

High incidence of *Aspergillus* and *Penicillium* spores in the atmosphere of the cave of Nerja (Malaga, southern Spain)

S. Docampo · M. M. Trigo · M. Recio · M. Melgar ·
J. García-Sánchez · M. C. Calderón-Ezquerro ·
B. Cabezudo

Received: 8 May 2009 / Accepted: 1 December 2009
© Springer Science+Business Media B.V. 2009

Abstract The cave of Nerja, which is visited annually by more than half a million people, is a karstic cavity situated in the east part of the province of Malaga (southern Spain), with internal conditions of humidity and temperature that make it a space highly conducive to the development of fungus. Fungal spores are of great interest in aerobiology and allergy due to their high incidence in both outdoor and indoor environments and their widely recognized ability to cause respiratory diseases and other pathologies. In this work, we focus on the seasonal and intradiurnal study of the *Aspergillus*/*Penicillium* spore type (conidia), which is especially abundant in the atmosphere inside the cave of Nerja. This study was carried out over an uninterrupted period of 4 years (2002–2005) with the aid of a Hirst-type volumetric pollen trap (Lanzoni VPPS 2000) situated in one of the halls of the cave. The results show that the spores of *Aspergillus*/*Penicillium* type represent 48.6% of the annual spore index, June, July and August being the

months with the highest rates of these spores, with an average incidence for the 4 years of 11, 63 and 15% of the annual total, respectively. However, the most interesting observation was the sudden increase in the concentration of these spores that occurred during 1 day of the year, when levels might reach nearly 300,000 spores/m³ of air as daily mean. Finally, it was concluded that these peaks were due to human activities carried out inside the cave, coinciding with celebration of the annual festival of dance and music. On the other hand, the intradiurnal study showed that the highest concentrations are reached between 1200 and 1400 hours. Although there are no standard indices related to the risk of exposure to spore concentrations, we think that the values obtained were high enough to be considered as a potential risk factor capable of producing harmful effects on human health. We therefore recommend taking the necessary measures to prevent such high increases in the spore levels inside the cave.

S. Docampo · M. M. Trigo (✉) · M. Recio ·
M. Melgar · J. García-Sánchez ·
M. C. Calderón-Ezquerro · B. Cabezudo
Department of Plant Biology, Faculty of Sciences,
University of Malaga, Campus Teatinos,
29071 Málaga, Spain
e-mail: aerox@uma.es

M. C. Calderón-Ezquerro
Centro de Ciencias de la Atmósfera, Universidad
Nacional Autónoma, México, D.F., Mexico

Keywords Aerobiology · *Aspergillus* spores ·
Cave · Health risk · *Penicillium* spores

1 Introduction

The cave of Nerja is a karstic cavity situated 4 km east from the locality of the same name, in the south edge of Sierra Almijara, 70 km east of Malaga, the provincial capital (southern Spain; Fig. 1). One of the

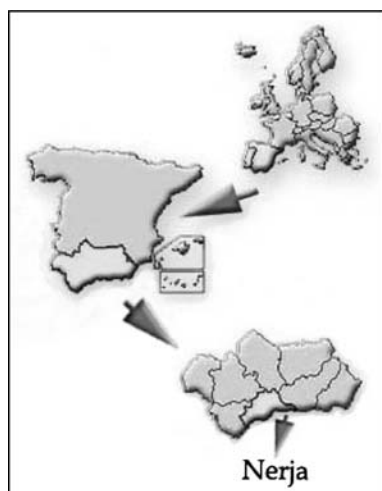


Fig. 1 Map of Nerja

most noteworthy features of this cave, situated 158 m above sea level, is the huge size of its chambers with a total volume of more than 250,000 m³. The cave was formed by the dissolution of the calcium–magnesium carbonate of the dolomitic rocks that comprise the mountain range of Almijara. The cave is divided into two zones: the low galleries, which can be visited by tourists and the general public (representing a third of the total), and the high galleries which, for the moment at least, cannot be visited. The genesis of the cave started in the Pliocene but the greatest lithogenic activity took place during the middle Pliocene (Carrasco 1993).

Although the cave of Nerja was discovered in 1959, evidence (archaeological remains and examples of rock art) indicate that it was inhabited by an uninterrupted succession of prehistoric cultures from the beginning of the upper palaeolithic (20,000 BC) to the Bronze Age (1,800 BC) (Sanchidrian 1994). Nowadays, the surroundings of the cave have been developed into a tourist complex that receives over 500,000 visitors a year.

The inner temperature in the Nerja Cave remains more or less constant throughout the year (18–19°C) and the small variations detected are due to external factors such as artificial lighting, which increases the temperature by up to 0.3°C, and the number of visitors. It is estimated that the temperature increases by 0.1°C per 1,000 people visiting the cave on the same day. The relative humidity values are high throughout the year, with an average ranging from 78 to 83%, although

values of 100% are not rare, especially in summer, when the maximum values are reached (Liñán et al. 2002). This phenomenon is due to a seasonal lag between the rainwater entering the rock and its dripping out—a consequence of its slow circulation through the marble that forms the ceiling of the cave. As with temperature, an increase in relative humidity of about 2–3% occurs during the day.

A feature of fungal spores is their high incidence in both indoor and outdoor environments. Moreover, their ability to cause problems in human health is also widely recognized. Many species of fungi produce toxins (Eaton 2002) and, although there is no evidence proving toxic effects by inhalation (Kelman et al. 2004), their potential as pathogenic and allergenic agents is known. Up to now, more than 70 fungal allergens have been isolated (Kurup et al. 2002) while the number of patients suffering from them has increased in recent decades (D'Amato et al. 2002).

In this work, we focus on the study of the *Aspergillus/Penicillium* spore type (conidia), which are the most frequent and abundant spores in both indoor and outdoor environments (Emberlin et al. 1995; Awad and Farag 1999; Chakraborty et al. 2000; Chandra and Chanda 2000; Portnoy et al. 2001; Adhikari et al. 2004), but especially indoors (Reponen 1995; Singh and Singh 1999). *Aspergillus* is one of the first settlers indoors, capable of growing even under conditions of nutrient deficiency (Korpi et al. 1997) and, due to the small size of its spores, many of them can penetrate the alveoli (about 70% in the case of oral breathing) (Reponen 1995). They are therefore of particular interest in clinical studies and are associated with a wide spectrum of respiratory diseases as well as other pathologies, including different forms of aspergillosis (Tomee and van der Werf 2001; Gupta et al. 2002; McDevitt et al. 2004). Studies carried out in contaminated indoor sites confirmed *Aspergillus* as an agent potentially capable of causing adverse health effects (Jussila and Komulainen 2002).

In addition to the impact that these genera of fungi have on human health, another factor to be considered is the importance of fungi as causal agents of geological changes (Burford et al. 2003). Fungi are involved in the precipitation of calcium carbonate (Northup 2001), as well as in the degradation of limestone materials. Many of them produce acid compounds and pigments that cause corrosion and stain substrates (Šimonovičová et al. 2004).

A preliminary study lasting only 1 year, Docampo et al. (2003) demonstrated the great diversity of airborne fungal spores in the atmosphere of the cave. When the study was continued in subsequent years, a high incidence of spores of *Aspergillus/Penicillium* type was observed. Due to the potentially adverse effects that these spores might have on human health, as well as on the cave itself, we decided to carry out a more thorough study in order to obtain more accurate data to see whether or not some methods of control should be applied to minimise their impact.

As mentioned above, many aerobiological studies have been carried out indoors, but very few of them were performed in natural caves. We found no reference at all to studies in caves that lasted so long and used an uninterrupted sampling method such as that used here, which allowed us to study seasonal variations over several years.

For all the above, we consider it to be of interest to carry out such a study in this much visited cave of great cultural and geological value.

2 Materials and methods

The sampling was carried out from 1 January 2002 to 31 December 2005, uninterruptedly, with the aid of a non-viable Hirst-type volumetric spore-trap (Hirst 1952), a 7-day recorder (model VPPS) manufactured by Lanzoni, where the vacuum pump is regulated to a constant suction flow of 10 l/min. The spore trap was screwed to the floor of the so-called “Sala del Belén”, the first chamber situated after the stairs of the main entrance, accessible from an artificial opening dug through a holocenic plug of sedimentary origin. For this study, the peak and the weathercock were removed from the spore-trap, since it was in a sheltered and fixed position, with the air inlet facing the corridor through which visitors pass. Silicone fluid (silicone dissolved in carbon tetrachloride at a concentration of 2%) was used as an adhesive substance, and glycerine jelly as the mounting medium. This sampling method allows a continuous, hour by hour, study over an extended period and makes it possible to carry out an almost immediate analysis after collecting the samples (Baxter et al. 2005). Counts of the fungal spores were made with the aid of a light microscope. For that, a longitudinal sweep per slide was made using a $\times 100$ objective, the

obtained data being expressed as numbers of spore/ m^3 of air (daily mean).

The spores were identified by comparison with samples taken from cultures in petri dishes, as well as using bibliographic resources (Grant Smith 1984, 1986). Nevertheless, due to similarities with other spore types and the difficulties of differentiation without other features, there exists the possibility that values could be slightly overestimated.

For the intradiurnal variation study, cumulative values of 2 h were taken, and the values were expressed as percentages of the daily total. Finally, the intradiurnal distribution index (IDI) was calculated, following the methodology proposed by Trigo et al. (1997).

3 Results

During the period studied, *Aspergillus/Penicillium* spores presented an annual mean index value of 430,032. The highest annual index was detected in 2003, with a total of 741,521 counts, followed by the years 2002 with a value of 389,916, and 2005 with a value of 307,972. In contrast, 2004 was the year with the lowest annual index: 280,719 (Table 1). Moreover, 2003 not only produced the highest spore concentration values but also had the highest percentage regarding annual total (Fig. 2).

Table 1 Monthly and annual values of *Aspergillus/Penicillium* spores over the years studied

<i>Aspergillus/Penicillium</i> monthly distribution (spores/ m^3)				
	2002	2003	2004	2005
Jan	3,588	5,905	2,212	3,925
Feb	2,904	4,099	2,728	1,245
Mar	6,847	4,702	4,476	2,885
Apr	3,700	3,968	1,978	2,223
May	3,882	6,322	5,719	1,225
Jun	17,667	20,447	63,839	38,857
Jul	265,190	526,090	134,171	204,173
Aug	55,756	134,303	37,237	39,938
Sep	4,169	14,694	6,025	2,235
Oct	8,591	9,902	9,486	7,733
Nov	9,272	8,393	7,452	2,749
Dec	8,350	2,696	5,396	784
Annual total	389,916	741,521	280,719	307,972

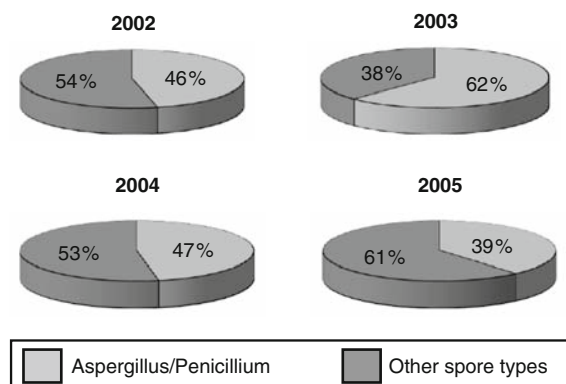


Fig. 2 *Aspergillus/Penicillium* spore percentage regarding other spore types in the different years studied

3.1 Seasonal behaviour

As regards the monthly spore index (Table 1), it should be noted that the highest levels of spore concentrations were registered in the summer months, especially in July, followed by August and June (Fig. 3). Of note is the fact that in July 2003 the monthly index of this spore type represented more than 70% of the monthly total. August was the second month as regards abundance in all the years studied, except in 2004, when it was June. October was the only other month that exceeded a rate of 2% of the annual total average. In general, the period in which the lowest percentages were detected ran from January to May. Nevertheless, the lowest index corresponded to December 2005, with a monthly value of 784 (Table 1).

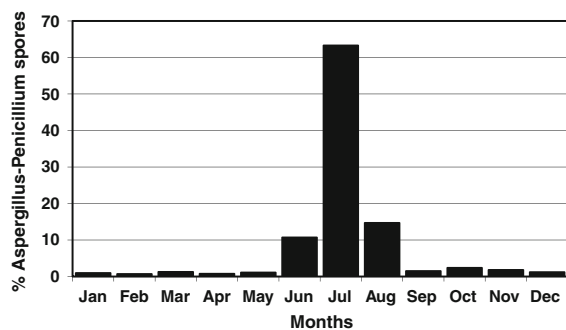


Fig. 3 Distribution of the monthly mean percentage of *Aspergillus/Penicillium* spores during the studied period (2002–2005)

In the seasonal distribution graphics (Fig. 4), the almost continuous occurrence of this spore type throughout the year can be seen. It can also be observed that, during a great part of the year, the daily mean concentrations hardly exceeded the value of 200 spores/m³, especially during winter. The number of days on which a daily mean concentration of 200 or fewer spores/m³ was obtained was 207 in 2002, 177 in 2003, 223 in 2004 and 218 in 2005 (59% out the days in which this spore type is present in the atmosphere of the cave; Table 2). The daily mean concentration range observed on the greatest number of days was 1–1,000 spores/m³, which ranged from 86.6% of the days when spores were present in 2003 to 88.1% in 2002 and 2005. In addition, although very high levels of daily mean concentrations (over 10,000 spores/m³) were reached, they were very isolated events and detected on a maximum of 11 days per year: 11 days in 2003 and 10 in 2005, while in the remaining years, this quantity was hardly exceeded on 4 or 5 days.

The maximum daily mean concentration was always detected in late July. As mentioned above, 2003 was the year in which the highest spore concentrations were detected but it was also the year in which the maximum daily peak was registered, with 282,064 spores/m³ reached on 29 July, corresponding to 38% of the total spores of *Aspergillus/Penicillium* detected in that year. In 2002, this maximum peak occurred on 30 July, with 101,569 spores/m³ while in 2004 and 2005, the peaks, which were also registered in July, took place on 26 and 28 July, with 35,683 and 47,704 spores/m³, respectively.

In Fig. 4, the shaded area represents the period in which a festival of dance and music was held in the cave of Nerja.

3.2 Intradiurnal distribution

The intradiurnal distribution study shows how the maximum concentration peak was detected between 12:00 and 14:00 hours, a period during which nearly 20% of the total daily spore content was registered, with an intradiurnal distribution index value of 0.18. Concentrations started to increase between 06:00 and 08:00 hours, and tended to decrease after midday, the lowest values being detected in early morning (Fig. 5).

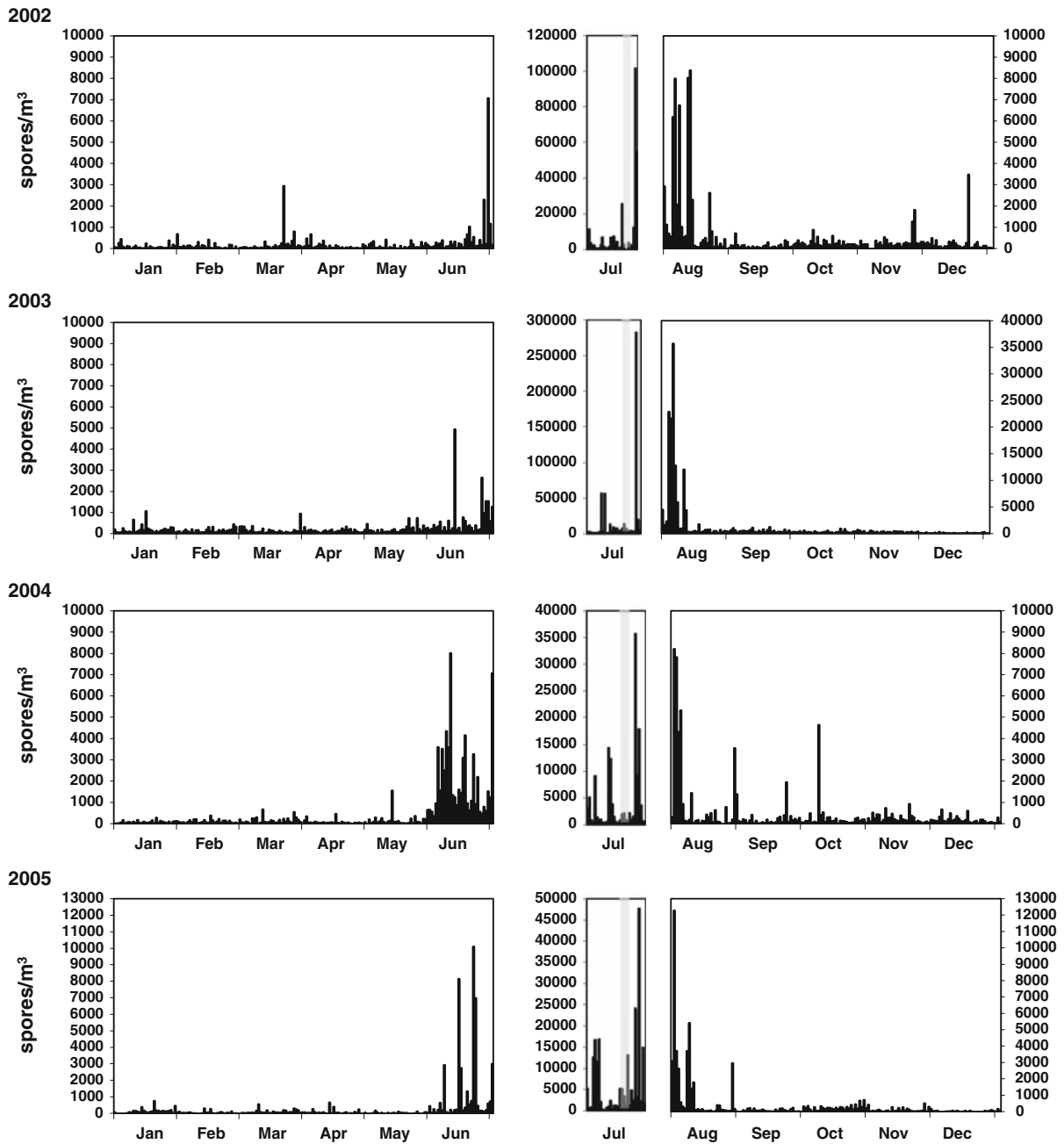


Fig. 4 Behaviour of the daily mean concentrations of *Aspergillus/Penicillium* spores during the studied years. Shaded areas correspond with the festival days. Note the different scales

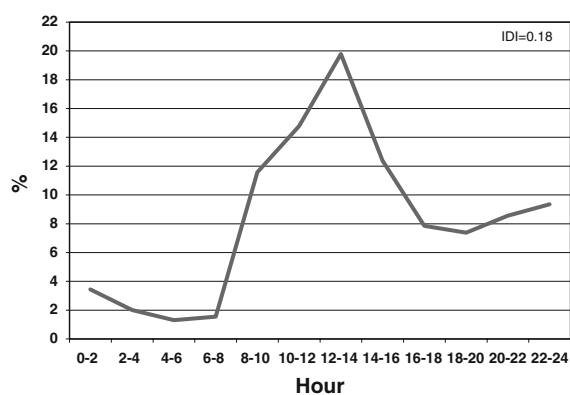
Finally, a comparison between the results of two samplers situated outside and inside the cave was carried out in order to clarify the origin of the spores detected. The data obtained are shown in Table 3.

4 Discussion

Aspergillus/Penicillium is, by far, the most abundant spore type in the atmosphere of the cave of Nerja, being present throughout the year. When the seasonal

Table 2 Number of days per year with different ranges of *Aspergillus/Penicillium* spore concentrations for the different years studied

Daily spore concentration (spore/m ³)	Number of days			
	2002	2003	2004	2005
1–200	207	177	223	218
201–500	87	104	62	46
501–1,000	23	35	31	16
1,001–2,000	12	14	16	8
2,001–5,000	17	18	17	14
5,001–10,000	9	6	8	6
>10,000	5	11	4	10

**Fig. 5** Intradiurnal distribution of *Aspergillus/Penicillium* spores (2002–2005). IDI Intradiurnal distribution index

distribution was studied, a similar behaviour for the 4 years could be observed: concentrations remained low most of the year with a considerable increase in the summer months. If we focus our attention on this summer period, it can be seen how a very high isolated peak only occurs on 1 day a year, after which the fungal spore levels sharply decrease again.

Some authors (Lehtonen et al. 1993) have reported that variations in spore levels indoors are mainly caused by day-to-day activities such as cleaning, object movement, or transport of spores on pets or clothes, and, although previous studies in the cave of Nerja had registered daily variations caused by this sort of activity (Docampo et al. 2003), they were not enough to explain the sharp increases mentioned above.

Besides the high increase detected in every year, of note is the fact that the date almost coincided: 30, 29, 26 and 28 July in 2002, 2003, 2004 and 2005,

respectively. To explain those sudden maximum concentration peaks, we analysed the different activities carried out in the cave, a much visited tourist complex, in which natural conditions may be quite altered. First, we rejected the idea that it could be an isolated incident caused by a series of hardly reproducible physical phenomena and, to a greater or lesser extent, the result of chance, since they were events periodically repeated in all the years studied. On the other hand, these situations could not be the product of a phenomenon occurring out of doors because, although there is evidence of the entry of particles from outside through openings in the cave due to air movements (Docampo et al. 2003), the spore concentrations registered outside the cave were significantly lower. To confirm this, for the first 2 years, a second spore trap was situated outside, near the entrance to the cave, showing that the number of *Aspergillus/Penicillium* spores during the peak days and the days before and after the peak, was much lower outside, never exceeding a concentration of 700 spores/m³ of air when more than 280,000 spores/m³ were detected the same day in the interior. So, the outside spore content barely represented 1% of the levels registered inside the cave (Table 3) and, for this reason, we centred our study on the activities carried out in the cave. The particular annual distribution of the *Aspergillus/Penicillium* spores can be attributed to the festival of dance and music celebrated inside the cave at the end of July. For this spectacle, a large wooden stage is set up and, as result of the high relative humidity prevailing in the cave, especially in summer, with an average of over 98%, a large number of colonies, which can be easily observable, develop on the planks of the stage. While the festival lasts for 5 days, the maximum

Table 3 *Aspergillus/Penicillium* outside/inside daily spore concentration comparison for the peak (in bold), pre-peak and post-peak days in 2002 and 2003, indicating relative percentage

	Outdoor	Indoor	%
29/07/2002	238	12,425	1.92
30/07/2002	275	101,569	0.27
31/07/2002	151	54,648	0.27
28/07/2003	113	3,456	3.27
29/07/2003	691	282,064	0.24
30/07/2003	124	19,602	0.63

concentration peak occurs a few days after it has finished, coinciding with the process of dismantling the stage, which leads to the spore dispersal and, therefore, to a strong increase in airborne spore levels.

Although a large number of aerobiological studies indoors and outdoors have been carried out to detect potential risks for human health, it is not possible to find standardised values which permit potential allergenicity ranges to be established for different spore types (Paugam et al. 1999; Fung and Hughson 2003). In fact, we found a wide range of values (Gots et al. 2003; Baxter et al. 2005) given by different organisations. Another problem is the difficulty of comparing the data obtained with other studies because of the use of different methods of sampling and the resulting presentation. Some results are given as CFU/m³ while other authors express their results as spore/m³. Other factors to take into account are the sampler type, the airflow aspirated (in case of volumetric traps) and the time of exposure. We were unable to find references in which similar studies have been carried out and which would allow us to compare the results obtained. So, we have restricted our comparison to the many studies conducted inside buildings, with the limitations outlined above.

In general, although there is little homogeneity in the results, *Penicillium* and *Aspergillus* spores are the most abundant in most of the papers consulted. Chakraborty et al. (2000) obtained, for five different indoor studies, levels that varied between 816 and 1,664 spores/m³. On the other hand, Baxter et al. (2005) registered quantities lower than 1,000 spores/m³ in what they classed as clean environments to levels of 40,000 spores/m³ as an average in buildings considered as spaces contaminated by fungi. In our study, the highest levels obtained were daily mean values of almost 300,000 spores/m³. Moreover, we have to take into account that the sampling methodology followed only allowed us to quantify the number of spores, while other fungal particles, such as hyphae fragments, were excluded. Studies developed in several species of *Aspergillus* and *Penicillium* have demonstrated that both hyphae fragments and spores share antigens that may cause allergy (Górny et al. 2002). Given this, the high allergenic potential of the air of the cave of Nerja revealed by this study might even be underestimated since such particles were not taken into account.

Unlike other studies that also found high levels of spores in different materials such as dust, surfaces or walls, the concentrations quantified in this work were already in the air, so all the spores are likely to be inhaled with the consequent increase in risk of causing health problems, while those that more or less adhered to a surface or other materials must pass to the air before they can penetrate the respiratory system (Gots et al. 2003). As commented above, there are no standardised criteria to determine whether the values obtained fall within a range of high risk, but what we can say is that the concentrations registered are high enough to cause problems in human health, especially during the summer time, since the high peaks occur during the period of increased influx of visitors.

As regards the intradiurnal variation observed, the different patterns found in the literature should be mentioned. For example, while Li and Kendrick (1995) did not find intradiurnal patterns, Millington and Corden (2005), similarly to our study, detected an increase in the concentrations of *Aspergillus/Penicillium* spores outdoors at around 11:00 hours. However, this behaviour is altered in the case of interior environments, where increases in the spore levels are directly associated with the activities carried out in these spaces. In our case, something similar could happen since the natural conditions of the cave of Nerja are altered by the presence of visitors and other anthropogenic activities since the cave opens at 10:00 hours and closes between 18:30 and 19:30 hours, depending on the season.

As to the sampling methodology used, although the technique sometimes does not enable different species or genera to be identified, as in this case in which it was not possible to differentiate between *Aspergillus* and *Penicillium* spores (Baxter et al. 2005), it presents advantages over viable methods in which only fungi capable of growing in a culture medium can be detected, and therefore the number of spores actually present in the atmosphere may be underestimated (Simmons 2003). Many comparative studies between spore-sampling devices were found in the literature (Burge 1990; Li and Lin 1999; Morris et al. 2000; Wang et al. 2001; Spurgeon 2003; Wüst et al. 2003). Although some authors consider Hirst-type samplers to be of little use for particles smaller than 5 µm (Spieksma 1995), this type of sampler allows a continuous study, hour by hour, for a long

period of time (4 years in this case), the results being available almost immediately after the sample collection (Baxter et al. 2005), unlike other types of spore traps, with time restriction records, and whose samples need to be incubated for several days.

With regard to the method of counting, different strategies were followed by different authors, including comparative studies (Sterling et al. 1999; Cariñanos et al. 2000). However, for this study, we finally chose to conduct a longitudinal sweep $\times 1,000$ to avoid the possibility that, because of their small size, a considerable number of spores would pass unnoticed (Burge 1989).

From the point of view of cultural heritage, fungi, bacteria and other microorganisms have been cited as responsible for the bio-deterioration of the surfaces of historic buildings, sculptures and even cave paintings (Gonzalez and Saiz-Jimenez 2004; Sáiz-Jiménez and González 2007), especially bacteria, for which fungal mycelia could constitute a substrate for growing. However, apart from those colonies detected developing on the planks of the stage during the festival, we have not detected fungal communities in the cave. What happens quite often is the emergence of colonies of algae, mainly chlorophytes and Cyanophyta growing on damp walls under artificial illumination (Ruiz Sánchez et al. 1991).

5 Conclusions

Finally, we conclude that high levels of *Aspergillus/ Penicillium* spore type occurs in the cave of Nerja, with isolated and dramatic increases in the concentrations due to human activity indoors. Due to the large number of people visiting the cave throughout the year, this phenomenon may represent a potential risk to human health. So, it would be advisable to study specific steps to reduce this high level of spores in the interior during the festival, especially in the period after the festival. One of the first steps might be to change the material from which the stage is made (wood) for another that impedes the development of the different fungal colonies or, at least, to treat the stage with antifungal substances.

We do not consider that the high number of visitors that the cave receives annually could substantially affect spore levels detected in the air. However, from the point of view of conservation of

cultural heritage, it must be taken into account that fungal spores deposited on different surfaces of the cave may serve as nutrient for the development of other microorganisms, which would be an interesting aspect to be addressed in future work.

Acknowledgment The authors want to thank the Patronato de la Cueva de Nerja for the support provided when carrying out this study through project 8.06/03.1773.

References

- Adhikari, A., Sen, M. M., Gupta-Bhattacharya, S., & Chanda, S. (2004). Volumetric assessment of airborne fungi in two sections of a rural indoor dairy cattle shed. *Environment International*, 29, 1071–1078.
- Awad, A. H. A., & Farag, S. A. (1999). An indoor bio-contaminants air quality. *International Journal of Environmental Health Research*, 9, 313–319.
- Baxter, D. M., Perkins, J. L., McGhee, C. R., & Seltzer, J. M. (2005). Aregional comparison of mold spore concentrations outdoors and inside “clean” and “mold contaminated” southern California buildings. *Journal of Occupational and Environmental Hygiene*, 2, 8–18.
- Burford, E. P., Kierans, M., & Gadd, G. M. (2003). Geomycology: Fungi in mineral substrata. *Mycologist*, 17, 98–107.
- Burge, H. A. (1989). Fungus aerobiology: A plethora of problems. In P. Comtois (Ed.), *Aerobiology health environment a symposium* (pp. 147–156). Canada: Université de Montréal.
- Burge, H. A. (1990). Bioaerosols: Prevalence and health effects in the indoor environment. *Journal of Allergy and Clinical Immunology*, 86, 687–701.
- Cariñanos, P., Emberlin, J., Galán, C., & Domínguez-Vilches, E. (2000). Comparison of two pollen counting methods of slides from a hirst type volumetric trap. *Aerobiologia*, 16, 339–346.
- Carrasco, F. (1993). *Geología de la Cueva de Neja*. Málaga: Patronato de la Cueva de Nerja.
- Chakraborty, S., Sen, S. K., & Bhattacharya, K. (2000). Indoor and outdoor aeromycological survey in Burdwan, West Bengal, India. *Aerobiologia*, 16, 211–219.
- Chandra, B., & Chanda, S. (2000). Aeromycoflora in the central milk dairy of Calcutta, India. *Aerobiologia*, 16, 367–372.
- D’Amato, G., Liccardi, G., D’Amato, M., & Cazzola, M. (2002). Outdoor air pollution, climatic changes and allergic bronchial asthma. *European Respiratory Journal*, 20, 763–776.
- Docampo, S., Trigo, M. M., Recio, M., & Cabezudo, B. (2003). Presencia de esporas fúngicas en la atmósfera del interior de la cueva de Nerja (Málaga): Estudio preliminar. *Polen*, 13, 279–288.
- Eaton, K. K. (2002). Mould, yeast, ascospores, basidiospores, algae and lichens: Toxic and allergic reactions. *Journal of Nutritional & Environmental Medicine*, 12, 321–335.
- Emberlin, S., Newman, T., & Bryant, R. (1995). The incidence of fungal spores in the ambient air and inside homes: evidence from London. *Aerobiologia*, 11, 253–258.

- Fung, F., & Hughson, W. G. (2003). Health effects of indoor fungal bioaerosol exposure. *Applied Occupational and Environmental Hygiene*, 18, 535–544.
- Gonzalez, J. M., & Saiz-Jimenez, C. (2004). Microbial diversity in biodeteriorated monuments as studied by denaturing gradient gel electrophoresis. *Journal of separation science*, 27, 174–180.
- Górny, R. L., Reponen, T., Willeke, K., Schemechel, D., Robine, E., Boissier, M., et al. (2002). Fungal fragments as indoor air biocontaminants. *Applied and Environmental Microbiology*, 68, 3522–3531.
- Gots, R. E., Layton, N. J., & Pirages, S. W. (2003). Indoor health: background levels of fungi. *American Industrial Hygiene Association Journal*, 64, 427–438.
- Grant Smith, E. (1984). *Sampling and identifying allergenic pollens and mold. Vol I*. Texas: Blewstone Press.
- Grant Smith, E. (1986). *Sampling and identifying allergenic pollens and mold*. Texas, USA: Volume II. Blewstone Press.
- Gupta, A. K., Ahmad, I., & Summerbell, R. C. (2002). Fungicidal activities of commonly used disinfectants and antifungal pharmaceutical spray preparations against clinical strains of *Aspergillus* and *Candida* species. *Medical Mycology*, 40, 201–208.
- Hirst, J. M. (1952). An automatic volumetric spore trap. *Annals Applied Biology*, 39, 257–265.
- Jussila, J., & Komulainen, H. (2002). Spores of *Aspergillus versicolor* isolated from indoor air of moisture-damaged building provoke acute inflammation in mouse lungs. *Inhalation Toxicology*, 14, 1261–1277.
- Kelman, B. J., Robbins, C. A., Swenson, L. J., & Hardin, D. (2004). Risk from inhaled mycotoxins in indoor office and residential environments. *International Journal of Toxicology*, 23, 3–10.
- Korpi, A., Pasanen, A. L., Pasanen, P., & Kalliokoski, P. (1997). Microbial growth and metabolism in house dust. *International Biodeterioration & Biodegradation*, 40, 19–27.
- Kurup, V. P., Shen, H.-D., & Vijay, H. (2002). Immunobiology of fungal allergens. *International Archives of Allergy and Immunology*, 129, 181–188.
- Lehtonen, M., Reponen, T., & Nevalainen, A. (1993). Everyday activities and variation of fungal spore concentrations in indoor air. *International Biodeterioration and Biodegradation*, 31, 25–39.
- Li, D.-W., & Kendrick, B. (1995). A year-round outdoor aeromycological study in Waterloo, Ontario, Canada. *Grana*, 34, 199–207.
- Li, C. S., & Lin, Y. C. (1999). Sampling performance of impactors for fungal spores and yeast cells. *Aerosol Science and Technology*, 31, 226–230.
- Liñán, C., Carrasco, F., Andreo, B., Jiménez de Cisneros, C., & Caballero, E. (2002). Caracterización isotópica de las aguas de goteo de la Cueva de Nerja y de su entorno hidrogeológico (Málaga, sur de España). In F. Carrasco, J. J. Durán, & B. Andreo (Eds.), *Karst and environment* (pp. 243–249). Málaga: Fundación Cueva de Nerja.
- McDevitt, J. J., Lees, P. S. J., Merz, W. G., & Schwab, K. J. (2004). Development of a method to detect and quantify *Aspergillus fumigatus* conidia by quantitative PCR for environmental air samples. *Mycopathologia*, 158, 325–335.
- Millington, W. M., & Corden, J. M. (2005). 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. *Aerobiologia*, 21, 105–113.
- Morris, G., Kokki, M. H., Anderson, K., & Richardson, M. D. (2000). Sampling of *Aspergillus* spores in air. *Journal of Hospital Infection*, 44, 81–92.
- Northup, D. E. (2001). Geomicrobiology of caves: a review. *Geomicrobiology Journal*, 18, 199–222.
- Paugam, A., Baixench, M. T., Mechour, B., & Dupouy-Camet, J. (1999). Comments on airborne *Aspergillus* and incidence of invasive aspergillosis. *Medical Mycology*, 37, 373–374.
- Portnoy, J. M., Flappan, S., & Barnes, C. S. (2001). A procedure for evaluation of the indoor environment. *Aerobiologia*, 17, 43–48.
- Reponen, T. (1995). Aerodynamic diameters and respiratory deposition estimates of viable fungal particles in mold problem dwellings. *Aerosol Science and Technology*, 22, 11–23.
- Ruiz Sánchez, J., Marín Girón, F., Ojeda F., Marín Olalla, F., Berros, J. & Marín Olalla, E. (1991). Estudio macroscópico “in situ” y microscópico-ecológico de pequeñas zonas de flora verde (algas verdes y verdeazuladas) del interior de la cueva de Nerja. *Trabajos sobre la Cueva de Nerja*, 2, 113–125. Patronato de la Cueva de Nerja.
- Sáiz-Jiménez, C., & González, J. M. (2007). Aerobiology and cultural heritage: some reflections and future challenges. *Aerobiologia*, 23, 89–90.
- Sanchidrian, J. L. (1994). *Arte rupestre de la Cueva de Nerja*. Málaga: Patronato de la Cueva de Nerja.
- Simmons, R. B. (2003). The role of microscopy in indoor air quality investigations. *Microscopy and Microanalysis*, 9, 1026–1027.
- Šimonovičová, A., Gódyová, M., & Ševc, J. (2004). Airborne and soil microfungi as contaminants of stone in a hypogean cemetery. *International Biodeterioration & Biodegradation*, 54, 7–11.
- Singh, A., & Singh, A. B. (1999). *Aspergillus* Spp. As an important occupational risk factor among susceptible individuals. *Aerobiologia*, 15, 233–240.
- Spieksma, F.Th.M. (1995). Outdoor atmospheric mould spores in Europe. XVI European congress of allergology and clinical immunology (pp. 625–630), Madrid: ECACI.
- Spurgeon, J. C. (2003). A method for detecting fungal contaminants in wall cavities. *American Industrial Hygiene Association Journal*, 64, 40–47.
- Sterling, M., Rogers, C., & Levetin, E. (1999). An evaluation of two methods used for microscopic analysis of airborne fungal spore concentrations from the Burkard Spore Trap. *Aerobiologia*, 15, 9–18.
- Tomee, J. F. Ch., & van der Werf, T. S. (2001). Pulmonary aspergillosis. *The Netherlands Journal of Medicine*, 59, 244–258.
- Trigo, M. M., Recio, M., Toro, F. J., & Cabezudo, B. (1997). Intradurnal fluctuation in airborne pollen in Málaga (S. Spain): a quantitative method. *Grana*, 36, 39–43.
- Wang, Z., Reponen, T., Grinshpun, S. A., Górny, R. L., & Willeke, K. (2001). Effect of sampling time and air

humidity on the bioefficiency of filter samplers for bio-aerosol collection. *Aerosol Science*, 32, 661–674.

Wüst, G., Friedl, H., Haas, D., Köck, M., Pichler-Semmelrock, F., Reinthaler, F. F., et al. (2003). A comparison between

andersen (ACFM) and reuter centrifugal sampler (RCS-plus) for indoor sampling of airborne molds. *Aerobiologia*, 19, 125–128.