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The lampenflora in show caves and its treatment: an emerging ecological problem

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Abstract: The artificial lighting of caves adapted for touristic visits, leads to the appearance and propagation of a complex community of phototrophic organisms known as “lampenflora”. Formed mainly by algae and cyanobacteria, they produce the degradation of the colonized substrates and decrease the show value of the caves. This phenomenon became famous worldwide in the 1960s due to the damage caused to the paintings in the Lascaux Cave (France). Since then it has become an issue of serious concern to both managers of show caves and to the international scientific community. Over time, the problem has been approached following two complementary strategies: preventing colonization by the invading organisms or eliminating them once they have become established through the use of chemical products, mainly biocides and strong oxidants. This kind of treatment generates pollutant effluents that can move the problem from the walls of the caves to the groundwater. This paper presents a critical literature review of the problem and the proposed solutions, and emphasizes the need for further study of the optimal doses of treatment chemicals and to develop quantitative methods to determine their effectiveness.

Keywords: lampenflora, show caves, biofilms, biodeterioration, groundwater pollution

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INTRODUCTION

Since prehistoric times, our ancestors have forged a special link with caves. They were initially used mainly for practical purposes as a temporary refuge or as spaces for permanent habitation, a source of water and certain minerals and even as places of worship and necropolises. Evidence of this early presence can be seen in the human remains found in numerous caves from the lower Pleistocene era through to the present day. Cave paintings are a special example of humans' close relationship with caves, of which some of the most spectacular are emblematic sites such as the Lascaux Cave and the Altamira Cave (Spain). Due to their outstanding natural and cultural importance, these caves, along with several dozen more such as the Las Manos Cave (Argentina), the Škocjan Caves (Slovenia) and the Mammoth Cave National Park (Kentucky, USA), are included on the list of World Heritage sites compiled by the UNESCO (United Nations Educational, Scientific and Cultural Organization).

In later centuries and until the present day, most caves have ceased to serve these functions; however, due to the natural beauty of their geological formations and their historic and cultural content, they have become a potent tourist attraction. In some cases, they also constitute a notable scientific resource, as many contain paleoenvironmental information and highly valuable geological records, such as is the case of the Nerja Cave (Spain) (Carrasco, 1993; Jordá Pardo et al., 2011).

Visiting caves is thought to be one of the oldest tourist experiences (Mulec & Kosi, 2009). The concept of show cave (Cigna & Forti, 2013) can be defined as “any cavity where a fee is paid to gain access and visit it” originates in the early 17th century in the Vilenica Cave (Slovenia), recognized since 1633 as the first show cave in the world; currently, practically every country in the world has at least one show cave.

It is estimated that there are around 500 large show caves worldwide with over 250 million visitors a year. If we include all the related activities (transport, accommodation, etc.), some 100 million people derive

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a direct or indirect income from this activity. Caves also provide income from speleotourism, religious tourism and even healthcare (Cigna & Forti, 2013). The increase in these practices means this type of tourism has become an important economic resource which in certain areas represents the main source of income for its inhabitants (Cigna & Burri, 2000; Bočić et al., 2006; Garofano & Govoni, 2012).

For centuries, what is known as “underground tourism” was done in a very rudimentary way using torches or oil lamps as a source of illumination. Over time, some caves fashioned an artificial entrance from the outside, marked out a safer route on their interior, and gradually incorporated lighting equipment. Although these actions enhanced the observation and enjoyment of the caves, it also represented a very significant modification of the natural environmental conditions (Hoyos et al., 1998; Gillieson, 2011), casting light on areas that had remained in total darkness for millennia, increasing the environmental temperature and decreasing the relative humidity in their interior, as occurred for example in the Las Maravillas Cave (Spain) (Pulido-Bosch et al., 1997).

The degree of anthropic alteration of a show cave varies depending on the actions undertaken and the type of tourism practised (del Rosal, 2015). The impacts tend to be greater in caves adapted for general tourism than in caves adapted exclusively for scientific activity or the practice of speleotourism, with limited groups of participants who only require a speleological installation and a minimal portable lighting system.

The first caves equipped with electric lighting (Mulec & Glažar, 2011) were the Luray Caverns (Virginia, USA) in 1881, followed by Kraushöhle (Austria) in 1883 and the Postojna Cave (Slovenia) in 1884. It was soon observed that the gradual installation of sources of artificial lighting inside the caves promoted the development and proliferation of a complex community of phototrophic organisms near the lamps. According to Cigna (2011a), this phenomenon was initially studied by Austrian (Kyrle, 1923; Morton & Gams, 1925) and French (De Virville, 1928) scientists; and it was not until

the 1960s when Dobat (1963, 1969) first introduced the word “lampenflora” (originally a German term coined in the English vocabulary) meaning “the flora of the lamps”. This term is currently used internationally to identify this phenomenon, although it is also known as “mal verde” in Spanish or “maladie verte” in French (Lefèvre, 1974).

The issue of lampenflora is occasionally overlooked in show caves, where economic interests sometimes take precedence over conservation (Saiz-Jiménez, 2012). In certain caves (Mulec & Glažar, 2011; Trinh & García, 2013) the lampenflora is even displayed to visitors in a somewhat irresponsible way, when it is highlighted as a tourist attraction without the managers considering its removal, as is the case for example in Natural Bridge Caverns (Texas, USA) or in the karstic caves of the bay of Ha Long (Vietnam).

However, lampenflora poses a serious problem, as it constitutes an invasive and opportunistic community in anthropized underground environments. This is because, with the exception of the cave entrance, these organisms use artificial light to develop and therefore grow in places where they would not occur naturally (Mulec, 2012). In addition, the new inhabitants of the caves that have developed thanks to artificial lighting compete successfully to occupy this ecological niche. The problem is aggravated in many caves, because the sites of greatest interest to tourists (cave paintings or high-value geological formations) tend to be better illuminated than the rest of the cave. This increases its attractiveness to the public, but means these areas are more likely to be colonized by lampenflora.

In recent decades numerous works have been published on lampenflora communities in the international scientific literature, and on the methods that have been used to prevent and control their growth in caves. This work is a review of the state of the art in the methods of lampenflora control and their pros and cons. Figure 1 shows a map of the locations of the caves mentioned in this work and offers an overview of their geographic distribution. Table 1 contains a list of the caves mentioned, and a brief summary of some of their most important features.

Fig. 1. Geographic distribution of the caves mentioned in this work. Numbers represent the caves in Table 1.

Table 1. Caves mentioned in this work and a brief summary of their most important characteristics.

Name		Country	Characteristics
1	Tito Bustillo Cave	Spain	Cave with prehistoric paintings included on UNESCO's World Heritage list since 2008 in the category of "Palaeolithic rock art of northern Spain". The development of lampenflora posed a serious problem and led to the closure of cave.
2	Altamira Cave	Spain	Declared a World Heritage site by the UNESCO in 1985, it contains one of the most extraordinary manifestations of Palaeolithic art in the world. The growth of photosynthetic microorganisms led to the closure of the cave in 2002.
3	Castañar de Ibor Cave	Spain	Located in the Villuercas-Ibores-Jara Geopark, this is a karstic cave whose importance and uniqueness derives from the fact that it is excavated out of Precambrian materials (540 Ma) and contains some spectacular speleothems. It has high radioactivity (radon). Since 2008, due to a fungal outbreak, access is limited to small groups of visitors wearing protective suits with hand-held or helmet-mounted torches.
4	Las Maravillas Cave	Spain	Cave developed on limestone and marble from the Cambrian period (over 500 million years old). It is located within the urban layout of Aracena (Huelva) and has been declared a Property of Cultural Interest. It contains karst formations of extraordinary beauty. The development of lampenflora communities has been controlled using hypochlorite.
5	Zuheros Cave	Spain	Also known as the Los Murciélagos cave. It is known for its archaeological excavations, and has been declared a Property of Cultural Interest and a Natural Monument. Studies have recently been done on the diversity and structure of its biofilm communities.
6	El Tesoro Cave	Spain	The only cave of underwater origin known in Europe. Of major scientific, historic and cultural interest: it is the site of discoveries of rock paintings and stone and ceramic remains from the Neolithic era. Declared a Property of Cultural Interest. Comparative studies have been done in this cave between hydrogen peroxide and sodium hypochlorite for the elimination of lampenflora. Liquid nitrogen has also been used and LED lighting has been installed (590 nm).
7	Nerja Cave	Spain	Declared a Property of Cultural Interest, it has 589 rock paintings dating from the upper Palaeolithic era. This is one of the most visited caves in Spain. Studies have been done on the emission spectrum of photosynthetic organisms, identifying their pigments and the least favourable range of wavelengths for their development. A new lighting system has been designed on this basis. Experiments have also been done to eliminate lampenflora with calcium hypochlorite.
8	Las Ventanas Cave	Spain	Declared a National Monument, camouflage methods have been used here to conceal the lampenflora communities.
9	Gelada Cave	Spain	Located in the Serra Gelada nature reserve, it was corroborated in this cave that the main stress factor for biofilms was the lack of light, followed by moisture deficit, lack of nutrients and temperature variations.
10	Ortigosa de Cameros Caves	Spain	Comprising the La Paz and La Viña caves, they have been the site of studies aimed at increasing the threshold concentration of hydrogen peroxide necessary to eliminate the lampenflora communities.
11	Collbató Cave	Spain	Also called the Montserrat Caves, they served as the inspiration for the artist Antoni Gaudí when he designed the Sagrada Família basilica (Barcelona). Studies have recently been done on the diversity and structure of their biofilm communities.
12	Lascaux Cave	France	Declared a UNESCO World Heritage site in 1979, this is one of the most important manifestations of Palaeolithic and cave art, and contains magnificent prehistoric depictions, including almost 1,500 etchings and 600 Palaeolithic paintings. This cave has suffered successive biological crises, leading to irreversible change in its ecological conditions and the discontinuation of tourist activity. It was the first cave in which a complex study was undertaken on the biodeterioration processes caused by photosynthetic microorganisms on its interior, and specifically on the cave paintings.
13	Moidons Cave	France	Located in the Jura massif, this cave has combined tourist activity with the assessment and practical application of a germicidal treatment with UV-C radiation applied to different types of biofilms.
14	Crypt of the Original Sin	Italy	A series of paleo-Christian Byzantine frescoes dating from the 9th century and located in the municipality of Mater. They represent different biblical scenes, particularly corresponding to the Creation. Affected by communities of phototrophic microorganisms, this is an example of the poor use of biocides to treat these communities: although these products eliminated the microorganisms, the colorimetric analyses also revealed changes in the colour of the substrate after treatment.
15	Frasassi Caves	Italy	Network of karstic caves located in the Gola della Rossa di Frasassi Nature Reserve. They constitute one of the most important speleological sites in all Italy. These caves were the site of one of the earliest tests to assess the corrosive action of sodium hypochlorite.
16	Grotta Gigante	Italy	Inaugurated in 1908, this is the largest show cave in the world. UV-C radiation is used here to limit and control the proliferation of photosynthetic microorganisms.
17	Vilenica Cave	Slovenia	Its importance is not due to its geological or archaeological worth, but to the fact that since 1633 it has been recognised as the most important show cave in the world.
18	Škocjan Caves	Slovenia	Included on the UNESCO's World Heritage list since 1986, this group of limestone caves in the Kras region is one of the best places in the world to study karstic phenomena.
19	Sezana Hospital	Slovenia	Healthcare facility with caves that are used as a speleotherapy centre for the treatment of patients with pulmonary ailments. It uses dispersed lighting that hinders the development of lampenflora communities.

20	Postojna Cave	Slovenia	This is the largest cave in the Kras region, in addition to one of the most visited caves in Europe. This was the third cave to be equipped with permanent electrical lighting, in 1884. Hydrogen peroxide is used to eliminate the lampenflora communities.
21	Kraushöhle	Austria	This cave is unique in Europe due to its speleothems with a sulphuric origin. Its importance also lies in the fact that it was the second cave equipped with permanent electric lighting, in 1883.
22	Moravian Karst Caves	Czech Republic	The Moravian Karst is a protected nature reserve of 92 km ² which includes over 1,100 caves, 14 of which are adapted for tourist use. Sodium hypochlorite is used to eliminate biofilms.
23	Katerinska Cave	Czech Republic	Part of the Moravian Karst, it was one of the first caves in which hydrogen peroxide was used to eliminate the problem of lampenflora.
24	Javoříčko Cave	Czech Republic	Underground system comprising a labyrinth of galleries and chasms that remained undiscovered until 1938. Dead bats were found after treatment with sodium hypochlorite.
25	Aggtelek or Baradla Cave	Hungary	Included on the UNESCO's list of World Heritage sites since 1995, it is the largest stalactite cave in Europe. Since they were adapted in 1920 as a tourist attraction, the lampenflora communities have spread quite fast, and there are now focuses of colonisation distant from the illuminated areas.
26	Alistrati Cave	Greece	In this cave, low-pressure sodium vapour lamps were not effective in preventing the development of lampenflora.
27	Ali-Sadr Cave	Iran	This is the largest aquatic cave in the world, and attracts millions of visitors each year. Studies have been done to increase the threshold concentration of hydrogen peroxide necessary to eliminate the lampenflora communities.
28	Cango Caves (South Africa)	South Africa	These are among the most important show caves on the African continent and attract numerous local and foreign visitors. They contain an extensive system of tunnels and chambers with a length of over 4 km. Hydrogen peroxide combined with UV-C radiation has been used to eliminate the lampenflora communities. The use of green light was also suggested (around 530 nm) to limit their growth.
29	Ha Long Bay Cave	Vietnam	Its karst formations and islands were declared a UNESCO World Heritage site in 1994, and since 2011 it has been known as one of the seven natural wonders of the world. The lampenflora growing in its formations is presented to visitors as a tourist attraction.
30	Jenolan Caves	Australia	They are located in the Blue Mountain region, and were declared a World Heritage site by the UNESCO in 2000. In the 1980s, as in many caves, pressurised water was used to clean speleothems covered by algae. This area was the venue for the 7th International ISCA Congress in November 2014, where the management document entitled "Recommended International Guidelines for the Development and Management of Show Caves" was agreed.
31	Waitomo Cave	New Zealand	Cave colonised by bioluminescent insects, where several experiments have been done to control the lampenflora communities: use of steam or hot water at low pressures, instead of cold water at high pressures, calcium hypochlorite and ultraviolet lamps.
32	Ruakuri Cave	New Zealand	Near the Waitomo Caves, tests using calcium hypochlorite were also done in the late 1970s to control the lampenflora communities.
33	Oregon Caves National Monument	Oregon, USA	Cave developed on marble and declared a National Monument. Exhaustive studies have been carried out on the composition of the lampenflora communities, and over 100 species have been identified.
34	Carlsbad Caves National Park	New Mexico, USA	Group of caves declared a UNESCO World Heritage site in 1995. This national park comprises 83 independent caves that emerged around 250 million years ago and attract hundreds of tourists and potholers. Exhaustive studies have been carried out on the composition of the lampenflora communities, and a total of 200 species have been identified.
35	Natural Bridge Caverns	Texas, USA	Declared a United States National Natural Landmark, the lampenflora in these caves is considered a tourist attraction, and its managers do not contemplate its removal.
36	Mammoth Cave National Park	Kentucky, USA	Declared a national park in 1941, a UNESCO World Heritage site in 1981 and a biosphere reserve in 1990. This is the longest known cave system in the world. It was successfully demonstrated that the development of lampenflora could be controlled through the use of LED illumination. An emission range of 595 nm (yellow) was used, preventing its growth for 1 1/2 years after its installation.
37	Luray Caverns	Virginia, USA	A UNESCO World Heritage site since 1973, this is the fourth largest cave in the United States and the first with permanent electrical lighting, installed in 1881.
38	Las Manos Cave	Argentina	This is one of the few archaeological sites in the Argentinian part of Patagonia and among the most beautiful and oldest artistic expressions of the South American peoples. It was declared a UNESCO World Heritage site in 1999.

General characteristics of lampenflora

The composition of lampenflora communities and their eco-physiological characteristics have been the subject of numerous studies. This section summarizes the main concepts in regard to these organisms.

Cyanobacteria, together with green (*Chlorophyta*) and golden (*Chrysophyta*) algae are the most common microorganisms identified in lampenflora communities (Padisák et al., 1984; Hoffman, 1989;

Rajczy, 1989; Mulec, 2012), but their abundance varies from one cave to another. For example, Aley (2004) compares the composition of two different lampenflora communities in the Carlsbad Caverns National Park in New Mexico and the Oregon Caves National Monument, both in the United States. In the first, out of a total of 200 species, it was estimated that 70% of the community were cyanobacteria and 20% green algae; the abundance percentages were

similar in the second, as out of a total of 100 species identified, 40% were cyanobacteria and 35% green algae. Golden algae were also present, but in very small percentages.

In a subsequent work, Mulec et al. (2008) identified 60 algae and cyanobacteria taxa colonising eight underground environments (six caves and two mines) in the karst region in Slovenia. Cyanobacteria were the most abundant (47%), followed by green (30%) and golden algae (23%). The cyanobacteria were represented mainly by the species *Aphanocapsa muscicola*, *Lyngbya* sp. and *Synechocystis* sp. In regard to algae, the authors found mainly the genera and species *Chlorocloster* sp. and *Navicula mutica* – golden algae – and *Chlorella* sp., *Stichococcus bacillaris* and *Trentepohlia aurea* – green algae.

In a more recent work, Mulec and Kosi (2009) showed that the most common communities of photosynthetic microorganisms in European caves are cyanobacteria (around 50% of the total), green and golden algae, the most common species are *Aphanothece castagnei*, *Gloeocapsa sanguinea* (cyanobacteria) and *Stichococcus bacillaris* (green algae). Other studies have reported the presence of *Chlorella* green algae genera in lampenflora communities in various caves around the world (Grobbelaar, 2000; Nugari et al., 2009; Urzi et al., 2010; Cennamo et al., 2012; Mulec, 2012).

Although this group of algae may live independently in nature, most of the microorganisms tend to form multicellular communities known as “biofilms”. Nikolaev and Plakunov (2007) state that between 95% and 99% of microorganisms in natural environments live in the form of biofilms which represent a privileged lifestyle for most microorganisms (Ragon, 2011).

Although the diversity of the lampenflora communities inside the caves is poor in comparison with those growing naturally at the entrances (Mulec, 2012), biofilms may constitute complex biocoenoses, in which cyanobacteria and algae coexist with bacteria, fungi and yeasts (Cooksey, 1992; Jones, 1995; Jurado et al., 2010). Kumar and Anand (1998) determined

that the biofilms with the greatest diversity are the most resistant to unfavorable external conditions.

Chemically, biofilms are composed mainly of water (~70-90%), organisms with a diverse metabolism and a hydrated matrix of extracellular polymeric substances (EPS) that they themselves segregate, composed of polysaccharides, lipopolysaccharides, proteins, glycoproteins, lipids, glycolipids, fatty acids and enzymes (Flemming, 1993; Warscheid & Braams, 2000).

This matrix confers a series of advantages that favour the survival of the community in hostile environments, increasing the resistance of its members to external agents or other organisms. Some of these benefits are: greater protection against sources of environmental stress (desiccation processes or exposure to UV rays, heavy metals and atmospheric contaminants), greater water retention and enhanced concentration and circulation of nutrients within the biofilm (Lawrence et al., 1991; Costerton et al., 1999; Watnick & Kolter, 2000; De Philippis et al., 2001; Roldán & Hernández-Mariné, 2009; Ragon, 2011). It has also been demonstrated that these single-cell microorganisms use a method of intracellular communication through chemical signals known as “quorum sensing”, which allows them to act in a coordinated way as multicellular organisms (Davies et al., 1998; Sharif et al., 2008; Annous et al., 2009).

Although at first sight the microscopic community that comprises the lampenflora (cyanobacteria and algae) (Fig. 2) appears to be static, this is not the case. The species' successional process is fairly dynamic, and early colonisers tend to grow rapidly before being slowly displaced by more persistent organisms (Mulec, 2012).

Although algae and cyanobacteria are the most common photosynthetic organisms identified in caves, other elements that may also form part of the lampenflora communities include mosses, lichens, and sometimes even ferns and higher plants (Merdenisianos, 2005; Cigna, 2011a). In the early phases of colonization, cyanobacteria and eukaryotic

Fig. 2. Diagram of the evolution and development of biofilms in show caves (based on Di Martino, 2016).

algae tend to play the most important role in the processes of forming the biofilm and are considered pioneering species in the ecological succession (Aleya, 1991; Gaylarde & Gaylarde, 2000; Whitton & Potts, 2000; Mulec et al., 2008). Subsequently, mosses and ferns develop, and the final successional phase may also include vascular plants, although almost always in the form of germinating shoots (Martincic et al., 1981).

The presence of cyanobacteria in lampenflora communities is very important from a metabolic point of view. These are the photosynthetic microorganisms that evolve most successfully in illuminated underground environments; they require no organic matter and are capable of absorbing a wide spectrum of light radiation thanks to the presence of accessory pigments (del Rosal et al., 2012). These qualities confer a significant advantage for the colonisation of new areas (Aleya et al., 1994; Herrera et al., 2004).

Thanks to their photosynthetic capacity and their nitrogen and CO₂ fixing function, biofilms induce the growth of mosses and ferns in the area around them, which benefit from environments enriched in organic matter and nutrients, in turn promoting the proliferation of certain heterotrophic microorganisms (Caneva et al., 2008; Scheerer et al., 2009). This last group of microorganisms is formed by bacteria and fungi (de la Torre et al., 1991; Singh et al., 2008; Dakal & Cameotra, 2012) and tends to be located in areas with an accumulation of organic matter such as animal excrement or detrital sediments dragged by water into the cave interior (Humpreys, 1991; Bottrell, 1996; Simon, 2012; Jurado & Saiz-Jiménez, 2016). Other elements of an organic nature that serve as a source of nutrients are the fluff and other detritus (hairs, dry skin, dust from shoes) introduced by visitors in the case of caves adapted for tourism (Aley, 2004; Cigna, 2012; Mulec, 2012).

Of all the microorganisms present in a cave, Porca et al. (2011) consider that fungi are to a large extent the cause of greatest concern due to their high rate of production of spores and dispersion in the air. It has also been demonstrated that certain species of arthropods can not only transport fungal spores, but that many of these fungi are parasites on certain groups of insects, and ultimately kill the host and use their body to produce spores and colonise any type of organic matter present in the cave (Jurado & Saiz-Jiménez, 2016).

Unlike plants, fungi do not require light to grow and can be found in areas of half-light or total darkness in the caves (Aley, 1972) at some distance from the point of artificial light. However, it is only in show caves that autotrophic plants produce organic matter in a sufficient concentration to allow significant development of fungal populations (Johnson, 1979). One of the best-known cases is that of the Lascaux Cave, where the development of the alga *Bracteacoccus minor* as a result of the presence of artificial light (Lefèvre, 1974) and the subsequent massive colonisation by the fungus *Fusarium solani* marked the start of a succession of biological crises in the cave, leading to an irreversible change in its ecological conditions and the

discontinuation of tourist activity (Bastian et al., 2010; Martín-Sánchez, 2012).

Environmental factors conditioning the presence and development of lampenflora

The microorganisms that form the lampenflora communities access the inside of the cave thanks to the mobility of the microorganisms themselves, or are accidentally dragged in by air currents, water flows, gravitational sedimentation through cracks and small cavities in the rock, or by the transit of cave fauna and visitors (Dobat, 1970; Vegh, 1989; Cigna, 2012; Mulec, 2012; del Rosal, 2015).

One important factor in the propagation of lampenflora is local air currents caused by the warm air generated near more powerful lamps, and particularly halogen lamps (500-1000 W) (Vegh, 1989). The growth of lampenflora has even been observed at distances of over 10 m when using lamps of this kind (Mulec, 2012).

Some of these factors, such as water seepage, air currents or the presence of animal and organic matter from the outside (or more generally, mass and energy flows) will in turn depend on the level of energy in the cave (Borderie et al., 2014a). Heaton (1986) introduced the concept of energy in caves, a cave that maintains high energy relations with the outside will therefore in principle be more susceptible to colonization by exogenous microorganisms, including lampenflora communities. High-energy show caves will be relatively unaffected by tourist activity, whereas caves with moderate and low energy levels are more vulnerable to anthropogenic perturbations (Grobelaar 2000).

One of the most widespread procedures in show caves to maintaining any additional energy levels as low as possible is to limit the number of visitors based on what is known as their "carrying capacity" (Hoyos et al., 1998; Cigna, 2011b). Carrying capacity is defined as the maximum number of acceptable visitors per unit of time that can be borne by a certain ecosystem (in this case underground environments) without causing permanent modification of its most important environmental parameters. This is done by reducing the number of people or the time they spend inside the cave. Merdenisianos (2005) and Cigna (2011b) also propose the installation of double doors and air curtains at the cave entrance, with the aim of protecting the cave from changes in the microclimatic conditions, avoiding air flows into the interior and reducing the dirt deposited by the visitors. During visits to highly unique caves with high levels of protection, Jurado and Saiz-Jiménez (2016) recommend wearing protective clothing and footwear, gloves and masks to prevent inhaling the microorganisms present in the air and depositing residues from the exterior. For example, these measures have been actively implemented in the Castañar de Ibor and Altamira caves, both in Spain.

Once the access to the cave has occurred, the microorganisms adhere to the rock through several different mechanisms (Hernández-Maríné & Roldán, 2005; Bellezza et al., 2006; Karsten et al., 2007) and

may remain on the exterior of the substrate (epilytic) or penetrate into it (endolytic); the latter may in turn be located in pores and cavities (cryptoendolytic) or in cracks (chasmoendolytic) (Golubic, 1981).

The colonization of the different parts of the cave depends on several factors, of which the main ones are the bioreceptivity of the substrate and the environment, and the conditions of the underground medium (lighting, moisture, nutrients, and temperature).

a) The bioreceptivity of the substrate defines its potential to be colonized by different microorganisms (Guillitte, 1995; Miller et al., 2009).

Based on texture, Gillieson (1996) determined that smooth substrates generally had a lower colonization by photosynthetic microorganisms compared to rough substrates. Warscheid and Braams (2000) emphasize the porosity of the substrate, and report that materials with large pores, due to their short water retention times, promote only temporary microbial contamination, whereas small pores retain moisture for longer and offer greater protection.

Another important factor affecting the growth of lampenflora is the presence of sediment in the substrate (Martincic et al., 1981; Chang & Chang-Schneider, 1991; Aley, 2004). Examples include the observations made by Johnson (1979) of discrete lampenflora populations in the Waitomo Caves (New Zealand). The presence of ferns, due to their very extensive root system

and their erect growth pattern, is limited to areas of clay, moist mud or slightly porous limestone substrates. Mosses, which require a large amount of moistures, are also found in mud, in areas of clay and in porous limestone substrates with a constant supply of groundwater. In contrast, algae are capable of supporting much wider fluctuations in available moisture than mosses and ferns, and can develop in practically any illuminated substrate.

Finally, and in regard to the type of materials, the presence of significant amounts of carbonate and/or mineral compounds like feldspars, clays and iron minerals means these substrates are particularly susceptible to the development of microorganisms (Warscheid & Braams, 2000). Artificial stone like bricks and mortar (which can be found inside caves that have been adapted for visitors) are also highly susceptible to microbial attack.

b) Environmental conditions. The light input, together with the availability of adequate levels of moisture, temperature and nutrients, are the most important environmental factors conditioning the presence and distribution of photosynthetic microorganisms in underground environments (Cigna, 2011a). Table 2 summarizes the environmental factors that condition the presence of lampenflora described in the preceding paragraphs.

Table 2. Environmental factors that influenced the presence of lampenflora.

Factor		Description	
Bioreceptivity of the substrate		Substrates with a rough texture and small pores.	
		Presence of sediments. Materials formed by carbonate compounds, minerals susceptible to meteorological elements and/or artificial stone.	
Environmental conditions of the underground environment	Lighting	Wavelength	The process of photosynthesis is enhanced at wavelengths in the following intervals: 430-490 nm (blue light) and 640-690 nm (red light).
		Duration and degree of intensity	The thickness and diversity of the biofilms decreases when the intensity of light is less than 10 to 50 lux in algae, 50 to 180 lux in mosses and 250 lux in ferns. Lampenflora communities can survive with much lower levels of lighting than described previously, and even in total darkness for long periods of time.
	Humidity	The development of lampenflora is generally limited to humid environments and wet surfaces. Duration of dry periods and number of humid periods. Algae are capable of withstanding much greater fluctuations in the available moisture than mosses and ferns, and some species may even recover their metabolic activity after prolonged periods of desiccation.	
	Nutrients	Obtained primarily from groundwater seeping into the cave interior. Secondary sources, excrement of the animals inhabiting the cave, detrital and clayey sediments dragged by water into the interior or residues introduced by visitors during tourist visits (fluff, dust, hair). The high levels of CO ₂ , generated by visitors contribute to the increase in photosynthetic capacity. Within the actual lampenflora population, there is also a cycle of nutrient utilisation, release and re-utilisation by autotrophic and heterotrophic organisms.	
	Temperature	The microorganisms are arranged according to temperature: extremely powerful illumination restricts the growth of lampenflora in the area around the artificial lighting point as a result of excess heat. However, lampenflora communities may continue growing in areas further away from the lamps. In comparison with the exterior, the temperatures in the underground environment are more uniform.	

Lighting

Three aspects of the lighting must be considered: intensity, duration, and wavelength, as the three influence the type of development of photosynthetic biofilms.

Chlorophyll (types A and B) has two absorption peaks in the wavelength intervals of 430-490 nm (blue light) and 640-690 nm (red light), and these wavelengths are more of a hazard for the proliferation of lampenflora (Bickford & Dunn, 1972; Caumartin, 1994; Olson, 2006; Cigna, 2011a). That is, if the lighting system used in a show cave contains these emission ranges, the process of photosynthesis will be enhanced.

Cyanobacteria, in addition to chlorophyll, possess phycobilins (phycoeritrin, phycocyanin and allophycocyanin), accessory pigments that expand the absorption spectrum of the primary pigments and act as a system of protection against high light radiation (del Rosal, 2015).

In studies on the diversity and structure of the biofilms in several Spanish caves (Zuheros Cave, Nerja Cave and Collbató Cave), Roldán and Hernández-Mariné (2009) observed that the thickness and diversity of the biofilms decreases if the intensity of the light also decreases. In fact, the availability of photosynthetic radiation was the parameter that determined whether the microorganisms forming a biofilm were predominantly photosynthetic (algae and cyanobacteria) or heterotrophic (fungi and bacteria). Thus, areas with poor illumination were dominated by heterotrophic communities, whereas others with strong illumination were occupied mainly by photosynthetic microorganisms. In any case, the organisms present in the least illuminated areas required a supply of organic matter generated by the organisms growing in the most illuminated areas.

The light intensity measurements taken by Johnson (1979) in New Zealand caves suggest that the minimal levels of light required for the continuous growth of the various organisms that comprise the lampenflora are the following: in algae, 10 to 50 lux; in mosses, 50 to 180 lux; and in ferns, 250 lux. Cigna (2011a) simplifies these intensity levels, and states that 85% of the lampenflora develop with an approximate minimum value of 40 lux.

Detailed studies carried out on stone surface, mural paintings in rocky indoor environments and subterranean archeological sites (Albertano et al., 2000; Hoffmann, 2002; Albertano & Bruno, 2003; Urzi et al., 2010; Albertano, 2012) also show light as the limiting factor for the growth of these phototrophic biofilms and the only parameter that can be controlled in situ. In this case, it has also been proven that the presence of artificial light with very low intensity provides a suitable environment for the development of phototrophic microorganisms (Albertano, 2012; Bruno & Valle, 2017). Monochromatic lights, with limited wavelength emission, have been successfully tested under laboratory conditions and inside a Roman hypogean site (Albertano & Bruno, 2003).

However, other studies suggest that established populations of lampenflora may survive for long

periods of time with much lower levels of light than those mentioned previously, revealing their capacity to survive in low lighting conditions. Some algae and cyanobacteria can survive and reproduce even at light intensities considerably below what is known as the "photosynthetic compensation point" (intensity of light at which the amount of CO₂ fixed in sugars during photosynthesis is the same as the CO₂ released during respiration) (Martincic et al., 1981; Mulec, 2005).

Some species of algae (such as *Chlorella* sp.) are capable of switching from an autotrophic to a mixotrophic and finally to a heterotrophic lifestyle, deploying their reserves according to changes in lighting conditions (Kermode, 1975; Roldán & Hernández-Mariné, 2009). Other organisms such as mosses may display etiolation, a physiological adaptation to low levels of light which involves exposing a greater surface area to capture the few photons available in periods of scarce light (Mulec, 2012).

In addition to withstanding long periods with very low levels of light, lampenflora populations can even survive in total darkness (Claus, 1962, 1964; Hajdu, 1966; Kol, 1967 in Aley, 2004). In the course of an experiment (Johnson, 1979) on the development of lampenflora under different light colors, one lamp failed after the growth of a lampenflora population. The lamp was not repaired for five months and after this period of essentially zero lighting, the associated lampenflora appeared to have suffered very little or no deterioration. In contrast, when the intensity of light is too high, some epilithic algae may switch to the endolithic phase to protect themselves from excess damage from light (Asencio & Aboal, 2001).

Moisture

The development of lampenflora is generally limited to moister or wetter surfaces, usually located in soft porous areas with a presence of water, which accelerates their growth (Martincic et al., 1981; Aley, 2004). However, for the development of biofilms in general, the duration of the dry periods and the number of humid periods appears to be more important (Gladis-Schmacka et al., 2014) than the total amount of water available (Büdel et al., 2009).

Some organisms have developed survival strategies during periods of prolonged drought, which include using the water retained in the substrate, forming protective compounds such as saccharose or trehalose (Potts, 1999, 2001; De Philippis et al., 2001), and the advantage gained by biofilms of growing in a thin layer directly on the substrate, thus being able to optimize all the available moisture (Johnson, 1979).

Algae are capable of withstanding much greater fluctuations in available moisture than mosses and ferns (Johnson, 1979). Grobbelaar (2000) observed that some species can recover their metabolic activity after long periods of desiccation. Specifically, some genera of cyanobacteria such as *Chroococcidiopsis* sp. and *Nostoc* sp. may become desiccated without dying (Alpert, 2006) and rapidly recover in favorable conditions, withstanding wide fluctuations in

moisture and surviving in the most extreme habitats for long periods of time (Potts, 2001; Wierzbos et al., 2006; Ramirez et al., 2011).

Nutrients

One of the characteristics of natural cave environments is their low nutrient content (Simon et al., 2007). The groundwater that seeps into the ground is the only source of almost all the nutrients necessary for the autotrophic growth of these communities. As it travels across the floor and the rock that forms the cave, the water picks up soluble inorganic nutrients that are introduced into the cave and absorbed by the lampenflora (Johnson, 1979).

As indicated above, due to their photosynthetic and nitrogen and CO₂ fixing capacity, biofilms induce the growth of bacteria, fungi, mosses and ferns in their surroundings, which benefit from an environment enriched in organic matter and nutrients. Hence within the actual lampenflora population, there is a cycle of nutrient utilization, release and reutilization by the autotrophic and heterotrophic organisms (Johnson, 1979).

Other sources of nutrients already mentioned are, for example, the excrements of animals who inhabit in the cave, detrital and clay-like sediments dragged by water into the interior, and the residues introduced by visitors.

Temperature

The rise in temperature is another factor with a significant influence on the growth of algae. Pulido-Bosch et al. (1997) used an incandescent lamp and estimated that at a distance of 50 cm from the light source the temperature was 8°C higher than in the surroundings, whereas Cigna (2011a) claims that at a distance of a few dozen centimeters this type of lamp produces a temperature increase in the order of 10°C and a decrease in relative humidity of 70-80%.

These conditions cause the cyanobacteria to adopt a mosaic or belt arrangement according to the environmental conditions (Roldán et al., 2004), in this case generating a central vegetation-free patch in the area around the artificial light point, as a result of the excessive heat and of the sharp decrease in moisture (Johnson, 1979; Mulec & Kosi, 2009; Esteban, 2014).

Mulec (2012) offers an interesting example of this issue: in the Aggtelek Caves (Hungary) green patches appeared after the snow melt in spring, but when the water evaporated the green covering nearest the lamps disappeared. The intense illumination had dried the surface and restricted the growth of the lampenflora, but in the area furthest from the lamps, the flora continued growing.

At a low flow-density of photosynthetic photons, a small increase in temperature can lead to a considerable increase in the production of biomass. For example, the green alga *Chlorella* sp., frequently identified in lampenflora communities, multiplied its biomass by 30 when the temperature rose from 9 to 11°C (Mulec, 2012). In comparison with the cave entrance the areas around the lamps offered more

stable conditions without any extreme environmental oscillations like melting-freezing processes.

Finally, the following are conditions that may exist in caves that could promote lampenflora colonisation (Grobelaar, 2000; Merdenisianos, 2005):

- 1) They are supplied with relatively constant illumination from an artificial light source.
- 2) Generally, the environment in caves maintains a humidity of around 100%.
- 3) The water that seeps through the floor and the geological formations often contain high concentrations of nutrients.
- 4) The temperatures in the underground environment are more uniform than on the exterior.
- 5) The high levels of CO₂ generated by visitors in the underground atmosphere contribute to increasing the photosynthetic capacity and as a result, to the more rapid growth of the lampenflora.
- 6) Fungi and other microorganisms are easily disseminated in the cave interior thanks to air currents and transport by cave fauna and visitors.

Problems generated by lampenflora

The colonisation and growth of biofilms normally involves a change in the properties of the substrate where they develop (Korber et al., 1994; Golubic & Schneider, 2003; Prakash et al., 2003). When an undesirable change occurs, this is known as "biodeterioration" (Hueck, 1965, 1968; Kumar & Kumar, 1999).

Numerous works have highlighted the relationship between the processes of deterioration of stone monuments in the presence of microorganisms such as bacteria, fungi, algae, mosses and lichens (Ortega-Calvo et al., 1991; Saiz-Jiménez, 1994; Warscheid & Braams, 2000; Papida et al., 2000; Crispim & Gaylarde, 2005; Caneva et al., 2008; Di Martino, 2016). Wakefield and Jones (1998) estimated that between 20% and 30% of the deterioration in rocks is a result of biological activity.

In underground environments, the proliferation of photosynthetic microorganisms can be considered a significant threat to the conservation of the natural and cultural heritage (Ciferri, 1999; Bastian & Alabouvette, 2009; Albertano, 2012).

The Lascaux Cave was the first in which a complex study was conducted on the processes of biodeterioration caused by photosynthetic microorganisms on its interior, specifically on its cave paintings (Lefèvre et al., 1974). Since then, this type of deterioration has been researched by several authors, both in caves and in other underground environments or hypogeous monuments.

Nugari et al. (2009) observed in the Crypt of the Original Sin (Matera, Italy) that the communities of phototrophic microorganisms not only formed a greenish layer on the surface of the Byzantine paintings (dating from the ninth century), but that they also colonized the interior of the rock substrate to a depth of several millimeters. Del Rosal (2015) gives as an example the case of several Spanish caves in which the "uncontrolled" development of microorganisms posed a serious problem that has sometimes led to the

closing of the cave. In the Tito Bustillo Cave, numerous speleothems were found to be abundantly colonized by photosynthetic microorganisms, including the cyanobacteria *Scytonema julianum* (Saiz-Jiménez, 1999). The Castañar de Ibor Cave had to be closed to the public in 2008 due to a fungal outbreak caused by a visitor's vomit which led to the development of *Mucor circinelloides* and *Fusarium solani* (Jurado et al., 2010). Finally, the growth of photosynthetic microorganisms due to intense artificial lighting caused the closure of the Altamira Cave in 2002 (Saiz-Jiménez et al., 2011).

To understand the different processes of biodeterioration in caves, Caneva et al. (2008) indicate that it is important to know whether the microorganisms use the material as a source of nutrients, as occurs with heterotrophic organisms (bacteria and fungi), or merely serves as a physical substratum for their development, as in the case of autotrophic organisms (cyanobacteria and algae). Whether for the purposes of nutrition or residence, microbial colonization significantly alters the physical and chemical properties of the mineral substrate, improving its bioreceptivity and allowing the development of different types of patina which range from thin films through to thick crusts (Warscheid & Braams, 2000). The negative effects of lampenflora therefore not only include changes in the aesthetic appearance of the caves (colouration), but these communities also cause a physical (disintegration) and chemical (dissolution) deterioration of the substratum.

In first place, the presence of lampenflora causes an aesthetic change to the substrate surface, producing a dirty greenish appearance (mainly due to the presence of chlorophyll-derived pigments), with the subsequent decline in aesthetic value of the colonized substratum (Warscheid & Braams, 2000; Caneva et al., 2008; De Muynck et al., 2009; Scheerer et al., 2009; Cutler et al., 2013). When the algae are grouped in biofilms, the presence of the extracellular matrix (EPS) acts as a viscous adhesive, providing sufficient resistance to adhere to the colonized surfaces (Karsten et al., 2007) and in turn promoting the retention of dust particles and certain atmospheric aerosols, which contribute to the change in color (Steiger et al., 1993; Warscheid & Braams, 2000; Mulec, 2012).

Physical biodeterioration includes all the mechanisms that produce structural changes, loss of cohesion, rupture or disintegration of the substrate through mechanical pressure due to the growth of microorganisms (Caneva et al., 2008; Warscheid & Braams, 2000). One example of this process is the penetration of the filaments of some endolithic species (certain algae and filamentous cyanobacteria) in the cracks in the substrate. The absorption of water and the cellular growth of these organisms exert pressure on the structure, which leads to the detachment and peeling of the surface layers of the substrate (Krumbein, 1988; Danin & Caneva, 1990; Bolívar & Sánchez-Castillo, 1997; Asencio & Aboal, 2001; Peraza-Zurita et al., 2005; Sarró et al., 2006). The extracellular matrix (EPS) may also play a role

in biodeterioration, generating an additional physical chemical stress due to the processes of hydration or dessication (Dornieden et al., 2000; Warscheid & Braams, 2000; Perry et al., 2004; Borderie et al., 2014a).

Chemical biodeterioration is mainly due to the metabolic activity of microorganisms, also known as "biocorrosion"; this is a process whereby the microorganisms excrete a series of organic acids (oxalic, citric, gluconic, fumaric, malic, and formic acid, among others) which cause the dissolution of materials and calcium carbonate formations in monuments and caves (Johnson, 1979; Warscheid & Braams, 2000; Herrera et al., 2004; Aley, 2004; Caneva et al., 2008; Scheerer et al., 2009). Electron transfer, the absorption of protons and cations from the substrate due to the formation of chelating complexes and phototrophic processes (including carbon dioxide transfer, oxygen production, and alkaline reactions with variations in pH), also contribute to the biodeterioration of the substrates and the precipitation of mineral mixtures (Albertano, 1993; Albertano et al., 2000; Warscheid & Braams, 2000; Hoffmann, 2002). All these biological activities lead to the alteration of the minerals and cause changes in the rock structure, involving greater porosity and permeability to water (Warscheid & Braams, 2000) and a weakening of its physical support function.

A particularly serious problem arises when the lampenflora, either living or dead, gradually becomes encrusted in the calcium carbonate substrate due to abiotic or biotic processes of precipitation and carbonate deposit. This amorphous mixture of dead phototrophs and carbonates irreversibly destroys the speleothems and other objects of cultural value (Mulec, 2012).

The presence of lampenflora also poses a problem for the fauna inhabiting the caves (Mulec & Glažar, 2011), as biofilms add a substantial amount of nutrients to the cave environment, available both to animals that are adapted to the underground environment and to other occasional inhabitants. It has been suggested that in this new environment, newcomers may become more competitive in this ecological niche and displace other autochthonous populations, affecting both their diversity and their abundance. However, these kind of effects have not been studied in a systematic and quantitative fashion yet and many conclusions may be merely speculative, particularly regarding higher order organisms. There are even works such as that by Meyer et al. (2017) where *T. celsus* is used as a biological model, that show no evidence of lampenflora-induced alteration of the subterranean food chain after the application of sodium hypochlorite.

Biodeterioration can be seen as a complex ecological process driven by numerous interactions between microorganisms, the substrate, and environmental factors described above (light, humidity and temperature, among others) (Nuhoglu, 2006; Dakal & Cameotra, 2012; Miller et al., 2012). Table 3 summarizes the problems generated by lampenflora in show caves.

Table 3. Problems generated by lampenflora in show caves.

Type		Causes	Consequences
Aesthetic changes		Presence of pigments derived from chloroform in the biofilms. The extracellular matrix (EPS) acts as a viscous adhesive, favouring the retention of dust particles and certain atmospheric aerosols.	Change of colour: dirty greenish colour. Loss of aesthetic value in the colonised substratum.
Biodeterioration	Physical	Mechanical pressure due to the cellular growth of microorganisms. Penetration of some endolithic species within the cracks in the substrate. Processes of hydration and/or desiccation of the extracellular matrix (EPS).	Peeling and exfoliation of the surface layers of the substrate. Structural changes: loss of cohesion, rupture or disintegration of the colonised substratum. Greater porosity and permeability to water.
	Chemical	Metabolic activity of the microorganisms and excretion of organic acids (oxalic, citric, gluconic, fumaric, malic and formic acids, among others). Transfer of electrons and absorption of protons and cations in the substrate due to the formation of chelating complexes. Phototrophic processes including carbon dioxide transfer, oxygen production and alkaline reactions with variations in pH.	Dissolution of the materials. Alteration and precipitation of mineral mixtures.

METHODS FOR CONTROLLING LAMPENFLORA

Historically the control of lampenflora communities involves two actions: the elimination of existing biofilms, and the prevention or delay of their development (Johnson, 1979; Hebelka, 2014). This control has led to the publication of an extensive bibliography on the work carried out in some of the world's most important show caves, where this problem has been studied and treated for decades. Mulec and Kosi (2009) highlight the contradiction of eliminating the phototrophic communities without removing or modifying the lighting in the caves, and thus without restricting their use by tourists, which would be unacceptable to their managers. These authors also say that in spite of the availability of several methods to control lampenflora, no definitive technique for preventing and avoiding their growth has yet been found.

The control methods tested can be classified into three main categories: physical, chemical and environmental. The following sections present and describe the main features of these methods.

Physical methods

Brushing and washing with pressurized water

Mechanical elimination using brushes and pressurized water are two mechanical techniques traditionally used to address the problem of lampenflora in show caves. The use of pressurized water injectors is very widespread among the managers of Australian show caves (Newbould, 1974; Anon, 1976) and, as indicated by the studies of Bonwick et al. (1986) in the Jenolan Caves (Australia), pressurized water is considered safe for cleaning speleothems covered by algae (cited in Werker & Hildreth-Werker, 2004).

Both brushing and washing with pressurized water have been found to be very efficient on hard surfaces, and offer instant results without generating

any toxic products (Ramírez-Trillo & González-Ríos, 2014). However, the use of these two techniques has currently been curtailed for the following reasons:

- Their long-term effectiveness is very low (Hebelka, 2014).
- Contrary to the declarations of Bonwick et al. (1986), it has been demonstrated that the repeated use of these techniques may cause damage to soft or earthy surfaces and alter the fragile crystalline structures of the speleothems (Ash et al., 1975; Spate & Moses, 1994; Merdenisianos, 2005; Mulec & Kosi, 2009; Mulec, 2012). The presence of calcified algae in many speleothems means that this type of mechanical method cannot be applied effectively without damaging the external calcareous structure (Esteban, 2014).
- The use of these methods causes the biological contamination to disperse more easily around the cave towards other unaffected surrounding areas (Rajczy, 1989; Hazslinszky, 2002; Hebelka, 2014).

The only exception in which highly pressurized water could be used is to eliminate dead plant matter and clean the surface of the cave after treatment with chemical products (Johnson, 1979). Werker and Hildreth-Werker (2004) recommend that if this method is applied, water from the interior of the cave or from the aquifer where it is located should be used, as the surface water may be chemically aggressive and damage the speleothems. Ramírez-Trillo and González-Ríos (2014) recommend a pressure of no more than two bars, and suggest the use of a liquid aspirator to collect remnants from the treatment.

Camouflage

Camouflage is designed to conceal the lampenflora communities and avoid their visual impact, but does not include any measures to prevent their subsequent development or eliminate them. This type of method has been applied on very few occasions. In the Las Ventanas Cave (Spain), colonies established in earthy

or sandy areas were disguised by mixing the surface with deeper materials. The more consistent substrates were rubbed with clay and earth and finally, the loose stones that were affected were turned over (Ramírez-Trillo & González-Ríos, 2014).

Radiofrequencies

The test carried out by Cennamo et al. (2013) based on the use of nonthermal effects of radiofrequencies seems to demonstrate that this kind of radiation produces visual disappearance of biofilms after a month of treatment. Treated stones, when transferred back to their original sites, did not show any microorganism re-growing after 6 months. It would be quite interesting to modify this technique only applicable in the laboratory for now, to test its efficiency in the show caves.

Chemical methods

Sodium hypochlorite

Sodium hypochlorite, NaClO, whose solution in water is known as bleach, is a strongly oxidative chemical compound that is often used as a disinfectant to produce drinking water and as a bleach in the textile industry or for domestic use (Hebelka, 2014). Commercial hypochlorite solutions (bleach) show variable concentrations depending on the manufacturer and the end use of the product. Household bleach sold for clothes laundering is a 3-8% sodium hypochlorite solution (OxChem, 2014). A 12% solution is widely used for water chlorination, and a 15% solution is more commonly used for disinfection of wastewater in treatment plants. The strength of these solutions decreases gradually with storage time, thus, actual concentration may differ from that specified in the container.

Since the late 1970s it has also been applied at a concentration of nearly 5% to restrict and eliminate lampenflora communities in show caves (Johnson, 1979; Aley et al., 1984; Zelinka et al., 2002; Aley, 2004; Cigna, 2011a; Mulec, 2012). Hebelka (2014) provides another example of the efficacy of this type of treatment based on the results obtained in the Moravian Karst (Czech Republic). An average cleaning efficiency of 80% was achieved by applying a 4% solution of sodium hypochlorite. The concentration of hypochlorite may be increased up to 8% in areas with a thick growth of biofilms however the efficacy of this method is highly variable, as it depends on numerous factors: a) Concentration of hypochlorite (chlorine) in the solution used. b) Density and type of plant matter to be eliminated. c) Type of substrate on which the lampenflora are growing. d) Amount of water present in the treated area. e) Age of the chemical agent used.

On the other hand, the most recent studies prove that usual NaClO concentrations to treat lampenflora are unnecessarily high (Meyer et al., 2017): “the success of NaClO is even more notable since the concentrations where an order of magnitude (0.5%) lower than the recommended guidance”. They suggest that it would be environmentally safe to use hypochlorite below 0.5%, although they do not provide experimental evidence.

Hypochlorite breaks down fast (between 5 minutes and 12 hours) in contact with organic substances, producing rapid oxidation of the substrate. When applied on lampenflora it leads to its degradation and whitening (Johnson, 1979; Hebelka, 2014). Treatment with sodium hypochlorite is approved and widespread in numerous caves, as it does not cause any significant damage to the formations and has a satisfactory final result. Nevertheless, some species of filamentous cyanobacteria trapped within the microcavities of the substrate, mainly *Scytonema julianum* and *Leptolyngbya* sp., are capable of resisting the treatment with hypochlorite (Iliopoulou-Georgoudaki et al., 1993).

One advantage of NaClO solutions over H_2O_2 solutions is that the former are much less corrosive. In tests to assess the corrosive action of sodium hypochlorite on some broken formations in the Frasassi caves (Italy), Bertolani et al. (1991) found that with ten minutes of treatment and after 17 hours, only 41 mg/m² of rock was dissolved.

The use of NaClO may represent a negative burden for the environment in the cave, as the products resulting from its oxidation are carbon dioxide, water and chloride ions, and it may also cause the release of chlorine gas (Faimon et al., 2003; Mulec & Kosi, 2009), a highly toxic compound when inhaled or in contact with the skin. It must be applied by personnel wearing adequate protective equipment (overalls with a hood, boots and rubber gloves, protective goggles and a respirator) and depending on the size of the area to be treated, using a manual spray device or a backpack sprayer (Hebelka 2014).

In 1981 a group of speleologists found dead bats in the Javoříčko Cave (Czech Republic) after cleaning operations with sodium hypochlorite solutions. The disappearance of some species of insects in Slovak caves may also be related to the biocidal effect of hypochlorite on underground fauna (examples cited in Faimon et al., 2003), the chlorine released contaminates the atmosphere, generates an unpleasant odor, and even acidifies the karstic waters, leading to the possible dissolution and erosion of calcium carbonate formations.

Iliopoulou-Georgoudaki et al. (1993) indicate that the use of sodium hypochlorite may also cause a reddish colouring in the carbonate substrates due to the oxidization of Fe^{2+} into Fe^{3+} , which precipitates as amorphous iron hydroxide $Fe(OH)_3$ (cited in Mulec & Kosi, 2009). In these cases, careful rinsing of the substrate with deionized water must be conducted after treatment in order to avoid undesirable effects and further damages.

Finally, other substances may be formed while spraying sodium hypochlorite, originated by the interaction with the biota. These subproducts are usually known as AOX (adsorbable organic halides) (Drewes & Jekel, 1998). For example, toxic chloramines are released when hypochlorite reacts with ammonia and nitrogenated compounds, (Greenwood & Aernshaw, 1984; Delalu et al., 2001) and may even form carcinogenic trihalomethanes (Faimon et al., 2003). All these products can significantly foul up

a cave environment and negatively influence native cave biota (Faimon et al., 2003).

However, these consequences can be minimised if the show caves where the treatment with NaClO is applied are adequately ventilated (Johnson, 1979; Cigna, 2011a), and the same recommendations are observed (Hebelka, 2014):

Calcium hypochlorite

Calcium hypochlorite, $\text{Ca}(\text{ClO})_2$, like sodium hypochlorite, is a fast-degrading compound with a strong oxidizing effect used as a disinfectant and bleach, and which has also been used to control lampenflora communities (Hebelka, 2014).

In large-scale tests carried out in the Ruakuri and Waitomo caves, Johnson (1979) indicates that the concentration of calcium hypochlorite necessary for the effective control of lampenflora depends on the composition and density of the biofilms to be treated. In places where there is a fine covering of algae, a 2% solution in water was sufficient to clean the surface, whereas if the lampenflora was constituted by a dense community of mosses and algae it was necessary to apply a 4% solution.

In terms of its effectiveness in cleaning speleothems, an exhaustive test was done in the Nerja Cave (Garrido et al., 2007). On this occasion, a solution of water saturated in calcite and calcium hypochlorite (2%) was used. The treated areas revealed an intense fungal colonization of the cleaned surface a few days after the treatment was applied. A further application of hypochlorite was made to eliminate the fungi and the remnants of dead microorganisms remaining on the substrate, which constituted a source of nutrition for these fungi. One year after the cleaning process, the recolonization of the substrate by photosynthetic microorganisms was defined as “practically unnoticeable”. However, in 2011, five years later, the areas that had been cleaned once again showed colonization by photosynthetic microorganisms.

The environmental effects of treatment with $\text{Ca}(\text{ClO})_2$ are similar to the use of NaClO, as both compounds are based on the action of chlorine (Faimon et al., 2003; Mulec & Kosi, 2009). However, Johnson (1979) downplays the problem of the strong odor and claims that 36 hours after treatment with four liters of calcium hypochlorite at 4% in an unventilated area of the Waitomo caves, the smell of chlorine was undetectable. Aley (2004) do not recommend its use, as calcium hypochlorite solutions leave a calcium residue that is difficult to eliminate. Mulec and Kosi (2009), Iliopoulou-Georgoudaki et al. (1993) noted that, as occurs with sodium hypochlorite, these substances are responsible for the development of a reddish coloring in carbonate substrates due to the oxidation of Fe^{2+} into Fe^{3+} that precipitates as an amorphous iron hydroxide, $\text{Fe}(\text{OH})_3$.

Hydrogen peroxide

In order to avoid the environmental drawbacks of the use of hypochlorite in the treatment of lampenflora, in the early 21st century several authors (Grobbelaar, 2000; Kubesová et al., 2000; Faimon et al., 2001,

2003) have proposed the use of hydrogen peroxide H_2O_2 as an alternative agent. Hydrogen peroxide is a colorless liquid, miscible with water in all proportions, and whose use does not leave residues that may negatively affect the environment, as it dissociates in oxygen and water (Grobbelaar, 2000), and does not release any toxic substance after its degradation.

This substance has long been used as an antiseptic and antibacterial agent in the healthcare sector and as a bleach and disinfectant in numerous industries. In its undiluted form it is a highly corrosive and aggressive oxygen that is difficult to handle. For this reason, Mulec and Kosi (2009) noted that it is crucial to estimate a sufficient concentration to destroy the lampenflora without deteriorating the speleothems. Based on the tests carried out in the Katerinska Cave (Czech Republic), Faimon et al. (2003) considered that the threshold concentration of 15%, applied three times during a period of between two to three weeks, is sufficient to destroy small developments of cyanobacteria, algae and mosses. However, this experiment is difficult to interpret. Authors started with a 5% peroxide concentration and gradually increased it up to 15% always applying the product onto the same area. This last concentration was considered effective as they observed lampenflora disappearing. Nevertheless, they did not take into account the cumulative effect of successive applications and the results just show the concentration but not the total amount of product applied per unit area or time.

Several authors (Faimon et al., 2003; Esteban, 2014; Mulec, 2014) highlight the difficulty of eliminating highly developed growth or lampenflora communities encrusted in a calcium carbonate substrate with a single application. There are two ways of increasing the effectiveness of treatment with H_2O_2 and reducing the number of applications:

- 1) Accumulated mosses and ferns must be removed before applying the peroxide, thus avoiding the need to repeat the treatment during humid periods after heavy rains (Mulec & Glažar, 2011).
- 2) The threshold concentration of H_2O_2 can be increased to 15% (Trinh et al., 2018). The tests carried out by Khanjani et al. (2014) and Esteban (2014) in the Ali-Sadr Cave (Iran) and in the Ortigosa de Cameros Caves (Spain) used concentrations of 20% and 25% respectively, without any adverse effects being detected on the natural biota of the cave and on the speleothems. However, these applications must be done in specific cases, as the use of H_2O_2 may ultimately end up significantly discoloring the calcareous structures (Esteban, 2014).

It has been verified that even a 15% solution produces a more aggressive attack on the underlying carbonate rock than karstic water. Mulec (2012) specifies that the 15% solution of H_2O_2 without a buffer has a pH of 4 and could therefore lead to the dissolution of the limestone formations (Caneva et al., 2008). To resolve this, Faimon et al. (2003) propose saturating the preliminary hydrogen peroxide solution with calcium carbonate and adding a few fragments of limestone rock at least ten hours after its application.

Once saturated, hydrogen peroxide is a chemical compound that provides good result and is more respectful with the environment than chlorated compounds, as it does not acidify groundwaters or generate unpleasant odors or harmful gases, and minimizes the aggression on the carbonate substrate (Grobbelaar, 2000; Faimon et al., 2003; Mulec, 2012).

In the Postojna Cave, Mulec and Glažar (2011) consumed an average of 1050 ml of buffered hydrogen peroxide solution for every 10 m² of treated surface in the cave. Each application took an average of 20 minutes. In successive treatments to prevent growths –normally two per year– the volume applied did not exceed the amount of 40 ml/m² recommended by Mulec (2014). The amounts of chemicals used in the solution applied is 15% (v/v) of hydrogen peroxide (pH 7.0-7.5) containing 500 ml of H₂O₂ at 30%, 75 ml of NaHCO₃/Na₂CO₃ buffer and 425 ml of distilled water. Due to the instability of the buffered H₂O₂ the solution must be sprayed as soon as possible. In this case, it was applied 20 minutes after preparation, three consecutive times, with a week between each application.

Due to the strong oxidizing power of H₂O₂, several authors (Grobbelaar, 2000; Cigna, 2011a; Mulec & Glažar, 2011; Hebelka, 2014) particularly emphasized the need to take special measures to protect the eyes and skin during spraying.

The efficiency of the hypochlorite may be due to the biocidal properties of the non-dissociated hypochlorous acid and the hypochlorite ion: both species damage cell membranes, are disseminated through the cell walls, alter enzymatic activity and may affect ion regulation (Claudi & Evans, 1993; Claudi & Mackie, 1994).

Faimon et al. (2003) therefore recommend that the show cave managers should opt for one of the following alternatives: (1) a rapid and effective elimination of lampenflora with hypochlorite, although it represents a negative burden for the cave environment; or 2) a more environmentally acceptable –albeit less effective– eradication technique, with hydrogen peroxide. However, Faimon et al. (2003) only tested the effectiveness of H₂O₂, and the comparison with sodium hypochlorite is based on an appreciation and not on quantitative data.

In some caves like the El Tesoro (Spain), studies have been done comparing hydrogen peroxide and sodium hypochlorite (Jurado et al., 2014). In this Spanish cave, both cleaning methods were very effective and maintained the walls and the speleothems clean for several months after the treatment. However, the administrators opted for treatment using hydrogen peroxide due to its harmlessness to the environment.

In the Congo Caves (South Africa), Grobbelaar (2000) suggested applying between 200-500 mg/L of H₂O₂ and washing and collecting the leachates after 5-30 minutes. If the lampenflora persists the washing must be repeated or combining it with the use of UV-C radiation. This procedure only needed to be used once every six months due to the low growth rate of the algae. It was also found that the use of H₂O₂ eliminated the fluff and part of the dirt introduced by visitors

that had accumulated over time on the surface of the formations.

Hebelka (2014) have reached the conclusion that although its application is slower (as it is less effective than hypochlorites), hydrogen peroxide is the least harmful compound for underground habitats from the environmental point of view.

Biocides

Due to their toxicity, biocides (Diquat, Diuron, Atrazina, Simazina Karmex, etc.) are unsuitable for the treatment of lampenflora in show caves (Caumartin, 1977; Cigna, 2011a; Mulec & Kosi, 2009; Mulec, 2012). However, there are some studies on the potential use of several herbicides such as Diquat and Diuron (Johnson, 1979) and Atrazine and Simazine (Grobbelaar, 2000) to eliminate lampenflora in show caves, although the latter are prohibited in the European Union: Simazine since 2003 and Atrazine since 2004 (European Biocides Directive 1998/8/EC). The results of further studies were disappointing and produced no apparent effect, as the greenish color persisted on the surface of the treated formations.

Nugari et al. (2009) tested the effectiveness of several biocides based on quaternary ammonium salts (Rocime 110, Preventol R80 and Umonium 38) on algae and cyanobacteria colonizing Byzantine paintings in the Crypt of the Original Sin (Matera, Italy). The products were effective in eliminating the lampenflora, but the colorimetric analysis revealed changes in the color of the substrate after treatment.

Other authors, cited by Mulec and Kosi (2009), have suggested the use of formaline (Cubbon, 1976; Caumartin, 1986; Merdenisianos, 2005), solutions of copper ammonia (Merdenisianos, 2005), butyl alcohol (Hill & Forti, 1997) and formaldehyde at a dilution of between 0.5% (Oriol et al., 2009) and 5% (Rajczyk et al., 1997).

In all cases, del Rosal (2015) notes that any cleaning protocol based on the use of biocides must include the subsequent removal from the area of the resulting dead organic matter in order to avoid the massive colonization of the medium by fungal microorganisms as occurred in the Lascaux Cave (Lefèvre, 1974; Bastian et al., 2010; Martín-Sánchez, 2012).

Borderie et al. (2014a) noted that special attention should be paid to monitoring the treated surfaces, as the biocides can be easily transported to other areas and contaminate the cave environment.

Intense and periodic cleaning with biocides over a period of years may give rise to the displacement of the autochthonous microbiota in the cave, and its replacement by communities of microorganisms that are resistant to biocides (del Rosal, 2015). In Lascaux Cave, for example, the invasion of *Fusarium solani* species complex was treated with Benzalkonium chloride from 2001 to 2004. As a result, the indigenous microbial community was replaced by microbial populations selected by the biocide (Bastian et al., 2009).

Natural Biocides

The use of natural substances has been proposed recently to control the development of biofilms over

natural substrate (Stupar et al., 2014; Sasso et al., 2016; Pfendler et al., 2018; Bruno et al., 2019). Among these substances, very different kind of compounds are mentioned such as glycoalkaloids, essential oils, Juglone, phenolic acids, flavonoids, terpenoids or alkaloids. These compounds show fungicide or insecticide properties, and some such as Juglone, can even interfere with the mechanism of photosynthesis and inhibit the growth of algae and it is, therefore, proposed to fight algae and cyanobacteria biofilms.

Any biocide, either natural or artificial, produces a similar effect: it selects resistant species and deeply modifies the original biota, therefore its use is questionable from an environmental point of view. Natural origin is not equal to less toxic. Toxicity depends on the chemical structure. In fact, most, if not all, biocides are toxic or otherwise polluting substances, and their degradation is frequently difficult, being persistent in the natural environment (Barresi et al., 2017). Moreover, when these substances are used inside a cave they will easily reach and pollute groundwater.

Nanoparticles

Many works have proven that metallic nanoparticles have powerful antimicrobial, antifungal and antialgae properties. Over the past few years, several experiments with these substances have been carried out for the mitigation of microbial colonization on stone surfaces specially in Cultural Heritage Restoration (Sierra-Fernández et al., 2017). The main limitation for their use is that almost all heavy metals containing these particles are highly toxic for most lifeforms including humans. Among the most studied metals there are silver (Bellissima, 2013; Bellissima et al., 2014; Zarzuela et al., 2018), mixtures of consolidants, water repellents and copper nanoparticles (Pinna et al., 2012), and some metal oxides, particularly titanium dioxide (Bellissima, 2013; Ruffolo et al., 2017). Both silver and titanium are considered metals with little toxicity, but they are not harmless, especially in the form of nanoparticles (Raymond et al., 2009; Hadrup & Lam, 2014). Their introduction in the natural environment must be avoided at any cost. Furthermore, those studies lack long-or medium-term in situ experimentation, since they deal mainly with laboratory tests aimed to assess the products' efficacy (Ruffolo et al., 2017). However, if the studies on the toxicity of these particles in humans are scarce, those on the effect on natural fauna in caves are entirely missing.

Liquid nitrogen

There are few studies published on the use of liquid nitrogen as a method of eliminating lampenflora in show caves. A test was done in the El Tesoro Cave (Jurado et al., 2014), where liquid nitrogen applied with a brush represented a cleaning which combined mechanical elimination (thanks to the effect of the brush) with the congelment of the biological structures, which would theoretically represent an advantage. However, cleaning with this procedure was much less effective than with other

chemical compounds (hydrogen peroxide and sodium hypochlorite), as it failed to totally eliminate the green patches. The complications involved in its transport, execution and handling, mean this treatment is not recommended.

Environmental control methods

Lighting control

In the natural cave environment, whether a show cave or not, adequate levels of moisture and nutrients are almost always achieved for the development of the biofilms that form the lampenflora (Johnson, 1979), and the limiting factor for the development of these communities is the lack of light. For example, Martínez and Asencio (2010) showed that in the Gelada Cave (Spain) the main stress factor for the biofilms was the lack of light, followed by moisture deficit, lack of nutrients and finally, temperature variations.

As lighting is precisely the factor that is easiest to control, managers seek to limit the development of lampenflora communities by modifying the design and the technology of the lighting system (Olson, 2006). The aim is to reduce the amount and intensity of the light emitted and adopt a light spectrum whose emission range does not coincide with the wavelengths that favor the growth of lampenflora (Johnson, 1979; Smith & Olson, 2007).

From a quantitative point of view, a discontinuous lighting regime theoretically restricts the growth of lampenflora, as plants require a series of chemical and physiological changes to adapt to different phases of light and darkness, which involves an extra energy input (Aley, 2004).

The simplest way of restricting the growth of lampenflora in show caves is by reducing the duration of the lighting periods, keeping the lights on only when visitors are present, which also achieves a reduction in the energy released into the environment and lowers electricity costs (Grobelaar, 2000; Mulec & Kosi, 2009; Cigna, 2011a; del Rosal, 2015). Planina (1974) estimates that lampenflora cannot develop if the illumination in the cave does not exceed 100 hours a year.

Lampenflora communities can survive and reproduce in conditions of low light and even tolerate its absence for varying periods of time (Dalby, 1966; Martincic et al., 1981; Mulec, 2005; Glime, 2007). Cigna (2011a) indicates that although the reduction in light for a prolonged interval of time (for example, a month) counteracts the proliferation of photosynthetic organisms in caves, it may favor the dissemination of other resistant organisms (generally cyanobacteria) due to reduced competition (Giordano et al., 2001; Montechiaro & Giordano, 2006).

The intensity of the light may be decreased by using less powerful lamps that emit the smallest possible amount of heat (Gurnee, 1994) or by establishing a safety distance between the light source and the cave surface. Cigna (2011a) indicates that a distance of approximately 1 m should be safe, whereas Byoung-Woo (2002) recommends extending this to at least 2 m.

Another option is to use disperse illumination (Mulec & Kosi, 2009), as lampenflora does not develop, or

develops very slowly under diffuse lighting methods, as occurs in the speleotherapy centre for patients with pulmonary ailments at the Sezana Hospital (Slovenia) (Mulec, 2005). Olson (2006) suggests that diffuse illumination can be supplied by corridor lamps directed towards the path surface.

The level of lighting must be sufficient for visitors to see the path and the low ceilings clearly, so they can enjoy the cave (Grobbelaar, 2000; Olson, 2006). However, Mulec and Kosi (2009) invite managers not to show off the caves in the brightest and most highly illuminated conditions possible, but to opt for a level of lighting that allows the natural or cultural heritage to remain partly concealed or in half light, so its beauty can be admired as it was at the beginnings of underground tourism.

From a qualitative point of view, when designing the lighting system, a light spectrum should be selected that is minimally absorbed by the photosynthetic pigments (Olson, 2006). This is done for example by using a green light (around 530 nm) as recommended by Harris (1981) in Mammoth Cave or by Oosthuizen (1981) and Grobbelaar (2000) in the Cango Caves. However, several authors (Aley, 2004; Olson, 2006; Mulec, 2014) say that for aesthetic reasons green lighting is a somewhat impracticable strategy and is normally rejected by the administrators of show caves as it does not give the underground environment a natural appearance. The use of green light would not be so successful against organisms that can modify their accessory pigments (Roldán et al., 2006).

Imprescia and Muzi (1984) and Olson (2002) have opted for the use of yellow light (around 580 nm) as a possible alternative. Lampenflora communities do not strongly absorb this type of light (Aley, 2004; Mulec, 2012) and the visual impact is minimal, giving the cave a natural appearance, as the walls are often bathed in earthy and yellowish tones. The human eye is also much more sensitive to yellow light than to the red and blue wavelengths that feed photosynthesis (Olson, 2006). For example, in the El Tesoro Cave, LED-type yellow light points were installed with an emission range of 590 nm, with good results (Jurado et al., 2014).

Del Rosal (2015) analyzed the emission spectra of the photosynthetic organisms in the Nerja Cave by monitoring the physiological state and the photo-acclimatization processes of the biofilms. She identified through the pigments the most unfavorable wavelength range for their development. A wavelength of around 560 nm was proposed as the most appropriate for the design of the new lighting system.

With regard to the type of lighting, halogen lamps are often still used in many show caves and other underground spaces open to the public. However, they are gradually being replaced by other types of lighting as they do not preserve the environmental conditions of the cave, but increase the temperature and decrease the environmental humidity when they are lit (Cigna, 2011a; Mulec, 2014; D'Agostino et al., 2015).

High- and low-pressure sodium steam lamps and LED-diode lamps are a more appropriate option,

as they have less impact on the atmosphere in the cave, and the emissions spectra of these lamps are largely located in the chlorophyll absorption peaks (Merdenisianos, 2005; Olson, 2006).

The problem with sodium steam lamps is that they take some time to achieve their total brightness, and repeatedly turning them on and off significantly reduces their useful life (Olson, 2006). Kartalis and Mais (2001) observed that in the Alistrati Cave (Greece), low-pressure sodium steam lamps were not active in preventing the development of lampenflora. Although LED lamps are not so bright, they have numerous advantages: they have a narrow emissions spectrum and can be adjusted according to need, they light immediately, are very energy efficient, emit very little heat, produce no noise and have a very long useful life (up to 100,000 hours or ten years of continuous use) (Olson, 2006; Toomey et al., 2009).

These characteristics have led numerous caves around the world to opt to install LED illumination at the start of the 21st century. Mulec and Kosi (2009) and Cigna (2011a) cite the work of Olson (2002) in Mammoth Cave as an example, where it was successfully demonstrated that the development of lampenflora could be controlled through the use of LED illumination. An emission range was used of 595 nm (yellow) with an intensity of 49.5 lx, preventing the growth of the lampenflora for a year and a half after its installation.

Several authors (Johnson, 1979; Mulec & Kosi, 2009; Cigna, 2011a; Hebelka, 2014; Ramírez-Trillo & González-Ríos, 2014), have suggested implementing a lighting system divided into two differentiated sectors or circuits:

- 1st circuit - illumination of the visitors' route on paths and platforms to allow their safe passage through the cave.
- 2nd circuit - scenic or artistic illumination to highlight the most spectacular formations. This can be turned on manually with switches activated by the guides during the visit, or they can be programmed to turn on for a limited period of time only when visitors pass nearby.

The first circuit must be equipped with emergency lighting batteries that guarantee safety in the case of an electrical failure, and the second circuit must be provided with infra-red sensors (or similar) that react to movement. Each of these circuits must have control units and automatic switching that operate separately and independently.

It's also very effective re-orienting or relocating the lights in areas susceptible to being colonized, avoiding the illumination of substrates covered by mud, sedimentary or earthy structures and damp surfaces (Rajczyk, 1989; Mulec, 2012; Ramírez-Trillo & González-Ríos, 2014). Mulec and Kosi (2009) also note that the placement of lamps in areas with a strong air circulation should be reconsidered due to the possible increase in the dispersion of the lampenflora.

A dramatic change in the lighting of areas colonized by photosynthetic biofilms could lead to the massive death of these organisms, and therefore to an increase in organic matter in the environment (del

Rosal, 2015). This would represent an important source of nutrients, and possibly cause a large-scale development of fungi generating a situation similar to what occurred in the Lascaux Cave. To avoid this the author proposes gradually replacing the illumination in a controlled way, and previously cleaning any intensely colonized speleothems in order to reduce the amount of organic matter.

Finally, Olson (2006) proposes the use of portable electric torches as an alternative. No problems with lampenflora have been reported in caves illuminated with hand-held or helmet-mounted torches, and many of the impacts related with the installation of a lighting system could be avoided. However, the author also concedes that in tourist caves with a high influx of public, the logistics of maintaining optimal operating conditions for hundreds of torches renders this idea somewhat impracticable. However, on a small scale, in caves where the influx of public is limited to small groups divided in shifts, this solution has been found to be the most effective, as is the case of the Castañar de Ibor Cave.

Ultraviolet radiation (UV-C)

Ultraviolet light is a form of electromagnetic radiation with a wavelength located between visible light and x-rays. It is divided into three categories based on its energy capacity and its effects on living matter: UV-A (400–315 nm), UV-B (315–280 nm) and UV-C (280–200 nm) (Yin et al., 2013).

UV-C radiation, unlike UV-A and UV-B, is completely filtered by the ozone layer and is considered to be the most harmful to living beings due to its highly energetic photons. Its strong germicidal effect causes irreversible damage to photosynthetic organisms (Adhikary & Sahu, 1998; Danon & Gallois, 1998; Jayakumar et al., 1999; Zvezdanović et al., 2009) and, in the case of algae and cyanobacteria, it has been demonstrated that this type of radiation has the potential to cause serious damage in the cell constituents involved in photosynthesis (Alam et al., 2001; Moharikar et al., 2006; Sakai et al., 2007; Tao et al., 2010, 2013; Ou et al., 2011, 2012) (works cited in Borderie et al., 2014a). It is therefore used mainly as biocidal and disinfectant lighting in several sectors such as medicine, the food industry and water treatment (Borderie et al., 2014a).

However, the use of UV-C radiation has also been incorporated in the field of conservation of heritage materials. The first to propose this alternative use was Dobat (1963) from his own observations in the Lascaux Cave (cited in Lefèvre, 1974). Among other measures, Dobat proposed the use of UV radiation as substitute for chemical products for the disinfection of algae. Subsequently, Van Der Molen and others (1980) successfully developed a mobile ultraviolet unit (MUVU) to treat walls colonized by algae and cyanobacteria in the Church of St. Stephanus in Pilsum (Germany).

However, exposure to UV-C radiation is not a suitable procedure to combat the proliferation of microorganisms on organic matter, as just as with living organisms, organic matter (paper, wood,

textiles, etc.) can be damaged by UV-C rays (Caneva et al., 2008).

In addition to Dobat (1963) other authors have proposed the use of UV-C radiation to limit and control the proliferation of photosynthetic microorganisms in the specific sector of show caves, such as for example in the Grotta Gigante (Italy) (Fabbriatore, 2009), where the research by Borderie and his collaborators deals more extensively with this method and its effects (Borderie, 2014; Borderie et al., 2011, 2012, 2014a, b, and c). These authors worked in the Moidons Cave (France) with the aim of assessing the efficiency of a germicidal treatment with UV-C applied to different types of biofilms.

The samples were irradiated with a UV-C box containing two 11 W lamps each with a wavelength of 254 nm and treated glass to avoid the formation of ozone. In each treatment the lamps alternated periods when they were on (30 min) and off (15 min) for 12 h (corresponding to 8 hours of exposure). The irradiation was done at night when the cave was closed to visitors, and the UV-C box was hermetically sealed with black plastic to avoid UV-C dispersion around the treated area. A photoradiometer was placed in the box to measure the effective irradiation. Each biofilm treated received a dose of 180 kJ m⁻². The treatment was able to kill the microorganisms and induce the degradation of the chlorophyll pigments responsible for the green appearance. This approach is very promising, as many chemical products are capable of killing the organisms, but fail to destroy the pigments so the greenish color persists in the substrate. However, treatment with UV-C did not always completely eliminate the green color, as its efficiency largely depends on the thickness of the biofilm: the underlying cells benefited from the protective shield of the superimposed cells, which absorbed the radiation. The application of two 8-hour UV-C treatments instead of one would have been sufficient to induce the complete whitening of a biofilm of thick green algae.

Another advantage of this method is that its use does not cause a negative impact on the materials exposed, nor does it generate any toxic residual element in the environment. The monitoring of the biofilms showed that the treatment was effective for at least one year, but the biological colonization did not cease, and the greening phenomena once again reappeared 16 months later.

Given that each cave is characterized by its own climate parameters, the results obtained in the Moidons Cave cannot be directly transferred to other sites. This cave is open to the public only six months a year (from April to October), so the absence of light in the closed period helps contain the colonization of algae, with no dynamic proliferation. It can be assumed that the recolonization time after UV treatment in the Moidons Cave (approximately one year) is probably longer than in other caves that are open all year round.

UV-C treatments can easily be applied in accessible areas such as on floors, walls and speleothems, but this method has some limitations, particularly in the case of remote areas (for example ceilings), and

indicate that new engineering developments are required to improve the effectiveness of the treatment and to limit energy expenditure (for example, through the use of UV-C LEDs).

Other authors have been less enthusiastic about this method. For example, Kermode (1975) and Johnson (1979) carried out several experiments with ultraviolet lamps to prevent the growth of lampenflora in the Waitomo caves (New Zealand), effective results were only obtained in the affected substrate using a 30 W germicidal lamp at a distance of 50-70 cm from the light source, when lampenflora generally tends to be located at distances of over 3 m. This would require the use of more powerful lamps, or the need to collect the existing carcasses in areas near the affected substrate. In any case the high costs of the work would render this method of control somewhat unprofitable.

Hebelka (2014) also identifies this problem in caves located in the Moravian Karst (Czech Republic) and recommends applying this method in smaller caves. Another disadvantage cited by the author is that in areas with a proliferation of stalactites and narrow cracks, shadows are generated that may reduce or significantly inhibit the effect of UV radiation.

Grobelaar (2000) tested the efficacy of UV-C lamps on the algae that thrive in the Cango Caves (South Africa), both in laboratory cultures and in the caves themselves. He concluded that this type of radiation is effective for killing and whitening the algae, and proposed a treatment in which the use of UV-C is combined with hydrogen peroxide. However, he also states that this process requires considerable time, as the lamps had to be turned off once the visitors accessed the caves. It should also be considered that UV light triggers the breakdown of peroxide, so both treatments cannot therefore act simultaneously.

Finally, Mulec (2014) indicates that UV-C irradiation is not a suitable procedure for caves unless due precautions are taken, particularly in places populated by fauna.

High temperatures

Kermode (1975) and Johnson (1979) suggested the use of low-pressure steam or hot water instead of cold water to eliminate lampenflora communities and clean the walls in the Waitomo Cave.

Tests and prior experiences have shown that these methods are effective, with the advantage that they have a highly localized effect and do not produce toxic residues (Aley, 1972). However, their repeated use may erode some fragile surfaces and this method also has some practical drawbacks, as it requires bulky equipment and continued access to a water and energy supply. Finally, this method is not fast, as it takes at least ten minutes to treat each square meters of surface area.

Biological control

One possible way of restricting the growth of lampenflora (Mulec & Kosi, 2009) is the use of antagonistic organisms such as genetically modified viruses. However, the authors acknowledges that the

introduction of exotic species for the control of other species often has unexpected consequences and can lead to far worse environmental problems. In any case these methods would kill the algae but leave a remnant of organic matter that would serve as a substrate for other organisms, mainly fungi.

CONCLUSIONS

The control of lampenflora communities poses two urgent questions: (1) how to prevent its growth, and (2) once it is established, how to eliminate it without damaging the substrate. Based on the advantages, drawbacks, limitations and recommendations of each method described in this work and synthesized in Table 4, it can be concluded that in spite of the variety of control methods available, no definitive solution has yet been found (Fig. 3).

Perhaps the main problem concerning research in lampenflora control is that in spite of the great number of experiments developed for over 40 years, many of the methods for assessing success were arbitrary, qualitative and non-replicable. There are no normalized methods to measure the efficacy of a lampenflora removing process and, more importantly, it is not possible to compare the results of different authors.

A similar problem has been observed regarding the information of the effects of cleaning treatments, particularly chemical methods, on the natural fauna of caves. The starting assumption is always that the use of products that are toxic in other environments will be also toxic for cave inhabitants, but there are almost no research where toxicity or unfavorable effects are measured quantitatively.

The most popular product for cave cleaning is sodium hypochlorite. However, there is no experimental study that sheds light on which hypochlorite concentrations are effective to kill the biofilm, which hypochlorite concentrations are effective to ensure a longer duration of the cleaning effect, and which hypochlorite concentrations are effective to achieve a whitening effect. Meyer et al. (2017) suggest concentrations 10 times lower than those usually applied are fully effective. It is essential to develop a methodology allowing for quantification and comparison between methods.

In the field of monument restoration, catacombs, stone walls, etc, new methods for biofilms removal through the application of biocides and metallic nanoparticles are being tested. In spite of the likely toxicity of these compounds, their use, effectiveness and real impact in caves should be studied.

The design of actions intended to prevent and –where necessary– combat the processes of biodeterioration caused by lampenflora requires three multidisciplinary actions:

- An integrated analysis of these biological communities to determine their diversity, physiology and relation with the colonized substrate.
- The implementation of combined methods of prevention and elimination. Current prevention

Table 4. Summary of methods proposed for the treatment of lampenflora communities in show caves.

Methods	Advantages	Drawbacks	Limitations	Recommendations
Physical	Brushing and washing with pressurised water	<ul style="list-style-type: none"> - Very low long-term efficiency. - Its repeated use may cause damage to soft or earthy surfaces and alterations in the structure of the speleothems. - Biological contamination can become dispersed in the cave interior. 	<ul style="list-style-type: none"> - The presence of calcified algae makes this method somewhat ineffective without damaging the external calcareous structure. 	<ul style="list-style-type: none"> - This method should only be used after treatment with chemical products. - Water from the cave interior must be used, as it is less chemically aggressive than water from the exterior. - Pressure of no more than two bars should be applied and the remnants must be collected with a liquid aspirator.
	Camouflage	<ul style="list-style-type: none"> - Conceals the lampenflora communities and avoids their visual impact. - Inexpensive method. 	<ul style="list-style-type: none"> - This method does not prevent the growth of biofilms or eliminate existing lampenflora communities. 	<ul style="list-style-type: none"> -
	Hypochlorites	<ul style="list-style-type: none"> - Chlorine gas is released as they break down, which can contaminate the atmosphere in the cave, produce unpleasant odours and act as a biocide to subterranean fauna and even acidify karstic waters. - May release toxic chloramines and cause a reddish colouring on the substrate due to the oxidation of Fe^{2+} into Fe^{3+}, which precipitates as amorphous iron hydroxide $\text{Fe}(\text{OH})_3$. - The residue left by the calcium hypochlorite solutions is difficult to remove. 	<ul style="list-style-type: none"> - The efficacy of the method depends on several factors such as the concentration of hypochlorite used, the density and type of plant matter to be eliminated and the type of substrate on which it develops, among others. 	<ul style="list-style-type: none"> - Concentrations of nearly 5% should be used for sodium hypochlorite and between 2% and 4% in the case of calcium hypochlorite. - The cave must be sufficiently ventilated. - The application must be done before the tourist season begins, at the end of the bat hibernation period and/or avoiding periods of heavy rainfall. - Due to its strong oxidising power, the staff tasked with its application must use adequate protective equipment.
Chemical	Hydrogen peroxide	<ul style="list-style-type: none"> - Compared with hypochlorites, it eradicates highly developed biofilms more slowly and less effectively. 	<ul style="list-style-type: none"> - The effectiveness of the method depends on several factors such as the concentration of peroxide used, the density and type of plant matter to be eliminated and the type of substrate on which it develops, among others. 	<ul style="list-style-type: none"> - Before its application, the preliminary solution must be saturated with calcium carbonate. - A threshold concentration of 15% must be applied. - Due to its strong oxidising power, the staff tasked with its application must use adequate protective equipment.
	Biocides and natural biocides	<ul style="list-style-type: none"> - Generally highly toxic products for the environment. - Depending on the biocide used, the green colouring may persist in the formations, and the original colour of the affected substrate may even change. - Intensive and periodic cleaning over a period of years may lead to the displacement of autochthonous biota and its replacement with biocide-resistant communities 	<ul style="list-style-type: none"> - Due to its toxicity, its use has been restricted by show cave managers. 	<ul style="list-style-type: none"> - The cleaning protocol must include the removal of the resulting dead organic matter to avoid massive colonisation by fungal microorganisms. - Special attention must be given to washing the treated areas, as the biocides may easily be transported to other areas of the cave.

Chemical Chemical	Liquid nitrogen	<ul style="list-style-type: none"> - When applied with a brush, this cleaning method combines mechanical elimination with the congealment of the biological structures. - Effective at very low concentrations. 	<ul style="list-style-type: none"> - Much less effective results compared to the use of other chemical compounds. - Complicated to transport and apply. - Hazardous to handle. 	<ul style="list-style-type: none"> - Few studies published on this method. 	<ul style="list-style-type: none"> - Expand research on ornamental stones and monuments to show caves. 	-
	Nanoparticles	<ul style="list-style-type: none"> - Alternative to using chemical products. - Numerous caves have opted for this method and there are many published studies. - It does not have a negative impact on the materials or generate any residual toxic element. 	<ul style="list-style-type: none"> - Possible toxic effects of heavy metals. - The effects on the environment is unknown 	<ul style="list-style-type: none"> - Studies of the application of this method are missing in Show Caves. 	<ul style="list-style-type: none"> - Reduction in lighting periods, maintaining the lights lit only during tourist visit. - Reorienting or relocating the lights located in areas susceptible to being colonised, or with strong circulating air currents. - Choice of a light spectrum that is minimally absorbed by photosynthetic pigments. - Use of LED-type lamps: their emission spectrum can be adjusted according to need, they switch off instantly, they are highly efficient, generate very little heat and noise and have a long useful life. 	-
Environmental	Lighting control		<ul style="list-style-type: none"> - The cost of renewing the lighting system may be high. - The lamps may produce variations in temperature and humidity rate inside the cave. 	<ul style="list-style-type: none"> - This method prevents the growth of biofilms in show caves, but does not eliminate existing lampenflora communities. - The lighting system must be replaced progressively, as a drastic change could induce the mass death of the biofilm populations and trigger the possible development of fungi. 		
	UV-C radiation	<ul style="list-style-type: none"> - Alternative to chemical products. - Eliminates the lampenflora communities and induces the degradation of chlorophyll pigments. - It does not have a negative impact on the materials or generate any residual toxic element. 	<ul style="list-style-type: none"> - Not a very efficient method in caves with large areas affected by lampenflora. - During the exposure to UV-C radiation there may be variations in temperature and humidity rate inside the cave. 	<ul style="list-style-type: none"> - The treatment is difficult to apply in certain areas (ceilings). - Areas that are rich in stalactites and narrow crevices cast shadows that significantly reduce the effect of UV-C radiation. 	<ul style="list-style-type: none"> - Treatments with UV-C must be done when the cave is closed to avoid exposing visitors to radiation. - In caves where the presence of animals has been detected in the area to be treated, an inventory must be taken of the fauna and due precautions should be employed. 	
	High temperatures	<ul style="list-style-type: none"> - Effective method. - Highly localised effect. - Does not generate toxic products. 	<ul style="list-style-type: none"> - Slow method. - Its repeated use can erode more fragile surfaces. 	<ul style="list-style-type: none"> - The equipment necessary is bulky and requires access to a continuous supply of water and power. 		-
	Biological control	-	<ul style="list-style-type: none"> - The introduction of exotic species to control other species often has unforeseen consequences and can lead to much more serious environmental problems than the original ones. 	<ul style="list-style-type: none"> - Few studies have been published on this method. 		-

Fig. 3. Time line showing the different methods proposed since the 1970s through to the present day to tackle lampenflora in show caves. FM: physical method; QM: chemical method; LC: light control; UV: ultra violet.

methods are based mainly on strict control of the lighting, reducing the duration of lighting periods and using inefficient wavelengths to restrict the process of photosynthesis. The most widespread method of elimination is the use of chemical products, specifically hypochlorites and hydrogen peroxide. However, in certain situations, other methods described in previous sections cannot be ruled out, including: UV-C radiation, brushing and others.

- In parallel it is necessary to determine the evolution of these organisms before and after carrying out these control actions.

Finally, from a management point of view it is recommended to adapt and follow current international guidelines. As described by Cigna (2011b), in 1997 the World Commission on Protected Areas at the International Union for Conservation of Nature (IUCN) published a leaflet (Watson et al., 1997) with a series of guidelines for the protection of karstic elements and wild caves. The principles included in this publication served as a good foundation, but it was considered necessary to create guidelines aimed specifically at show caves. After many recommendations and suggestions received over a period of 20 years, a final text was agreed at the seventh International Congress of the International Show Caves Association (ISCA) held in the Jenolan Caves in November 2014, with the title "Recommended International Guidelines for the Development and Management of Show Caves", available at the following address: www.uis-speleo.org.

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