

Fungal content of air samples from some asthmatic childrens' homes in Mexico City

Beatriz Escamilla Garcia^{a,c}, Paul Comtois^{a,c}, Pablo Cortes Borrego^b

^aDépartement de Géographie, Université de Montréal, C.P. 6128, Montréal, H3C 3J7, Canada

^bServicio de Pediatría, Instituto Nacional de Enfermedades Respiratorias, Calzada de Tlalpan 4502, Tlalpan, C.P. 1408, Mexico, D.F., Mexico

^cRespiratory Health National Center of Excellence, Montréal, Canada

Received 24 June 1994; accepted 11 August 1994

Abstract

Fungal particles can be considered a potential risk factor in children causing asthma, rhinitis, and allergy. However, a direct relationship between mold exposure and respiratory symptoms is difficult to establish, particularly if volumetric results are not well documented, as is often the case in Mexico. In order to assess mold exposure in some asthmatic childrens' homes, eight asthmatic children were selected. For each child, lung function was measured by spirometry and the flow expiratory volume drop in the first second (FEV₁) as well as the CVF were estimated at 5, 10 and 15 min, after a running period on a treadmill. Exercise induced asthma (EIA) was defined as a FEV₁ drop > 10% or FEV₁/CVF drop > 10%. Atopy was estimated by skin testing of allergen extracts. Each room was volumetrically sampled using Burkard Personal samplers, every week for a month and with different degrees of activity. Fungal composition was mostly dominated by allergenic molds, spores smaller than 10 µm and non-seasonal molds. Concentrations ranged from 0 to 2087 CFU/m³ and 102 553–29 522 200 spores/m³, being significantly higher in indoor air, but the differences between rooms or weeks were not significant, either for CFUs ($P > 0.27$) or for total particles ($P > 0.80$). In general, mold counts were dominated by *Penicillium* spp. and *Cladosporium* spp. especially *P. aurantiogriseum* and *C. cladosporioides*. We observed that the median concentration values were in general higher for children with exercise induced asthma (EIA). But the differences were significant only for EIA and degree of asthma particularly for total ($P = 0.038$), mycotoxin producers ($P < 0.009$), seasonal molds ($P < 0.006$) and *Penicillium* spp. ($P = 0.012$). Total spore counts showed the highest median values in children without EIA, moderate asthma degree and no atopy. Significant differences were found for almost all spore type groupings when comparing the presence of EIA ($P < 0.05$) or asthma degree categories ($P < 0.030$), but no differences were observed for atopy ($P > 0.21$). We conclude that respiratory symptoms as described by EIA, asthma severity and atopy may give a good correlation with fungal concentrations and especially comparisons based on their physical and chemical properties.

Keywords: Indoor air; Asthma; Children; Fungi

1. Introduction

Fungal spores have long been considered a potential risk in asthma and allergy. Those respiratory illnesses are most frequently found in children (Suliaman and Townley, 1987). It was also observed that asthma incidence and mortality caused by asthma are increasing in many countries (Ayres, 1986; Mao et al., 1987; Sly, 1988; Burney, 1988). In tropical and subtropical areas, allergies and asthma caused by fungal spores are considered more important than the same illness caused by pollen grains. In Mexico, 66–88% of asthmatics were found to be allergic to molds (Cueva, 1960; Baeza-Bacab et al., 1987; Cadena Bustamante, 1990). However, a

direct relationship between mold exposure and respiratory symptoms is difficult to establish, notably because studies made in Mexico, especially volumetric results, are non-existent. We consider that, to optimize asthma and allergy treatment and prevention, two different but interrelated aspects should be studied: (1) the determination of a relationship between biological air content and asthma symptoms and (2) the characterization of the indoor environment where the child is spending most of his/her time, usually the home. These two aspects are in fact the last and first phases of the aerobiological pathway (impact and sources).

In Mexico, indoor mold surveys have been available since the early 50s, most of them using gravitational deposition on agar plates (Backaller, 1955; Cueva and Tellez, 1958; Cueva et al., 1967; Venegas et al., 1969).

* Corresponding author.

No results of previous indoor volumetric sampling were found in the literature by the authors. This study was carried out in order to assess mold exposure in some asthmatic childrens' homes in the southwestern part of Mexico City. This area was chosen based on the fact that a specialized hospital for respiratory illness is located in this district (National Institute of Respiratory Diseases, INER), and a high number of outpatients coming from the same area can easily be followed.

2. Materials and methods

In our study, eight asthmatic children, aged from 7 to 11 years were selected. For each child, lung function was measured by spirometry (Flowmate, T.M., Spirometrics, Inc.). The flow expiratory volume drop in the first second (FEV₁) and the vital capacity forced (CVF) were estimated at 5, 10 and 15 min after a running period on a treadmill. During the exercise, each patient was allowed to attain 85% of their maximal cardiac fre-

quency, as described by Jones et al. (1982). Exercise induced asthma (EIA) was defined as a FEV₁ drop > 10% or a FEV₁/CVF drop > 10%. Atopy was also estimated by skin testing with seven different extracts, including those most frequently found indoor: *Dermatophagoides farinae*, *D. pteronyssinus*, Mold mix, *Aspergillus* mix, *Penicillium* mix, *Alternaria alternata* and *Cladosporium cladosporioides* (Miles, Canada).

The eight houses were thoroughly investigated during November 1922, this being one of the months when the highest rate of hospital visits for asthma attacks has been reported in the INER (National Institute of Respiratory Diseases), in the past 7 years (Chapela, pers. commun.). Samples were taken once a week in every room inside the house, and outside. Samples of 5 min each were collected, and the hour and day of sampling was varied in order to evaluate mold exposure with different degrees of activity. Volumetric sampling was carried out using Personal Burkard air samplers. Viable particles (CFUs) were collected by drawing 15 l/min for 5 min, onto Petri dishes containing 35 ml of malt extract agar and were later incubated at 24°C for a week. After incubation, viable samples were counted and identified. Total particles (spores) were impacted on a vaseline-hexane mixture (1:5 w/v) directly on a 75 × 25 mm microscope slide using a personal sampler set at 10 l/min, also for 5 min. During the sampling period, parameters such as temperature, humidity and home characteristics were also recorded.

Table 1
Isolation frequencies (%) for taxa in indoor and outdoor air

Spore types	Indoor	Outdoor
<i>Cladosporium</i>	100	100
Other ascospores > 10 µm	88	90
<i>Aspergillus/Penicillium</i>	85	93
<i>Coprinus</i>	80	90
<i>Alternaria</i>	60	53
<i>Epicoccum</i>	49	93
Other basidiospores > 10 µm	46	36
Other ascospores < 10 µm	41	46
<i>Torula</i>	39	57
<i>Camarosporium</i>	33	30
<i>Ustilago</i>	33	53
Other basidiospores < 10 µm	28	40
Rust	27	40
<i>Nigrospora</i>	21	56
<i>Ganoderma</i>	21	40
<i>Myxomycetes/Smuts/Periconia</i>	20	100
<i>Agrocybe</i>	20	30
<i>Drechslera/Helminthosporium</i>	13	47
<i>Leptosphaeria</i>	9	20
CFUs		
<i>Penicillium</i>	97	100
<i>Cladosporium</i>	95	100
<i>Alternaria</i>	60	80
<i>Penicillium aurantiogriseum</i>	58	56
<i>Phoma</i>	39	30
<i>Cladosporium cladosporioides</i>	32	36
<i>Alternaria citri</i>	28	33
<i>Aspergillus</i>	32	37
<i>Fusarium</i>	22	16
<i>Penicillium viridicatum</i>	20	16
<i>Monilia sitophila</i>	17	37
<i>Alternaria alternata</i>	15	20
<i>Penicillium chrysogenum</i>	15	20
<i>Aspergillus niger</i>	10	20
<i>Nigrospora</i>	9	20

Table 2
Isolation frequencies of taxa with ability of mycotoxin production^a

Species	Indoors	Outdoors
<i>Penicillium aurantiogriseum</i>	57.8	56.7
<i>Penicillium viridicatum</i>	19.8	16.7
<i>Alternaria alternata</i>	15.5	20.0
<i>Penicillium chrysogenum</i>	14.7	20.0
<i>Penicillium brevicompactum</i>	12.9	3.3
<i>Aspergillus niger</i>	10.3	20.0
<i>Aspergillus flavus</i>	8.6	0.0
<i>Penicillium citrinum</i>	8.6	6.7
<i>Aspergillus ochraceus</i>	6.0	0.0
<i>Aspergillus versicolor</i>	4.3	3.3
<i>Penicillium griseofulvum</i>	4.3	3.3
<i>Aspergillus fumigatus</i>	3.4	3.3
<i>Fusarium oxysporum</i>	3.4	10.0
<i>Aspergillus candidus</i>	2.6	0.0
<i>Penicillium purpurogenum</i>	2.6	0.0
<i>Trichoderma viride</i>	2.6	3.3
<i>Aspergillus parasiticus</i>	1.7	3.3
<i>Penicillium janthinellum</i>	1.7	3.3
<i>Penicillium verrucosum</i>	1.7	0.0
<i>Aspergillus glaucus</i>	0.9	0.0
<i>Aspergillus wentii</i>	0.9	0.0
<i>Penicillium crustosum</i>	0.9	0.0
<i>Penicillium oxalicum</i>	0.9	0.0
<i>Pithomyces chartarum</i>	0.9	0.0

^aWillie and Morehouse, 1977; Betina, 1989.

3. Results

The air we breathe contains an assemblage of different mold species and the different properties of these particles could have an impact on health. For that reason, our results were analyzed using some physical and chemical properties of fungal spores. Those properties were allergenicity, ability of mycotoxin production, spore size and seasonal characteristics of mold assemblages (Willie and Morehouse, 1977; Koivikko, 1984; Ellis and Ellis, 1985; Betina, 1989).

Seventeen different mold genera and 47 spore types were identified. Table 1 shows the decreasing order of taxa with an isolation frequency greater than 20% either in indoor or outdoor air. It can be seen that mold composition in both sites shows no significant differences, either for colonies (CFUs) or total spore counts. There was also a strong association between rooms for both CFU ($P = 0.003$) and spore ($P = 0.0029$) concentrations.

Table 2 shows the frequencies of isolation for all the possible mycotoxin producers found in Mexican houses.

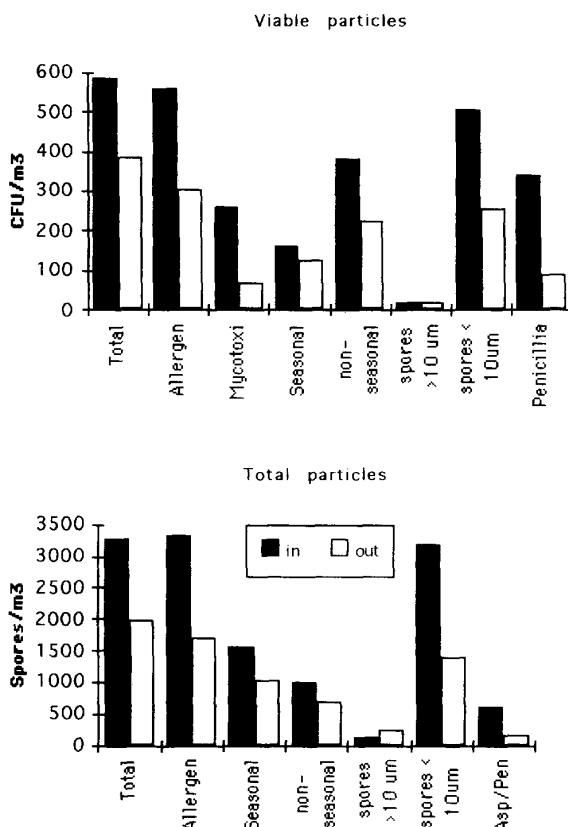


Fig. 1. Median concentration of different mold groupings found indoors and outdoors.

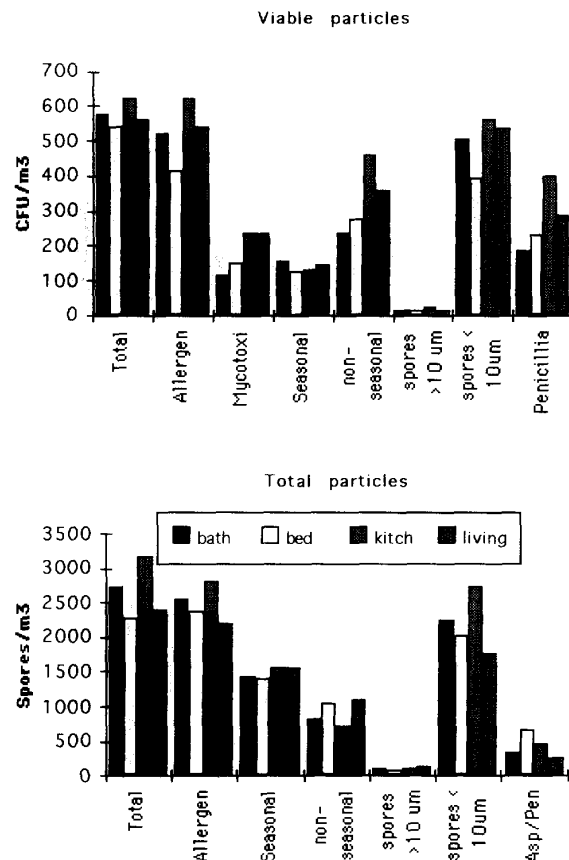


Fig. 2. Median concentration for each mold type recorded in the different rooms.

It can be seen that *Penicillium* spp. were the most frequent, and showed the highest diversity of species.

In general, mold counts were dominated by *Penicillium* spp. (54.7%) and *Cladosporium* sp. (26.8%), *P. aurantiogriseum* (15.6%) and *C. cladosporioides* (11.7%) being the predominant species.

CFUs mold assemblages were mostly dominated by allergenic molds (94.6%), spores smaller than 10 µm (89.5%) and non-seasonal molds (65.5%). These three groups were also the most important for total particles, especially the spores smaller than 10 µm (97.6%).

Fungal concentrations ranged from 0 to 2087 CFU/m³ and 102 553–29 522 200 spores/m³, being significantly higher indoors. Fig. 1 shows the median concentration values recorded for each mold group in both environments. The median was significantly higher inside the houses for all mold types ($P < 0.027$) except seasonal molds ($P > 0.06$) and spores >10 µm ($P > 0.53$). This difference was also of a higher magnitude in CFUs than in spore counts (all $P < 0.027$).

By comparing fungal concentrations between rooms

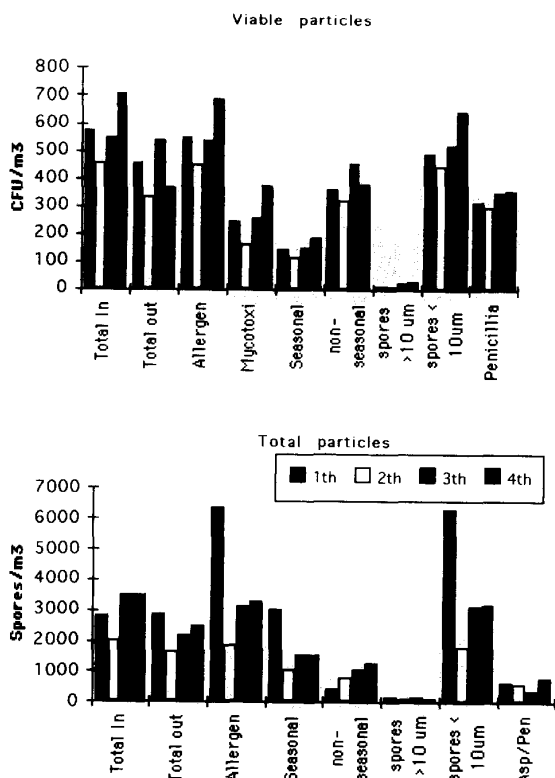


Fig. 3. Week-to-week variation of median values for the different grouping of fungal particles.

(Fig. 2), we observed that the lowest median was found in the child's bedroom, and the highest in the kitchen, but differences between rooms were not significant, either for CFUs ($P > 0.27$) or for total particles ($P > 0.80$).

We also analyzed the week-to-week variation of airborne fungal particles in the house (Fig. 3). For both viable particles and total spores, the lowest concentrations were recorded in the second week of the study, rising to a concentration 1.5 times higher in the last week of the study. Despite this variability, no significant differences were found for CFUs ($P > 0.18$) or for total spores ($P > 0.12$) for any of the mold types.

4. Relationship to symptoms

We observed that the median concentration values were in general higher in houses of children with exercise induced asthma (EIA) defined by a FEV_1 drop $> 10\%$, and in atopics. For all the criteria used to separate asthmatics (Table 3), we noted that mycotoxin producers ($P < 0.009$) and seasonal molds ($P < 0.006$) showed significant differences. For total counts, the difference was significant only by using a FEV_1 drop $> 10\%$ while for *Penicillium* spp. and non-seasonal molds ($P < 0.030$), the different asthma degrees were important.

Total spore counts (Table 4) showed the highest median values in children without EIA (FEV_1 drop or FEV_1/CVF drop $> 10\%$), moderate asthma degree and

Table 3
Median values for mold counts according to respiratory symptoms

Mold type	FEV ₁ drop > 10%			FEV ₁ /CVF drop > 10%			
	(+)	(-)	P-value	(+)	(-)	P-value	
Total	595	497	0.038	563	454	0.455	
Allergenic	573	483	0.054	534	536	0.531	
Mycotoxins producers	160	206	0.002	160	286	0.000	
Seasonal	190	97	0.002	180	83	0.001	
Non-seasonal	366	265	0.914	334	353	0.860	
Spores > 10 μm	13	13	0.491	13	13	0.178	
Spores < 10 μm	540	458	0.061	514	506	0.433	
<i>Penicillium</i>	320	227	0.754	266	270	0.100	
	Asthma degree				Atopy		
	Severe	Medium	Low	P-value	(+)	(-)	P-value
Total	488	613	657	0.181	606	562	0.520
Allergenic	441	612	644	0.14	606	539	0.418
Mycotoxins producers	229	544	197	0.001	367	197	0.009
Seasonal	183	5	225	0.006	122	175	0.051
Non-seasonal	342	593	396	0.03	440	354	0.540
Spores > 10 μm	14	4	23	0.117	12	24	0.320
Spores < 10 μm	425	595	580	0.061	510	504	0.490
<i>Penicillium</i>	247	544	370	0.012	367	327	0.450

Table 4
Median values for total spores according to respiratory symptoms

Spore type	FEV ₁ drop > 10%			FEV ₁ /CVF drop > 10%		
	(+)	(-)	P-value	(+)	(-)	P-value
Total	2334	3620	0.001	2490	5146	0.005
Allergenic	2040	3353	0.001	2164	4800	0.006
Seasonal	1500	1417	0.413	1500	1205	0.125
Non-seasonal	755	1717	0.000	765	3738	0.001
Spores > 10 μm	93	139	0.006	98	139	0.020
Spores < 10 μm	1872	3399	0.002	1967	4259	0.007
<i>Aspergilli</i> / <i>Penicillia</i>	347	2330	0.000	320	1350	0.001

Asthma degree				Atopy			
Severe	Medium	Low	P-value	(+)	(-)	P-value	
Total	1973	6453	3382	0.048	2344	3845	0.210
Allergenic	1700	16 171	3543	0.012	2197	3552	0.490
Seasonal	1376	1487	2176	0.592	1388	2176	0.213
Non-seasonal	757	14 485	1207	0.003	757	1207	0.386
Spores > 10 μm	119	185	148	0.229	119	148	0.410
Spores < 10 μm	1485	15 667	3413	0.011	2105	3113	0.430
<i>Aspergilli</i> / <i>Penicillia</i>	458	4842	847	0.003	458	847	0.380

no atopy. Mann-Whitney and Kruskal-Wallis Tests showed significant differences for almost all spore type groupings when we compared EIA ($P < 0.05$) or asthma degree ($P < 0.030$), but none were observed for atopy ($P > 0.21$).

5. Discussion and conclusions

Indoor fungal concentrations are generally considered to be lower than those found outdoors and some authors suggest that they represent a subproduct of the outdoor exposure (O'Rourke et al., unpublished data). In our study, the indoor concentration was significantly higher than outdoor. Indoor and outdoor mold compositions were represented by the same taxa, but their contributions showed large differences. In Mexico, November 1992 was a particularly rainy month, and unusually wet for that period of the year (Jauregui Ostos, 1975). This change in the seasonal pattern may explain the low fungal concentrations recorded outdoors, especially when compared with those recorded for a rainy season in previous studies, where mold concentration reached 3000 CFU/m³ (Rosas et al., 1990). As for the indoor concentrations found, we can emphasize the fact that the indoor environment of our population was a homogeneous entity that showed no major changes from room to room or from week to week. However, we cannot generalize because volumetric indoor surveys are not well documented in tropical areas and especially in Mexico.

In the different indoor mold assemblages, we observed the same genera found elsewhere in the world

(Hirsch and Sosman, 1976; Kozak et al., 1980; Vergoheoff et al., 1990). Indoor fungal species and spore types are not always described in previous studies, but the taxa collected in our study seem to follow the same pattern as those collected at other sites in North America (Comtois, 1990).

Some other researchers have observed more eye irritations, rhinitis and chest tightness or cough/sputum in asthmatics' households with high fungal concentrations (Lebowitz et al., 1982; Kodama and McGee, 1986).

In our study, we were able to relate mold assemblages or airborne fungal counts to some respiratory symptoms categories described either by EIA or asthma severity, even relying only on a small sampling size. As mentioned above, November 1992 was an extremely rainy period, and previous studies have shown that, in Mexico City, the highest number of hospital visits for asthma are during the rainy season (Chapela, unpublished data). We can therefore consider that our sampling period may represent the situation where asthma crises are usually taking place. Moreover, our results suggest that EIA can be related to fungal exposure, in conjunction with other criteria such as atopy or asthma severity. Finally, fungal grouping comparisons based on their physical and chemical properties can give more information than the sole consideration of total or individual genus counts.

Acknowledgements

The authors would like to thank Dr Irma Rosas (C.C.A., UNAM) for the technical facilities provided

for our analysis, Dr Rocio Chapela (INER), who made contact possible between U.N.A.M. and I.N.E.R. and Dr Alicia Rojas Gonzalez for her technical assistance in the spirometry tests.

References

- Ayres, J.G. (1986) Trends in asthma and hay fever in general practice in the United Kingdom, 1976–83. *Thorax* 41, 283.
- Backaller, F.A. (1955) Hongos atmosféricos de la region Medio Occidental de la República Mexicana. *Alergia* 2, 149.
- Baeza-Bacab, M.A., Ginebra-Cabral, F.A. and Bastarrachea-Sosa, G. (1987) Alergia respiratoria inducida por hongos. *Bol. Med. Hosp. Infan. Mex.* 44, 214.
- Betina, V. (1989) Bioactive Molecules: Mycotoxins, Chemical and Environmental Aspects, Vol. 9. Elsevier, Amsterdam, 437 pp.
- Burney, P. (1988) Asthma death in England and Wales, 1931–85: evidence for a true increase in asthma mortality. *J. Epidemiol. Community Health* 42, 316.
- Cadena Bustamante, J. (1990) Aeroalergenos: Importancia y comportamiento anual en el estado de Tabasco. *Alergia* 37, 215.
- Comtois, P. (1990) Indoor mold aerosols. *Aerobiologia* 6(2), 165–176.
- Cueva, V.J., Venegas, C.M. and Juarez, A.X. (1967) Hongos contaminantes y alergia respiratoria en Zamora, Michoacan. *Rev. Fac. Med.* 9, 159.
- Cueva, V.J. (1960) Características del asma bronquial en México. *Gaceta Médica de México* XC, 197.
- Cueva, V.J. and Tellez, G. (1958) Hongos del interior de la habitación de la Ciudad de Mexico. *Alergia* 6, 243.
- Ellis, M.B. and Ellis J.P. (1985) Microfungi on Land Plants. An Identification Handbook. Croom Helm, London.
- Hirsch, S.R. and Sosman J.A. (1976) A one year survey of mold growth inside twelve homes. *Ann. Allergy* 36(1), 30–38.
- Jauregui Ostos, E. (1975) El clima urbano de la Ciudad de México. *Bol. Inst. Geo.* 6, 47.
- Jones, N.L., Campbell, E.J. and Edwards, R.H.T. (1982) *Clinical Exercise Testing* (2nd edn.). Saunders, Philadelphia, PA.
- Kodama, A.M. and McGee, R.I. (1986) Airborne microbial contaminants in indoor environments. Naturally ventilated and air conditioned homes. *Arch. Environ. Health* 41(5), 306–311.
- Koivikko, A. (1984) In: T. Foucard and S. Dreborg (Eds.), *Mould Allergy Workshop*, Uppsala, Sweden. Ord and Form Pharmacia Diagnostics AB, 1984.
- Kozak, P.P., Gallup, J., Cummins, L.H. and Gillman, S.A. (1980) Currently available methods for home mold surveys II: Examples of problem homes surveyed. *Ann. Allergy* 45(9), 167–176.
- Lebowitz, M.D., O'Rourke, M.K. and Dodge, R. (1982) The adverse health effects of biological aerosols, other aerosols and indoor microclimate on asthmatics and non-asthmatics. *Environ. Int.* 8, 375–380.
- Mao, Y., Semencio, R., Morrison, H., MacWilliam, L., Davies, J. and Wingle, D. (1987) Increased rates of illness and death from asthma in Canada. *Can. Med. Assoc. J.* 137, 620–624.
- Rosas, I., Escamilla, B., Calderon, C. and Mosiño P. (1990) The daily variations of airborne fungal spores in Mexico City. *Aerobiologia* 6, 153–158.
- Sly, R.M. (1988) Mortality from asthma in children 1979–1984. *Ann. Allergy* 60, 433.
- Suliaman, F. and Townley, R.G. (1987) The risk of allergy in asthma. *Prim. Care* 14, 475–490.
- Venegas, C.M., Cueva, V.J. and Juarez, A.X. (1969) Hongos atmosféricos de la ciudad de la Piedad, Michoacan. *Alergia* 16, 18.
- Vergohoeff, A.P., Wijnen, J.H., Boleij, J.S.M., Brunekreef, E.S. and Van Rienen-Hoekstra Samson, R.A. (1990) Enumeration and identification of airborne viable mould propagules in houses. *Allergy* 45, 275–284.
- Willie, T.D. and Morehouse, L.G. (1977) *Mycotoxic Fungi and Chemistry of Mycotoxins*, Vol. 1. Marcel Dekker, New York.