

Fungal Air Spora at Ibadan, Nigeria

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Received for publication 31 July 1974

The fungal air spora at Ibadan, Nigeria, was investigated by using Casella Slit Samplers. Three sites, incorporating three locations at each site, were selected for the exposure of replicate plates during sampling. To provide data on a wide range of saprophytic and pathogenic fungal spores, isolations were made on Sabouraud dextrose agar and malt agar plates incubated at 26 and 37 C. Altogether over 60,000 fungal colonies were isolated and counted during the 12-month sampling period. The prevalent fungal genera recorded were: *Cladosporium*, *Curvularia*, *Fusarium*, *Aspergillus*, *Penicillium*, *Pithomyces*, *Aureobasidium*, *Geotrichum*, *Phoma*, *Nigrospora*, *Epicoccum*, and *Neurospora*. The wet and dry seasons (indicated by the temperature, relative humidity, and rainfall data) caused seasonal periodicity in colony numbers. The influence of culture media on the isolated colonies was not significant when the total number of isolated colonies were considered on a monthly basis, but in reviewing a few of the fungal genera there were marked differences between the two media, especially with *Pithomyces*. Attempts were made to identify some of the isolated colonies by species, e.g., *Aspergillus carneus*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Curvularia geniculata*, *Fusarium oxysporum*, *Penicillium herquei*, *Pithomyces chartaum*, *Rhizopus arrhizus*, and *Syncephalastrum racemosum*. Such identifications provide a basis for further studies on the role of these fungal species in the frontier problem of contamination and biodegradation of drugs and pharmaceuticals, allergies and other problems in the local environment.

The microbiology of the atmosphere has, during the past two decades, produced more and more challenging studies mainly because of its relevance to various ecological and economic problems. Ludlam (10) discussed the meteorological variations affecting the circulation of fungal spores and the correlation of the observations with the involvement of particle sizes and heat. A census of fungal spore population in the atmosphere in the United Kingdom was carried out by Hyde and Williams (9), and Flensberg and Samsome-Jensen (6) showed the broad distribution of airborne organisms in Copenhagen. Qualitative studies on airborne fungal spores in Dunedin were carried out by Di-Menna (4), and the seasonal changes in three common constituents of the air spora in southern Nigeria were reported by Cammach (1). Cammach studied the epidemics of plant diseases that were due to airborne fungi. The significance of fungal spores in the air, in relation to allergy, was discussed by Hyde et al. (8) and similarly by Sandhu et al. (16) in reference to the air in Delhi. To provide reliable information concerning the relative concentrations of all microorganisms (both pathogens and

saprophytes) in hospital air Greene et al. (7) carried out qualitative and quantitative studies in Minneapolis. They reported on the factors which influence the level of contamination and the particle sizes with which microbial contamination is associated. Noble and Clayton (12) have also investigated the fungal flora of the air in hospital wards, but they were particularly concerned with the flora that grew at a 37 C incubation temperature. Moving away from specific studies of flora, Morrow et al. (11) published a summary of airborne mold surveys in the United States, and certain general distribution patterns emerged. Area or district investigations have also been carried out in the United Kingdom since Pawsey and Heath (15) reported on the results of petri-dish trappings over a 1-year period at Nottingham. Dransfield (5) reported similar studies at Samaru, northern Nigeria, in which it was indicated that there was seasonal periodicity in colony numbers and that air spora originate from vegetation rather than from soil.

Such area or district investigations can produce a considerable amount of information on the distribution and airborne fungal spora es-

sential for varying needs. Since we are interested primarily in the frontier problems of contamination and biodegradation of drugs and pharmaceuticals in our environment, it would be appropriate to provide detailed information from the southern parts of Nigeria to supplement the studies of Dransfield (5). These studies may be further extended to studies on evaluating appropriate air disinfectants in rooms, as well as on possible allergens.

Whereas the studies show airborne fungal spora over a 12-month sampling period, it is recognized that, in addition to spores, detached fragments of fungal mycelia might be viable, as reported by Pady and Gregory (14).

MATERIALS AND METHODS

Air-sampling equipment and techniques. A Casella Slit Sampler (Casella & Co. Ltd., London) adjusted to sample 1 cubic foot (0.028 m³) of air per min was used for a period of 2 min. The air was sampled on 4-inch (10.2-cm) petri dishes. It was noted from preliminary studies that overcrowding of the petri dish was avoided if exposure was for 2 min. It was also observed that the lower and upper limits of colonies counted were 40 and 100, respectively. These values were obtained after incubation at 26 C for 3 days.

Two media, sabouraud dextrose agar (SDA) and malt agar (MA), were used for collecting samples to observe any possible effect of media on the collection of the fungal spores. Preliminary studies indicated the need to suppress bacterial growth, especially in the MA plates; consequently, 20 U of penicillin per ml and 40 U of streptomycin per ml were added to each of the media. Noble et al. (13) have also achieved the suppression of the growth of actinomycetes in media by adding 0.5 mg of Acti-Dione per ml. This was similarly added to some of the media used.

Various precautions were taken to minimize contamination either from the equipment or the operators. A petri dish containing formaldehyde solution (30 to 35%, wt/vol) was left in the sampling chamber of the slit sampler overnight before sampling. The sampler parts and slit were swabbed with ethanol before any sampling sequence. The movements and activities at the sampling sites were reduced to a minimum before and during sampling processes. These precautions reduced the possibility of contamination.

The sampler was left at the site of sampling for a period not less than 30 min before the sampling operation was started. The sampler was invariably kept at a height of 3 to 3.5 feet (91.4 to 106.7 cm) from the elevation at each of the sites of sampling. The significance of such standard height had been indicated by Daws (3), who pointed out that it is difficult to suppress the tendency of convection causing recirculation of air in the upper portions of an environment while air in the lower portions remains relatively undisturbed.

Sampling locations. Ibadan is in the southern part

of Nigeria. It is situated on lat. 7.17 N and long. 3.30 E, about 70 miles (112.6 km) from the coast. The town is set on seven hills at an average elevation of 748 feet (228 m). During the year there are two distinct seasons, wet and dry. The wet season is from April to October and the dry season is between November and March. The mean annual rainfall is 1.2 m, and the temperature varies between 18.3 and 35 C.

Since the concentrations of airborne fungal spores generally differ from location to location and even fluctuate with time in a given location, as indicated by Greene et al. (7), we chose three locations at each of three sites, thus working at a total of nine locations. We used the same hour of the day (11:00 a.m. to 12:00 noon) for each of the three sites. The study was conducted over a 15-month period, but the results of a 12-month period (July, 1971 to June, 1972) are presented here. Three replicate plates of each of the two media were exposed at each incubation temperature (26 and 37 C) at each of the locations once a week.

Laboratory studies. The exposed plates were incubated initially for 3 days at 26 and 37 C, the colonies were counted, and the plates were left for further incubation, with daily colony counts made for up to 7 days. This was found necessary from preliminary studies, which indicated a need for continued incubation up to 14 days at 26 C for the development of slow-growing organisms.

The fungal colonies on each plate were counted and examined for morphological characteristics, and the mean number of colonies on each medium for each incubation temperature at each site were recorded. Representative plates from each of the locations were used in the characterization study. Each colony was coded before subculturing for identification purposes.

Various standard methods were used in identifying the organisms, and it was also found to be convenient to prepare slide cultures using the method of Chauhan and Walters (2). Confirmation of the identification of some of the fungal colonies was carried out at the Commonwealth Mycological Institute, Kew, England. Most of the fungal colonies were identified by genera only, but some were fully identified by species. Records of the mean maximum and minimum temperatures, relative humidity, and rainfall for each month of sampling were made.

RESULTS

The mean numbers of colonies growing from plates exposed at each site during each month of sampling are indicated in Fig. 1 and 2 for incubation at 26 and 37 C, respectively. The variations of the two media, SDA and MA, are indicated. The graphs in Fig. 1 show values including those of July, 1972 mainly to compare the data for the same months in 1971; there appears to be no significant difference. Since the records of the mean minimum and maximum temperatures, relative humidities, and rainfalls during the 12-month period are shown in Fig. 3, the effects of these factors on the air spora can be evaluated. The rainfall data devi-

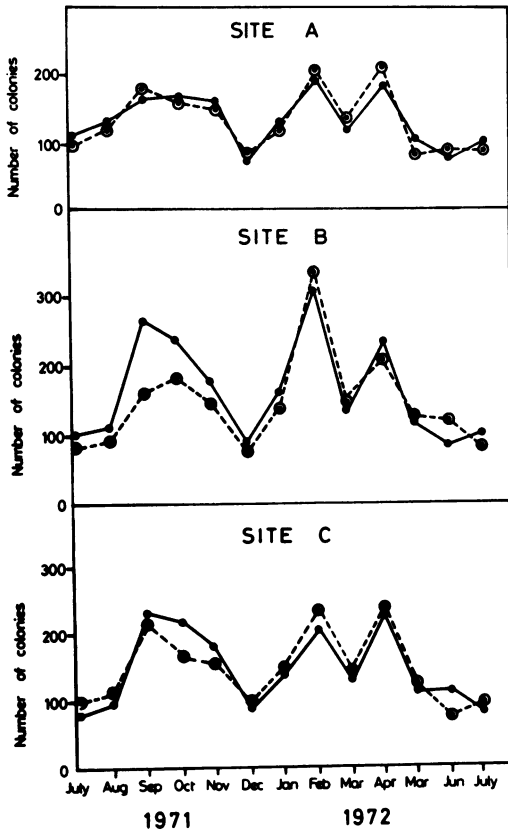


FIG. 1. Mean number of colonies isolated at three different sampling sites per month on incubation at 26 C. Symbols: ○, MA; ●, SDA.

ate slightly from the expected, since the months of December, February, and March, which are generally dry, had an appreciable amount of rainfall which affected the relative humidity for those months. It is also noted that the lowest minimum temperature and relative humidity was in the month of December, which may account for the lowest counts of colonies at the two incubation temperatures from samples at all sites. The lowest total number of colonies on incubation at 26 and 37 C was also obtained in December, 1971 for the two media (Fig. 4).

There is a marked seasonal variation shown in Fig. 2 and 4(B) with respect to organisms growing at 37 C, but this is not so marked in Fig. 1 except that there are relatively higher numbers of colonies towards the beginning and end of the wet and dry seasons. Consequently, troughs are produced at the middle of the seasons.

Table 1 shows the percentage of occurrence of the prevalent fungal spores, and their distribu-

tion on the two test media at 26 C is indicated in Fig. 5.

The identification of some of the fungal colonies by species was carried out, including the following: *Penicillium citrinum* Thom; *Penicillium herquei* Bain. & Sart; *Penicillium clavigerum* Demelius; *Fusarium semitectum* Berk. & Rav; *Fusarium oxysporum* Schlecht; *Aspergillus flavus* Link ex Fr.; *Aspergillus fumigatus* Fres.; *Aspergillus luchnensis* Inui; *Aspergillus niger* van Tiegh.; *Aspergillus sclerotiorum* Huber; *Aspergillus aculeatus* Iizuka; *Curvularia pallescens* Boedijn; *Curvularia geniculata* (Tracy & Earle) Boedijn; *Syncephalastrum racemosum* (Cohin) Schroet; and *Pithomyces chartarum* (Berk & Curt.) M. B. Ellis. (incubation at 26 C). The following fungal species were obtained after incubation at 37 C: *Aspergillus niger* van Tiegh.; *Aspergillus nidulans* (Eidam) Wint; *Aspergillus fumigatus* Fres; *Aspergillus carneus* Blochw.; *Aspergillus flavus* Link ex Fr.; *Mucor pusillus* Lindt; *Neurospora sitophila*

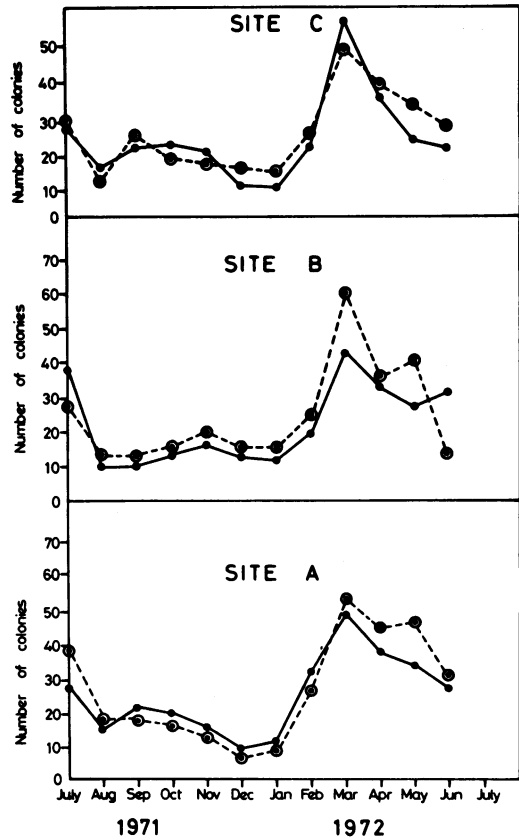


FIG. 2. Mean number of colonies isolated at three different sampling sites per month on incubation at 37 C. Symbols: ○, MA; ●, SDA.

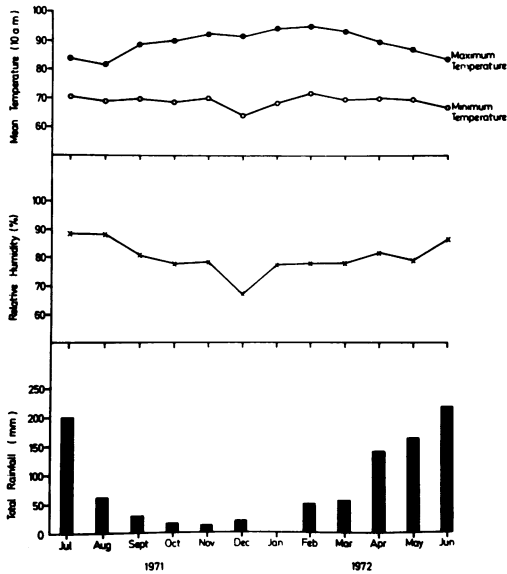


FIG. 3. Mean values of temperatures (maximum and minimum), relative humidity, and rainfall at Ibadan during each month of sampling.

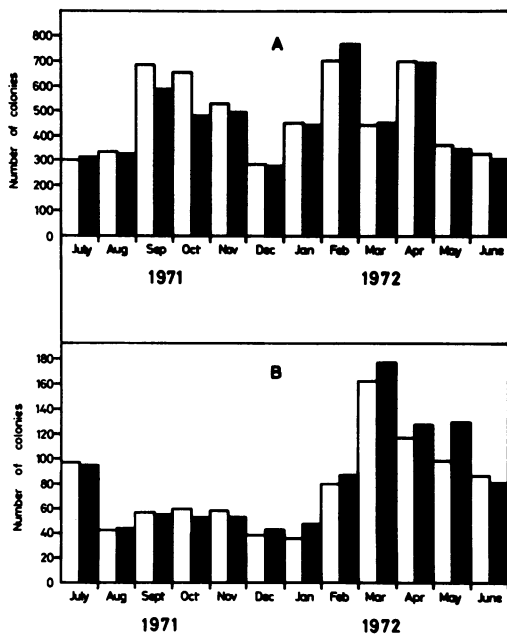


FIG. 4. Total number of colonies isolated on each of the media at all sites after incubation (A) at 26 C and (B) at 37 C. Symbols: ■, MA; □, SDA.

Shear & Dodge; and *Rhizopus arrhizus* Fischer.

There were no marked differences observed with respect to the two media, SDA and MA, in the graphs indicating the total number of colo-

nies isolated at all sites after incubation at 26 and 37 C (Fig. 4). However, there are marked differences shown by a few of the fungal genera (Fig. 5), especially fungal group no. 5, *Pi-*

TABLE 1. Isolations of fungal genera at incubation at 26 C

Fungal genera	Isolations (%)
<i>Aspergillus</i>	13.2
<i>Cladosporium</i>	18.5
<i>Curvularia</i>	16.6
<i>Epicoccum</i>	2.2
<i>Fusarium</i>	12.1
<i>Geotrichum</i>	3.6
<i>Neurospora</i>	1.6
<i>Nigrospora</i>	2.8
<i>Penicillium</i>	7.5
<i>Phoma</i>	3.0
<i>Pithomyces</i>	8.8
<i>Aureobasidium</i>	5.0
<i>Stemphyllium</i>	0.4
<i>Syncephalastrum</i>	0.5
Yeasts	0.3
Sterile mycelia	3.7
Others	0.2

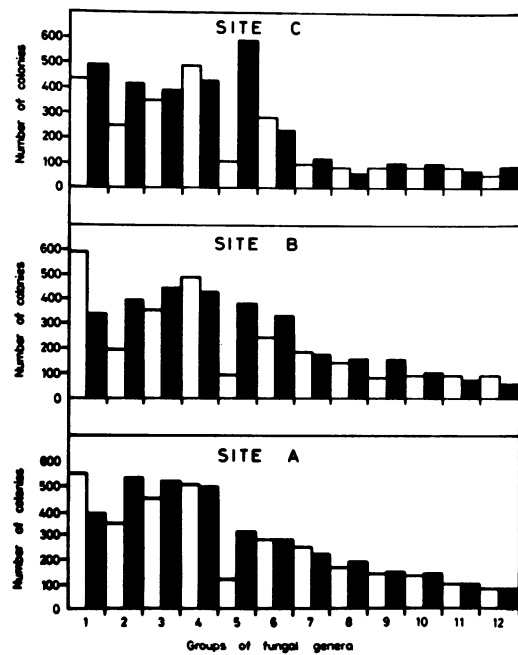


FIG. 5. Variation in the number of colonies of the prevalent fungal genera isolated on each of the media at each site after incubation at 26 C. Symbols: ■, MA; □, SDA. Group 1, *Curvularia*; 2, *Fusarium*; 3, *Aspergillus*; 4, *Cladosporium*; 5, *Pithomyces*; 6, *Penicillium*; 7, *Aureobasidium*; 8, *Geotrichum*; 9, *Phoma*; 10, *Nigrospora*; 11, *Epicoccum*; 12, *Neurospora*.

thomyces spp., which grew better in the MA medium.

The various species of *Cladosporium* isolated constituted the highest percentage of the prevalent spores; these were followed by *Curvularia* spp. There were some sterile mycelia which could not be identified even after near ultraviolet treatment for several weeks. In some cases there were failures in growing subcultures and identification could not be made from the originals.

DISCUSSION

In the quantitative evaluation of the fungal spores isolated at the three sites, the values indicated in the figures were the sum of mean values of replicate plates at the three locations on each site for each month. Consequently, the standardized volume of sampled air coupled with the identification tests would provide a basis for comparing the air spora at various locations. During the 12-month period of sampling, over 60,000 colonies were isolated on the two types of media used. There were no marked variations observed with the total monthly numbers of isolated colonies between the two media, SDA and MA, which were employed, but there was a particularly marked difference with the fungus *Pithomyces*, which was better isolated with MA than with SDA.

The isolations at 26 and 37 C were useful in supplementing information on the fungal air spora. The colonies isolated at 37 C were very few in number in comparison with those at 26 C, but it is interesting to note that a few known pathogenic organisms were identified. However, the peak of colonies at 37 C was obtained just about the end of the dry season (Fig. 2 and 4B). The lowest number of colonies at any site for both incubation temperatures coincide with the month having the lowest relative humidity and minimum temperature.

In the qualitative determination of the fungal colonies it has been stated that some of the organisms were identified by species. It was not possible to do this effectively with the isolated *Cladosporium* sp., as one of our collaborators confirmed that some of the *Cladosporium* sp. did not match exactly any of the *Cladosporium* sp. known to him in culture. It may reflect on the point that relatively little work has been published on members of that genus from the tropics. The genus *Alternaria* was not identified, but *Curvularia* sp. have considerably replaced *Alternaria* sp., which are moderately abundant in the temperate countries. Both *Cladosporium* and *Curvularia* showed high inci-

dence during the dry months, even though *Cladosporium* was still higher. There were more *Fusarium* sp. identified during July and August, and the same was noted for *Aspergillus* and *Penicillium* sp. (5, 15). *Pulkularia* (*Aureobasidium*) was isolated throughout the year and appears to be of common occurrence. This genus is found on painted walls in rather damp conditions. The observations on *Aureobasidium* are similar to those on *Thoma*. *Pithomyces* was isolated throughout the year, but the peak was distinctly in August and September. However, it was noted that the high incidence of growth in MA medium put it at a relatively higher incidence rate. *Geotrichum* was identified mainly at the beginning and middle of the rainy season; they were found with some yeasts. *Epicoccum* was isolated in mid-August to September. *Nigrospora* gave peak isolations during the months of December to February and low values were obtained during the remainder of the year.

Most of the isolated organisms are of economic significance either in medicine or in agriculture. Consequently, further studies on their role in contamination of drugs and pharmaceuticals are being studied.

There are observations that the spore population of the atmosphere was high at the beginning of the dry season, i.e., October; thereafter there was a gradual decrease to the middle of the dry season, i.e., December, after which an upward trend was observed. In recognition of the possibility of viable hyphal fragments, as reported by Pady and Gregory (14), it is conceivable that hyphal fragments are included in this study of the airborne fungal population. What would emerge, however, is that most of the viable units of fungi, namely, spores and hyphal fragments, would have been isolated. This study has therefore been able to supplement the work of Dransfield (5) in northern Nigeria in approaching a detailed survey of fungal air spora in Nigeria.

ACKNOWLEDGMENTS

This work was supported by a research grant from the University of Ife research funds.

I am grateful to the Director of the Commonwealth Mycological Institute, Kew, England, for making the services of his staff available in confirming the detailed identification of some of the isolated organisms.

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