



A META-ANALYSIS OF ENVIRONMENTAL FACTORS INFLUENCING THE ALGAL COLONISATION IN CAVES AND ROCKSHELTERS WORLDWIDE

METAANALIZA OKOLJSKIH DEJAVNIKOV, KI VPLIVAJO NA NASELITEV ALG V JAMAH IN SPODMOLIH PO VSEM SVETU

Andrea BELDA¹, Laura GARCÍA-ABAD¹ & Antonia Dolores ASENSIO^{1*}

Abstract

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Andrea Belda, Laura García-Abad & Antonia Dolores Asencio: A meta-analysis of environmental factors influencing the algal colonisation in caves and rockshelters worldwide

Microclimate conditions, mainly radiation, temperature and relative humidity vary according to cavities' configurations and determine the microorganism's colonisation. A meta-analysis was performed of environmental factors influencing the algal species colonisation in caves and rockshelters. For this purpose, the results of studies about algal colonisation in 82 caves and rockshelters in 11 European, Asian and American countries were analysed. Firstly, 412 species were counted of which Cyanobacteria predominated, followed by Chlorophyta and Bacillariophyta, and finally by Rhodophyta. The Shannon Index determined that the diversity of the algal species developing in these places was very high. The most diverse Cyanobacteria genera to appear in the different studied caves and rockshelters are *Leptolyngbya* with 28 different species, *Gloeocapsa* with 24 and *Phormidium* with 23. They are followed by *Chroococcus* with 18, *Aphanothece* with 14, *Oscillatoria*, *Nostoc* and *Scytonema* with 10 each and *Schizothrix* and *Tolypothrix* with 9 each. The most diverse Chlorophyta and Bacillariophyta genera are *Chlorella* with 9 different species and *Diademesis/Humidophila*, *Luticola* and *Nitzschia* with 4, respectively. The principal component analysis revealed that both photosynthetically active radiation and relative humidity more actively conditioned the development of some algal species in cave environments than temperature.

Keywords: algae; Cyanobacteria; caves; diversity; environmental factors; meta-analysis.

Izvleček

UDK 582.27:551.435.84(100)

Andrea Belda, Laura García-Abad & Antonia Dolores Asencio: Metaanaliza okoljskih dejavnikov, ki vplivajo na naselitev alg v jamah in spodmolih po vsem svetu

Mikroklimatske razmere, predvsem sevanje, temperatura in relativna vlažnost, se spreminjajo glede na konfiguracijo votlin in vplivajo na naselitev mikroorganizmov. Opravljena je bila metaanaliza okoljskih dejavnikov, ki vplivajo na naselitev vrst alg v jamah in spodmolih. V ta namen so bili analizirani izsledki študij o naselitvi alg v 82 jamah in spodmolih v 11 evropskih, azijskih in ameriških državah. Najprej je bilo naštetih 412 vrst, med katerimi so prevladovala modrozeleni ceppljivke ali cianobakterije (Cyanobacteria), sledile so zelene alge (Chlorophyta), diatomeje (Bacillariophyta) in nazadnje rdeče alge (Rhodophyta). Shannonov indeks je pokazal, da se na teh območjih razvijajo zelo raznovrstne vrste alg. Najbolj raznovrstni rodovi modrozeleni ceppljivk ali cianobakterije, ki se pojavljajo v proučevanih jamah in spodmolih, so *Leptolyngbya* z 28 vrstami, *Gloeocapsa* s 24 in *Phormidium* s 23 vrstami. Sledijo jim *Chroococcus* z 18 vrstami, *Aphanothece* s 14, *Oscillatoria*, *Nostoc* in *Scytonema* s po 10 ter *Schizothrix* in *Tolypothrix* s po 9 vrstami. Najbolj raznovrstni rodovi zelene alge in diatomeje so *Chlorella* z 9 vrstami ter *Diademesis/Humidophila*, *Luticola* in *Nitzschia* s po 4 vrstami. Iz analize glavnih komponent je razvidno, da tako fotosintetično aktivno sevanje kot relativna vlažnost aktivneje vplivata na razvoj nekaterih vrst alg v jamskem okolju kot pa temperatura.

Ključne besede: alge, cianobakterije, jame, raznovrstnost, okoljski dejavniki, metaanaliza.

¹ Departamento de Biología Aplicada (Botánica), Universidad Miguel Hernández, Avenida de la Universidad s/n 03202, Elche, Alicante, Spain

¹ Andrea Belda, e-mail: a.belda@umh.es

¹ Laura García-Abad, e-mail: laura.garciaa@umh.es

* Corresponding author: Antonia D. Asencio, e-mail: aasencio@umh.es

1. INTRODUCTION

1.1 ALGAE IN CAVE ENVIRONMENTS

1.1.1 CAVES AND ROCKSHELTERS

Algae are distributed in different aquatic and terrestrial environments, and caves and rockshelters stand out in the latter. Both the terms caves and rockshelters refer to cavities. Their difference lies in depth, and the word rockshelter is employed when depth is shallow (Figure 1).

Microclimate conditions, mainly photosynthetically active radiation (PAR), temperature (T) and relative humidity (RH), vary according to these caves' locations and configurations. Two environments can be distinguished in caves: the cave interior, where the RH and temperature values remain constant all year long and the entrance, where conditions seasonally vary, as with PAR. In rockshelters, the fluctuation in relative humidity and temperature, and in light intensity, is not notable between the innermost part and the outer part due to the shallowness that characterises these cavities.

Algal survival in such environments, where water and nutrient availability is scarce, has been possible thanks to the morphological and physiological adaptations that algae have undergone (Hoffmann, 1989). One example is the presence of sheaths in Cyanobacteria cells, which are considered a water reservoir that maintains metabolic activity under drought conditions, and also protects from drying and UV-radiation when pigmented (Asencio, 2022).

Caves and rockshelters occupy a special place in humanity's history because they present much natural and cultural interest. This is why many of these cavities are found on the World Heritage List of the United Nations Educational, Scientific and Cultural Organization (UNESCO). In recent decades, organised tourist visits to many caves and rockshelters have intensified (Mulec & Kosi, 2009), which is deteriorating their walls and damaging both their aesthetic beauty and any pictorial remains with a high cultural value that may remain in them.

1.1.2 TERMINOLOGY

The different actions performed by algae on substrates began to be defined at the end of the 19th century by distinguishing between the microorganisms that live on a rock, known as epilithic, and those that develop in cracks of rocks, known as endolithic.

Later when defining the organisms that live on rocks, Golubic et al. (1981) used the term lithobionts, which they subdivided into:

- Epilithic; they colonise outer rock surfaces
- Endolithic; they colonise the interior of rocks
 - o Chasmoendolithic; they develop in cracks, fissures and pores on the surface of rocks
 - o Cryptoendolithic; they colonise the structural cavities inside porous rocks
 - o Euendolithic; they actively penetrate the interior of rocks by forming tunnels that adapt to the shape of their bodies

Khomutovska et al. (2021) deal with the lithobiontic habitat by differentiating six types: epilithic, chasmoendolithic, cryptoendolithic and euendolithic with a similar definition to that of Golubic et al. (1981); hypolithic and hypoendolithic, which are terms respectively defined as organisms occupying the ventral side of a rock and organisms colonising the rocky matrix in the lower rock part.

The present work follows the terminology of Golubic et al. (1981).

1.1.3 HISTORIC BACKGROUND

Towards the end of the 18th century, research into the flora of caves commenced and they were restricted to heterotrophy organisms. Early in the 20th century, the first references to the presence of algae in caves were made (Asencio, 1997).

Nowadays, data are known about algal flora in caves from countries all over the world. In some cases, research

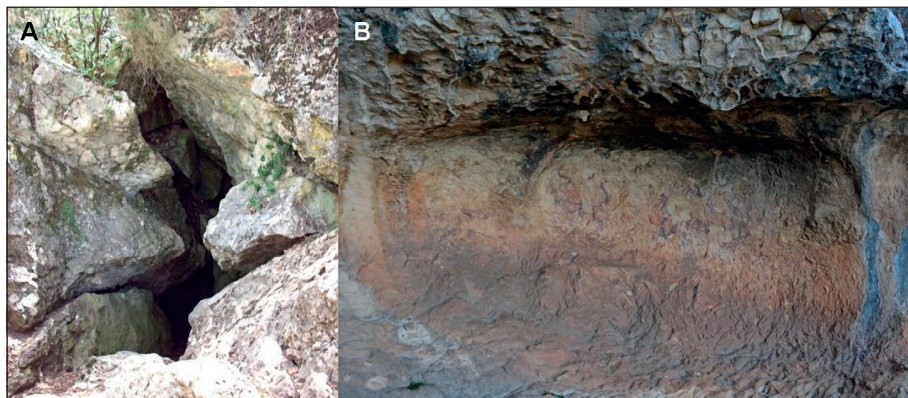


Figure 1: Gelada cave, Spain (A) and La Sarga rockshelters, Spain (B).

works centre on certain species that develop in cave environments, such as *Asterocapsa divina* (Aboal et al., 2003), *Chroococcidiopsis kashayi* (Friedmann, 1962), *Cyanidium chilense* (Cinigliaet al., 2017, 2019), *Geitleria calcarea* (Friedmann, 1979; Davis & Rands, 1981; Couté, 1982, 1989), *Geitleria floridana* (Friedmann, 1979), *Hapalosiphon intricatus* (Davis & Rands, 1982; Moore et al., 1984) and *Scytonema julianum* (Aboal et al., 1994; Asencio & Aboal, 2011).

Given the peculiarity of these habitats, records of rare or new species are relatively frequent. Some examples are: *Chalicogloea cavernicola* (Roldán et al., 2013); *Herpyzonema pulverulentum* (Hernández-Mariné & Canals, 1995); *Iphinoe spelaebios* (Lamprinou et al., 2011), *Loriella* sp. (Hernández-Mariné et al., 1999); *Loriellopsis cavernicola* (Lamprinou et al., 2011); *Symphyonema caverniculum* (Asencio et al., 1996); and *Toxopsis calypsus* (Lamprinou et al., 2012).

In addition, in recent years, new genus and species that have been found in caves have been described using combined molecular and cytomorphological criteria. It should be noted the genus *Timaviella* represented by two different species *Timaviella circinata* and *Timaviella karstica* (Sciuto et al., 2017), the genus *Oculatella* represented by *Oculatella subterranea* (Zammit et al., 2012), the genus *Jenufa* represented by four distinct species *Jenufa perforata*, *Jenufa minuta*, *Jenufa aeroterrestica* and *Jenufa lobulosa* (Němcová et al., 2011; Prochazkova et al., 2015; Song et al., 2018). Moreover, it should be noted the species *Diploneis mawsmatii* (Bhatt & Karthick, 2020), *Brasilonema geniculatum* and *Calothrix dumus* (Villanueva et al., 2019), *Phormidesmis nigrescens* (Raabová et al., 2019), *Nephrococcus serbicus* (Popovic et al., 2016), and *Leptolyngbya corticola* (Johansen et al., 2011).

1.2 ALGAE TYPES

1.2.1 PROKARYOTIC ALGAE: CYANOBACTERIA

Cyanobacteria are organisms characterised by presenting properties of both bacteria and algae. Their main bacterial characteristics include not having organelles enclosed in membranes and the cell wall structure, whereas their main algae characteristics are chlorophyll *a*, and the thylakoid structure, and organisms act as primary producers in nature (Asencio, 1997).

Cyanobacteria are a morphological diverse group of prokaryotes that successfully colonise and inhabit almost every kind of terrestrial and aquatic habitat, including extreme microhabitats like caves, rocks, external walls on monuments and buildings, etc. The microorganisms that inhabit caves have had to undergo a series of adaptations to survive the more or less extreme conditions of their habitats (Asencio & Aboal, 2004). They also play a key

role as colonisers, nitrogen-fixers or deterioration agents in relation to several environmental aspects (Czerwik-Marcinkowska et al., 2015). Regarding nitrogen fixation, some filamentous species have been able to develop heterocytes, which are the cells that specialise in this process. This has enabled them to occupy very nutrient-poor places (Asencio, 2010).

1.2.2. EUKARYOTIC ALGAE, CHLOROPHYTA, RHODOPHYTA AND BACILLARIOPHYTA

The most outstanding groups of eukaryotic algae are Chlorophyta, Rhodophyta and Bacillariophyta, which appear in aquatic and terrestrial environments. Very little knowledge is available about the latter groups, specially Rhodophyta because only few studies have been conducted and published on it than those that live in aquatic environments like rivers, lakes and oceans (Falasco et al., 2014).

Chlorophyta comprise one of the biggest groups of algae given their large number of species and variety of shapes. They have a very broad distribution because they can be found in aquatic habitats, on the surface of rocks, and on wet land and tree trunks, but they need light and relative humidity to develop (Peña-Salamanca et al., 2005). They also include a wide range of organisation levels because they come as free cells that are either flagellated or not, and as colonies in many forms (Romero, 2010).

Rhodophyta range from unicells and uni- or multiseriate (arranged in rows) filaments, to large pseudoparenchymatous, branched or unbranched, cylindrical to foliose thalli, including crustose and erect forms, some of which are calcified. They can be found in many different environments – marine, freshwater, and terrestrial (Yon et al., 2016).

All Bacillariophyta are unicellular, and play an important role in the general carbon and silicon cycle. It is believed that they emerged from a secondary endosymbiotic event between two eukaryotes: a Rhodophyta and a heterotrophic flagellates. Thus Bacillariophyta possess a diversity of characteristics that make them different from the classic cell structures of higher plants (Lopez et al., 2005). Bacillariophyta can be found in virtually any kind of environment from salty water to zones where high temperatures predominate. They also stand out for being well able to interact with other organisms like Cyanobacteria (Nieves-Mori6n et al., 2020).

The aim of this paper is to analyse research on caves and rockshelters, where microclimatic conditions and microalgae growth have been studied, in order to identify the environmental factors that favour algae growth in caves. This work will make it possible to create a database of cave algal species and the environmental conditions under which they develop.

2. MATERIALS AND METHODS

2.1 LITERATURE REVIEW

A literature review was conducted according to information about algal flora in caves and rockshelters worldwide.

The articles that included algae analysed in caves around the world were selected first. Then, specific articles of each algal group in caves were searched. Finally, a selection was made of those articles who presented a more complete study, those that presented studies of light intensity ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; included values converted from lux according to $1 \text{ klux} = 19.5 \mu\text{mol s}^{-1} \text{ m}^{-2}$) temperature ($^{\circ}\text{C}$), and humidity (%) in caves where the different type of algae grows. On the other hand, the articles of the latest new species found in caves were studied and those which included environmental data were chosen.

This literature review was done in 2022 by performing a multiple search following the methods indicated below:

- The research group's database: 11 papers about the study theme were selected.
- Public Internet databases:
 - o PubMed (<https://pubmed.ncbi.nlm.nih.gov/>). The following search equations were used:
 - (Algae [Title/Abstract]) AND (cave [Title/Abstract]) with 26 results
 - (Cyanophyceae) AND (cave) with 49 results
 - (Cyanobacteria) AND (cave) with 49 results
 - (Cyanophyta) AND (cave) with 49 results
 - (Cyanoprokaryota) AND (cave) with 0 results
 - (Green algae) AND (cave) with 15 results
 - (Chlorophyceae) AND (cave) with 1 result
 - (Chlorophyta) AND (cave) with 11 results
 - (Diatom) AND (cave) with 11 results
 - (Bacillariophyceae) AND (cave) with 1 result
 - (Bacillariophyta) AND (cave) with 9 results
 - (Rhodophyta) AND (cave) with 4 results
 - (Rhodophyceae) AND (cave) with 4 results
 - (Red algae) AND (cave) with 6 results
 - o Google Academic-Google Scholar (<https://scholar.google.es/>). The employed keywords were "cave" and "algae", and 40,700 results were obtained. To facilitate the search, the presence of the keywords only in the title were added.
 - allintitle: cave and algae with 16 results
 - allintitle: cave and cyanophyceae with 1 result
 - allintitle: cave and cyanophyta with 0 result
 - allintitle: cave and cyanoprokaryota with 0 result

- allintitle: cave and cyanobacteria with 12 results
- allintitle: cave and "green algae" with 2 results
- allintitle: cave and chlorophyceae with 0 result
- allintitle: cave and chlorophyta with 0 result
- allintitle: cave and diatom with 1 result
- allintitle: cave and bacillariophyceae with 2 results
- allintitle: cave and bacillariophyta with 0 result
- allintitle: cave and rhodophyta with 0 result
- allintitle: cave and rhodophyceae with 0 result
- allintitle: cave and red algae with 0 result

Articles containing the keywords in the title were selected.

Of the 280 papers obtained from Internet databases (Figure 2), 198 were excluded because no direct relation was found to the specific study herein conducted or because were repeated in more than one search. Therefore, 82 articles were analysed in this review.

- Interesting websites:
 - o Algaebase. It is a world database with information about the taxonomy, nomenclature and distribution of algae groups, including terrestrial, marine and freshwater organisms, and a group of marine plants with flowers (<https://www.algaebase.org/>)

The following books were also consulted:

- "*El conjunto prehistórico y de arte rupestre de El Milano. Mula, Murcia*" (2009) by Miguel San Nicolás del Toro
- "*El abrigo de Ciervos Negros (Moratalla, Murcia)*" (2010) by Miguel Ángel Mateo Saura & Esteban Sicilia Martínez.
- "*Ecology of Cyanobacteria II. Their Diversity in Space and Time*" (2012) by Brian A. Whitton.

2.2 STATISTICAL ANALYSIS

The analysis of the data obtained in this study was performed with the help of the Microsoft Excel 2019 computer programme and version 11.2 of the STATA statistics software (StataCorp LP, TX, USA). The Shannon Index (H') was calculated to know the diversity of the algal species that develop in cave environments. A principal component analysis (PCA) (with standardization) was run to determine if any influence existed between 412 algal species (Cyanobacteria, Chlorophyta, Bacillariophyta, and Rhodophyta) that develop inside the caves and rockshelters selected from the literature review and the recorded environmental parameters (temperature, relative humidity and photosynthetically active radiation).

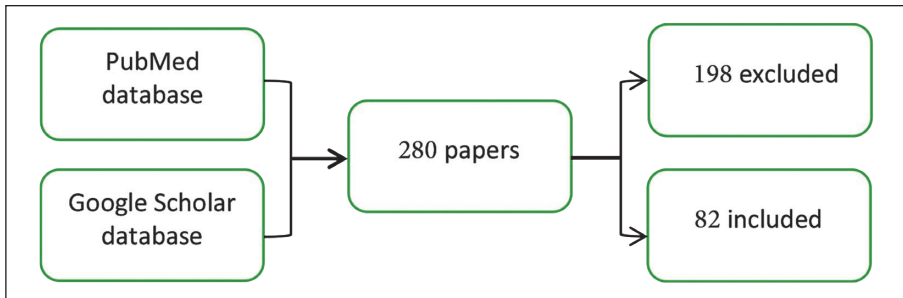


Figure 2: Flowchart of the search carried out in internet databases.

3. RESULTS AND DISCUSSION

To conduct this work, several studies into caves and rockshelters from different European countries were selected (Figure 3), such as: Spain (1, 2, 3, 4); Italy (5, 6); Slovenia (7), the Czech Republic (8); Poland (9); Hungary (10); Serbia (11); Greece (12, 13, 14); Russia (15). Studies from other parts of the world were also included: the USA (16), Chile (17) and India (18).

3.1 ENVIRONMENTAL DATA

Cave interiors are generally characterised by presenting a stable environment all year round as far as temperature and relative humidity are concerned. However, the environmental conditions in cave entrances and rockshelters vary similarly to those outside (Asencio et al., 1996).

Light intensity is the main factor that determines

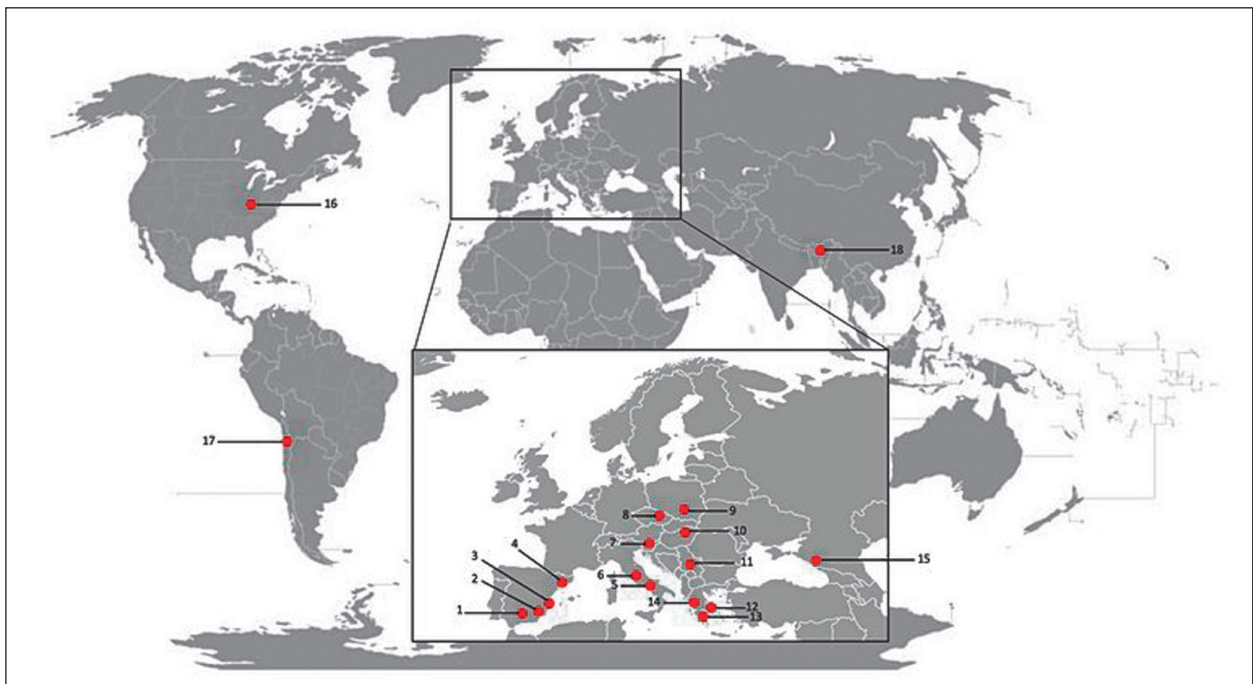


Figure 3: Geographic distribution of the studied caves. (1) Murciélagos cave; (2) Serreta chasm, rockshelters, Vapor chasm, Peliciego cave, Enredaderas rockshelters, Pozo rockshelter, Cañaica del Calar rockshelter, Buen Aire rockshelter, Grajos rockshelters, Pucheros cave; (3) L'Aigua cave, Gelada cave; (4) Papallona chasm, Corral Nou chasm, Puigmoltó chasm, Salpetre cave; (5) Fornelle cave; (6) Holy Saviour's cave; (7) Račiške ponikve cave, Postojnska cave, Kostanjeviška cave, Pekel pri Zalogu cave, Pivka cave, Škocjansjje cave, Zupanova cave; (8) Mladeč cave, Javoičko cave, Zbrašov cave; (9) Sąspowska cave, Labajowa cave, Nietoperzowa cave, Nad Marką Boską cave, Zarska cave, Krakowska cave, Mamutowa cave, Dzika cave, Twardowskiego cave, Jasna cave, Głęboka cave, Tomaszówkach cave, Za Kratą cave, Lopiankach cave, Szachownica cave, Biala cave, Zbójecka cave, Schronisko Male cave, Pustelnia cave, Koziarnia cave, Lokietka cave, Sypialnia cave, Ciemna cave, Złodziejska cave, Wielka Dolna cave, Ostrężnicka cave; (10) Ice-cave in Zemplén Mountains, Baradla cave at Aggtelek II, Mátyás Mount cave, Beremendi-ördöglyuk, Nagy Vizes-barlang, Kis Vizes-barlang; (11) Bozana cave, Ribnička cave, Hadzi Prodanova cave, The Rčanska cave, The Degurić cave, Vernjickica cave, Petnica cave; (12) Leontari cave; (13) Kastria, Selinita, Francthi; (14) Perama cave; (15) Akhshtyrskaya Excursion cave; (16) Mammoth cave; (17) Atacama Desert Coastal cave; (18) Arwah cave, Mawjmbuin cave, Krem Dam cave, Krem Puri cave, Krem Traw cave.

if the microbial communities living on walls of caves or rockshelters are autotroph (Cyanobacteria and algae) or heterotroph (bacteria and fungi) (Albertano, 2012). This factor varies from the cave entrance to the inner of caves because of their depth, but does not apply to rockshelters given their shallowness.

Photosynthetic communities tend to be found on the surface of entrances, but also appear inside tourist caves where artificial light allows them to grow. It is also known that caves with little natural light house photosynthetic microorganisms (Martínez & Asencio, 2010).

Caves barely contain autochthonous resources for algal flora to proliferate, which is why they are considered extreme environments with low nutrient availability despite them receiving allochthonous resources transported by water, wind and animals. Nevertheless, many groups of organisms have been able to grow and proliferate under such conditions (Czerwik-Marcinkowska, 2013). The primary source of energy is generally decomposing organic matter from plants and guano, whose bioavailability depends on their chemical properties and environmental factors, such as temperature and light intensity (Smith & Benner, 2005).

The majority of the microorganisms that have colonised these environments are distributed on the surface layer of the minerals that rocks are composed of; that is, they are epilithic. However, some have been able to develop under this layer (Albertano, 2012), which could have led to small rock fragments coming away in caves and rockshelters. The development of algal communities on surfaces of rocks forms different kinds of structures that can be macroscopically seen thanks to their colouring, which varies from grey to black, and with brown, green and blue tones (Asencio, 2022).

The environmental data (T, RH, PAR) collected from the different caves and rockshelters found in the various papers selected to conduct this work are included (Appendix 1). Mineralogical composition is also included (Figure 4) because it is considered another important factor when contemplating the biodeterioration of caves

and rockshelters caused by algal flora because substrate type determines the composition, distribution and structure of species in algal communities (Uher, 2010). Mineralogical composition data do not appear in 55 % of the analysed studies, but do in the remaining 45 % of caves and rockshelters where most caves are composed of limestone.

According to the analysed data, the interior of most caves presents stable relative humidity all year long owing to their depth and being scarcely exposed to external environmental factors. Nonetheless, we come across some extreme cases, such as: the L'Aigua cave (Spain), which is a broad shallow cave where minimum and maximum relative humidity is respectively 47.13 % and 75.1 %; the La Serreta chasm (Spain) with its two openings that allow contact between the inside of the cave and the outside, where minimum and maximum relative humidity is respectively 49.15 % and 75.1 %. The same applies to the studied rockshelters because they are very shallow and are exposed to external environmental conditions (Appendix 1).

Most of the caves also have a high maximum RH, which can be as high as 100 % in some cases, such as the Perama cave (Greece), Ice-cave in Zemplén Mountains (Hungary), Baradla cave at Aggtelek II (Hungary), Zbrašov (Czech Republic), and the Mammoth cave (USA). RH rarely drops to 41.5 %, except in Andragulla rockshelters (Spain), and in Papallona chasm (Spain), and in Ribnička cave (Serbia) and Hadzi Prodanova cave (Serbia), with minimums of 6.9 % because of their location (Appendix 1).

As with RH, the temperature inside caves tends to remain stable all year long. However, in the L'Aigua cave (Spain), the La Serreta chasm (Spain), the Gelada cave (Spain), the Cave in Frantchi (Greece), the Atacama Desert Coastal cave (Chile) and the Akhshtyrskaya Excursion cave (Russia), a difference of more than 10 °C appears between their recorded minimum and maximum temperatures. In the L'Aigua and La Serreta chasm (both in Spain), this variation is due to their characteristics for

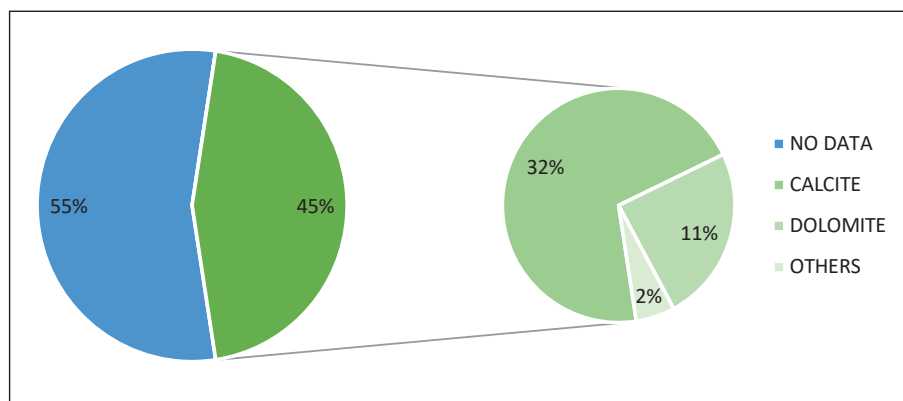


Figure 4: Mineralogical composition of the caves and rockshelters.

being more in contact with the exterior environment, as previously mentioned. Conversely, the difference in temperature in all the other caves is because values come from different points, one of which is the entrance. Both their minimum and maximum temperatures correspond to entrances, and they vary according to the exterior environment. In our studied rockshelters, this variation in maximum and minimum values is even bigger with a difference between them of 38.3 °C because all-year-round temperatures vary according to their environment (Asencio & Aboal, 1996).

As Mulec & Kosi (2008) discovered that algal community composition notably varies according to PAR levels, it is important to analyse them. In most caves, the difference between the maximum and minimum PAR values is large because data are taken from different points, ranging from the entrance to the end of caves. Thus the maximum value corresponds to the value taken at the entrance and the minimum one to the value taken at the end of caves. The maximum values recorded from almost all the caves are below 300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Nonetheless, the value recorded at the L'Aigua cave (Spain) is 690.1 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ because of its morphology. In rockshelters, light intensity largely depends on the zone where they are located and their orientation because solar radiation in shaded zones is much lower than in sunny spots. This is why maximum PAR values up to 753 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ appear in the rockshelters Andragulla (Spain), while the minimum PAR value of 0.03 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ is for Cova Gelada cave in Spain (Appendix 1).

Cyanobacteria and algae are strongly impacted by not only temperature, PAR, and relative humidity conditions (Pouličková & Hašler, 2007), but also by many other factors like nutrient input, the type and physico-chemical properties of substrate (pH, rock composition, porosity), cave morphology (size, location, dimension, orientation) and water availability. These factors mainly affect the composition of microbial communities (Lamprinou et al., 2012; Czerwik-Marcinkowska, 2013). The importance of substrate may have something to do with its calcareous and alkaline nature favouring Cyanobacteria proliferation whenever light is adequate (Popović et al., 2017).

3.2 ALGAL FLORA

The Shannon Index was determined with a value of 4.49, which indicates that the diversity of the algal species that develop in the studied cave environments is very high. This coincides with Czerwik-Marcinkowska & Massalski (2018), who point out that caves and rockshelters represent centres of biodiversity for different kinds of microorganisms, particularly for Cyanobacteria, which can be widespread inhabitants on surfaces of rocks in caves (Albertano, 2012). The present study confirms this be-

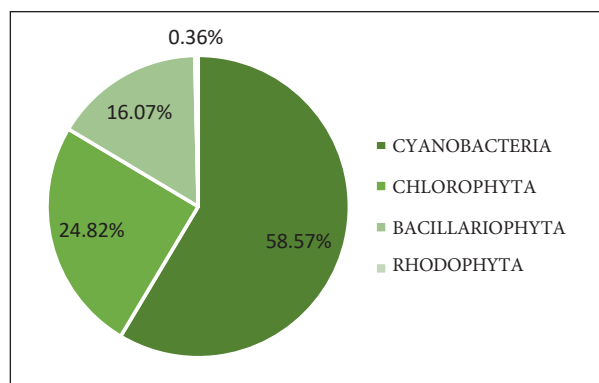


Figure 5: Algal flora of the studied rockshelters and caves.

cause, of all the analysed works, the dominant algal group in number of species terms is Cyanobacteria (Figure 5). Therefore, cave environments can be considered under-examined environments as regards biodiversity according to Asencio & Espinosa (2013).

On the contrary, Rhodophyta division presents a lower proportion of the total algal flora (Figure 5) because only two genera were found. *Lemanea torulosa* abundantly appeared on an old trunk in the Mammoth cave, which surprised researchers because this genus tends to live in small streams. Although *L. torulosa* can tolerate a certain degree of pollution, it needs suitable ventilation to grow (Jones, 1965). In the Atacama Desert Coastal cave, *Cyanidium* sp. that formed a microbial mat was identified. This was another unexpected finding because most of the known species of the Cyanidiales order live in acidic hot spring waters (Azúa-Bustos et al., 2009).

Of the 412 algal species analysed, the most diverse Cyanobacteria genera to appear in the different studied caves and rockshelters are *Leptolyngbya* with 28 different species, *Gloeocapsa* with 24 and *Phormidium* with 23. They are followed by *Chroococcus* with 18, *Aphanothece* with 14, *Oscillatoria*, *Nostoc* and *Scytonema* with 10 each and *Schizothrix* and *Tolypothrix* with 9 each. The most diverse Chlorophyta and Bacillariophyta genera are *Chlorella* with 9 different species and *Diadesmis/Humidophila*, *Luticola* and *Nitzschia* with 4, respectively (Appendix 1).

The most abundant Cyanobacteria taxa are *Aphanocapsa muscicola*, *Aphanothece saxicola*, *Chroococcus minor*, *Gloeocapsa biformis*, *Gloeocapsa punctata*, *Leptolyngbya foveolarum*, *Nostoc commune*, *Pseudocapsa dubia* and *Scytonema julianum* (Figure 6). Chlorophyta are *Desmococcus olivaceus*, *Klebsormidium flaccidum*, *Stichococcus bacillaris* and *Trentepohila aurea*. The most frequent Bacillariophyta species are *Diadesmis/Humidophila contenta*, *Hantzschia amphioxys* and *Orthoseira roseana* (Appendix 1).

Cyanobacteria are considered to be the oldest cellular organisms on Earth, and are very resistant to extreme cave conditions (Czerwik-Marcinkowska & Massal-

ski, 2018), and even to low-light environment (Asencio, 2022). Barton & Jurado (2007) suggest that this group is able to adapt to cave interiors by interacting with minerals on cave walls and ceilings. Despite Cyanobacteria being pioneers in inhabiting these environments, the rapid growth of Chlorophyta in places with better environmental conditions enables them to outcompete Cyanobacteria to proliferate in this habitat and invade it (Czerwik-Marcinkowska et al., 2015). Small Cyanobacteria and eukaryote algae have also been found to live under small drops of water (Mulec & Kosi, 2008).

Both Cyanobacteria and Chlorophyta prefer humid places while they develop, but have shown considerable resistance to dry environments (Mulec & Kosi, 2008, 2009). These extreme environments significantly impact colonisation processes, which is why the algae and Cyanobacteria that occupy such places need to undergo specific adaptations (Czerwik-Marcinkowska et al., 2015).

According to Pouličková & Hašler (2007), Bacillariophyta are found mainly on rocky walls close to cave entrances, or in areas surrounding electric lights. Such conditions provide photoautotroph organisms with a

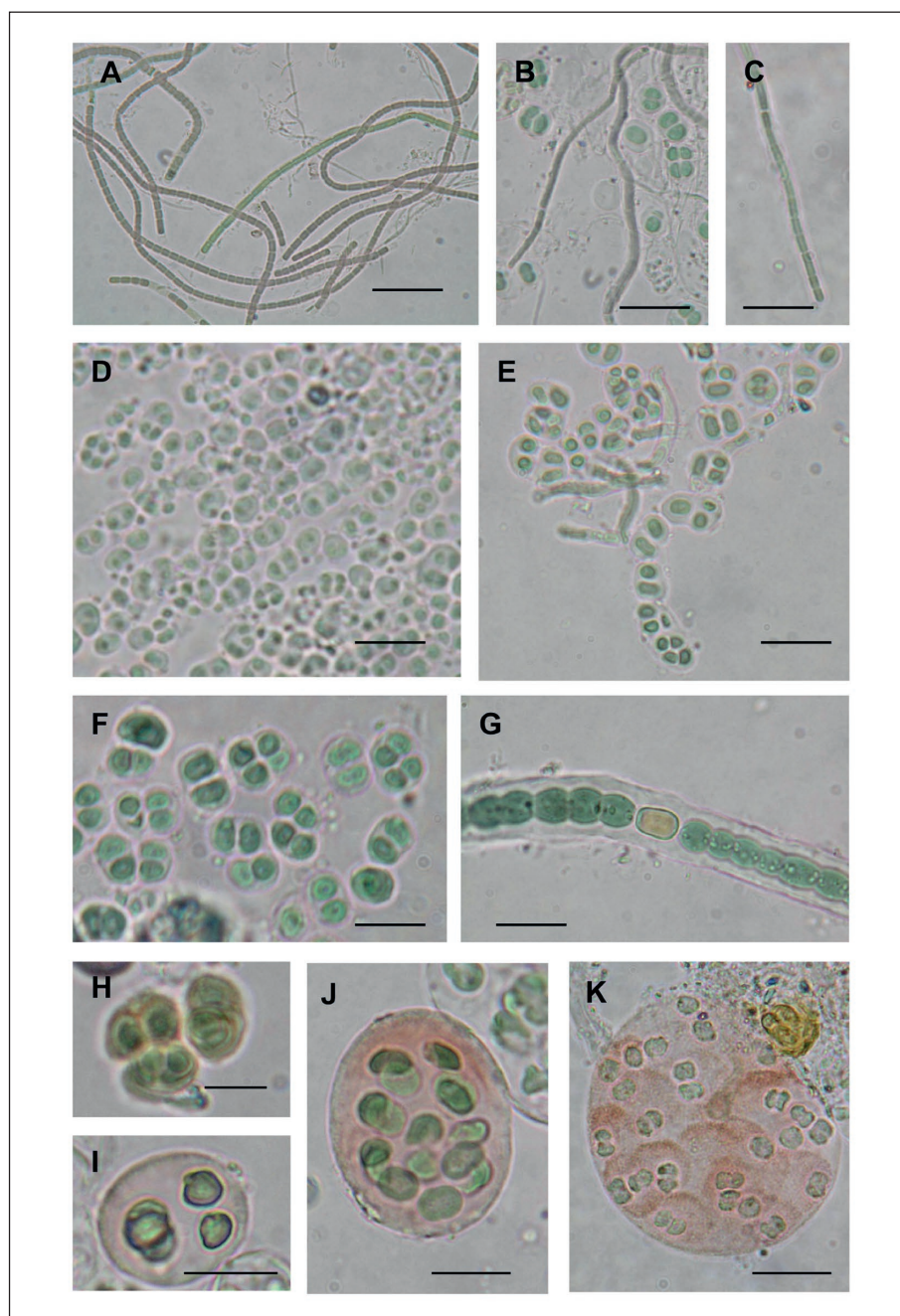


Figure 6: Light micrographs [scale bar: 10 μm] of some of the most abundant Cyanobacteria taxa in caves environments: A- *Leptolyngbya* "Albertano/Kováčik-red", B- *Leptolyngbya carnea*, C- *Leptolyngbya leptotrichiformis*, D- *Pleurocapsa* sp., E- *Pleurocapsa minor*, F- *Pseudocapsa dubia*, G- *Scytonema julianum*, H- *Gloeocapsa bififormis*, I- *Gloeocapsa nigrescens*, J- *Gloeocapsa novacekii*, K- *Gloeocapsa rupicola* (Martínez & Asencio, 2010).

sufficient source of energy. Therefore, whenever there is light, whether natural or artificial, a relatively high number of Bacillariophyta species will be able to colonize it. Bacillariophyta also tend to grow in humid places characterised by the presence of mosses (Falasco et al., 2014). Coinciding with Falasco et al. (2014), *Hantzschia amphioxys* is one of the typical Bacillariophyta found in most caves (Appendix 1).

According to the PCA (Figure 7), the first three axes explain 100 % total variance (PC1, PC2 and PC3 with 57.37 %, 30.26 % and 12.37 %, respectively). These axes are linear functions of the cave species and the environmental parameters according to which they develop. Despite considerable overlapping, algal species tend to be positioned along the first axis, which appears to capture the gradients of RH and PAR. The second axis is associated mostly with temperature. This analysis reveals that both PAR and RH condition, more actively than temperature, the development of algal species in cave environments.

Algae are often found to form microbial mats with other microorganisms. The microbial mat provided a protective barrier and an improved chance of survival for cells growing in a low-nutrient and low-light environment (Asencio, 2022). The variety of microbial mats in caves can be quite vast (Mulec & Kosi, 2009) because the microbial mat structure is generally related to light availability. Thus in cave entrances, rockshelters and artificially lit places, microbial mats are characterised by presenting a series of thick layers. This thickness proportionally narrows with decreasing light (Asencio et al., 1996).

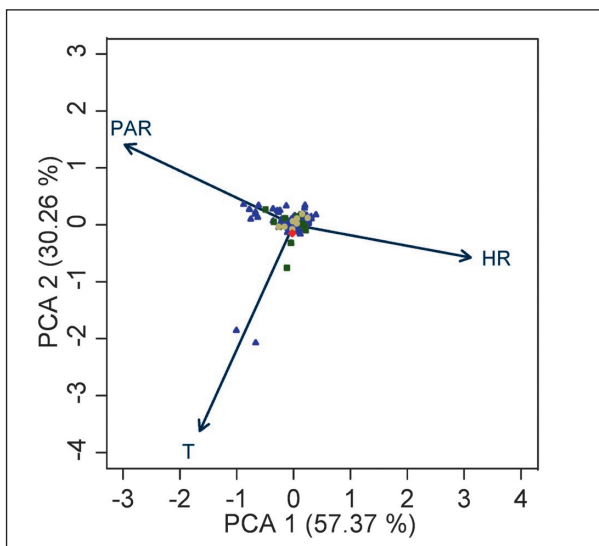


Figure 7: Principal component analysis (PCA) (with standardization) of 412 algal species (blue triangles included Cyanobacteria, green squares: Chlorophyta, golden circles: Bacillariophyta, and red circles: Rhodophyta) and microclimate conditions inside caves and rockshelters as temperature ($^{\circ}\text{C}$), relative humidity (%) and photosynthetically active radiation ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

Pouličková & Hašler (2007) suggest that most walls in cave entrances are covered with a microbial mat of greenish-bluish Cyanobacteria. However, this study observes that the microbial mats in cave entrances are composed partly of Cyanobacteria, and partly of Bacillariophyta and Chlorophyta. Even ferns, mosses, lichens and fungi can be identified in some. Not only do they have greenish-bluish colouring, but they also display a yellow, brown, red, black and grey colouring, among others, and even combinations of them.

Photosynthetic algae, along with epilithic Cyanobacteria, play a key role in microbial mat generation, and can produce exopolymers (EPS) that allow them to adhere to rocks and a microbial community to be established (Falasco et al., 2014). Apart from substrates' colonisation and pigment production, which are responsible for both colour effects on rocky cave walls and stone substrate erosion, they may also serve as a source of animal food. Cyanobacteria have adopted survival strategies to environmental stress like drying, extreme temperature and UV radiation by producing photoprotective pigments and bioactive compounds (Czerwik-Marcinkowska & Massalski, 2018).

Most of the Cyanobacteria living in caves and rockshelters possess a mucilaginous extracellular sheath, which varies in terms of number of layers and consistency, but this diversity is more marked in endolithic Cyanobacteria. This structure surrounds a cell wall (Romero, 2010) and, thus, plays a crucial role in substrate adhesion, and to such an extent that it is sometimes impossible to distinguish between biological and non-biological material (Asencio & Aboal, 2001). Moreover, the production of thick multilayered sheaths aided adherence to the cells in microbial mat formation (Asencio & Espinosa, 2013). These sheaths also act as a water tank, which allows Cyanobacteria to survive drought periods (Keshari & Ashikary, 2013). This could be an important factor in the deterioration process of caves and rockshelters because the water absorbed and released by sheaths confers them expansion and compression strengths, which can fragment substrate (Asencio & Aboal, 2011).

As previously reported by Asencio & Aboal (2004), the cell wall, cellular sheaths and thylakoids are features that might play a role in adaptation to chasmoendolithic environments.

3.3 ANTHROPOGENIC IMPACT

The growth of photoautotroph organisms in caves is limited by the area where light enters (Johnson, 1979). Nonetheless, the impact of tourism on these environments has altered the natural light gradient when artificial lighting is fitted. This has relevant repercussions on lampenflora (community of autotroph organisms that colonise lit up

cave walls, including Cyanobacteria, algae, mosses, lichens, and even higher plants) composition inside caves (Mazina & Maximov, 2011) because original populations and communities could be displaced (Falasco et al., 2014).

Cyanobacteria compete with some algae and mosses for light at cave entrances, in rockshelters and on surfaces surrounding the artificial light fitted inside caves to benefit visitors (Czerwik-Marcinkowska & Massalski, 2018). This artificial lighting is a source of energy, and not only a stimulus, to photosynthetic algae and Cyanobacteria, which are unsightly and can harm speleothems and other cave surfaces (Albertano et al., 2003; Falasco et al., 2014; Halvena et al., 2021). In the Baradla cave (Hungary), photosynthetic algae and Cyanobacteria communities spread and have doubled only 7 years after fitting artificial light (Mulec & Kosi, 2009). However, artificial light can also have a negative effect by considerably lowering RH, which can be adverse for microorganisms living in caves (Saiz-Jimenez et al., 2012). Likewise, lampenflora severely damages cave paintings (Baquedano et al., 2019) because it can completely or partly cover pictorial representations by growing on the surface of rock. This occurred with the painting called the Serreta Idol (Figure 8) (Asencio & Aboal, 2001).

Despite Cyanobacteria being the phototroph organisms that are best able to adapt to extreme environments, the habitats under less environmental stress, such as points lit up by lights, are easily covered by rapidly growing eukaryote algae (Mulec & Kosi, 2009).

The tourists who visit caves are responsible for transferring lampenflora (Ivarsson et al., 2013), which leads to unintentional biological pollution (Albertano, 2012). Consequently, this alteration to a natural environ-

ment might also modify microorganism communities because artificial lighting influences the water content of substrate and air (Czerwik-Marcinkowska & Massalski, 2018). The presence of tourists also leads to rises in the temperature and CO₂ concentration inside caves, which intensifies the erosion of walls (Mulec & Kosi, 2009).

Based on the obtained results, it would be interesting to extend this research work to possibly apply it particularly to caves and rockshelters that have been adapted to increasingly more frequent tourist visits. In recent years, anthropogenic activities have become more frequent in caves and rockshelters. These cavities have been adapted for tourist visitors by fitting lighting systems. This artificial light brings about changes in the T and RH in cave environments, which benefits the invasive growth of photosynthetic algae and Cyanobacteria, both of which cause biodeterioration and aesthetically harm cavities, hence the importance of preventing this or eliminating communities. To prevent these microorganisms from proliferating, an in-depth study should be performed before fitting lighting systems, and the microorganisms present on all cave elements should be periodically monitored. However, eliminating communities is not that simple because, despite several methods having been studied, no ideal solution presently exists. So, it is still necessary to identify optimum solutions to remove these communities, but without destroying or causing harm to cave and rockshelter environments. Moreover, visits paid to caves and rockshelters must be organised so that they minimise effects on these microorganisms, such as avoiding visitors contacting speleothems because this introduces nutrients and all kinds of microorganisms.

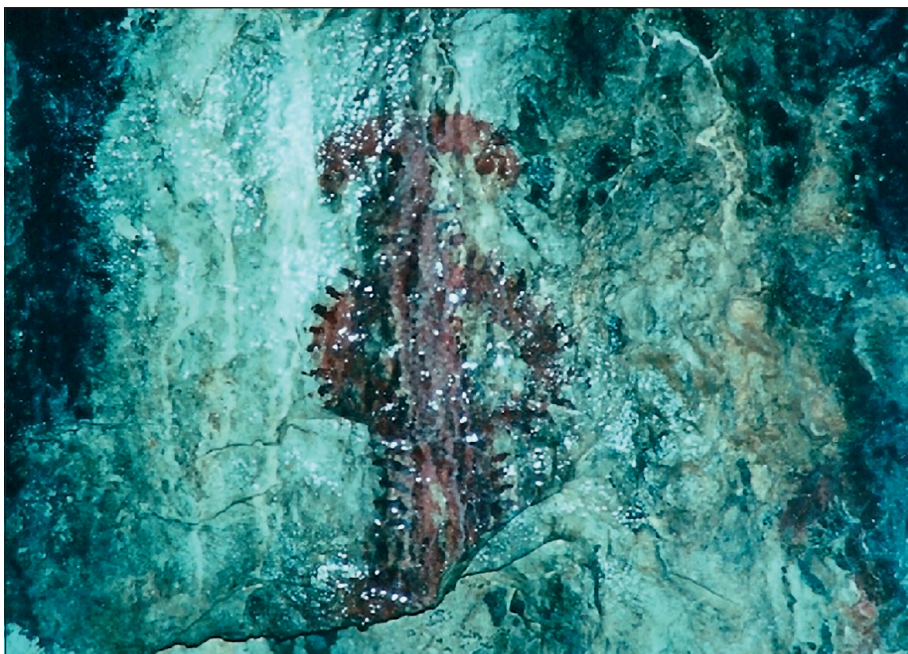


Figure 8: Ídolo of Serreta chasm covered by communities of epilithic algae (Asencio & Espinosa, 2013).

4. CONCLUSIONS

This work counted 412 algal species. The most diverse Cyanobacteria genera to appear in the different studied caves and rockshelters are *Leptolyngbya* with 28 different species, *Gloeocapsa* with 24 and *Phormidium* with 23. They are followed by *Chroococcus* with 18, *Aphanothece* with 14, *Oscillatoria*, *Nostoc* and *Scytonema* with 10 each and *Schizothrix* and *Tolypothrix* with 9 each. The most diverse Chlorophyta and Bacillariophyta genera are *Chlorella* with 9 different species and *Diadesmis/Humidophila*, *Luticola* and *Nitzschia* with 4, respectively.

The microclimate conditions of T, RH and PAR in-

side caves where darkness dominates tend to remain constant all year long. However, they vary in cave entrances and rockshelters in accordance with the microclimate conditions outside. There are, however, some exceptions given the morphology and orientation of these cavities. Microclimate conditions determine the composition, distribution and structure of algal flora in caves and rockshelters to a great extent, as demonstrated by the PCA. The PCA indicated that both PAR and RH condition the development of algal species in cave environments more actively than T.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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AUTHORSHIP STATEMENT

A.D.A. conceived, designed and directed the study. A.B. & A.D.A. wrote the paper. L.G. & A.D.A. analysed the data. All the authors contributed to the general discussion, revision, and manuscript editing.

A META-ANALYSIS OF ENVIRONMENTAL FACTORS INFLUENCING THE ALGAL COLONISATION IN CAVES AND ROCKSHELTERS WORLDWIDE

	Ts	RH	PAR	SPAIN	SLOVENIA	SERBIA	HUN-GARY	ITALY	GRECIA	USA CHILE	CZECH REPUBLIC	RUSSIA
<i>Hassalia byssoides</i>	12.84	85.55	1.56									
<i>Herpyzonema pulverulentum</i>	15.48	70.97	3.3									
<i>Heteroleibinia kuetzingii</i>	8.5	99		X								
<i>Homeatrix varians</i>	13.93	85.95	4.14						X			
<i>Hormothoe cylindrocellulare</i>	22.2	53.1	627.15						X			
<i>Hydrocoleum homeotrichus</i>	11.41	86.99	3.3		X				X			
<i>Hydrocoleum stankovicii</i>	11.41	86.99	3.3						X			
<i>Iphinoe spelaebios</i>	11.41	86.99	3.3					X				
<i>Jaaginema sp.</i>	26.5	71.5	8.59									
<i>Leibierina epiphytica</i>	8.5	99						X				
<i>Leptolynghya africana</i>	26.5	71.5	8.59									
<i>Leptolynghya "Albertana Kováčik-red"</i>	11.7	75	0.03		X							
<i>Leptolynghya baryana</i>	15.01	81.33	2.59						X		X	
<i>Leptolynghya carnea</i>	15.53	73.95	2.09						X			
<i>Leptolynghya cebennensis</i>	16.38	78.39	4.96						X			
<i>Leptolynghya compacta</i>	18.86	74.1	5.79						X			
<i>Leptolynghya ercegovicii</i>	16.38	78.39	4.96						X			
<i>Leptolynghya foveolarum</i>	17.91	76.16	15.5		X				X			X
<i>Leptolynghya fragilis</i>			1.07									
<i>Leptolynghya gracillima</i>	17.31	68.33	1.34						X			
<i>Leptolynghya henningsii</i>	19.36	72.89	4.13						X			
<i>Leptolynghya lagerheimii</i>	15.39	79.95	3.72						X			
<i>Leptolynghya leptotrichiformis</i>	11.7	75	0.03									
<i>Leptolynghya lurida</i>	13.34	82.75	1.5						X			
<i>Lepolynghya nana</i>	19.36	72.89	4.13									
<i>Leptolynghya norvegica</i>	26.5	71.5	8.59						X			
<i>Leptolynghya nostocarum</i>	8.5	99										
<i>Leptolynghya palikiana</i>	16.38	78.39	4.96						X			
<i>Leptolynghya perelegans</i>	15.46	57.86	253						X			
<i>Leptolynghya perforans</i>	20	61.38	18.34						X			
<i>Leptolynghya purpurascens</i>	19.36	72.89	4.13									
<i>Leptolynghya subtilissima</i>	14.5	100								X		
<i>Leptolynghya tenuis</i>	13.53	84.4	4.45						X			
<i>Leptolynghya tenuissima</i>	17.03	77.48	3.3							X		
<i>Leptolynghya truncata</i>	19.36	72.89	4.13									
<i>Leptolynghya undosa</i>	18.35	75.28	7.42									
<i>Leptolynghya sp.1.</i>	14.65	72.6	9.02									
<i>Leptolynghya sp.2.</i>	20	68.25	21.16									
<i>Loniella osteophila</i>	16.25	72										
<i>Lyngbya palikiana</i>	11.41	86.99	3.3									

	Ts	RH	PAR	SPAIN	SLOVENIA	SERBIA	HUN-GARY	ITALY	GRECIA	USA	CHILE	CZECH REPUBLIC	RUSSIA
<i>Lyngbya perelegans</i>	19.95	41.55	753										
<i>Lyngbya pusilla</i>	14.5	100								X			
<i>Lyngbya truncicola</i>	14.5	100								X			Zbrašov
<i>Lyngbya sp.</i>			1.07		X								Javoričko
<i>Microchaete tenera</i>	18.35	75.28	7.42										Mladeč
<i>Microcoleus chthonoplastes</i>	11.41	86.99	3.3						X				
<i>Microcoleus steenstrupii</i>	18.35	75.28	7.42						X				
<i>Microcoleus sp.</i>	26.5	71.5	8.59					X					
<i>Microcystis stagnalis</i>	14.5	100								X			
<i>Myxosarcina s.p.</i>	14.64	77	3.3										
<i>Nadosilinea bijugata</i>	26.5	71.5	8.59					X					
<i>Nadosilinea nodulosa</i>	26.5	71.5	8.59					X					
<i>Nadosilinea sp.</i>	26.5	71.5	8.59					X					
<i>Nostoc commune</i>	17.09	77.82	20.3										
<i>Nostoc letestui</i>	18.35	75.28	7.42			X			X				
<i>Nostoc linckia</i>	18.35	75.28	7.42						X		X	X	
<i>Nostoc microscopium</i>	14.41	68.47	18.24										X
<i>Nostoc minutissimum</i>	14.5	100								X			
<i>Nostoc muscorum</i>	14.5	100											
<i>Nostoc paludosum</i>	11.5	70											
<i>Nostoc punctiforme</i>	17.73	74.04	6.87							X			
<i>Nostoc sphaericum</i>	15.4	57.73	381.1										
<i>Nostoc sp.</i>	8.12	74.04	3.97										
<i>Oculatella subterranean</i>	26.5	71.5	8.59									X	
<i>Oscillatoria angusta</i>	26.5	71.5	8.59										
<i>Oscillatoria animalis</i>	14.5	100						X					
<i>Oscillatoria clausiana</i>	14.5	100						X					
<i>Oscillatoria limnetica</i>	14	82.5								X			X
<i>Oscillatoria mauchiana</i>	18.35	75.28	7.42										
<i>Oscillatoria neglecta</i>	8.4	100											
<i>Oscillatoria rupicola</i>	16.56	82.67	5.79							X			X
<i>Oscillatoria subtilissima</i>	14.5	100								X			X
<i>Oscillatoria ucirenica</i>	26.5	71.5	8.59					X					
<i>Oscillatoria sp.</i>			0.65										
<i>Phormidochaete nordstedtii</i>	18.35	75.28	7.42										
<i>Phormidium ambiguum</i>	19.93	74.05	5.79										
<i>Phormidium animale</i>	11.41	86.99	3.3			X							
<i>Phormidium articulatum</i>	18.86	74.1	5.79										
<i>Phormidium autumnale</i>	13.96	63.3	4.96										
<i>Phormidium cebennense</i>	14.5	100											
<i>Phormidium corium</i>	12.44	88.7	4.96										

	TS	RH	PAR	SPAIN	SLOVENIA	SERBIA	HUN-GARY	ITALY	GRECIA	USA	CHILE	CZECH REPUBLIC	RUSSIA
<i>Gloeocystis botryoides</i>	2.25									X			Akhshtyrskaya Excursion cave
<i>Gloeocystis vesiculosa</i>	17.13	83.75	9.74				X						Zbrašov
<i>Gaeotila protogenita</i>	2.25						X						Javoríčko
<i>Hormidiopsis crenulata</i>	2.25						X						Mladeč
<i>Hormidium flaccidum</i>	2.25						X			X			
<i>Harpochytrium hyalothecae</i>	14.5	100					X			X			
<i>Kirchnerella lunaris</i>	14.5	100								X			
<i>Kirchnerella sp.</i>		1.94											
<i>Klebsormidium crenulatum</i>	14.92	88.13			X							X	
<i>Klebsormidium flaccidum</i>	15.74	78.73	9.03									X	
<i>Klebsormidium sp.</i>	11.3	73.9	9.02				X					X	
<i>Leptosira obovata</i>	21	74											
<i>Leptosira sp.</i>	11.3	73.9	9.02										
<i>Marvania coccoides</i>	26.5	71.5	8.59					X					
<i>Micractinium reisseri</i>	26.5	71.5	8.59					X					
<i>Microthamnion kützingianum</i>	2.25						X						
<i>Muriella decolor</i>	15	87.5										X	
<i>Muriella terrestris</i>	13.4	67.84	9.03									X	
<i>Muriella sp.</i>	18.5											X	
<i>Myrmecia astigmatica</i>	18.5												
<i>Myrmecia biatorellae</i>	21	74											
<i>Myrmecia bisecta</i>	11.3	73.9	9.02										
<i>Myrmecia sp.</i>	14.75	88.75									X		
<i>Netrium oblongum</i>	14.5	100								X			
<i>Oocystis lacustris</i>	14.5	100								X			
<i>Oocystis sp.</i>	14.75	88.75									X		
<i>Penium curtum</i>	2.25												
<i>Pediastrum boryanum</i>			1.07										
<i>Planophila laetevirens</i>	14.5	100								X			
<i>Pseudoclonium basilense</i>			0.65										
<i>Scenedesmus abundans</i>	14.5	100								X			
<i>Scenedesmus sp.</i>	18.5												
<i>Scottellopsis terrestris</i>	14.75	88.75											
<i>Stichococcus bacillaris</i>	17.04	66.55	210.5				X				X		
<i>Stichococcus minor</i>	8.13	82.5											X
<i>Stichococcus minutus</i>	14.92	88.13					X				X		
<i>Stigeoclonium terrestre</i>	14.5	100											
<i>Tetracystis excentrica</i>	15	87.5								X			
<i>Tetracystis inconstans</i>	14.5	100								X			
<i>Trebouxia decolorans</i>	11.5	87.5										X	

	Ts	RH	PAR	SPAIN	SLOVENIA	SERBIA	HUN-GARY	ITALY	GRECIA	USA	CHILE	CZECH REPUBLIC	RUSSIA
<i>Trebouxia glomerata</i>	11.5	87.5											
<i>Trebouxia</i> sp.	20.98	60.3	318.5									X	
<i>Trentepohlia aurea</i>	38.8	77	4.18										X
<i>Trentepohlia umbrina</i>	2.25						X						
<i>Trentepohlia</i> sp.	12.89	68.54	9.03							X			
<i>Ulothrix tenerima</i>	14.5	100											
<i>Ulothrix variabilis</i>		1.06											
BACILLARIOPHYTA													
<i>Achnanthes coarctata</i>	14.92	88.13											
<i>Achnanthes</i> sp.	11.5	70										X	
<i>Achnanthydium minutissima</i>	11.58	85.92										X	
<i>Adiafia bryophila</i>	11.5	70											
<i>Amphora pediculus</i>	11.5	70											
<i>Cocconeis placentula</i>	14.5	100											
<i>Craticula halophila</i>	11.5	70											
<i>Cyclotella meneghiniana</i>	18.5												
<i>Cymbella silesiaca</i>	14.75	88.75											X
<i>Denticula tenuis</i>	19.55	54.95	3.3										
<i>Diadesmis/Humidophylla aerophila</i>	12.52	83.38	9.03									X	X
<i>Diadesmis/Humidophylla contenta</i>	15.06	77.86	135									X	X
<i>Diadesmis/Humidophylla gallica</i>	11.5	87.5										X	
<i>Diadesmis/Humidophylla</i> sp.	11.3	73.9	9.02										
<i>Diploneis oblongella</i>	11.5	70											
<i>Diploneis ovalis</i>	11.5	70											
<i>Encyonopsis microcephala</i>	11.5	70											
<i>Gomphonema angustatum</i>	14.75	88.75											X
<i>Gomphonema clavatum</i>	11.5	70											
<i>Gomphonema parvulum</i>	14.75	88.75											
<i>Hantzschia abundans</i>	19.75	67.5	14.43										X
<i>Hantzschia amphioxys</i>	16.71	72.88	35.55									X	X
<i>Luticola mutica</i>	16.63	88.75										X	X
<i>Luticola nivalis</i>	14.67	78.38										X	X
<i>Luticola nivaloides</i>	18.5											X	X
<i>Luticola paramutica</i>	18.5											X	X
<i>Melosira granulata</i>	14.5	100										X	X
<i>Melosira varians</i>	16.5	100										X	X
<i>Meridion circulare</i>	14.5	100										X	X
<i>Navicula cryptocephala</i>	14	82.5											X
<i>Navicula tenelloides</i>	18.5												X
<i>Neidium binodis</i>	20.5	49.15	35.55										X

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