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Detection of human-induced environmental disturbances in a show cave

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Detection of human-induced environmental disturbances in a show cave

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Abstract
Purpose We investigated the effects of human-induced disruption in a subterranean stable environment containing valuable Palaeolithic paintings and engravings (Ardales Cave, Southern Spain) using a double analytical approach.
Methods An environmental monitoring system was installed in the cave to record temperature, relative humidity, carbon dioxide (CO_2) and radon (^{222}Rn) concentrations in air. In the same stations, an aerobiological sampling was conducted to quantify the level of airborne microorganisms.
Results The combination of different methods allowed us to detect the extent of human-induced changes, confirming that these can be very hazardous in certain cave areas that should be apparently outside the scope of human disturbances, either by their remoteness to the visitor entrance or by being briefly visited.
Conclusions The detection of evident anomalies in the environmental parameters and airborne microorganism concentration in the cave area housing the high density of paintings and engravings helps to control human disturbances and supports the direct application of this double approach for cave management purposes.

Keywords Aerobiology · Caves · Environmental control · Fungi · Bacteria

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1 Introduction

Many subterranean sites, such as caves, mines, tunnels or catacombs, house geological, biological and/or historical elements which are considered natural and cultural heritages (Hill and Forti 1997; Sanchez-Moral et al. 2005). Caves usually show a stable and fragile confined environment that makes it very susceptible to disturbances resulting from human activities. In the case of subterranean sites impacted by tourism, it is necessary to determine the areas affected by human activity and the type and level of disturbance. Previous studies indicate that a scientific knowledge of the environmental parameters (temperature, humidity, CO_2) are key factors in the conservation of the environment being crucial the separation between environmental changes due to natural causes and those resulting from human action (Huppert et al. 1993; Fernandez-Cortes et al. 2006; Faimon et al. 2006). One of the most evident impacts of visitors in subterranean environments is the increase of CO_2 concentration, water vapour and temperature (Andrieux 1988; Hoyos et al. 1998). This results in the interruption of the fragile geochemical/environmental balance and provokes mineral corrosion (Sanchez-Moral et al. 1999; Dreybrodt et al. 2005) and microbial colonization of rock substrata (Sanchez-Moral et al. 2005; Bastian et al. 2010). However, less known are the direct impact of visitors regarding microbial dispersion, the replacement of

the natural microbial communities by alien human-induced microbial populations (Bastian et al. 2009) or the pool of pathogenic microorganisms thriving inside the caves (Jurado et al. 2010).

Recently, some studies on airborne bacteria and fungi have been carried out in indoor environments (Rintala et al. 2008; Wang et al. 2010; Chen et al. 2010). The number of visitors in subterranean environments has an influence on both microorganism concentrations and compositions (Salmon et al. 1995; Docampo et al. 2011; Wang et al. 2010) as normal indoor and enclosed conditions, with high relative humidity, provide a suitable environment for the colonization and growth of bacteria and fungi (Bastian et al. 2010). However, there are no previous works combining aerobiological studies with detailed monitoring of the underground environment.

Here, we propose a double analytical approach to assess the extent of anthropogenic impacts on a cave atmosphere: first, a spatiotemporal monitoring of the main microclimatic parameters during daily visits, including the control of trace gases (CO_2 and ^{222}Rn) as indicators of cave ventilation and air movement within cave, and second, aerobiological samplings, conducted to quantify the concentration and diversity of airborne microorganisms and their spatial distribution. This study is focused for understanding microorganism dispersion processes and reinforces the importance of an environmental monitoring programme in order to ensure adequate conservation strategies in caves.

2 Material and methods

2.1 Site

Ardales Cave was discovered in 1821, open to visitors in 1852, and the visits discontinued in 1896. The cave was declared a National Monument in 1931, but no protection was guaranteed. A cave visit was resumed in a controlled way in the year 1992 and historically ranges around 1,000 visitors per year, on average, although 2,640 people visited the cave in the year 2007. This cave is characterized by a moderate to low visitor impact when compared with other caves nearby: Nerja 500,000 visitors, Tesoro 26,000 (Fernández-Cortés et al. 2008). The cave walls and rocks have 1,009 paintings and engravings of animals, humans, and different signs distributed among 251 panels (Cantalejo et al. 2006). Recent studies have been previously reported the presence of metabolically active bacteria on whitish colonizations present in the rock, sediments and speleothems, with a highly significant role of species of the genus *Pseudonocardia* (Stomeo et al. 2008).

2.2 Microclimate monitoring

The microenvironmental monitoring system operating in the cave was installed to record the microclimate at different locations (end of the entrance stair, Great Hall and Calvary Gallery, see Fig. 1) and also at the exterior. Each monitoring station consisted of a set of OPUS-200 two-channel datalogger/transmitter (Lufft, Fellbach, Germany), used for resistance, current and voltage measurements with high accuracy. Measurements were taken at 1 m from the floor and recorded every 5 min. Temperature and relative humidity of the air were measured by a humidity and temperature probe (Lufft, model 8160.TFF10), which combines a Pt1000 temperature sensor (measuring range −30 to 70°C, accuracy ±0.2°C and resolution 0.05°C) and a capacitive sensor (measuring range 0% to 100% RH, accuracy ±2% and resolution 0.1%). Cave air CO_2 concentration was measured using an infrared absorption sensor (Lufft 8520) configured over the range 0–3,000 ppm, with

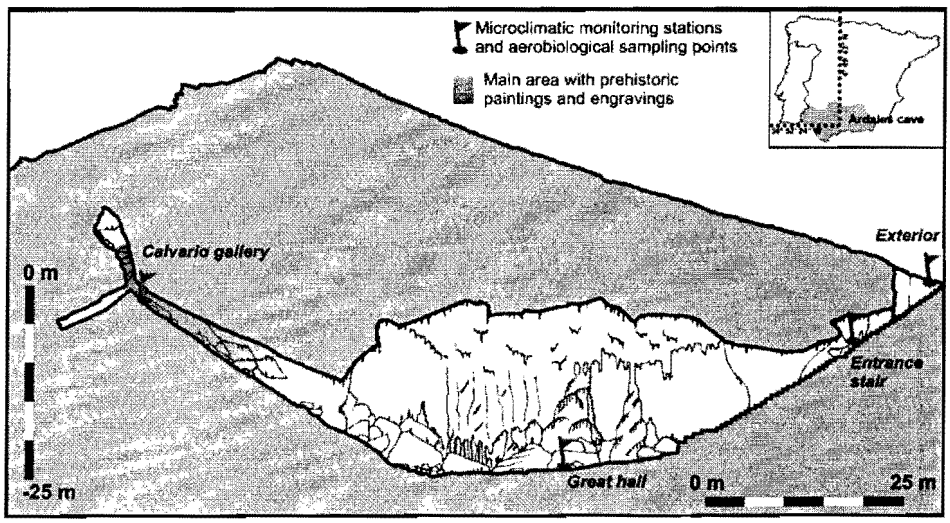

Fig. 1 Cross section of Ardales Cave in relation to surface and locations of the microclimatic monitoring stations and aerobiological sampling points

an accuracy of ±2% of reading and resolution of ±1 ppm (operating range 0–45°C and avoiding condensation with a vent microsystem).

The average ^{222}Rn concentration in air was measured at the entrance stair and Calvary Gallery by means of two Radim 5WP radon monitors (GT-Analytik KG, Innsbruck, Austria). The lowest activity detectable is 80 Bq/m^3, for 1-h measurements with a statistical error equal to ±20%, and the maximum is 150 kBq/m^3. The instrument response is 0.4(imp/h)/(Bq/m^3).

The microclimatic disturbance of the cave atmosphere by human presence was assessed by controlling key parameters (temperature, relative humidity and carbon dioxide) during six winter-days (from 2 to 7 February 2008). Thus, four groups visited the cave around the midday during the second day (14 people, 110 min), third day (10 people, 120 min) and fifth day (7 people during 95 min, and 4 people during 30 min and after 60 min from the preceding visit). During tourist visit, the groups (including a cave guide) are 25 min in the area of the Great Hall (over two times) and about 15 min in the area of Calvary. The rest is dedicated to the visit of the Lake Hall and Archer area and 10–15 min to the entrance and exit of the groups (Fig. 1).

2.3 Aerobiological study

An aerobiological sampling was conducted, inside and outside the cave, to quantify the level of airborne bacteria and fungal spores in the same places where the microclimatic system was installed. A Duo SAS 360 sampler (bpInternational, Milan, Italy) containing Petri dishes with Dichloran Rose Bengal Agar was used for the sampling of fungi. This medium facilitates the counting of fungal spores than otherwise would be impossible (King et al. 1979). For bacteria, the medium Tryptone-Soya Agar (TSA) was used. Air volume sampled was 100 L. This volume was selected, among several others tested, as the most appropriate for an easy counting in this cave. Samples were taken in duplicate. The dishes were incubated at 25°C, and the fungi were isolated as pure culture in malt extract–agar and kept at 5°C until further study. For bacteria, the dishes were incubated at 28°C and isolated in TSA medium.

2.4 Molecular methods

DNA extraction, PCR amplification of DNA, sequencing and phylogenetic analysis have been extensively described elsewhere, either for bacteria (Laiz et al. 2009) or for fungi (Jurado et al. 2010). Accession numbers of strains described in this paper are FR848405-FR848426 for bacteria and FR799474-FR799479 and FR799505-FR799510 for fungi.

3 Results and discussion

An experimental approach involving the control of microclimatic parameters during the visit of a selected number of people to Ardales Cave was carried out during winter in three different cave areas. In response to the cave geomorphology, the sensors were installed at the end of the entrance stair, Great Hall and in the top of the Calvary Gallery (Fig. 1). An increase in air temperature and CO_2 concentration due to visits was only noticed in the Calvary Gallery, the highest cave level in occasion of the visits, and was proportional to the number of people (14 visitors: +0.17°C and +203 ppm CO_2; 10 visitors: +0.10°C and +162 ppm CO_2; and 7 visitors: +0.06°C and +115 ppm CO_2). A small group of four visitors during 30 min do not seem to cause a significant microclimatic impact. Recovery time after daily visits reaches the early morning of the next day (Fig. 2).

One of the most important data is the temperature difference between the three areas monitored. The air temperature in Calvary Gallery (17.45°C) remains well above the air temperature in the entrance (16.78°C) and the Great Hall area (16.55°C). The warming of Calvary coincides with a higher and more stable concentration of ^{222}Rn during the period monitored (12,000–14,000 Bq/m^3), higher than the 6,000–9,000 Bq/m^3 in the entrance area (Fig. 3).

Calvary Gallery is nearby to the surface and topographically higher than the rest of areas. Microenvironmental data show that it operates as a motionless atmosphere that practically does not take part in the aerodynamic process between cave and the external atmosphere. The trap of CO_2 gas in the Calvary Gallery is essentially due to its accumulation near a gas source identified as the CO_2 exhalation of the successive groups of visitors in this area of the cave (Fig. 3a). The coeval air thermal stratification that creates a motionless trap of warm and less dense air contributes to the gas entrapment process in the Calvary Gallery, and it is controlled by the cave geomorphologic features. Here, diffusivity must play the major role in the exchange of CO_2 between the air parcels from Calvary Gallery and other deeper and nearby areas of the cave as the Great Hall. A gradient in the molar fraction of gases in both air parcels favours to launch a net transport of gas from high to low CO_2 concentrations. Consequently, the recovery of the CO_2 levels in the Calvary's atmosphere after the presence of visits is followed, with a certain delay, by an upward trend of the CO_2 level in the air parcel of the Great Hall (Fig. 2).

Contrarily to the time evolution of CO_2 levels, no thermal impact was noticed in the Great Hall's atmosphere after the tourist visits. Warmer and moist air breathed out by visitors tends to move towards the Great Hall's roof, so none rise of air temperature was registered by the monitoring station. Likewise, the anthropogenic air parcel

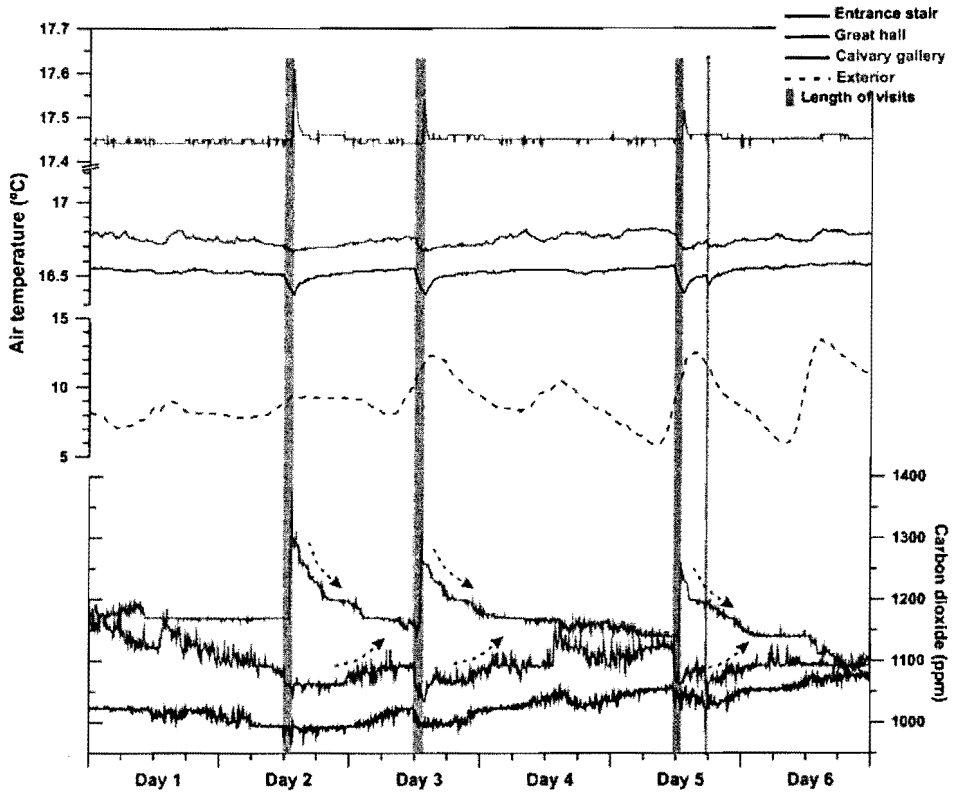

Fig. 2 Detailed time series of the microclimatic conditions in Ardales Cave during a sequence of daily visits. Several environmental impacts are distinguished (see text); CO_2 entrapment thermal stratification of the atmosphere in Calvary Gallery and CO_2 diffusion from upper to deeper areas (represented by *dashed black arrows*)

of Calvary's Gallery do not displace by thermal convection to deeper areas because the inversion of air density gradient was never established (Fig. 3a).

On the other hand, cave air ventilation is forced during time intervals with tourist visits due to the door opening. This effect is marked at the beginning of the visits and

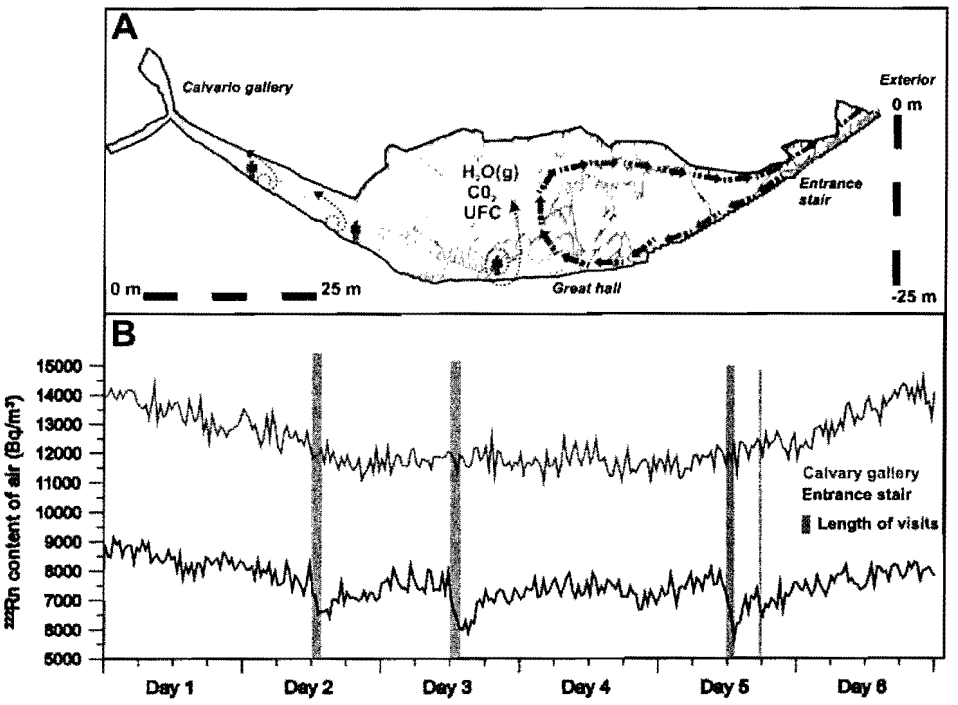

Fig. 3 **a** Scheme of human-induced disturbance of the cave environment (forced ventilation by opening of the cave entrance and local impact of visitors by extra contribution of CO_2, water vapour, and dispersion of airborne fungal spores and bacteria). **b** Detailed time series of ^{222}Rn content of air during a sequence of daily visits

while the cave air temperature is above the temperature of the external atmosphere. A convective air circulation along a thermo-density gradient is established. Accordingly, the influx of colder and denser air from the exterior into the cave is favoured, and simultaneously, the warmer, less dense and inner air parcels evacuate the cave. This human-induced ventilation provokes a short-term shift of the air temperature, CO_2 and ^{222}Rn levels in the cave atmosphere, mainly in those areas with direct connection to the cave entrance: entrance stair and Great Hall (Fig. 3b).

Aerobiology measurements (Tables 1 and 2) in two different days, one immediately after a visit of 32 people during 90 min and the second after a period of 2 days of cave resting, without visits, illustrate the impact of the visitors on the concentration of bacteria and fungal spores in the cave air. Regarding fungal spores, and according to what was observed in the microenvironmental monitoring, the highest impact was noticed in the Calvary Gallery, while in the Great Hall was very low. It is noteworthy that a visit multiplied the concentration of fungal spores by a factor of 100 in the Calvary, which revealed to be the most disturbed area, and by a factor of 4 at the end of the entrance stair, while remained constant in the Great Hall due to the high size and volume of this hall. These data agree well with those obtained for CO_2 concentration and temperature and are of importance because the main rock art paintings and engravings are located in the proximity of the Calvary Gallery, which is the area denoting the most disturbing conditions after visits.

The main fungal genera in cave air corresponded to *Aspergillus*, *Penicillium* and *Cladosporium*. These are also the most abundant fungi in indoor and outdoor samplings (Docampo et al. 2011). The presence of *Cladosporium* at the end of the cave points to a strong influence of the opening of cave door and the establishment of air flow from outside to inside, during the visit events, sweeping along the fungal spores. The *Cladosporium* spore number decreases from the entrance to the end of the cave. On the contrary, *Penicillium* spore number is high inside the cave and concentrates in the Calvary Gallery, the end of the cave. Supporting our observations, Docampo et al. (2011) reported that *Aspergillus* and *Penicillium* are the most abundant fungal genera indoor. This was also the trend found in this cave, particularly when the number of visitors was high and the stream of air maximized, and explains the presence of *Alternaria*, an outdoor fungus, in the Calvary Gallery, as well. The two most abundant species inside the cave, in terms of spore concentrations, were *Penicillium chrysogenum* and *Penicillium corylophilum*. Both *Penicillium* species have been reported in caves all over the world (Grishkan et al. 2004; Semikolennykh et al. 2005; Nováková 2009; Vaughan et al. 2011). *Cladosporium cladosporioides* was comparatively less frequent inside the cave.

The fungal spores identified after the days without visits show a different behaviour. In fact, the amounts of *Aspergillus*, *Penicillium* and *Cladosporium* are low at the end of the entrance stair and in the Great Hall. However, the identification of *Arthrinium* in the undisturbed Calvary Gallery the days without visits is of interest. This is a cosmopolitan fungus found in soils and decomposing plant material, which spores are dispersed by wind. *Arthrinium* was previously reported in the air, sediment, bat guano and earthworm casts of Slovakian caves (Nováková 2009), and was abundantly represented in Kartchner Caverns, Arizona (Vaughan et al. 2011). In Ardales Cave, the presence of bats and rodents as well as small animals was registered in the frequent surveys, and a similar origin (animal excrements) for this fungus is possible.

In general, fungal aerobiology data reflect the importance of the opening of the door for the visits, and the time that remains open, in the cave bioaerosol composition. In addition, this contributes to the emission/dispersal mechanisms of fungi through conidiophores or asci.

The aerobiological behaviour of bacteria is different. We might expect caves to have a significant influence on the airborne bacteria due to the specific nature of the communities found on speleothems and rocks. Bacteria are found in this cave coating stalactites, stalagmites, rocks and sediments (Stomeo et al. 2008). The data show that after an opening of the door and visit, the concentration of outdoor bacteria (lower than inside) does not justify the increasing amount found in the entrance stair and Great Hall. This increase is likely produced because the visits when descending the stair steps, which by the way showed abundant bacterial colonization, as well as the stalactites and stalagmites, originate great turbulences and erosions involving mechanical removal of sediment particles, which are much larger than the size of single bacterial cells or fungal spores. When the cave was non-visited for a few days, the higher bacteria concentration is found in the Great Hall. We have observed in this hall that the area where the sensors were installed has a rodent maximum activity (up to 20 excrements by square metre were found there). In absence of visits, rodent activities would maximize.

The composition of the culturable airborne bacteria inside the cave is remarkable. These are mainly represented by Gram-positive bacteria, ranging from 82.4% to 100%. Outdoors, the range is 50.4–81.2%. It has been reported that spore-forming organisms, such as *Bacillus* species and other Gram-positives, tend to dominate airborne microbial diversity (Mancinelli and Shulls 1978). While practically *Bacillus* spp. are represented in all the sampling stations, there are differences regarding the presence or absence of visitors. In fact, the higher percentages of spores can be found the days with visits, particularly in the Great Hall. After the visits, the Calvary Gallery shows a concentration

Table 1 Concentration of fungal spores as colony forming units CFU/m^3 during two sampling surveys with different scenarios, one immediately after a visit of 32 people and the second after a period of 2 days resting without visits

Hall/Gallery	After a visit					Without visits				
	CFU/m^3		Fungi identified	% Similarity	% Abundance	CFU/m3		Fungi identified	% Similarity	% Abundance
	Average	SD				Average	SD			
Calvary gallery	1,010	100	*Penicillium chrysogenum*	100	97.8	10	7.0	*Arthrinium* sp.	99	100
			Alternaria sp. 1	98	1.6					
			Cladosporium sp. 1	100	0.6					
Great hall	40	0	*Aspergillus* sp. 1	100	75.0	40	21.2	*Penicillium* sp. 1	99	35.0
			Cladosporium cladosporioides	100	25.0			*Cladosporium cladosporioides*	100	32.5
								Aspergillus sp. 1	100	32.5
Entrance stair	330	28.2	*Penicillium corylophilum*	100	71.8	80	84.9	*Aspergillus* sp. 1	100	62.5
			Penicillium chrysogenum	100	18.8			*Penicillium chrysogenum*	100	37.5
			Cladosporium cladosporioides	100	9.4					
Outdoor	620	40.0	*Cladosporium cladosporioides*	100	51.6	50	7.0	*Cladosporium* sp. 2	100	30.0
			Cladosporium cucumericum	99	42.6			*Cladosporium cladosporioides*	100	30.0
			Penicillium digitatum	100	5.8			*Alternaria alternata*	98	10.0
								Penicillium sp. 2	99	10.0
								Alternaria sp. 1	98	10.0
								Chalara microchona	99	10.0

SD standard deviation

The numbers represent the average value and standard deviation of two repeats

Table 2 Concentration of bacteria as colony forming units CFU/m^3 during two sampling surveys with different scenarios, one immediately after a visit of 32 people and the second after a period of 2 days resting without visits

Hall/Gallery	After a visit					Without visits				
	CFU/m^3		Bacteria identified	% Similarity	% Abundance	CFU/m^3		Bacteria identified	% Similarity	% Abundance
	Average	SD				Average	SD			
Calvary gallery	60	70.7	*Bacillus* sp. 1	99	65.0	10	7.1	*Arthrobacter* sp.	98	100
			Escherichia coli	99	11.7					
			Streptomyces sp. 1	98	11.7					
			Streptomyces zaomyceticus	99	11.6					
Great hall	330	205.1	*Bacillus* sp. 1	98	35.1	580	572.8	*Streptomyces avidinii*	99	80.5
			Bacillus sp. 2	98	29.7			*Paracoccus* sp.	99	9.3
			Micrococcus luteus	99	17.6			*Escherichia coli*	99	1.7
			Hydrogenophaga intermedia	98	17.6			*Bacillus* sp. 1	99	1.7
								Pseudomonas vancouverensis	99	1.7
								Streptomyces zaomyceticus	99	1.7
								Bacillus simplex	99	1.7
								Bacillus sp. 3	98	1.7
Entrance stair	430	367.7	*Streptomyces zaomyceticus*	99	37.2	150	127.3	*Streptomyces avidinii*	99	41.3
			Bacillus simplex	99	28.5			*Microbacterium phyllosphaerae*	99	24.0
			Escherichia coli	99	4.9			*Streptomyces* sp. 2	99	17.3
			Streptomyces avidinii	99	4.9			*Bacillus simplex*	99	8.7
			Bacillus idriensis	99	4.9			*Arthrobacter methylotrophus*	98	8.7
			Bacillus sp. 2	98	4.9					
			Bacillus weihenstephanensis	99	4.9					
			Arthrobacter methylotrophus	99	4.9					
			Paenibacillus lautus	98	4.9					
Outdoor	120	42.4	*Acinetobacter* sp.	99	35.5	170	120.2	*Oerskovia paurometabola*	99	25.3
			Lysinibacillus sphaericus	99	21.5			*Escherichia coli*	98	20.0
			Streptomyces sp. 2	97	21.5			*Bacillus* sp. 1	99	14.1
			Shigella sonnei	99	14.1			*Bacillus simplex*	100	13.5
			Bacillus simplex	100	7.4			*Rothia amarae*	99	12.9
								Planococcus sp.	99	7.1
								Arthrobacter tumbae	99	7.1

SD standard deviation

The numbers represent the average value and standard deviation of two repeats

lower than the Great Hall, but four times higher than the Great Hall without visits. It is remarkable that the number of *Bacillus* spores is low when the cave was non-visited. This behaviour mimics the one reported for fungi and can be interpreted as that *Bacillus* spores suffer a similar dispersion pattern than fungal spores, while other bacteria are likely dispersed by particles.

On the other hand, the species of the genera *Arthrobacter*, *Micrococcus*, *Pseudomonas*, etc. have no relevant patterns and can be occasionally scattered in a hall or gallery. Most bacteria have no other dispersal mechanism than that derived from wind speeds, turbulences, impacts or splashes of falling drops from the stalactites and the ceiling on the sediments, etc. The activity of small animals (rodents, bats) and humans inside the cave can be a source of particles removal and emission to the atmosphere as well. Shaffer and Lighthart (1997) stated that the majority of airborne bacteria are associated with particles and they may occur as agglomerations of cells. In addition, it has been reported that high concentrations of airborne bacteria can be found after a simulated rainfall (Robertson and Alexander 1994).

Escherichia coli merits some comments. This bacterium has a relative abundance outdoors (20%) as corresponds to an area of cattle farming in the topsoil and cave surroundings. This has been found randomly distributed in all the stations, irrespective of the visits. Also, rodents and bats activities inside the cave can contribute to its dispersion.

In a previous paper, it was stated that the phylum *Actinobacteria* was the most frequently found in the analysed 16S rRNA gene library of the speleothems of this cave, reaching 44% of sequences out of a total of 25 clones (Stomeo et al. 2008). Within the *Actinobacteria*, most of the clones corresponded to microorganisms belonging to *Streptomyces*, the *Rubrobacteridae*, and a high proportion of them to the genus *Pseudonocardia*. Unfortunately, the number of clones used by Stomeo et al. (2008) was too low for deriving ecological consequences or for comparing with the aerobiology data. Anyway, the presence of species of *Streptomyces*, and particularly *Streptomyces avidinii* and *Streptomyces zaomyceticus* in different cave compartments, is of interest and suggests that members of this genus are actually involved in the colonization of speleothems. No culturable *Pseudonocardia* were found in the aerobiological study. It is well known that the vast majority of environmental bacteria are non-culturable. This may be particularly true for airborne bacteria since the culturability of bacteria rapidly decreases following aerosolization (Heidelberg et al. 1997).

4 Conclusions

This paper shows for the first time data obtained from a combination of microclimate and aerobiological monitoring in caves and reveals that the human presence in Ardales Cave results in the entrapment of warmer, less dense and CO_2-enriched air at the Calvary Gallery, as well as a strong increase of airborne fungal spores.

The energy released in the air parcel of Calvary Gallery due to visit's impact creates a thermal sedimentation that traps air in the hot bubble and only diffusion can evacuate CO_2 from there, since the cave entrance is far and the convective air circulation is mitigated.

Human-induced variations of the cave microclimate by opening of the entrance entail the local air movement and, therefore, reinforce the role of the atmosphere as a vehicle for the transport and dispersion of airborne microorganisms and nutrients inside the cave. Furthermore, visits originate the transfer of airborne fungal spores to endangered areas such as the Calvary Gallery.

The mobilization of airborne microorganisms and the extra contribution of water vapour, temperature and CO_2 caused by visitors could activate rock surface weathering, including those with prehistoric paintings and engravings.

The detection of evident anomalies in the cave area housing the high density of paintings and engravings helps to control human disturbances and supports the direct application of this double approach for cave management purposes.

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