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Fungal spore content of the atmosphere of the Cave of Nerja (southern Spain): Diversity and origin

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ABSTRACT

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 study the spore content of the atmosphere of the Cave of Nerja, a karstic cavity and an important tourist attraction situated on the eastern coast of Malaga (southern Spain), which receives more than half a million visitors every year. This study was carried out over an uninterrupted period of 4 years (2002–2005) with the aid of two Hirst-type volumetric pollen traps (Lanzoni VPPS 2000) situated in different halls of the cave. In the atmosphere of the Cave of Nerja, 72 different spore types were detected during the studied period and daily mean concentrations of up to 282,195 spores/m^3 were reached. Thirty-five of the spore types detected are included within Ascomycota and Basidiomycota (19 and 16 types, respectively). Of the remaining spore types, 32 were categorized within the group of so-called imperfect fungi, while Oomycota and Myxomycota were represented by 2 and 3 spore types, respectively. *Aspergillus*/*Penicillium* was the most abundant spore type with a yearly mean percentage that represented 50% of the total, followed by *Cladosporium*. Finally, the origin of the fungal spores found inside the cave is discussed on the basis of the indoor/outdoor concentrations and the seasonal behaviour observed.

1. Introduction

Large karstic caves have always aroused the curiosity of both scientists and the general public, so many have become tourist attractions visited by large numbers of people each year. Moreover, during recent years, the study of fungal allergens associated with allergies has become a matter of great importance (Kurup et al., 2002). Many indoor studies have been carried out in Spain and throughout the world in different buildings, such as hospitals (Sautour et al., 2009), homes (Basilico et al., 2007; Crawford et al., 2009), offices (Baxter et al., 2005; Law et al., 2001), schools (Meklin et al., 2003; Scheff et al., 2000), factories (Awad et al., 2010), farms (Miao et al., 2010), stables (Beck et al., 2007), markets (Arya and Arya, 2007), museums (Camuffo et al., 1999; Niesler et al., 2010), wineries (Li and LaMondia, 2010), churches (Aira et al., 2007), etc. However, after an exhaustive search of the literature, there seem to be few works that address the study of biological airborne particles inside natural cavities. Although some references were found such as Bastian et al. (2010), Borda and Borda (2006), Borda et al. (2009), Groth et al. (1999), Jurado et al. (2009), Koilraj et al. (1999), and Mulec et al. (2002), most studies used viable methods and counting of colony forming units (CFUs), usually over a short period of time, which means that the results of our study at Nerja, which involved a non-viable uninterrupted sampling over 4 years, cannot be compared with similar studies.

The aerobiological study was carried out inside the Cave of Nerja, situated near the village of the same name, southern Spain. This coastal town, (21 m above sea level), is located 65 km east of the capital of the province, Malaga, on the border with the province of Granada.

The surrounding area of the Cave of Nerja is situated in the thermo-Mediterranean belt (Rivas-Martínez, 1981) and is characterized by mild frost-free winters. The rainfall is unevenly distributed throughout the year, the highest levels occurring in early autumn and late winter–early spring. This leads to a prolonged drought that causes a large water deficit in the soil from June to October, which is further accentuated by the high temperatures reached during the summer.

The cave was formed by the dissolution of the calcium–magnesium carbonate of the dolomitic rocks that comprise the mountain range of Almijara. It is situated 158 m above sea level and 800 m from it. It is thought that the genesis of the cave started in the Pliocene although the greatest lithogenic activity took place during the Middle Pliocene (Carrasco, 1993). Although the Cave of Nerja was re-discovered in 1959, evidence (archaeological remains and examples of rock art) indicate that it was inhabited by members of an uninterrupted succession of prehistoric cultures from the beginning of the Upper Palaeolithic (20,000 B.C.) to the Bronze Age (1800 B.C.) (Sanchidrian, 1994). Nowadays, the surroundings of the cave have been developed into a tourist complex that receives over 500,000 visitors a year. This natural

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cave in which one of the most noteworthy features is the huge size of its halls with a total volume of more than 250,000 m^3 and a topographic development of 4823 m, 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 and new galleries which, for the moment, cannot be visited (Carrasco et al., 1998). The low galleries (zone in which our study was carried out) have a major axis 250 m in length and consist of a succession of big halls and small diverticula separated by substantial speleothem deposits. Nowadays, the cave is accessible from an artificial opening dug through a holocenic plug of sedimentary origin.

The inner temperature in the Nerja Cave remains more or less constant throughout the year (18–19 °C), while the relative humidity is high, varying between 78 and 100%, the maximum values being recorded in summer. This phenomenon is due to a seasonal lag between the rainwater entering the rock and its dripping out, as a consequence of its slow circulation through the marble that forms the ceiling of the cave (Liñán et al., 2002).

Several works carried out on the concentration of radon in the atmosphere of the cave have revealed that it is relatively well ventilated. In autumn and winter, when the outside temperature is lower than inside the cave, the air flow is better and the air is replaced in 1 day, while in spring and summer the same process lasts up to 5 days (Cañete, 1997; Dueñas et al., 1993).

The aim of this study was to analyse the airborne spore content in a frequently visited natural cave, to assess the incidence of the same in its atmosphere and the variations that occur throughout the year, a topic to which we have found no references in the literature. In addition to the impact that the spores of these fungi have on human health, another factor to be considered is their importance as causal agents of geological changes (Burford et al., 2003). Fungi are involved in the precipitation of calcium carbonate (Northup and Lavoie, 2001), as well as in the degradation of limestone materials. Many of them produce acid compounds and pigments that cause corrosion and stain substrates (Bastian et al., 2010; Šimonovičová et al., 2004).

2. Materials and methods

2.1. Type and location of the samplers

The sampling was carried out with the aid of two non-viable Hirst-type volumetric spore traps (Hirst, 1952), a seven-day recorder (model VPPS 2000) manufactured by Lanzoni, in which the vacuum pump was regulated at a constant suction flow of 10 l/min. For this study, the peaks and the weather vanes were removed from the spore traps, since they were in a sheltered and fixed position, with the air inlet facing the corridor through which visitors pass.

The first sampler was screwed to the floor of the so-called "Hall of the Manger", the first chamber after the stairs of the main entrance, near the hall entrance and the exit, 1.5 m from the cement pathway along which the visitors pass. This chamber is a vaulted corridor, which is 20 m long, 10 m wide and 5 m high.

The second sampler was situated in another hall, the so-called "Hall of the Cataclysm", further from the entrance and exit. This is a huge chamber with a large central column 32 m in height, situated in the lowest point of the cave and at the end of the visited area (Carrasco et al., 1998). In this case the sampler was also screwed to the floor but at a height of about 3 m above the pathway.

2.2. Sampling treatment and spore counting

The data obtained from the sampler in the "Hall of the Manger" were used for both the qualitative and quantitative study of the fungal spores inside the cave. The sampling was carried out from 1 January 2002 to 31 December 2005, uninterruptedly, except for the period of 24–25 July, from 4 to 6 November 2002 and from 2 to 5 January 2005 because of a failure in the electrical power supply. The second sensor, located in the "Hall of the Cataclysm", was operational from 1 April 2003 to 31 December 2005. Silicone fluid (silicone dissolved in carbon tetrachloride at a concentration of 2%) was used as adhesive substance, and glycerine jelly as mounting medium.

As regards the spore counts a longitudinal sweep at 1000× was made because of the large number of small fungal spores that would otherwise go undetected and a second sweep was performed at 400×, which allows a wider microscopic field, reducing the error when extrapolating the total number of spores counted to the whole sampling. Finally, the data obtained were expressed as the number of spores per cubic metre of air (daily mean concentration).

In this paper we have followed the classification proposed in the IX edition of Ainsworth and Bisby's Dictionary of Fungi (Kirk et al., 2001). However, although there is a tendency to ignore the category "imperfect" or "mitosporic" in favour of including such fungi in previously existing groups, we prefer to use this artificial group in order to facilitate the grouping of the spores on the basis of their sexual or asexual origin.

2.3. Comparative study

After the qualitative and quantitative study, we proceeded to compare the results obtained by the two samplers installed inside the cave with the aim of analysing the heterogeneity of the spore spectrum in the atmosphere of the cave. Although both samplers operated uninterruptedly, for this study we only took the data corresponding to 1 day a week at a time interval of 8 days in order to avoid the bias that might arise from using the same day of the week. In this way, we took into account the daily mean concentrations of the overlapping sampling period for the years 2004–2005, in which the two samplers operated simultaneously.

The study is accompanied by a statistical analysis, using a Spearman's correlation test, searching for degrees of association between the concentrations of spores obtained by both samplers.

However, since the cave is not a totally isolated environment, we could observe the presence of pollen in the interior whose provenance cannot be other than outdoors. So, if we compare the outdoor/indoor concentrations of pollen, it is possible to estimate the percentage of biological particles that enter the cave due to the replacement of air. For this study we used the daily and monthly total pollen concentrations from a third sampler situated outside, near the entrance to the cave (Docampo et al., 2007), as well as the data obtained from the sampler located in the "Hall of the Manger" during the years of simultaneous sampling (2002 and 2003). Besides the pollen concentrations, we also compared the concentrations of two spore types (*Cladosporium* and *Alternaria*) with the levels reached by these types in the atmosphere outside the cave.

Finally, we analysed the monthly indices obtained for the three samplers (2 inside and 1 outside), setting a rate that gave a value of 1 to the level found outside (point of origin), while for the other two samplers the rates were obtained as a function of the first. Thus, the rates of monthly indices of the samplers in the "Hall of the Manger" and "Hall of the Cataclysm" were calculated by dividing their respective indices by the monthly index obtained outdoors. For this, the data of 2003 were taken, because this was the only year in which the samplings of the 3 spore traps coincided. No data are available for the first 3 months in the "Hall of the Cataclysm", because sampling did not start until April.

3. Results

3.1. Spore diversity

In the study carried out with the aid of the sampler located in the "Hall of the Manger" a total of 72 spore types were identified, 35 of