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AIRBORNE FUNGI ISOLATED FROM RAIN WATER COLLECTED IN MEXICO CITY

IRMA ROSAS, CARMEN CALDERON, SARA GUTIERREZ AND PEDRO MOSIÑO

Departamento de Química Atmosférica y Estudios del Agua, Centro de Ciencias de la Atmósfera, UNAM, Coyoacán, 04510, México, D. F.

RESUMEN

Se llevó a cabo una investigación sobre los hongos presentes en la atmósfera de la Ciudad de México, a través del análisis del agua de lluvia colectada durante los meses de junio a septiembre de 1982. Para lograr el aislamiento de una amplia gama de hongos se utilizaron los medios de Sabouraud dextrosa agar y papa dextrosa agar, incubados a temperatura ambiente (20-26°C) por 48-72 h. Durante el muestreo se registraron entre 600 y 6000 colonias por ml. Los hongos que aparecieron con más frecuencia fueron *Cladosporium, Alternaria, Penicillium* y levaduras. Se estableció la relación que existe entre la abundancia de estos organismos en el aire y los factores ambientales.

Se observó que la velocidad del viento estuvo relacionada con la abundancia de los hongos en el agua de lluvia con una r de 0.80 ($p < 0.01$). La identificación de algunas colonias a nivel de especie, tales como *Penicillium citrinum, P. clavigerum, Fusarium semitectum, Aspergillus niger, A. candidus, A. glaucus* y *A. tamarii,* permitió señalar la presencia de hongos patógenos o alergenos.

Esta información puede servir de base para estudios futuros sobre la implicación de los factores ambientales en la abundancia y dispersión de hongos importantes para los sectores médico y agrícola.

ABSTRACT

A research was carried out on the fungi present in the atmosphere of Mexico City, through the analysis of rain water collected from June to September of 1982. To obtain data on a wide range of fungi, isolations were made on Sabouraud dextrose agar and potato dextrose agar plates incubated at the room temperature of 20-26°C for 48-72 hours.

During the sampling period 600 to 6000 colonies per ml were recorded. The most frequent fungi were *Cladosporium, Alternaria, Penicillium* and yeasts. The relationship between these airborne organisms and weather elements was established.

It was found that wind speed was related to fungal counts through a correlation coefficient of $r = 0.80$ ($p < 0.01$). Identification at the species level of some fungi, such as *Penicillium citrinum, P. clavigerum, Fusarium semitectum, Aspergillus niger, A. candidus, A. glaucus* and *A. tamarii,* allowed to show the presence of both pathogenic and allergenic fungi.

This information could be used in further studies of a medical or agronomical character about the effect of environmental factors on the abundance and dispersion of airborne fungi.

INTRODUCTION

It has been shown by several research papers that the atmosphere bears microorganisms which are able to survive in this adverse, gaseous environment for a considerable lenght of time (Schlichting 1969, Edmons 1979, Jones and Cookson 1983, Imshenetskii *et al.* 1984). Among these microorganisms, about 1 200 species of both bacteria and actinomycetes have been identified, more than 100 000 different types of pollen grains, and abundant spores from aproximately 40 000 species of fungi, many mosses, liverworts and ferns (Gregory 1973).

A great diversity of fungi has been isolated from the atmosphere either through direct sampling of the air or by the collecting of rain water (Overeem 1937, Gregory *et al.* 1955, Pady 1957, Maguire 1963, Goodman *et al.* 1966). Gregory (1960) suggests that an efficient method for the sampling of microorganisms present in the air is to collect water and culture it on certain agar media. Thus a great amount of algae, protozoa and fungal spores have been isolated from samples of rain water (Schlichting 1961, Davies 1961, Mc Donald 1962, Maguire 1963, Starr and Masson 1966).

It is interesting to know the identity and amount of the fungal spores, because they may produce disease in man and plants, as some of them have been reported as phytopathogenic (Nutman *et al.* 1960, Vander Plank 1967), and as producers of mycotoxins which may contaminate food and storaged products (Moreno 1977, Horsfall and Cowling 1978). Also, it has been reported that diverse fungi are pathogenic for both animals and man (Jungerman and Schwarts 1972, Rippon 1974, Alexopoulos and Mims 1979). The inhalation of these type of microorganisms may induce allergenic reactions in man, so the exposure to high concentractions of them implies a risk to health (Hyde *et al.* 1956, Sandhu *et al.* 1964).

To acquire a knowledge of the behaviour of fungal spores in the atmosphere, it is not enough to obtain information on its biology, but also it is necesasary to generate information on the environmental factors that affect both their release and distribution (Rich and Waggoner 1962). In the present paper it was considered important to isolate the fungi present in rain water, as a first stage in the research of the airborne mycoflora of Mexico City.

MATERIALS AND METHODS

The sampling site is at the Center of Atmospheric Sciences in the campus of the National University of Mexico, located southewest of downtown Mexico City. The Federal District plus its surroundings represent a population of about 17×10^6 inhabitants, in 3.1×10^6 habitational units, 130 000 factories and 3×10^6 vehicles. The valley in which Mexico City is located is considered one of the most contaminated areas of the world.

The rain water samples were collected by means of a sampler, which consisted of an iron bars frame, 2 m in height which supports a funnel of 28 cm in diameter seated on an sterilized Erlenmeyer flask (Maguire 1963). The funnel was covered with aluminium foil up to the moment the rain started.

For isolating the fungi from rain water samples, a medium of potato dextrose agar (PDA) with streptomycin was used. The rain water was subjected to dilutions of 0.1, 0.01 and 0.001. With these subsamples the culture medium was inoculated and incubated for 3 days at room temperature; afterwards, the colonies that developed different characteristics were counted and transfered to new Petri dishes containing sterile Sabouraud dextrose agar to identify the developed fungal cultures.

To observe the fungal structures under the microscope, microcultures were made which were fixed and stained with lactophenol cotton blue. To further identify the fungi at the genus level the following works were consulted: Fitzpatrick (1930), Raper and Fennel (1965), and Barnet and Hunter (1972). In some cases the identification was made at the species level using the books of Raper and Fennel (1965), and of Pitt (1985).

RESULTS

Twenty three samples of rain water obtained from the Mexico City area were taken, and a total of 23 genera of fungi were identified belonging to the classes Blastomycetes, Hyphomycetes, Coelomycetes and Zygomycetes. The fungi most frequently found during the sampling period were species of *Cladosporium, Alternaria* and *Penicillium,* as well as yeasts (Table I). The identification of some fungal colonies at the species level was done, including the following: *Penicillum citrinum* Thom, *P. clavigerum* Demelius, *Fusarium semitectum* Berk & Rav., *Aspergillus niger* van Tiegh., *A. candidus* Link, *A. flavus* Link, *A. glaucus* Link, and *A. tamarii* Kita. From Fig. 1 it can be deduced that in the rainy months (from June through September) the most abundant fungi were *Cladosporium* and yeasts. The numbers obtained show that the heavy rainfall of June carry few fungal spores, whereas the August rains contain great amounts of fungal spores, in spite of the lesser rainfall recorded in this month (Table II). It is important to point out that hourly counts of fungi show two peaks related to the starting time of rain, one at 12:30 and another at 16:30; the former peak is the larger one (Fig. 2). Weather ele-

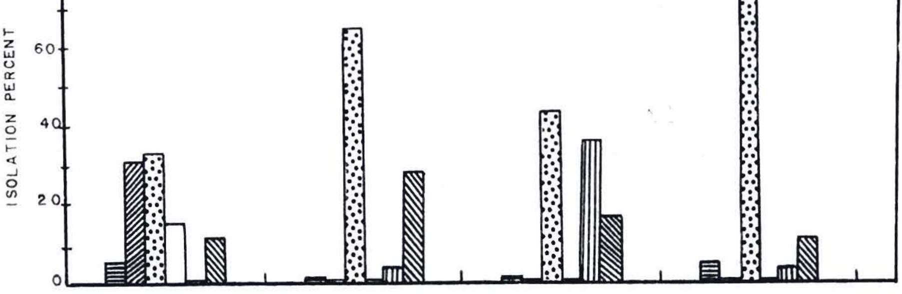

FIG. 1. Fungi isolated from rain water collected at the University of Mexico City.

TABLE I. PERCENT FREQUENCY OF OCURRENCE OF FUNGI ISOLATED FROM RAIN WATER COLLECTED AT THE UNIVERSITY OF MEXICO

Taxa	*Frequency*
CLASS HYPHOMYCETES	
Alternaria	68.4
Aspergillus	29.4
Botrytis	26.6
Chalara	3.8
Cladosporium	100.0
Epicocumm	39.2
Fusarium	41.8
Geotrichum	15.2
Gonotrichum	3.8
Monilia	38.0
Penicillium	64.6
Pyricularia	3.8
Phynchosporium	3.8
Scopulariopsis	3.8
Trichoderma	41.8
Verticillium	3.8
CLASS COELOMYCETES	
Asteromella	15.2
Chaetophoma	15.2
Dothiorella	3.8
Phoma	3.8
Peyronellaea	11.4
Phyllosticta	3.8
CLASS ZYGOMYCETES	
Rhizopus	7.6
CLASS BLASTOMYCETES	
Yeasts (unidentified)	39.5

ments recorded during the sampling periods are shown in Table III. As to temperature and relative humidity, these ranged from 13.9° to 19°C and 53.2 to 78.2%, respectively.

TABLE II. FUNGAL COUNTS FROM RAIN SAMPLE AT THE UNIVERSITY OF MEXICO CITY

Date	*Colonies/ml*	*Rainfall mm*	*Rain starting time*
JUNE			
14	688	3.0	19.00
23	1 251	4.3	11.10
29	605	7.5	20.45
30	813	1.8	13.50
JULY			
02	707	10.9	15.25
12	1 658	18.7	17.00
27	2 620	11.6	16.30
AUGUST			
02	2 071	6.6	19.50
03	4 444	1.2	16.30
09	782	11.6	18.40
10	3 958	1.0	18.45
11	1 329	21.0	21.00
12	5 025	0.1	13.00
16	792	0.1	19.00
18	1 403	0.6	18.55
19	1 746	23.2	15.30
24	6 256	0.2	16.30
25	1 325	4.3	20.55
SEPTEMBER			
08	750	16.2	16.20
13	1 700	21.0	21.00
20	3 754	14.2	14.20
21	2 730	16.2	16.20
22	1 328	13.6	13.50

The sultriness index was low in general, its values ranging between 116.3 and 302.7 mb °C. The winds were light and from the NE. In correlating further fungal counts with the weather parameters above mentioned, it was found that a relationship exists with wind speed $r = 0.81$ ($p < 0.01$). However, the correlation coefficients for temperature and relative humidity were low (Table IV). The sultriness index and the rainfall show a good relationship with $r = 0.40$ ($p < 0.05$).

Fig. 3 shows that the highest counts were obtained with the highest recorded wind speeds, mostly with a northerly wind. However, it must be pointed out that the lighter winds were the most frequent.

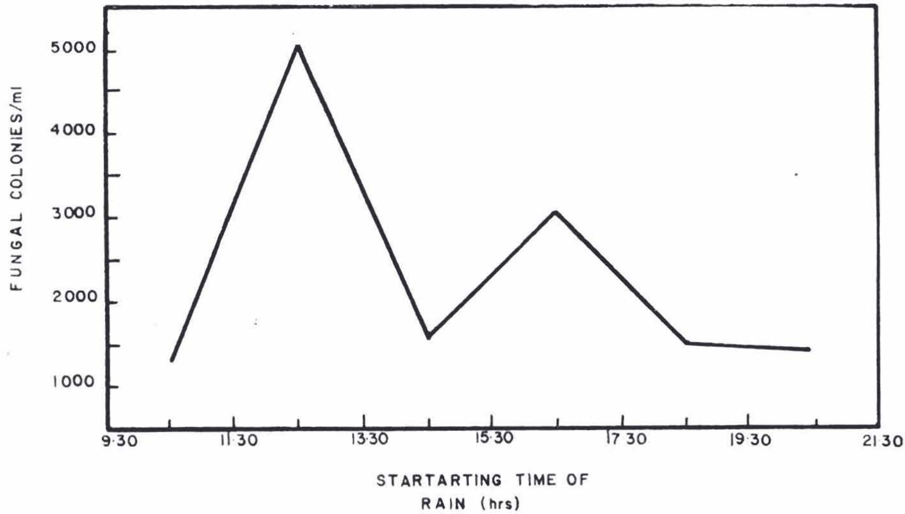

FIG. 2. Relationship between fungal colonies in rain water and starting time of the rain.

DISCUSSION

Through the mycological analysis of rain water, several types of fungi were found in the atmosphere of Mexico City. The atmospheric washout that rainfall exerts is able of removing spores and other fragments of fungi. It was also observed that some environmental factors exert an influence on the concentration of these microorganisms in the air. The measure of the abundance of isolated fungi is referred to as colonies per ml, regardless of the quantity of spores suspended in the air, since it has been proved that hyphal fragments are present in the air and that they are able to develop into colonies (Pady and Kramer 1960).

Research done in different parts of the world on some qualitative aspects of airborne fungi, show that there are basic groups of fungi of universal distribution (Harris 1950, Dye and Vernon 1952, Morrow *et al.* 1964, Ogunlana 1975). Most of the isolated fungi reported in the present paper fall within such a group.

The 23 genera isolated through the 23 samplings belong to the airborne fungi more commonly found in tropical areas (Kramer *et al.* 1963, Goodman *et al.* 1966). Among the fungi more frequently isolated are species of *Cladosporium, Alternaria, Fusarium, Penicillium* and *Aspergillus,* as well as unidentified yeasts. The first genus was the most abundant.

Pady *et al.* (1962) and Rich and Waggoner (1962) report *Cladosporium* as one of the most common fungi found in the air, which is probably due to its high resistance to atmospheric conditions (Pady 1957). It has also been observed that there are environmental factors that determine both the release of spores and their introduction in the atmosphere, to the extent that being a soil fungus it is more frequently found in the air (Gregory 1973). The amount of *Cladosporium* recorded could be due to the fact that the University campus is surrounded by green areas which provide adequate substrata for colonization; Richards (1956) and Davies *et al.* (1963) refer to it as an open field fungus. Not so for *Penicillium* which was less abundant during the sampling periods, confirming that it is a fungal species characteristic of urban areas and common in home dust (Davies 1960). *Alternaria* and *Fusarium* were also present but never in great amounts. Despite the determinations were made in rain water samples and not referred to as countings per cubic meter, it was posible to detect some relationships between abudance and the weather conditions.

TABLE III. WEATHER CONDITIONS AT THE UNIVERSITY OF MEXICO DURING THE SAMPLING TIME

Date	*Air Temperature °C*	*Relative Humidity %*	*Sultriness Index mb °C*	*Wind Direction*	*Wind Speed m/s*
JUNE					
14	19.6	58.3	196.0	N-NE	0.8
23	18.5	66.7	156.8	SE	0.9
29	17.1	69.2	171.0	N-NE	0.8
30	16.3	73.9	166.3	S-SW	0.8
JULY					
02	16.3	63.6	143.4	E-NE	1.6
12	16.9	66.9	163.9	NE	1.0
27	13.9	78.2	132.0	N-NE	1.3
AUGUST					
02	17.6	67.3	179.5	NW	1.3
03	15.7	68.2	144.4	NW	2.5
09	12.1	76.1	96.8	SE	1.2
10	16.4	69.0	159.0	NE	1.8
11	15.6	68.6	143.5	NE	0.9
12	15.2	62.9	124.6	N	1.6
16	17.4	53.2	135.7	SE	0.9
18	16.7	62.6	150.3	NE	0.9
19	15.6	72.0	151.3	NE	1.0
24	17.5	59.5	152.2	NE	2.0
25	18.4	59.0	174.8	NW	1.0
SEPTEMBER					
08	16.1	64.1	302.7	NW	1.0
13	16.9	65.6	160.5	W	0.9
20	15.3	60.6	116.3	NW	2.0
21	16.7	53.9	130.8	N	1.5
22	15.9	68.3	152.6	NE	0.8

Regarding the influence that rain exerts on the fungal content of the atmosphere there exist several opinions. It is important to point out that most of the data refer to *Cladosporium,* and considering that in the present study it was the most abundant, this information may be interesting to discuss. The results show that there is a good inverse relationship between the fungal concentration and the rainfall amount, indicating that the light rains bring down a larger amount of spores than the heavy ones (Mc Donald 1962, Rich and Waggoner 1962).

On relating the fungal content to the starting hour of the rain a double peak was observed coinciding this with the known behaviour of *Cladosporium* during the hours of light. It is considered that the first peak coincides with the start of mechanical turbulence; with the spores reaching great heights between 13:00 and 15:00 hours, so that spore amounts dilute into a larger volume of air by thermal turbulence, giving low counts in the samples of rain water within that lapse of time. The second peak coincides with the reduction of thermal turbulence that permits the descent of the spores down to the collectors's height. Similar findings have been reported by Cammack (1955) and Rich and Waggoner (1962).

Among the most frequently isolated fungi there were those with dry spores, for which there is no mechanism for their active release, responsible for the introduction of these fungi into the air, but environmental factors such as strong wind, thermals and the splashing up of the rain may be the only factors responsible for both the release and dispersion of this kind of fungi in the atmosphere. There are some data on the fluctuation of the quantity of spores because of weather elements (Hirst 1953, Hamilton 1957, Zoberi 1964, Gregory 1973).

TABLE IV. CORRELATION COEFFICIENTS BETWEEN FUNGAL COUNTS AND WEATHER

DEPENDENT VARIABLE	Fungal colonies/ml			
		r[a]		p
INDEPENDENT VARIABLE				
	Relative humidity	0.13	>	0.05
	Temperature	0.20	>	0.05
	Sultriness index	0.41	<	0.05
	Wind speed	0.81	<	0.01
	Rainfall	0.40	<	0.05

[a]Pearson's correlation coeficcient

The present paper shows that wind speed influenced the spore concentration in rain water. It was observed that fungal spore counts increased as wind speed also increased, and that during the sampling hours prevailed the winds from the north; the winds from the south had little influence because of their low velocity, coinciding with the data of Kramer *et al.* (1963), and Hammet and Manners (1971).

Among the fungi identified at the species level, some of them are known to be pathogenic to human and/or animals, such as *Aspergillus flavus, A. niger* and *Penicillum citrinum* (Emmons *et al.* 1970), and as phytopathogenic *Aspergillus candidus, A. flavus, A. niger,* and *Fusarium semitectum* (Moreno 1977).

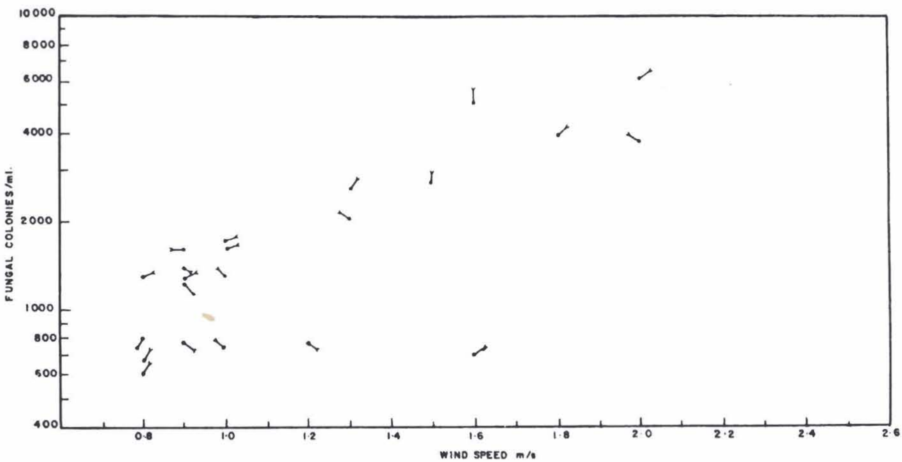

FIG. 3. Relationship between fungal colonies and wind speed. (Arrows show wind direction).

Together with the above it can be pointed out that fungi are a prominent component of aerial biota and it is very important to generate both qualitative and quantitative information through both the diurnal and seasonal variation of these microorganisms in the atmosphere of Mexico City. It is believed that the information obtained on airborne fungi brought down by rainwater can serve as a base for the future evaluation of the relationships between the weather and the mycology of the air.

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