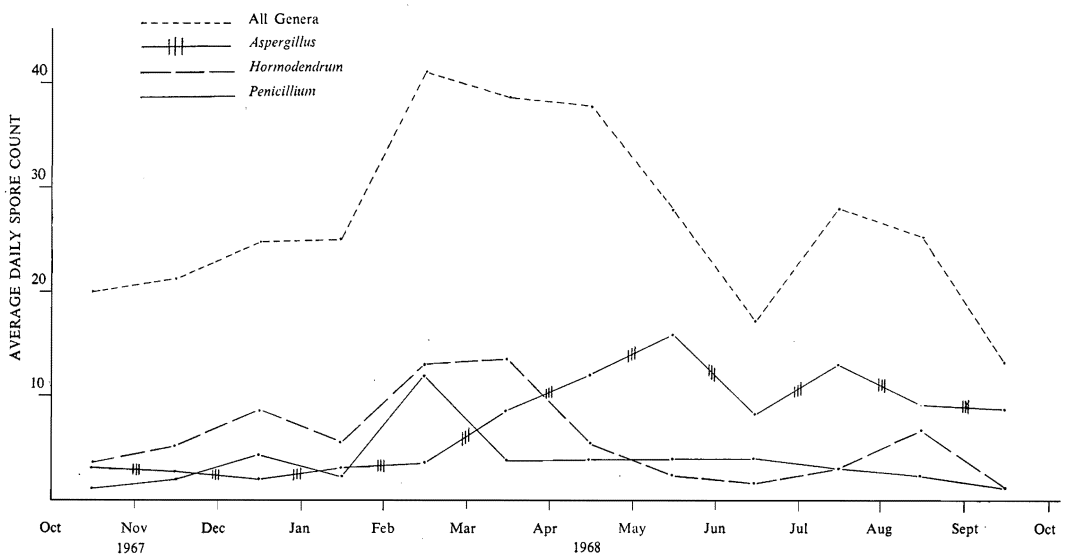

Fig. 1—Average Daily Spore Count, Cebu City October, 1967 to September, 1968.

The survey showed that the predominating airborne fungi in Cebu City were essentially the same as in Manila. *Aspergillus*, *Hormodendrum* and *Penicillium* constituted the first 3 most predominating fungi in Manila and Cebu Atmosphere. It is interesting to note that the genus *Alternaria*, which predominates in most surveys in the United States and Canada, was encountered infrequently in Cebu City. This fungus appeared in the atmosphere 11 times in 236 exposure days only and that it produced less than 0.5% of the total colonies isolated. The 2 surveys in Manila also demonstrated the infrequency of appearance of *Alternaria* in the air. A similar situation is found in many tropical countries including Puerto Rico, Costa Rica, and Honolulu where *Alternaria* in the air constitutes less than 1% of the total colony count (Pons and Belaval, 1961).

The climatological data of Cebu City was obtained from the Weather Bureau Station located at the Cebu Airport at Lahug, Cebu City. An attempt was made to determine the effect of the common meteorological variables on the average daily spore count*. The meteorological observations considered were

i) the mean wind velocity in knots (X_1)
ii) the total rainfall in mm (X_2)
iii) the mean relative humidity in per cent (X_3)
iv) the mean monthly temperature in degrees Celsius (X_4)

Accordingly in the analysis, the average daily spore count is as the Y variable or the dependent variable.

There were 12 sets of observations used in this aspect of the study, one set for each month of the year. (Table 4).

* Analysis was made by Prof. Ildefonso T. Cruz of the Institute of Hygience, Department of Epidemiology and Biostatistics.

Table 4

Multiple Regression Study of Average Daily Spore Count on Mean Wind Velocity, Total Rainfall, Mean Relative Humidity, and Mean Monthly Temperature.

Let Y = average daily spore count
X_1 = mean wind velocity (knots)
X_2 = total rainfall (mm)
X_3 = mean relative humidity (%)
X_4 = mean monthly temperature (degree Celsius)

	Y	X_1	X_2	X_3	X_4
	16.1	4	107.1	80	27.07
	22.4	5	139.3	80	26.76
	25.5	5	59.6	79	25.56
	24.9	6	57.1	79	26.43
	41.5	6	56.4	76	25.95
	39.0	7	58.1	75	27.35
	38.0	7	32.6	70	28.25
	27.9	6	5.1	69	29.32
	17.3	4	144.6	77	28.65
	28.0	5	115.7	80	27.83
	26.0	5	134.9	82	27.76
	13.4	4	117.3	79	27.85
Totals	320.0	64	1027.8	926	328.78

$\Sigma Y = 320$ $\Sigma X_1 = 64$ $\Sigma X_2 = 1{,}027.8$

$\Sigma X_3 = 926$ $\Sigma X_4 = 328.78$

$\Sigma YX_1 = 1{,}801.5$ $\Sigma YX_2 = 24{,}594.21$

$\Sigma YX_3 = 24{,}488.4$ $\Sigma YX_4 = 8{,}750.076$

A multiple regression analysis was made on the data to determine if a functional relationship existed between these meteorological observations and the spore count, and if so, to assess the degree of such a relationship. In the process it would be possible also to determine to what extent the latter variable is influenced by the group of meteorological variables, and whether any meaningful prediction of the average spore count maybe made from the data. The analysis yielded the following multiple regression equations, or estimating equation:

$$Y = \overline{Y} + \underline{b_1}(X_1 - \overline{X}_1) + \underline{b_2}(X_2 - \overline{X}_2) + \underline{b_3}(X_3 - \overline{X}_3) + \underline{b_4}(X_4 - \overline{X}_4)$$

$$Y = 26.667 + 7.881(X_1 - 5.333) + .067(\overline{X}_2 - 85.650) - .7266(X_3 - 77{,}167) - 2.438(X_4 - 27.398)$$

From this it maybe seen that the predictor variable most strongly related to the average daily spore count is the wind velocity. In other words, in the neighborhood of wind velocities observed in this study, a marked positive relationship was found to exist between these variables, i. e. high spore counts are associated with correspondingly high wind velocities and vice versa.

To assess the significance of the relationship of average daily spore count with all four meteorological factors, the reduction in sum of squares due to regression (SSR) was computed following the standard formula of multiple regression analysis.

$$SSR = \Sigma b_i SP_{x_i y} = (7.88149)(94.83) + (.067604)(-2813.79) + (-.72655)(-204.93) + (-2.43771)(-17.39) = 748.46$$

The results are organized in the following analysis of variance table:

Source of variation	DF	SS	MS	Variance ratio
Regression on X_1, X_2, X_3, and X_4	4	748.46	187.11	8.509*
Residual	7	153.95	21.99	
Total	11	902.41		

* Highly significant.

As a single numerical index describing the intensity of the observed association, the multiple correlation coefficient R was also computed:

$$R^2 = \frac{SSR}{SS_y} = \frac{748.46}{902.41} = 0.8294$$

$$R = \sqrt{0.8294} = 0.91$$

The above analysis indicates a highly significant relationship between the average spore count and the meteorological variables considered in the study.

SUMMARY

A one-year survey of airborne fungi in Cebu City using the culture plate technic was conducted in 1967-1968. A total of 6,223 colonies representing 41 genera of fungi were isolated. The 10 most common genera which constituted 89.18% of the total colonies were *Aspergillus*, 26.4%; *Hormodendrum*, 20.87%; *Penicillium*, 15.75%; *Curvularia*, 5.74%; *Helminthosporium*, 4.21%; *Fusarium*, 3.89%; *Nigrospora*, 3.25%; *Mycelia sterila*, 3.17%, *Pullularia*, 3.12%, and *Yeast*, 2.78%. *Aspergillus* spores predominated in the air from April through Septem ber; *Hormodendrum* spores from November through March. *Alternaria* was isolated infrequently. Statistical analysis showed a highly significant relationship between the average spore count and the meteorological variables considered in the study. The variable most strongly related to the average spore count was wind velocity. In the neighborhood of wind velocities observed, a marked positive relationship was found to exist between these two variables, i.e., high spore counts were associated with correspondingly high wind velocities and vice versa.

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