

Long term trends in outdoor *Aspergillus*/*Penicillium* spore concentrations in Derby, UK from 1970 to 2003 and a comparative study in 1994 and 1996 with the indoor air of two local houses

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Abstract

Aspergillus/*Penicillium* spore concentrations have been monitored in Derby since 1970 using a volumetric spore trap, with full year data from 1991. In addition a short comparative study with the indoor air was undertaken at two local houses in 1994 and 1996. *Aspergillus*/*Penicillium* spores were present in the Derby air throughout the year and often reached maximum monthly cumulative concentrations in the autumn, although they were occasionally the dominant spores in the winter when total spore concentrations were low. Very high daily concentrations could occur at any time of year with a count of over 5000 recorded. Peak days in the autumn and winter of 2002–2003 were examined on a two hourly basis showing higher concentrations in the middle of the day. There was a positive correlation of cumulative monthly *Aspergillus*/*Penicillium* totals with maximum temperature. Indoor data from the two houses was examined on a daily basis and compared with simultaneously sampled outdoor daily spore concentrations. The elevated *Aspergillus*/*Penicillium* spore levels found in the older of the two houses occurred on all of the days sampled. Compared to the modern house, the *Aspergillus*/*Penicillium* spore concentrations in the old house represented a much higher percentage of the total spore count than in the modern one. The correlation between outdoor *Aspergillus*/*Penicillium* spore concentrations and the indoor air of the old house was 0.62, whereas in the modern house it was 0.31. Peak hourly samples of *Aspergillus*/*Penicillium* spore counts occurred at times of greatest activity.

1. Introduction

Aspergillus and *Penicillium* spores are known to cause a range of serious respiratory conditions, including allergic asthma and rhinitis, whilst *Aspergillus* is known to cause allergic broncho-pulmonary aspergillosis (Stevens et al., 2000). Raper and Fennell (1965) state that the main external factor in establishing infection in man is the number of *Aspergillus* conidia taken into the body; Mullins and Seaton (1978) found *Aspergillus*

and *Penicillium* spores in sputum and lung tissue and they thought immunosuppressed patients and post open-heart surgical cases may be more prone to invasion by fungal pathogens. Infante Garcia-Pantaleon et al. (1995) reported that 30% of fungus sensitive patients known to the Cordoba University Hospital Allergy service were sensitive to *Aspergillus*.

There have been few all year round studies of *Aspergillus*/*Penicillium* spores in the outdoor UK air but early work by Hudson (1969) on outdoor

air found a tendency for a winter rise in spore concentrations. Studies by Mullins et al. (1984) in Cardiff found *Aspergillus fumigatus* spore concentrations peaked in November. Outdoor *Aspergillus/Penicillium* spore concentrations have been monitored in Derby since 1970, and all year round from 1991 to the present day, making Derby the only site in the UK able to carry out a long-term evaluation. Any increase in understanding of elevated concentrations of *Aspergillus/Penicillium* spores in the outdoor air will be beneficial for both clinicians and patients.

Aspergillus/Penicillium spores have also been found in significant numbers indoors (Lacey, 1981; Gallup et al., 1986; Levetin et al., 1995), and recent work by Reed and Kita (2004) investigates the role of protease activation of inflammation in allergic respiratory disease suggesting that fungi growing in damp, water damaged buildings might increase the prevalence in these buildings of asthma, rhinitis and other respiratory disease. In order to increase our knowledge of *Aspergillus/Penicillium* spore concentrations indoors, we decided to re-investigate the indoor air at two local contrasting houses (Stern et al., 1999), to assess the effect of household activity, and to compare the outdoor and indoor levels of concentration that allergy patients may encounter.

2. Materials and methods

Derby being an industrial city more than 100 km from any coast and bounded on three sides by large areas of arable farming is the third largest city in the East Midlands, with a population of about 230,000. It is located on the River Derwent, to the south and east of the Derbyshire uplands, and on the northern flanks of the Trent valley. From 1970 to 1989 volumetric spore traps (Hirst, 1952) were sited on a second storey roof 10 m above ground level in central Derby, building developments necessitated the site change in 1990 to the roof of Derby University Mickleover site, 10 m above ground level, in a suburb 100 m above sea level and approximately 4 km S.W. of the city centre. The Mickleover site has improved accessibility but maintained optimum sampling height. Monitoring of fungal spores in Derby began in 1970 and for many years extended throughout most of the year (Table 1) but from 1991 pollen and spore monitoring has taken place throughout

Table 1. Daily outdoor *Aspergillus/Penicillium* spore monitoring periods for the years 1970–1990

Year	Date started	Date finished
1970	21st April	15th December
1971	5th May	12th November
1972	25th March	18th November
1973	9th March	31st December
1974	15th March	9th December
1975	25th February	3rd December
1976	3rd April	22nd November
1977	14th April	8th December
1978	11th March	28th November
1979	1st April	14th November
1980	1st April	29th November
1981	1st May	18th November
1982	11th April	31st August
1983	1st May	24th October
1984	20th May	15th October
1985	12th May	13th October
1986	20th May	20th August
1987	31st March	27th October
1988	19th April	31st October
1989	4th February	18th October
1990	3rd February	31st December

the whole year with either daily slides or seven-day drums used, mounting and staining techniques have followed the methods described by Morrow Brown and Jackson (1978). It is not possible to distinguish *Aspergillus* and *Penicillium* spores to species level under the light microscope so we monitored both genera under a joint heading (Li and Kendrick, 1995; Stern et al., 1999). Average daily outdoor concentrations (spores/m³) were calculated by counting one microscope field along the length of the slide at either $\times 400$ or $\times 500$ magnification and then applying the appropriate conversion factor. Monthly cumulative totals were calculated for all years, and correlated with meteorological data. Statistical analysis was carried out using EXCEL, descriptive statistics were calculated for 1991–2003 and trend lines were obtained from available data from 1970 to 2003 and whole year data from 1991 to 2003. Peak daily counts were identified for all years from 1970, and daily slides with the highest *Aspergillus/Penicillium* spore concentrations in September 2002 to February 2003 were chosen and transverse traverses were counted every two hours to observe diurnal variation.

Table 2. *Aspergillus/Penicillium* monthly spore concentrations for 1991–2003

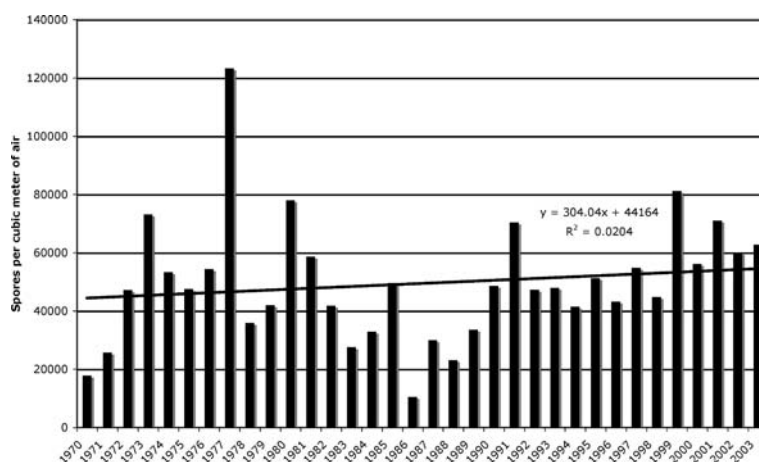
	January	February	March	April	May	June	July	August	September	October	November	December
1991	5810	3520	4805	3640	3580	2782	5790	7335	11505	9395	9145	2985
1992	3602	4380	3730	2400	3005	3975	7149	4800	5490	3622	2527	2456
1993	2655	2753	3202	3097	2675	4320	5201	3345	2767	7322	5745	4678
1994	4607	2805	3074	1917	2591	2991	3803	3246	2220	3384	7446	3236
1995	2404	2957	5469	3944	3205	2807	5389	5752	6375	4176	6578	2078
1996	3344	2047	3092	5220	2068	3227	4504	5246	3607	5164	4583	956
1997	2081	2703	2898	3347	2471	4165	5360	8833	6143	8498	4178	3986
1998	888	2049	2242	2143	3265	4903	4140	5222	6308	4689	5376	3508
1999	8788	6759	4350	3376	9582	6197	6643	3600	6012	13940	8725	2116
2000	1660	1749	6108	4533	4655	4991	7886	6753	6679	4633	3724	2616
2001	10420	6310	4238	4327	3260	5735	7755	5450	3827	8499	8151	2908
2002	4900	3435	4680	3748	2680	4075	5025	8642	7144	6347	6314	2960
2003	5330	5488	6311	4745	2992	4183	5799	4574	7130	5290	6975	3818
Total 1991–2003	56,489	46,955	54,199	46,437	46,029	54,351	74,444	72,798	75,207	84,959	79,467	38,301

The indoor air was sampled by a portable 24 hour continuous recording volumetric sampler (Stern et al., 1999) in a modern (1968) suburban house 5 km S.W. of the city centre and a country cottage (ca. 1810) 18.5 km west of Derby in 1994. The modern property had central heating and double-glazing and sampling took place on 21 days in September and October, whereas the older cottage had neither central heating nor double-glazing and sampling took place on 21 days in October and November. This sampling period was chosen because outdoor *Aspergillus/Penicillium* spore concentrations were usually high in the autumn. In the modern suburban house, the spore trap was in the dining room, 1.29 m above the floor and in the older cottage the spore trap was sited 1.35 m above the floor, in a hall where

the door to the outside was not used. Daily indoor counts from both houses were compared with simultaneous outdoor spore concentrations and the percentage of total spores for *Aspergillus/Penicillium*, *Cladosporium*, *Sporobolomyces* and other spores in each house were compared. In 1996, indoor air in both houses was sampled again in each house and hourly transverse traverses counted to investigate household activity.

3. Results

Aspergillus/Penicillium spores were present in the Derby air all year round from 1991 to the present day. During this time *Aspergillus/Penicillium* spores were only absent from our daily micro-

Figure 1. Derby *Aspergillus/Penicillium* spore concentrations for 1970–2003.

scopically examined sample on a maximum of eighteen occasions in any one year.

When considering 1970–2003 yearly data a slightly increasing long-term trend is observed (Figure 1). The highest yearly concentration was 81,088 in 1999, although a total concentration of 122,585 was reached in only nine months in 1977. Full year data from 1991 to 2003 showed a more apparent upward trend. The highest monthly cumulative totals 1991–2003 often occurred in the autumn (Table 2), the highest monthly count being 13,940 in October 1999. Descriptive Statistics were calculated for the years 1991–2003 (Table 3).

The highest daily count ever recorded in Derby was 5560 spores per cubic meter of air on 28th May 1999 when *Aspergillus*/*Penicillium* spores were present in large numbers between 08.00–10.00 and nearly all the spores appeared to be the same species. High counts were recorded in most years but in 1977 there were 27 days with counts over 1000 (Table 4) including every day from May 28th until June 4th. In the winter, when total spore total concentrations were low, *Aspergillus*/*Penicillium* spores were sometimes the dominant spore type for example *Aspergillus*/*Penicillium* spores were the most abundant spore type on 24 days in the whole of January and February 2001. On peak days, sampled two-hourly from September 2002 through to February 2003, the highest spore concentrations usually occurred towards the middle of the day (Figure 2). The highest two-hourly concentration was 12,654 spores/m³ at 11.00 h 13 February 2003.

Statistical analysis of maximum temperature, minimum temperature, relative humidity and rainfall using monthly data revealed a positive correlation with maximum temperature $r = 0.57$ ($p = 0.05$). The correlation with rainfall $r = 0.39$ was not significant.

In the autumn of 1994, *Aspergillus*/*Penicillium* spore concentrations in the modern house were low when compared with the outdoor air. However, in the old cottage they were always much higher than in the outdoor air and represented a much higher percentage of the total indoor spore concentrations than in the modern house (Figure 3). The correlation between the outdoor air and the indoor air of the old house was $r = 0.62$ ($p = 0.002$) whereas the correlation with the air in the modern house was $r = 0.31$ ($p = 0.002$). In early summer 1996, household activity was always found to be associated with an increase in spore concentrations in

both houses (Figures 4 and 5), with the highest hourly concentration 951 spores/m³ at midnight in the old house on 30 May 1996.

4. Discussion

Aspergillus and *Penicillium* spores are important aeroallergens, which because of their small size can easily penetrate the lungs (Mullins et al., 1976; Mullins and Seaton, 1978). This preliminary long-term study of the outdoor air is the first one undertaken in the UK in order to assist understanding of exposure to these spores. Hudson (1969) over two years showed the highest outdoor concentrations of *Aspergillus*/*Penicillium* spores were in the winter but Morrow Brown and Jackson (1978) looking at June–October data from sites within a 60 km radius of Derby found no seasonal pattern. Our study of all year round data showed Derby outdoor *Aspergillus*/*Penicillium* spore concentrations were highest in the autumn agreeing with the work of Infante Garcia-Pantaleon et al. (1995) in Cordoba, and Larsen and Gravesen (1991) in Copenhagen. Li and Kendrick (1995) in Ontario, Canada reported no distinct diurnal pattern from May to October, but a small rise in the middle of the day in the November to April period which is similar to the rise in Derby in the late morning during the autumn and winter months.

This study of *Aspergillus*/*Penicillium* spore concentrations in Derby showed considerable variation in spore concentrations over a thirty-year period with very high daily concentrations occurring on certain days in the year. These occasional sporadic surges in concentration, (for instance large numbers of spores appeared on 28th May 1999), have implications for patients with asthma, aspergillosis or who are immunosuppressed. *Aspergillus* has been detected in African dust samples (Kellog et al., 2004) and as this dust is regularly transported to Europe it is possible that the sudden spore surges seen in Derby could be partly caused by *Aspergillus*/*Penicillium* transported in Saharan dust. *Aspergillus*/*Penicillium* spores were present in the air throughout the whole year and were the dominant spore in the winter on occasions, especially in recent years, so any patient will be exposed to *Aspergillus*/*Penicillium* spores at all times of the year. It is known that temperatures of between 10 and 12 °C favor high counts of

Table 3. Descriptive statistics of *Aspergillus/Penicillium* spore concentrations 1991–2003

	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003
Mean	193.12363	129.13973	132.29917	114.77778	140.09315	121.83099	150.58678	126.72238	224.6205	154.23416	198.54342	167.4581	172.54821
Standard error	10.554183	6.4248794	6.2294919	6.0242984	6.2575289	6.6376195	7.4406295	6.7350984	18.24952	7.3931903	9.6509563	6.9335266	7.2187527
Median	140	95	104	80	105	80	115	84	165	115	150	130	135
Mode	55	50	44	24	16	0	120	16	12	8	115	55	120
Standard deviation	201.36099	122.74715	118.36035	114.30303	119.54992	125.06233	141.76303	126.54101	346.74089	140.85919	182.34945	131.18848	137.53571
Sample variance	40546.246	15066.862	14009.171	13065.182	14292.184	15640.587	20096.757	16012.627	120229.24	19841.312	33251.322	17210.417	18916.072
Kurtosis	11.029244	5.0290372	6.1939863	7.8769286	3.661802	3.9602164	3.2164108	2.2312835	168.42023	3.5550101	11.78326	2.1456747	7.3436789
Skewness	2.8206753	1.9900743	2.0993515	2.3435362	1.6886825	1.7365333	1.6192656	1.5413356	11.070271	1.5484656	2.6967732	1.3743529	1.9757036
Range	1390	760	804	776	680	775	825	630	5660	900	1445	750	1125
Minimum	0	0	0	0	0	0	0	0	0	0	0	0	0
Maximum	1390	760	804	776	680	775	825	630	5660	900	1445	750	1125
Sum	70297	47136	47760	41320	51134	43250	54663	44733	81088	55987	70880	59950	62635
Count	364	365	361	360	365	355	363	353	361	363	357	358	363
Largest(1)	1390	760	804	776	680	775	825	630	5660	900	1445	750	1125
Smallest(1)	0	0	0	0	0	0	0	0	0	0	0	0	0
Confidence level (95.0%)	20.755019	12.634541	12.250765	11.847348	12.305446	13.054125	14.632286	13.246094	35.889057	14.538995	18.980052	13.635689	14.195957

Penicillium species (Lacey, 1981) so it is likely that they can sometimes dominate the winter air.

Our study of the indoor air reflected the striking differences between the two houses not only of age but also in insulation and heating. The modern house was virtually hermetically sealed when the owners were out because of complete double-glazing, whereas the old cottage had no double-glazing and was not centrally heated. The owners of the modern house were out at work all day during the week, whereas the old cottage was rarely empty, with activity there most of the time. The differences in the correlation between the outdoor and the indoor air at the two houses highlighted the ease with which these small spores entered the old house from outside. Maunsell (1954) found that disturbance could increase *Penicillium* spore concentrations by more than twenty times, and our results agreed with this finding in that the peak counts observed in *Aspergillus/Penicillium* spore levels in both houses were associated with increased activity nearby. The increases in spore levels in the modern house could have been associated with passage in and out via the conservatory, filled with plants, agitation of which is cited by Burge et al. (1982) as likely to cause spores to become airborne. Passage through the hall in the old house increased before people retired to bed, which explains the large increase in spore concentrations around midnight.

The greatly elevated *Aspergillus/Penicillium* spore levels in the old cottage were probably consistent with one or more spore sources in the hall where conditions were favourable for mould growth Lacey (1981) cites handling of organic matter (in this case vegetables and animal feed) as likely to increase airborne spores. Levetin et al. (1995) said that elevated spore levels indoors might indicate an indoor source, that *Aspergillus/Penicillium* spores were often associated with contamination, and were more frequent when disturbed by activity. Dampness, especially of carpets, was cited as an important factor by Gallup (1986), water damage and visible mould by Green et al. (2003), and also dust by Emberlin et al. (1995).

The high concentrations of *Aspergillus/Penicillium* spores sometimes found both in the old house and outdoors in Derby would have implications for allergic and immunosuppressed patients and warrant further study.

Table 4. Frequency of high *Aspergillus*/*Penicillium* spore counts in Derby 1970–2003

Year	Days >50	Days >100	Days >500	Days >1000	Days >2000	Days >3000	Days >4000	Days >5000	Peak count	Peak date
1970	129	60	0	0	0	0	0	0	435	5-Jun
1971	146	104	5	0	0	0	0	0	660	10-Sep
1972	220	179	8	2	0	0	0	0	1730	15-Oct
1973	277	231	14	1	0	0	0	0	1825	15-Oct
1974	258	210	11	1	0	0	0	0	1735	31-Mar
1975	240	194	7	1	0	0	0	0	1030	27-Jun
1976	194	171	20	2	0	0	0	0	1145	13-Oct
1977	228	219	84	27	6	0	1	0	4380	3-Jun
1978	195	146	3	1	0	0	0	0	1820	9-Jul
1979	180	157	12	0	0	0	0	0	760	5-Aug
1980	237	203	47	14	2	0	0	0	2600	4-Aug
1981	176	160	36	5	0	0	0	0	1200	17-Nov
1982	154	129	23	4	0	0	0	0	1400	27-Mar
1983	137	111	11	1	0	0	0	0	1000	17-Jul
1984	114	95	20	3	0	0	0	0	1550	9-Oct
1985	148	141	26	8	1	0	0	0	2145	28-Jun
1986	60	40	2	0	0	0	0	0	520	28-Jun
1987	146	99	6	2	0	0	0	0	1356	14-Jun
1988	124	73	5	2	0	0	0	0	1630	24-Oct
1989	175	117	5	0	1	0	0	0	2550	5-Oct
1990	236	161	18	1	0	0	0	0	1505	24-Aug
1991	304	232	21	5	0	0	0	0	1390	5-Nov
1992	268	179	6	0	0	0	0	0	760	30-Sep
1993	276	192	8	0	0	0	0	0	804	5-Dec
1994	237	145	6	0	0	0	0	0	776	7-Jan
1995	282	197	10	0	0	0	0	0	680	11-Mar
1996	232	158	5	0	0	0	0	0	775	22-Jan
1997	263	201	10	0	0	0	0	0	825	29-Aug
1998	236	159	10	0	0	0	0	0	630	9-Oct
1999	288	237	25	4	0	0	0	1	5560	28-May
2000	272	197	10	0	0	0	0	0	900	17-Nov
2001	310	246	20	3	0	0	0	0	1445	18-Jan
2002	305	228	11	0	0	0	0	0	750	11-Sep
2003	316	239	11	1	0	0	0	0	1125	23-Mar

5. Conclusions

This is the first study to look at long term *Aspergillus*/*Penicillium* spores concentrations in the UK and showed a slightly increasing long-term trend. Higher monthly concentrations are often found in the autumn but very high daily concentrations can be found at any time of year. Continuous monitoring of the indoor environment accurately pinpoints increased concentrations of *Aspergillus*/*Penicillium* spores and possible spore sources within the home. This paper highlights the importance of clinicians having detailed knowledge of both outdoor and

indoor *Aspergillus*/*Penicillium* spore concentrations because of their allergenicity and known effect on patients' health.

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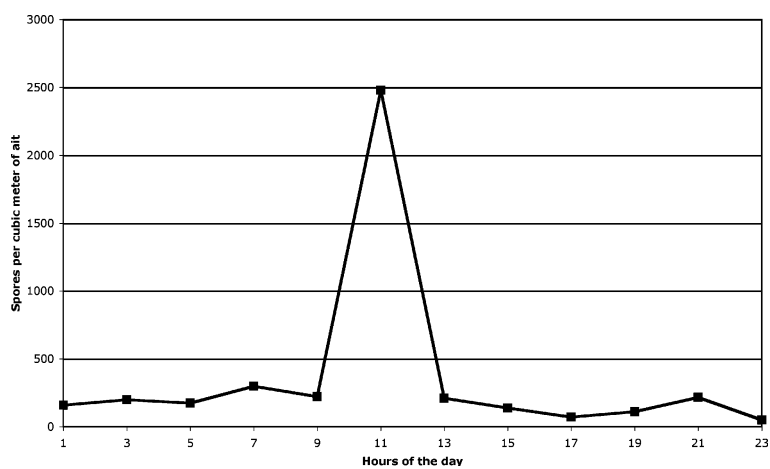
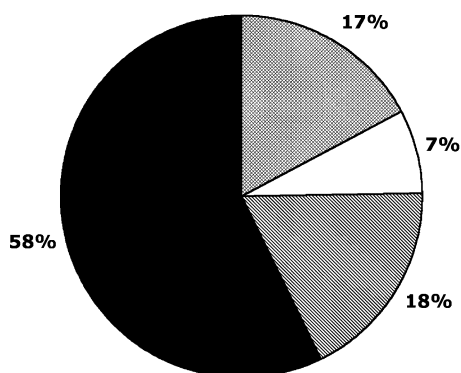


Figure 2. Diurnal periodicity of *Aspergillus/Penicillium* spores 2002–2003.

(a) % OF TOTAL SPORES IN MODERN HOUSE



(b) % OF TOTAL SPORES IN OLD HOUSE

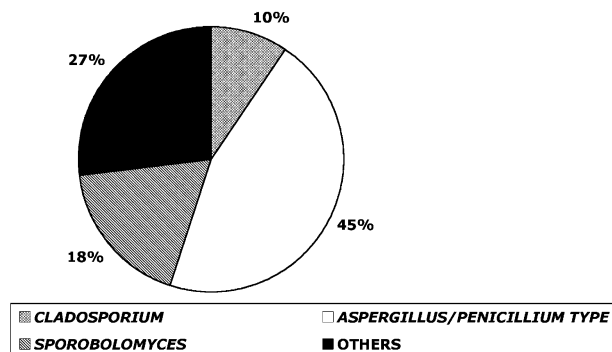


Figure 3. (a) Composition of indoor airspora in modern house for 21 days in September and October 1994; (b) Composition of indoor airspora in old house for 21 days in October and November 1994.

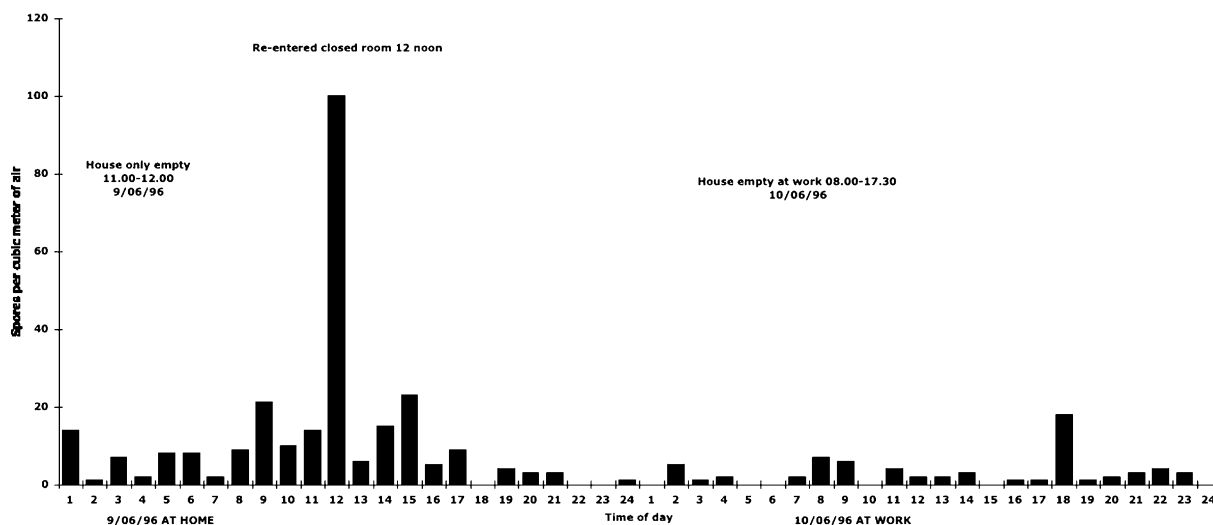


Figure 4. Hourly *Aspergillus*/*Penicillium* spore concentrations in the modern house.

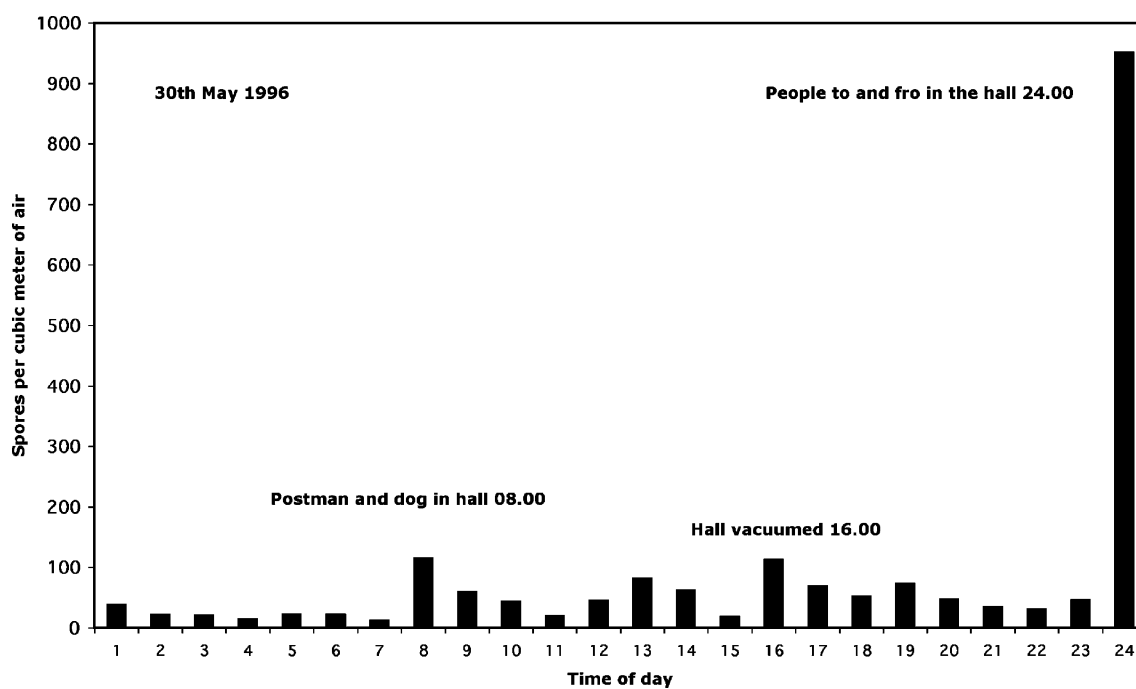


Figure 5. Hourly *Aspergillus*/*Penicillium* spore concentrations in the old house.

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