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Identification of threats on geodiversity and biodiversity
In Pena Cave, Portugal: contributions to improve cave management

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Universidade do Minho
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Identification of threats on geodiversity and biodiversity in Pena Cave, Portugal: contributions to improve cave management

Abstract

Karst caves have significant geodiversity and biodiversity values that lead to a basis for educational, scientific, and touristic uses. So, it became necessary to develop a management plan to preserve the intrinsic values of the cave and minimize the impact inevitably brought by humans. This work provides concepts about identifying and mitigating threats in caves applied to Pena Cave, a natural cave in Serras de Aire e Candeeiros Natural Park in central Portugal with an interpretation centre and infrastructure for public visitation.

In order to achieve the goals of the dissertation, the research method followed an integrated approach: (1) literature review of the cave's geo and biodiversity and visitation patterns, (2) fieldwork, interviews with staff members, photography of Pena Cave, (3) data analysis to identify temperature and visitor relationship patterns; and (4) graphical representation of the results in the form of maps and schemes. The results show that six cave-adapted species (troglobionts) live in Pena Cave. One of them is endemic to this cave, *Cylindroiulus villumi* Reboleira and Enghoff 2018. The central values are landforms (speleothems) and chemical and physical processes responsible for the cave's landforms. An increase in visitors raises the temperature inside the cave; however, the main hitting trigger is artificial lighting, which may lead to a cumulative effect. Furthermore, the temperature rise affects the process responsible for the natural growth dynamics of the speleothem, and more research is needed to understand its potential impacts on biological communities. Finally, the definition of mitigation measures in the form of a list of proposals was compiled to optimize the management of Pena Cave.

Overall, future efforts should be placed on monitoring temperature and CO_2 simultaneously with tracking the number of visitors, replacing existing sodium lights with new generation LEDs, and monitoring cave-adapted species' population trends. Finally, the priority proposal for optimizing the management of the cave is to take an inventory of the essential elements in the Pena Cave, starting with an inventory of all potential points of interest and mapping the most critical areas for faunal communities.

Keywords: karst cave, geodiversity, biodiversity, threats, management optimization.

Identificação de ameaças à geodiversidade e biodiversidade no Algar do Pena, Portugal: contributo para melhorar a gestão de grutas

Resumo

As grutas cársicas têm importantes valores no que respeita à geodiversidade e biodiversidade, o que permite um uso educacional, científico e turístico destes espaços naturais. Assim, torna-se necessário desenvolver um plano de gestão para preservar os valores intrínsecos das grutas e minimizar o impacto inevitavelmente trazido pelos seres humanos. Este trabalho apresenta contributos para a identificação e mitigação de ameaças em grutas, aplicados ao Algar do Pena, uma cavidade natural localizada no Parque Natural das Serras de Aire e Candeeiros (centro de Portugal), dotada de um centro de interpretação e infraestruturas para facilitar a visitação pública.

Para atingir os objetivos da dissertação, a investigação seguiu uma abordagem integrada que incluiu: (1) revisão bibliográfica sobre a geo e biodiversidade da gruta e sobre os padrões de visitação; (2) trabalho de campo, entrevistas com pessoal técnico, recolha de fotografias; (3) análise de dados para identificar as variações de temperatura e sua relação com a visitação; e (4) representação gráfica dos resultados sob a forma de mapas e esquemas. Os resultados evidenciam, até ao momento, a existência de seis espécies adaptadas à gruta (troglóbios). Uma delas é endémica desta gruta, *Cylindroiulus villumi* Reboleira and Enghoff 2018. Os valores abióticos principais correspondem às geoformas (espeleotemas) e aos processos químicos e físicos responsáveis pela sua formação. Foi igualmente verificado que a presença de visitantes provoca um aumento da temperatura da gruta, embora o principal fator para este aumento seja a iluminação artificial, o que pode levar a um efeito cumulativo. Além disso, o aumento da temperatura afeta o processo responsável pela dinâmica natural de crescimento dos espeleotemas, sendo necessária mais investigação para compreender os seus potenciais impactos nas comunidades biológicas.

Foram propostas medidas de mitigação para otimizar a gestão do Algar do Pena. De um modo geral, devem ser envidados esforços futuros para monitorizar a temperatura e o CO_2, simultaneamente com o rastreio do número de visitantes, substituir as luzes de sódio existentes por LEDs de nova geração e monitorizar as tendências populacionais das espécies adaptadas à gruta. Finalmente, para otimizar a gestão, é prioritário fazer um inventário pormenorizado dos elementos essenciais do Algar do Pena, começando por todos os potenciais pontos de interesse e mapeando as áreas mais críticas para as comunidades faunísticas.

Palavras-chave: gruta cársica, geodiversidade, biodiversidade, ameaças, otimização da gestão.

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1. Introduction

Around 20.3 million km^2 of the Earth's land surface is characterized by the presence of carbonate rocks, which are potentially karst. These areas have distinctive surface landforms and over 10.000 km of cave passages (Gunn, 2022). Karst systems have a special landscape of soluble rocks, such as limestone, gypsum and dolomite. These rocks are characterized by dissolution processes, which are responsible for a special landscape represented by depressions, caves and particular surface and underground rock formations. The same can be said about the originality of the karst cave ecosystem, which dictates the conditions for special endemic organisms, which in turn are highly adapted and endangered (Van Beynen and Townsend, 2005).

People have been using caves for various purposes for tens of thousands of years and only recently realized that karst caves have high aesthetic, educational, scientific, and touristic values (Gillieson, 2011; Gunn, 2022). However, the degradation of caves caused by anthropogenic activities is still a problem in most countries. The pressure on caves is not the same for all caves, the fact requires individual consideration for each case, which complicates a systematic approach to cave management and conservation (Gillieson, 2011).

Show caves deserve special attention because they require additional infrastructure, such as artificial lighting, paths, platforms, interpritaion centres that can damage geo- and biodiversity. In addition, the presence of visitors in caves can provoke changes in temperature, humidity, carbon dioxide content, among others, causing impacts on rocks and on biota.

There are many works done is several countries about the relationship between the presence of visitors and changes in abiotic factors, such as in Italy (Cigna, 1993), Spain (Pulido-Bosch et al., 1997; Calaforra et al., 2003), China (Song et al., 2000), or Portugal (Leal et al., 2009).

The abiotic factors are critically important for the life of endemic animals because, by itself, without human presence, a cave ecosystem is quite stable. From the geological point of view, carbon dioxide gas and temperature play an essential role in the dissolution of limestone (Gilli, 2015). Calcium carbonate ($CaCO_3$) is slightly soluble in water. However, with an increase in CO_2 pressure and temperature, the solubility rate of limestone increases as well as aggressive condensation, which is accompanied by corrosion processes (Gilli, 2015). This justifies the need for constant monitoring of the atmosphere in show caves, where changes in CO_2 content and temperature are often provoked by the presence of visitors and artificial lighting.

Moreover, visitors can carry undesirable elements and bacteria on shoes and clothing, which leads to degradation of biodiversity and speleothems (Leal et al., 2009; Reboleira et al., 2021; Reboleira et al., 2011; Jones, 1965; Viles, 1987; Cooks and Otto, 1990). In addition, with the growth of tourist activity, the risk of vandalism towards the geological elements in the cave increases, such as graffiti or attempts to take a sample of speleothems. Such cases are common in all geotouristic areas, and karst caves are no exception (Osborne, 2019; Woo and Worboys, 2019; Gillieson, 2011; Prosser et al., 2006).

Along with tourist activity, other anthropogenic stressors such as mining and quarrying, industrialization, farming with all the ensuing domestic, pollution, and vibrations can negatively impact caves. The work of Castaño-Sánchez et al. (2020) is devoted to this topic, emphasizing the impact on subterranean fauna. However, unwanted vibrations can also damage speleothems. Even scientific research can generate impacts in caves. Therefore, the proposed research projects must be implemented with minimal damage to the cave environment (Gillieson, 2011).

Thus, the manifestation of pressure from human activities has two main ways: inside the cave, as a result of a direct visit for various purposes, and from the outside due to industrial development (Castaño-Sánchez et al., 2020).

Karst caves, especially show caves, require proper management and monitoring. In order to minimize the human pressure on karst caves, and follow the consistent and practical management, many works have been written, such as Watson (1997), Gillieson (2011), Crofts et al. (2020), Gillieson (2021), Gunn (2022) and Gilleson et al. (2022). All these books sufficiently maintain a balance of attention to the geo- and biodiversity of karst caves. However, cave management regimes still require new paradigms, the primary purpose of which will be to preserve the caves as close to it as possible to the origin state, which was before human arrival.

This dissertation aims to make an analysis of general problems associated with the impact on karst cave, considering the main threats to geo- and biodiversity. The object of the dissertation research is a cave known as Algar do Pena Cave located in the Estremenho Limestone Massif–Central Portugal, for which possible impacts are described, current management is analysed, and options for mitigating adverse consequences are proposed.

Objectives

1. To characterize the geodiversity and biodiversity of Pena Cave;
2. To identify the main threats and map the most sensitive sites of the cave;
3. To present proposals for the mitigation of pernicious impacts and to optimise the cave management;

Methods

As a first step, a large amount of literature review concerning the karst cave features: the formation of a karst cave, geomorphology, processes, ecology, and cave-adapted fauna.

In order to achieve the goals of the dissertation, the literature review of the cave's geodiversity, biodiversity, possible threats, geoconservation, management, visitation visit and temperature patterns was made. In addition, in order to better understand the current management situation and characterize geo- and biodiversity for the case study, fieldwork, interviews with staff members, review of reports and photos were done in Pena Cave. The data analysis method is used to identify temperature and visitor relationship patterns: the temperature data available for 2000, 2001, and 2019 and data on visitors in 2001 are analyzed.

Maps were built to graphically demonstrate the information using QGIS, Surfer, Google Earth Pro, and CorelDRAW programs.

2. Pena Cave

2.1. General characterisation

Pena Cave is located in the Serras de Aire e Candeeiros Natural Park (PNSAC), nearby Tagus Valley, Santarém district, at about 90 km NW of Lisbon (39° 27 '30' N; 8° 48 '40''W)[1] (figure 1).

Before the designation of this natural park as protected aria in 1979, limestone was already quarried in several places for industrial purposes. During these activities, Pena Cave was discovered in 1983 by the owner of one of the quarries (Mr. Pena), exposing the original entrance of that cavity. Nowadays Pena Cave is controlled and managed by the Institute for Nature Conservation and Forests (ICNF).[2]

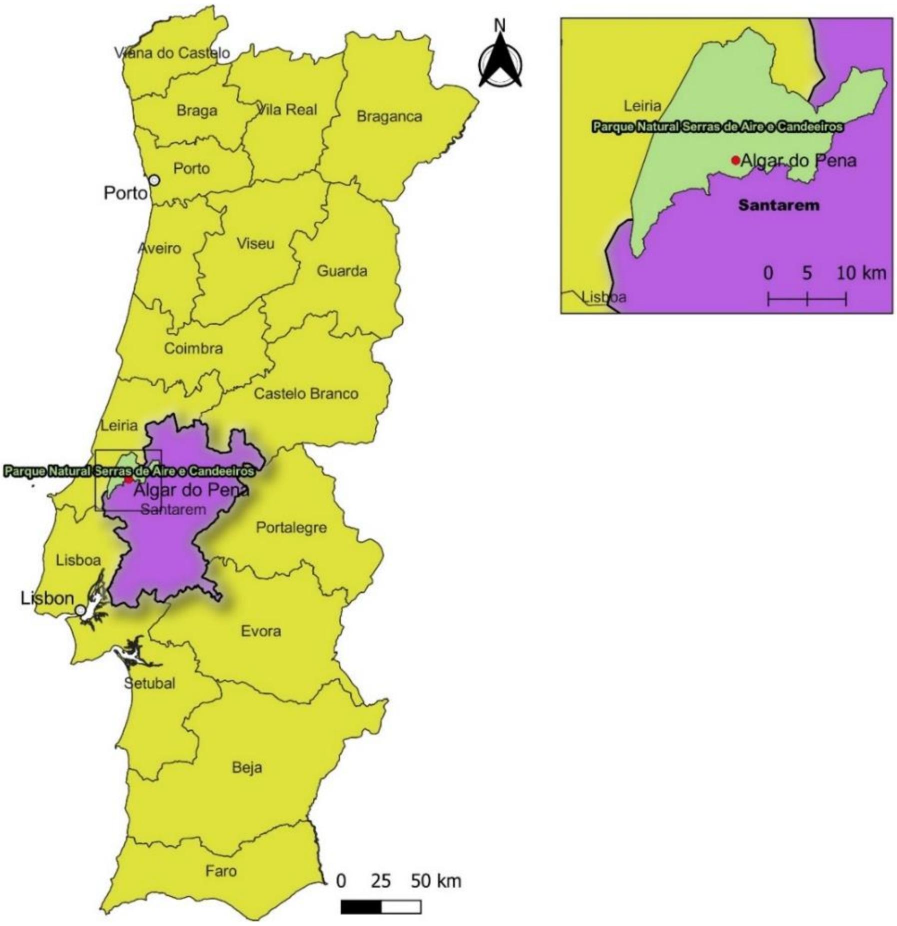


Figure 1 – Map with the location of Pena Cave (red dot). The divisions correspond to the different districts in Portugal mainland. The Serras de Aires e Candeeiros Natural Park is represented in green.

Pena Cave is considered to have the larger underground volume in Portuguese territory and a high aesthetic value. The geological setting of the cave is associated with the Estremenho Limestone Massif of the southern border of the plateau of Santo António (see section 2.3), one of the references of the Mesozoic geology in Portugal. The cave is associated to a Middle Jurassic (Bathonian) limestone

[1] https://natural.pt/protected-areas/parque-natural-serras-aire-candeeiros/geosites/penas-shaft?locale=en

[2] https://natural.pt/search?locale=en&q=Pena%2520Cave

formation and has a typical cavernous morphology with irregular walls, full of stalactites, stalagmites and some other speleothems.

After a first vertical descent to its interior along 35 meters, there is a large room with an estimated volume of about 125.000 m^3. This large room is elongated in the NE-SW direction and has about 20 m of maximum width, 70 m long and 50 m high, reaching a depth of 85 m (figure 2) (Simões, 2015).

To better characterize the morphology of Pena Cave, this cavity was divided into four sectors: initial, intermediate, lower, and upper sectors (table 1 and figure 2).

Table 1– Division of Pena Cave into four sectors (Simões, 2015).

Initial sector	Where the structures to support visitors are located, and from where is possible to have a whole view of the cavity. This is where the connection to the surface is made.
Intermediate sector	Where is possible to inspect all other sectors and the elongated structure of the cave. This is the flattest sector, contracting only with the depression that corresponds to the lower sector.
Lower sector	Corresponds to the lowest part of the cave and can be divided into two subsectors: the first upstream receives the materials transported or fallen from the other sectors, and the second, where the maximum depth of the cavity is reached (about 85m) being a place of accumulation to where these materials will tendentially move. It is in this sector where the influence of the dripping water is smaller; it is also where we can see an accumulation of blocks not totally consolidated but already partially enveloped in a covering of calcite crystals.
Upper sector	Located in the NE part of the cavity at an altitude of about 8 m above the intermediate sector, it has a floor of softer slopes, and also where there is a greater chaos of speleothems, both on the floor and on the ceiling.

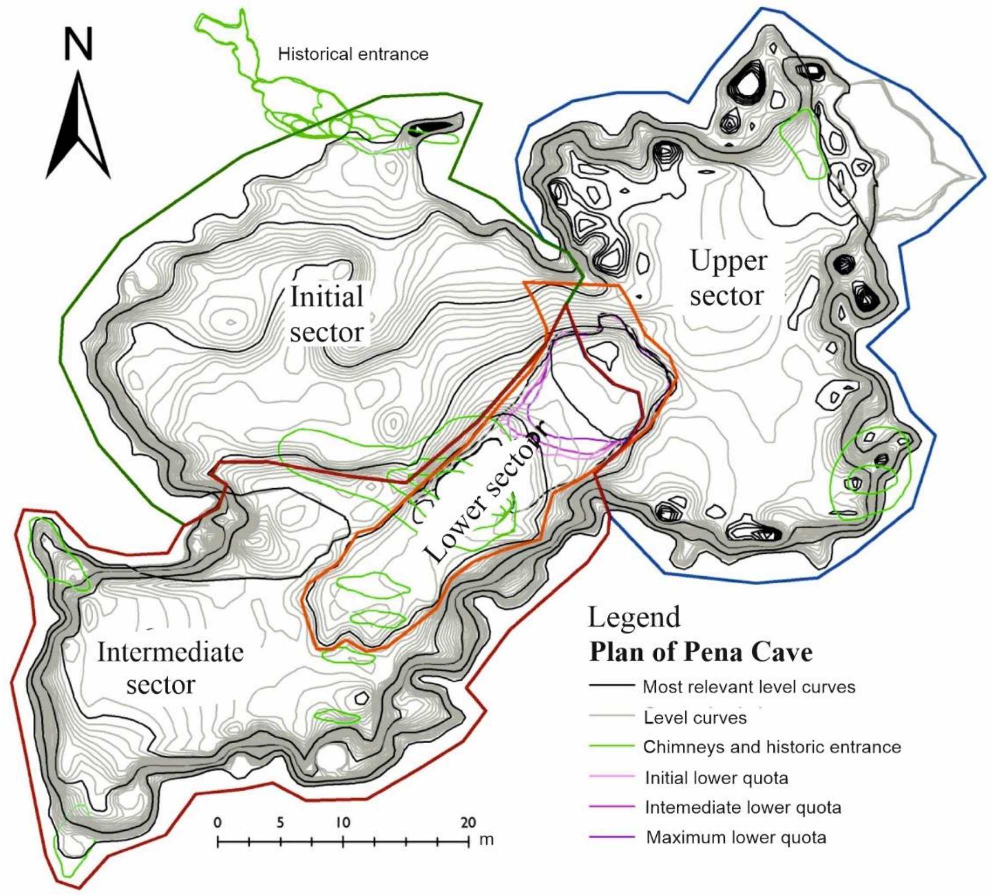


Figure 2 — Internal sectors defined for the Pena Cave (equidistant: 1 m), in Simões (2015).

From an ecological point of view, the temperature of the cave is between 13-14°C and a relative humidity of about 99.8%. There is a diversified fauna inside that cave-adapted to the darkness, from bats to small invertebrates (Simões, 2015). There are six species of cave-adapted animals, one of which, *Cylindroiulus villumi*, is an endemic of the cave, i.e., found only in Pena Cave:

Zoological group	**Scientific name**
Spider	*Domitius lusitanicus* (Fage, 1931)
Millipede	*Cylindroiulus villumi* Reboleira and Enghoff, 2018
Woodlouse	*Trichoniscoides meridionais* Vandel, 1946
Springtail	*Onychiurus confugiens* Gama, 1962
Dipluran	*Podocampa* cf. *fragiloides* Silvestri, 1932
Beetle	*Trechus gamae* Reboleira and Serrano, 2009

A barren landscape, sparse vegetation and mostly herbaceous and shrub strata is a general motto throughout the outer area of the cave from which stands out the strawberry tree (*Arbutus unedo*) and olive tree (figure 3). The flora also takes advantage of the stratification joints of the limestones, through which the roots are inserted in search of water at depth.

Soils are difficult to find far from the valleys, except for the rare places where there are active erosion processes on the surface, especially reported near CISGAP (Interpretation Centre of Pena Cave) by the accumulation of *terra rossa* in dissolution spaces (figure 3) (Simões, 2015).

Figure 3 – Near the entrance to Pena Cave is possible to see traces of old quarrying and accumulation of terra rossa that develop along SW-NE dissolution spaces (Simões, 2015).

2.2. Infrastructure and availability

Pena Cave is managed by the Institute of Nature Conservation and Forests (ICNF), the official national agency responsible for the implementation of nature conservation policies in Portugal. Since the discovery of Pena Cave that several studies were carried out by PNSAC speleologists and by researchers from various universities. Later, the cave was equipped with some infrastructures to facilitate the research and public visits for touristic and educational purposes. The visits require a prior booking and are guided by certified speleologists, in order to maintain as much as possible the cave with pristine conditions.

The first karst Interpretation Centre in Portugal opened on June 5th, 1997 at Pena Cave. The centre provides a technical support building and all necessary infrastructure to provide a comfortable and safe visit to all types of visitors. Access by the historical entrance is still possible but requires speleological training (figures 4 and 5).

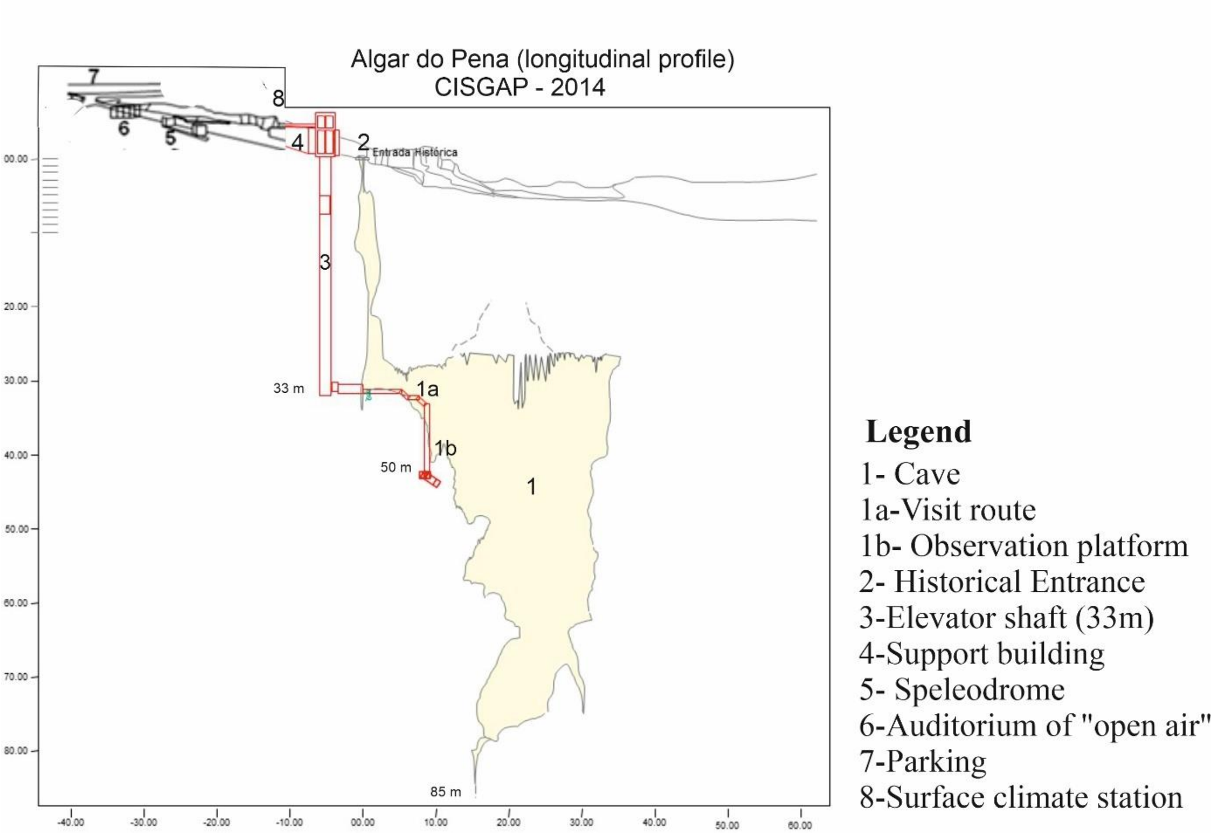


Figure 4 –Longitudinal profiles of Pena Cave (source: PNSAC).

Figure 5 – CISGAP support building (a); Original entrance to the cave (b).

The CISGAP support building contains an office for managers, toilets, laboratory and room for lectures with projector and speleology equipment (figure 6). In order to allow a comfortably and safe entrance to the cave gallery, a staircase and an elevator were installed descending to a depth of 35 m. Along the stair walls there are explanations about the formation of stalactites and stalagmites, as well as examples of how the light of incandescent lamps is responsible for the growth of undesirable fauna (figure 7). Inside the gallery, there is another staircase to the observation platform from where visitors can appreciate the cave's landforms. While on the platform, visitors can also observe some transparent boxes with living inhabitancies of the cave (figure 8). The information given to visitors aims to explain the processes of cave formation, the biodiversity and geodiversity of the cave, and the dangers to which this environment is subjected.

Figure 6 – Inside of the support building: a) speleological equipment, b) lab-room, c-d) room for lectures.

In general, there are two possible types of visits. One is a "simple route" that includes a walk to the observation platform and a short lecture about the cave's geo- and biodiversity features and cave conservation. The second type is the "integral route" (figure 8), which includes a visit through the main room using with speleological equipment.

Pena Cave is frequently visited by students from different universities and schools. Usually, their visit is limited to a "simple route" and a small lecture about the cave and its management. However, the cave staff and researchers are always ready to cooperate with students to develop further research on the cave. From the scientific point of view, the cave allows biologists to collect samples of troglobionts and carry out research on biodiversity and ecology. For geologists, the scientific interests consist of paleoclimate, paleoenvironment, neotectonics events, and karst geomorphology.

Regarding to geodiversity and biodiversity conservation actions and in order to minimize the negative impacts of visitors, cave managers and professors of the University of Coimbra calculated a carrying capacity for Pena Cave (Martins O. pers. com., 2022). The permanence in the room is limited to 20 minutes and to a maximum of 120 visitors per day in groups of 12 persons for the "simple route" and 6 persons for the "integral route". However, usually less than 50% of the allowed number of visitors came to see the cave.

In addition, to avoid the introduction of bacteria, dust particles, and flora on shoes, visitors are required to use a disinfection carpet before entering the cave. Stairs and handrails are built in non–oxidizable and removable materials, visitors' paths are very restricted and it is forbidden to touch speleothems.

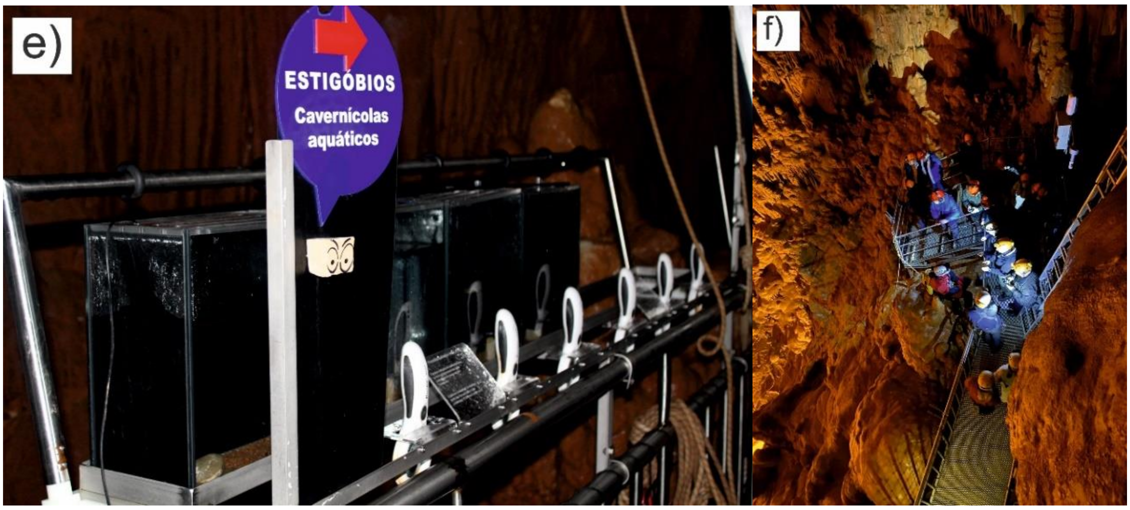


Figure 7 – Different infrastructure in Pena Cave: a) staircase and elevator; b-c) explanation about cave processes in the stair walls; d) entrance to the observation platform; e) boxes with troglobionts; f) observation platform.

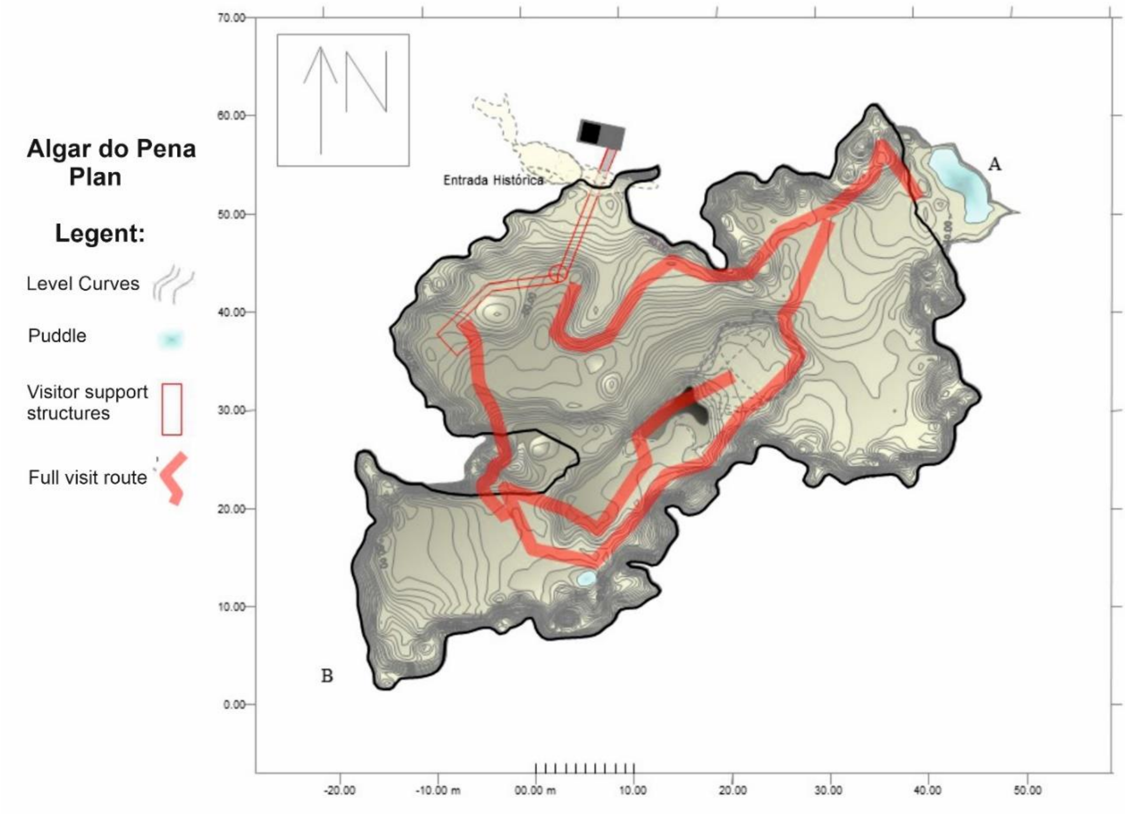


Figure 8 – Mapping of Pena Cave based on vector information prepared by extrapolation of the topographic data collected after its discovery (source: CISGAP).

2.3. Geological and geomorphological setting

Serra de Aire e Candeeiros Natural Park is located in the Estremenho Limestone Massif as part of the Mesozoic Lusitanian Basin (figure 9) (Simões, 2015). This Atlantic marginal basin is related with the Mesozoic distention and subsequent opening of the Atlantic Ocean (Ferreira, 2000). The initial phase of "rifting" (Upper Triassic) created an irregular topography of blocks of normal faults, grabens and semigrabens. The second active phase of "rifting" was initiated in Upper Jurassic. The interval between these two phases of extensive activity, i.e., the period corresponding to most of Lower Jurassic and Middle Jurassic, in contrast, was characterized by relative tectonic stability (Ferreira, 2000).

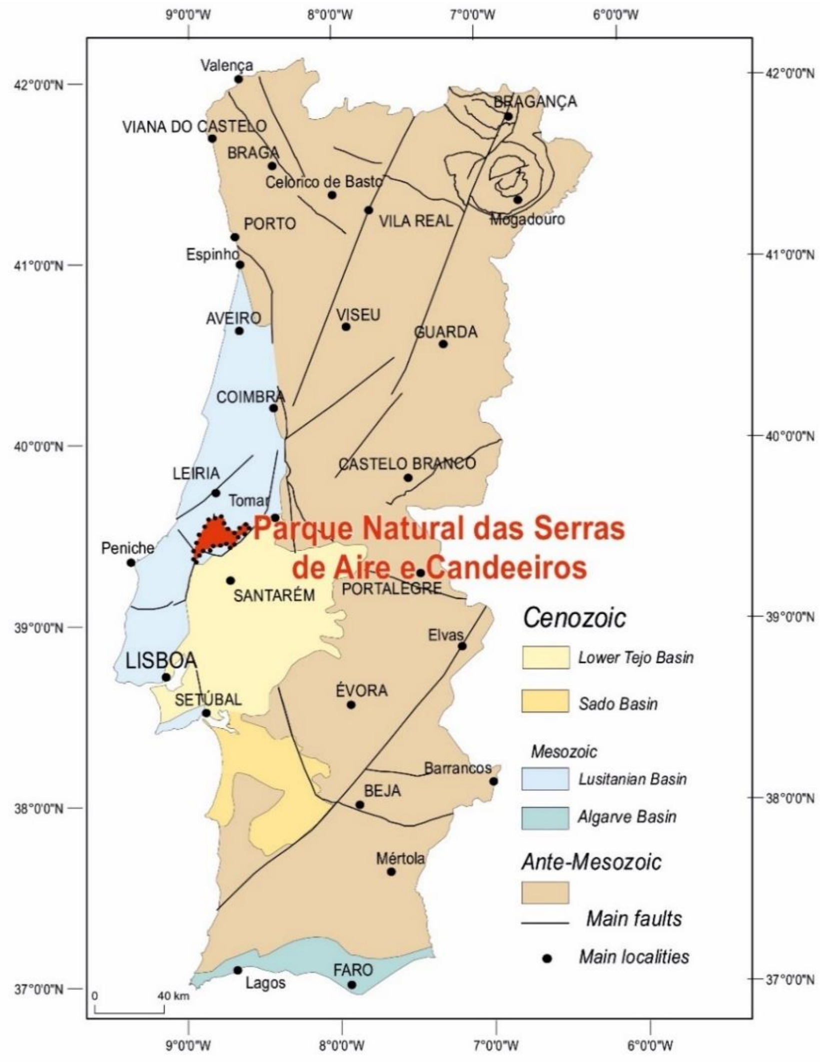


Figure 9 – General geological setting of Serra de Aire e Candeeiros (source: ICNF).

The Estremenho Massif is elongated to the NE-SW direction and includes four main morphostructural units: Aire Mountain, Candeeiros Mountain, Santo António Plateau and São Mamede Plateau (figure 10). In the higher zones of the Massif crops out Middle Jurassic formations, while Upper

Jurassic rocks occur in the depressed zones, where the Lower Jurassic also emerges in narrow bands (figure 11) (Ferreira, 2000).

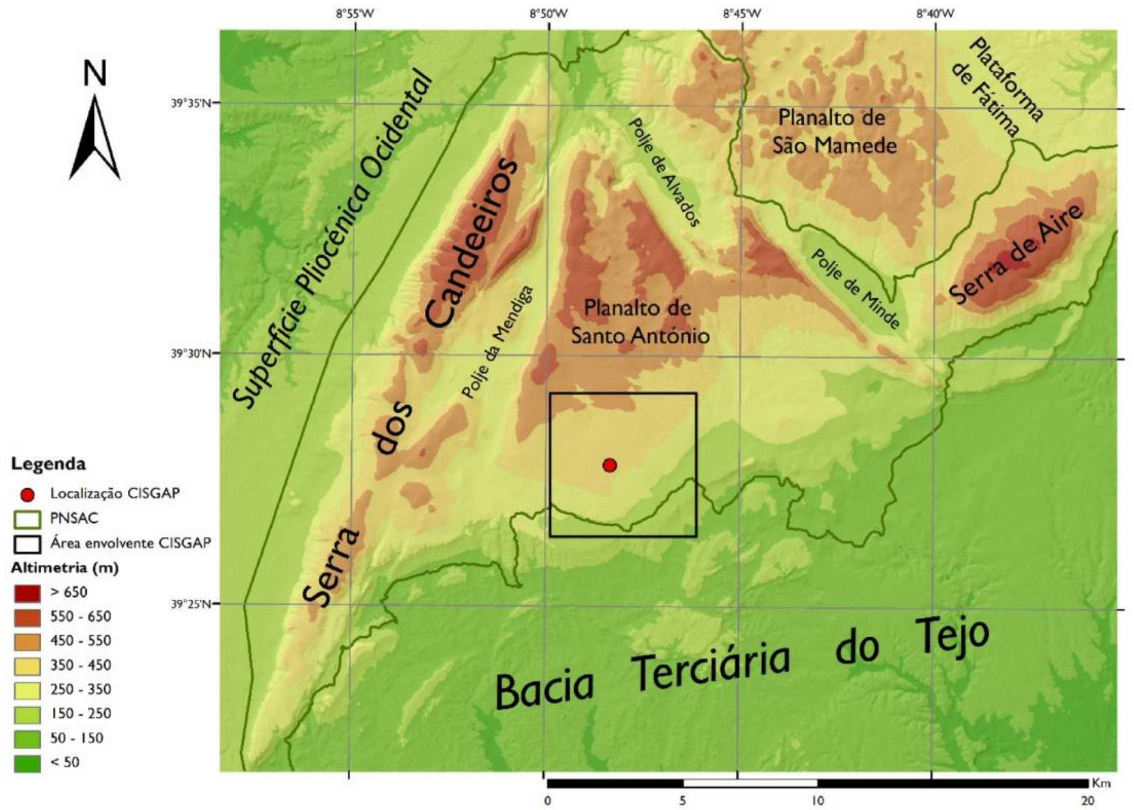


Figure 10 – Main morphostructures of the Estremenho Limestone Massif (Simões, 2015).

Pena Cave is located at Santo António Plateau. The Plateau was raised by tectonic movements along faults bounded to the NE by a set of tectonic structures that include the Alvados-Minde depression with NW-SE orientation, and at the west by Serro Ventoso – Mendiga – Valverde graben (figure 10) (Ferreira, 2000).

Pena Cave occurs in carbonate rocks of Serra de Aire Formation, Middle Jurassic (J_2SA), namely Bathonian Age (166.1–168.3 Ma). The formation is composed by limestone pelmicritic and biomicritic, mudstone and wackstone carbonate rocks (table 2) with a global thickness of 360 m (Ferreira, 2000).

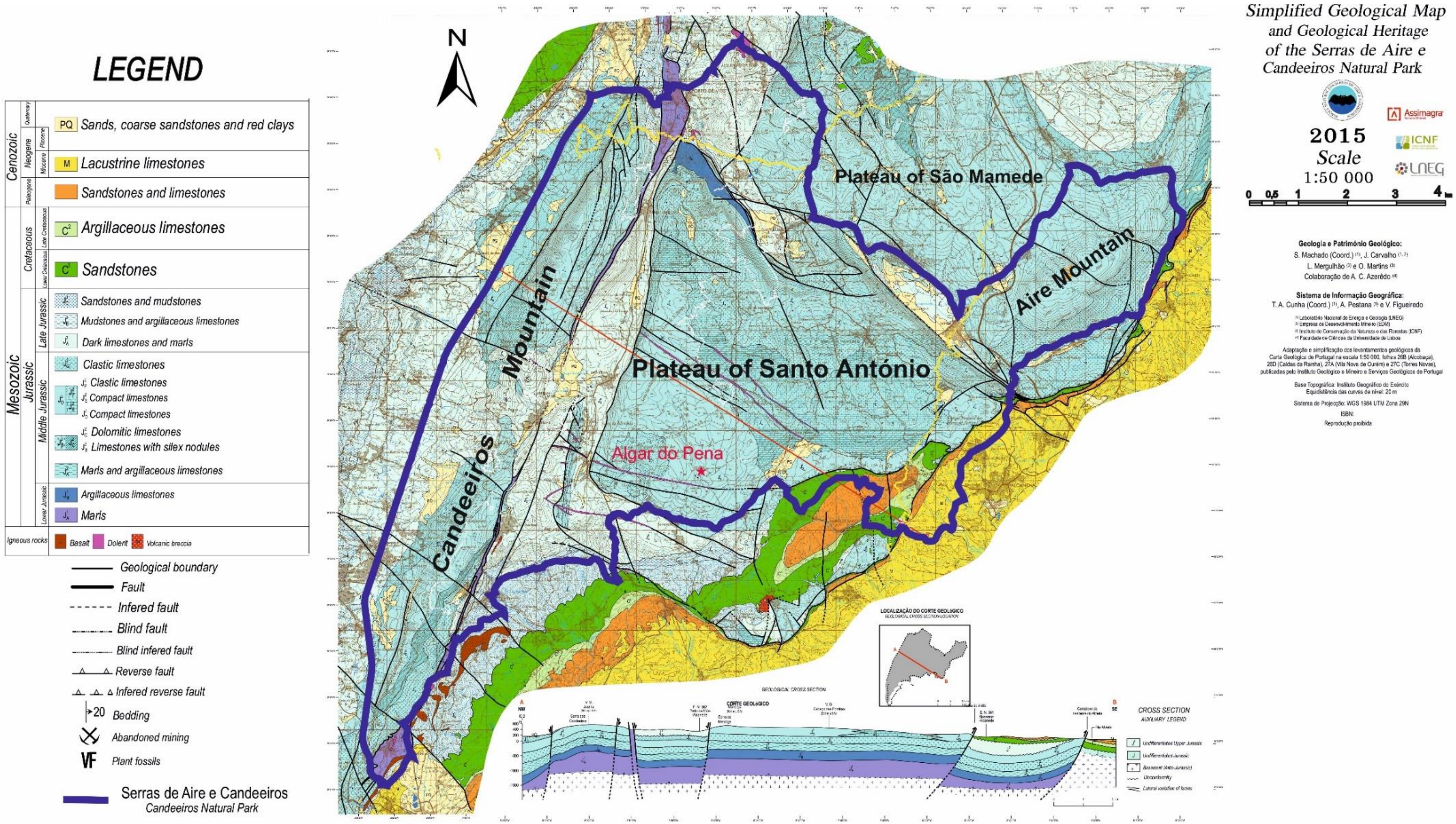


Figure 11 – Geological map of Serra de Aire e Candeeiros Natural Park (source: ICNF).

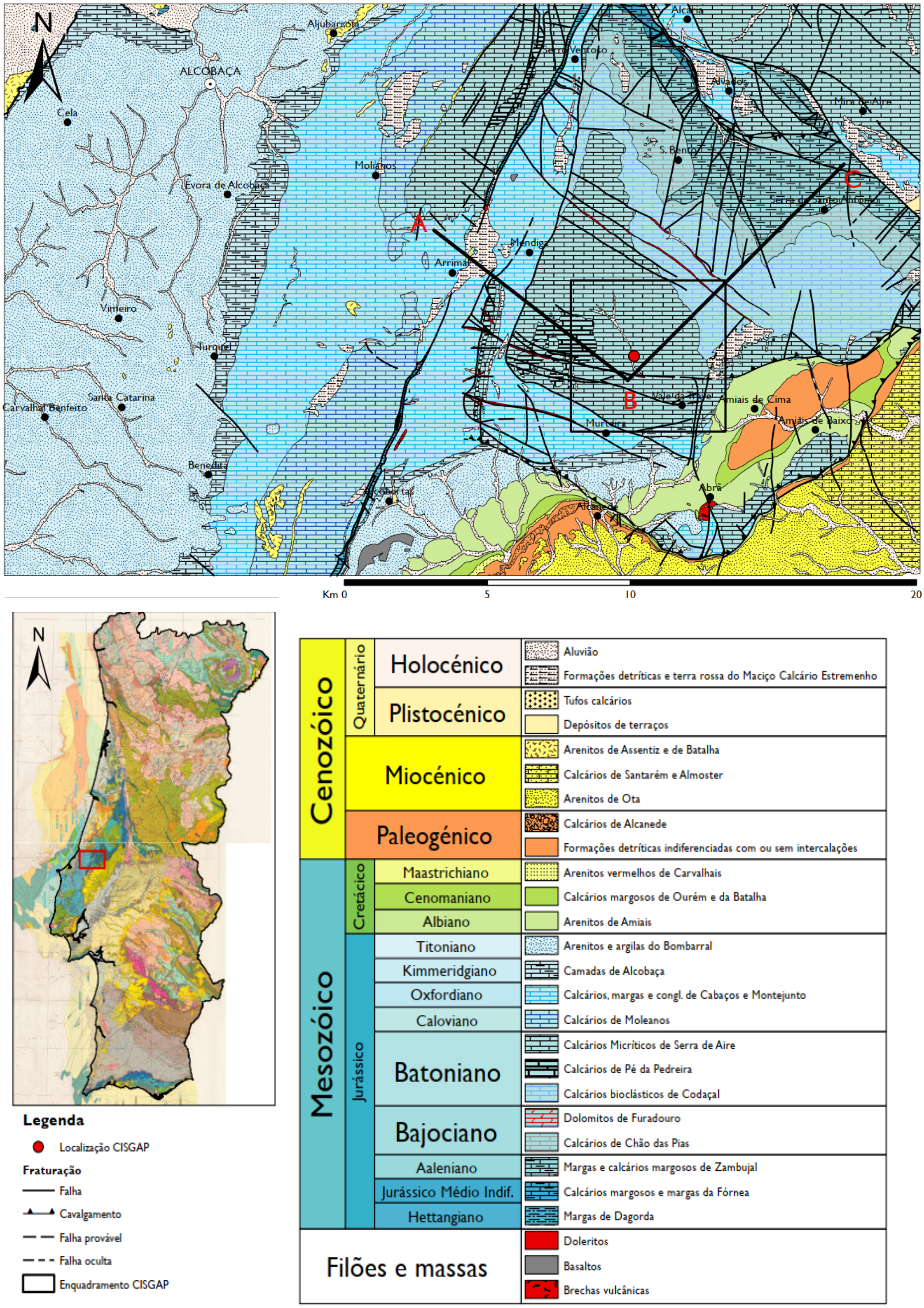
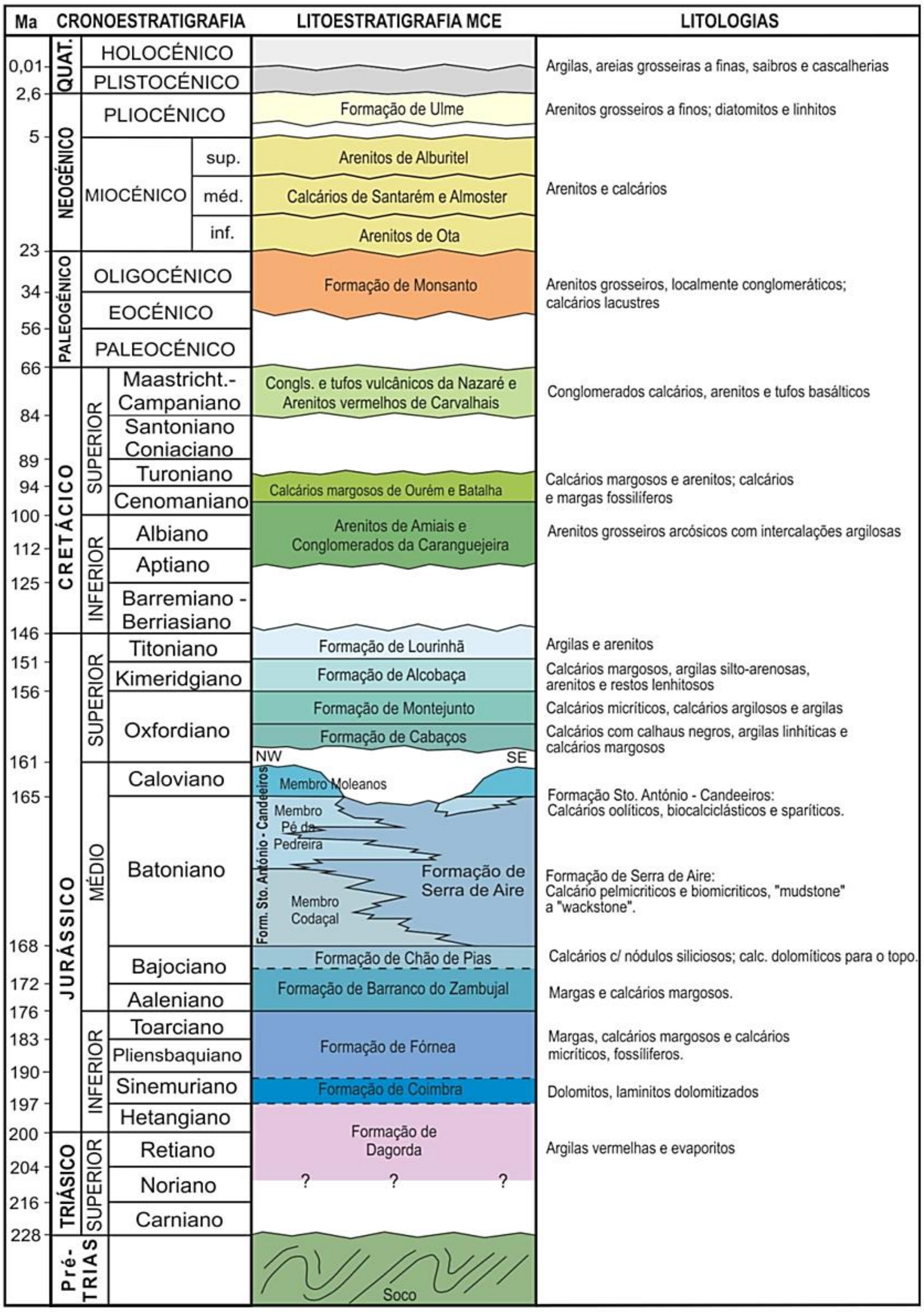


Figure 12 – Geological map of the study area. Pena Cave is located with a red dot (from Simões, 2015).

Table 2 – Lithostratigraphy of the Estremenho Limestone Massif (Carvalho, 2013).



Under the geomorphological point of view, Pena Cave is located on the slightly steep slope of Vale do Mar to the south of Santo António Plateau, drawing the karstic platform of Murteira-Vale da Trave-Cortiçal (figures 13 and 14) (Simões, 2015).

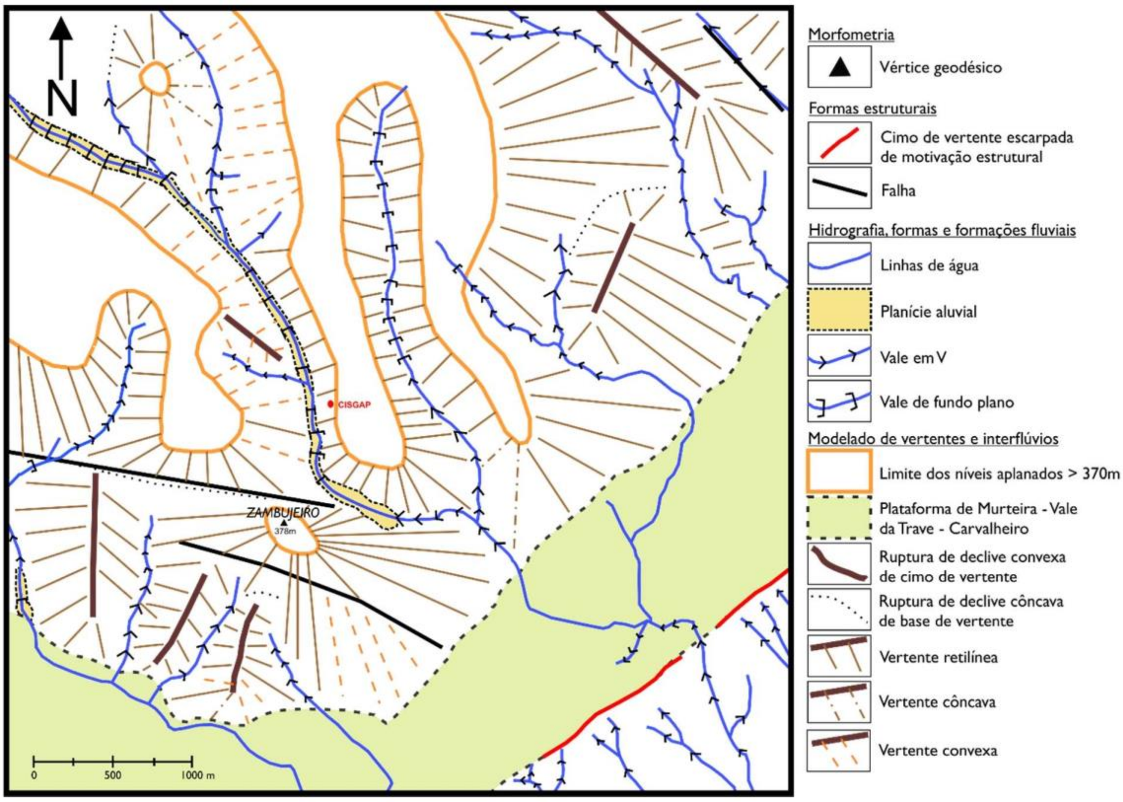


Figure 13 – Geomorphological sketch of the area around Pena Cave (red dot) (from Simões, 2015).

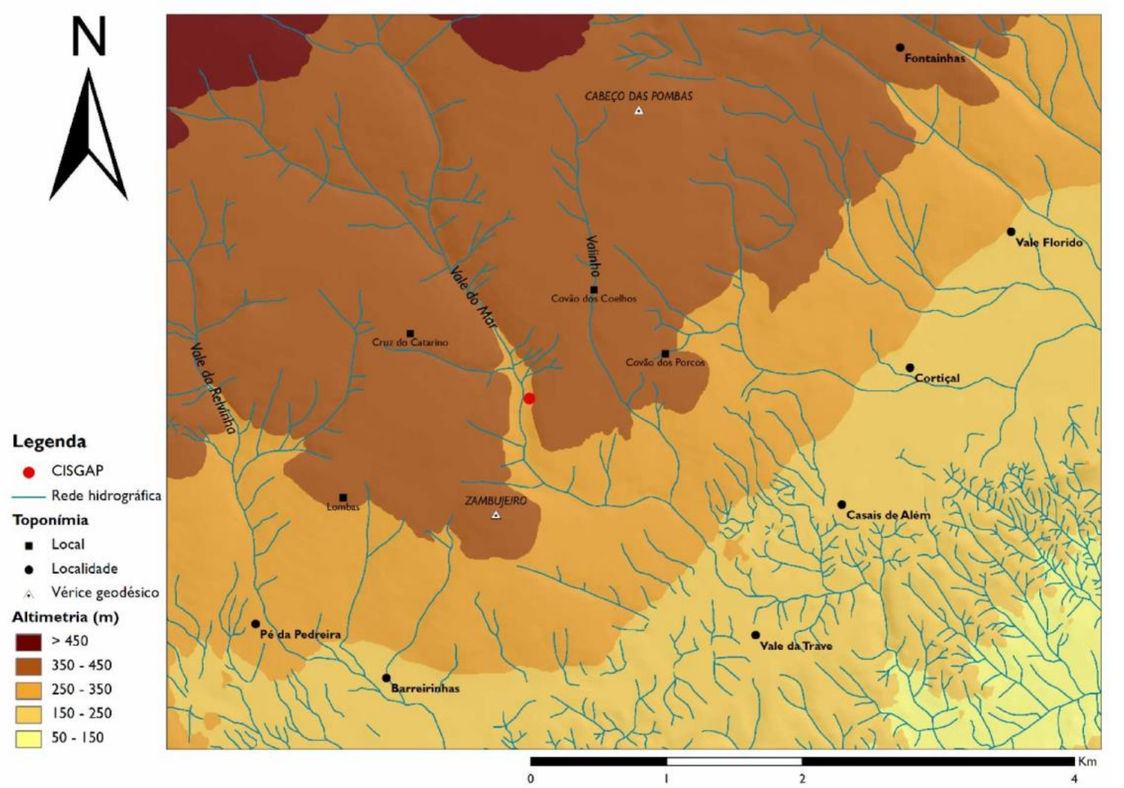


Figure 14 – Location of Pena Cave (red dot) in the context of the southern slope of Santo António Plateau (from Simões, 2015).

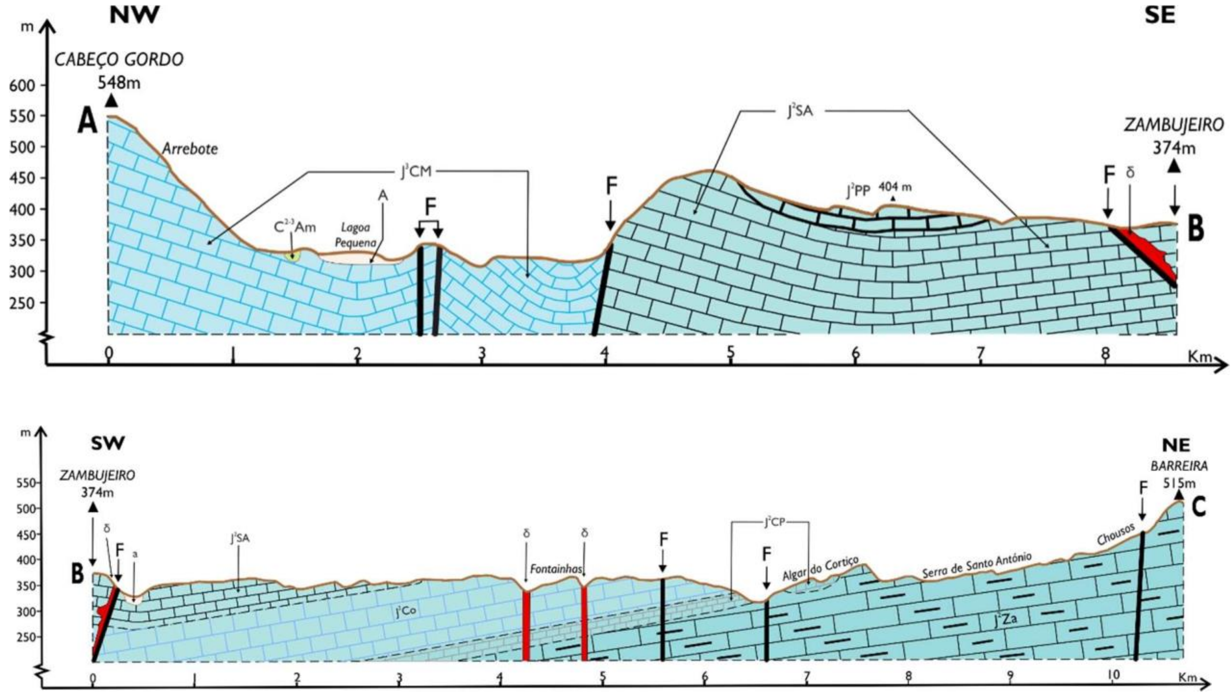


Figure 15 – Geological profile of Mendiga depression (A—B), with emphasis on the SE slope of Serra dos Candeeiros and to the NW of the plateau of Santo António. Geological profile of the plateau of Santo António (B–C), corresponding B to Pena Cave and C to one of the highest elevations of the fault escarpment adjacent to the Minde depression (from Simões, 2015).

The Pé da Pedreira limestone (J_2PP) reveal a higher resistance to mechanical action, observing a tendency for the valleys to be eroded on their edge. In contrast, on the opposite side, the materials corresponding to the Codaçal bioclastic limestone (J_2Co) are notoriously eroded forming V-shaped valleys, where the plains are practically non-existent (figure 15).

In the area of Pena Cave, the local geomorphological structure is a bit different of the regional trend. The primary NE-SW orientation of the cave appears to be related with movements perpendicular to one of the main orientations of the limestones, perhaps with direct influence of a fold and consequent alteration of the slope of the limestones. In the vicinity of Pena Cave there are two other caves, namely the Algar das Gralhas (to the west) (see figure 16) and the Algar do Pipas (to the south), separated from CISGAP by a few hundred meters. The genesis of both caves is associated with dissolution processes and fractures in the rock, which assume a construction very similar to dissolution sinks along a fracture, but which develop in-depth (just over a dozen meters) (Simões, 2015). Thus, it can be assumed that Pena Cave was formed in the same way by dissolution processes and fractures in the rock. Caves in carbonate rocks exhibit a wide range of geometries and passage morphologies related to their formation and subsequent developmental history. Carbonate rocks can be subject to erosion by water containing dissolved CO_2 that infiltrates from the surface by gravity. This process of cave formation is called epigenetic (Gunn, 2022).

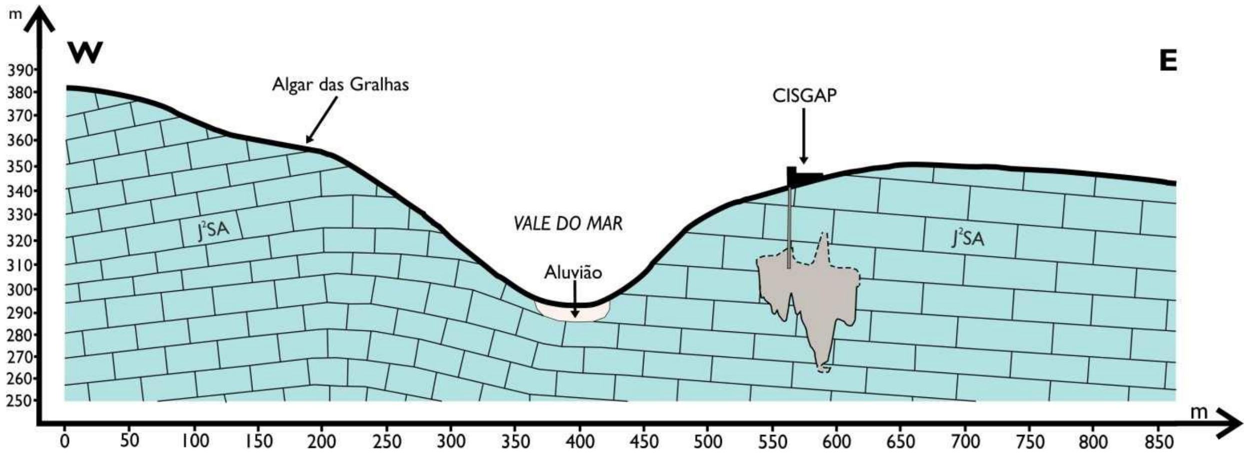


Figure 16 – Topographic profile around CISGAP (from Simões, 2015).

The surface part (crust) of the array undergoes decompressing and creates discontinuities in the first place (bedding or unloading joints). This stage forms the epikarst (figure 17) (Gilli, 2015). Then the water seeps through the gaps of the epikarst and begins to dissolve the bedrock (massive limestone). Usually, the bedrock can be exposed or covered with a variable layer of sediments (clay, sand). Thus, water can contact massive limestone by opening vertical channels called shafts.

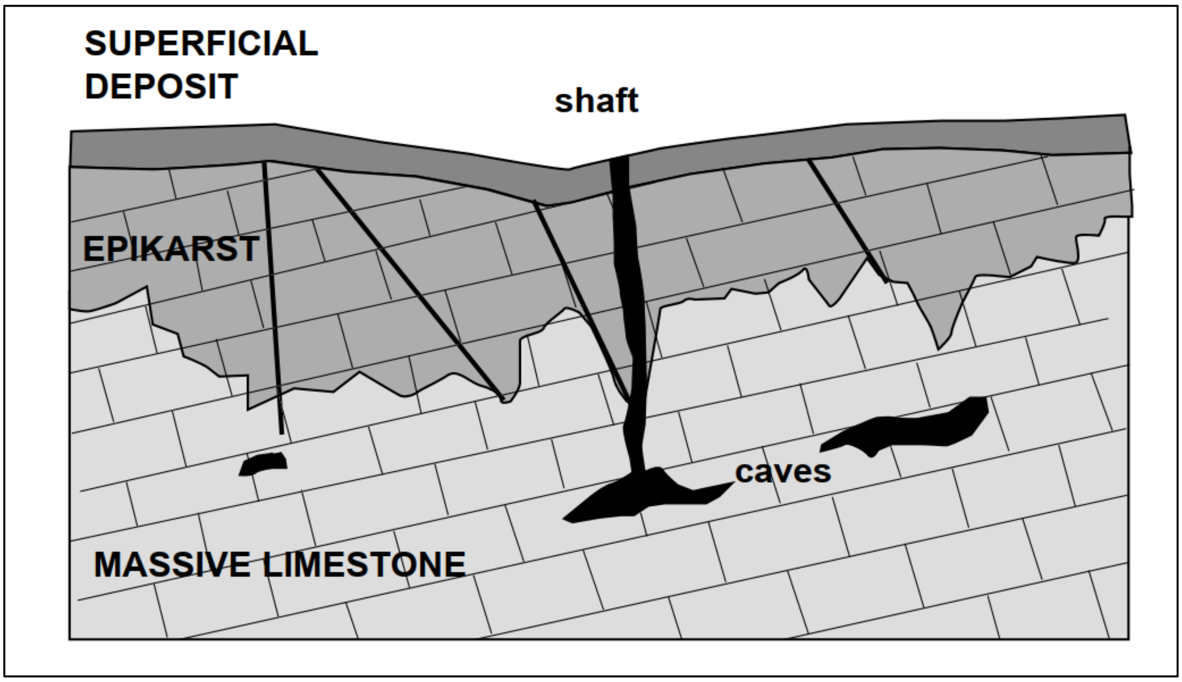


Figure 17 – Cross-section showing the development of the epikarst on the Hortus Causses (Gilli, 2015).

3. Ecosystem context of karst caves

The environmental conditions of caves differ from the surface ecosystems mainly by absence of light, temperature, and humidity conditions (Ravn et al., 2020; Moldovan et al., 2018; Tercafs, 1988). The diurnal and seasonal temperature variation is less pronounced in caves than on the surface, and the humidity is higher (Ravn et al., 2020). The total darkness of caves prevents photosynthesis and implies the complete absence of primary production through photosynthesis (Ravn et al., 2020; Moldovan et al., 2018; Tercafs, 1988). Therefore, subterranean ecosystems depend on organic matter transported from the surface to maintain heterotrophic processes (Ravn et al., 2020). Organic matter and nutrients, comes into caves from allochthonous sources, such as water percolation, or floating into the cave, transported by wind, by the movement of animals in and out or in the most superficial caves by root penetration (Ravn et al., 2020).

The biodiversity of karst caves is expressed in typical cave fauna: (1) animals that spend their life cycle underground and are fully adapted to the cave environment, mainly invertebrates such as arachnids (spider and scorpions), crustaceans and insects, but also vertebrates such as salamanders, and fish as well (2) animals that spend part of life cycle in subterranean - partially adapted, for example bats, insects and spiders. And (3) animals are not adapted, but visiting a cave such as mice, rats, lizards and birds (Howarth and Moldovan, 2018; Komerički and Deharveng, 2018).

Subterranean fauna has striking differences from other species of fauna expressed in morphological, physiological, and behavioural traits. Their main characteristics of morphological adaptations are the absence of pigmentation, regression of their visual system, leading to the complete disappearance of the eyes and body hardness, wing degradation, and thinning of the cuticle of arthropods (Langecker, 2000; Christiansen, 2012). In physiological traits, their reduced metabolic rate is often accompanied by an increase in longevity, low fertility, lack of circadian rhythm, tolerance to starvation and high CO_2/low O_2, dietary and water balance mechanisms changes (Langecker, 2000; Christiansen, 2012). For behavioural features, loss of circadian rhythm, changes in mating behaviour, and random walks can be distinguished (Moldovan and Paredes Bartolome, 1998). Next, this chapter will describe all the adaptation traits of subterranean fauna and cave environment features in detail.

3.1. Abiotic conditions

3.1.1. Atmosphere

The climate in caves is very stable compared to the exterior climate conditions. The cave atmosphere is constantly saturated with humidity and variation in temperature results in evaporation or condensation that engenders enough energy to reduce the temperature variations. The air temperature is less and less variable as one gets further from the entrance, becoming approximately the mean annual temperature of the cave's area (Howarth and Moldovan, 2018).

The composition of the air is different from the outside air. During the deposition of calcite and the formation of speleothems, carbon dioxide is released, and its proportion becomes higher than it is outside the cave and is compensated by a reduction of oxygen. The level of the radioactive gas, radon, is also superior, threat for people working in caves (Allegrucci et al., 2015).

3.1.2. Darkness

A characteristic of underground environments is the absence of light. Total darkness affects almost all aspects of the adaptation of organisms and their response to environmental conditions. The behavioral and physiological characteristics of cave species are closely related to the absence of light. This part of the evolutionary process allows the species to avoid harmful circumstances, find food, and reproduce in cave conditions (Moldovan, 2018; Ravn et al., 2020).

3.1.3. Humidity

In deep cave environments, where troglobionts live, they remain at or near 100% relative humidity (Howarth, 1980; Ravn et al., 2020). A saturated atmosphere is stressful for most terranean organisms, and cavernicolous have altered their water balance mechanisms to cope. Since saturated air is above the equilibrium humidity of bodily fluids, troglobionts must deal with excess water rather than desiccation (Moldovan et al., 2018).

3.1.4. Organic Matter

The decomposition of caves organic matter plays an important role in the ecosystem carbon cycle and the net ecosystem CO_2 emission (figure 18), but it is poorly studied (Ravn et al., 2020). The main source of organic matter in caves originates from plant material from the surface, carrion and animal droppings. Surface organic matter decomposition is controlled by abiotic factors such as temperature, water availability and lack of light and substrate alongside biotic factors as the decomposer community (Ravn et al., 2020). The rates and main factors of decomposition of organic matter are very different in caves from how the same decomposition occurs on the surface. The question of the causes and specific differences is poorly understood (Ravn et al., 2020).

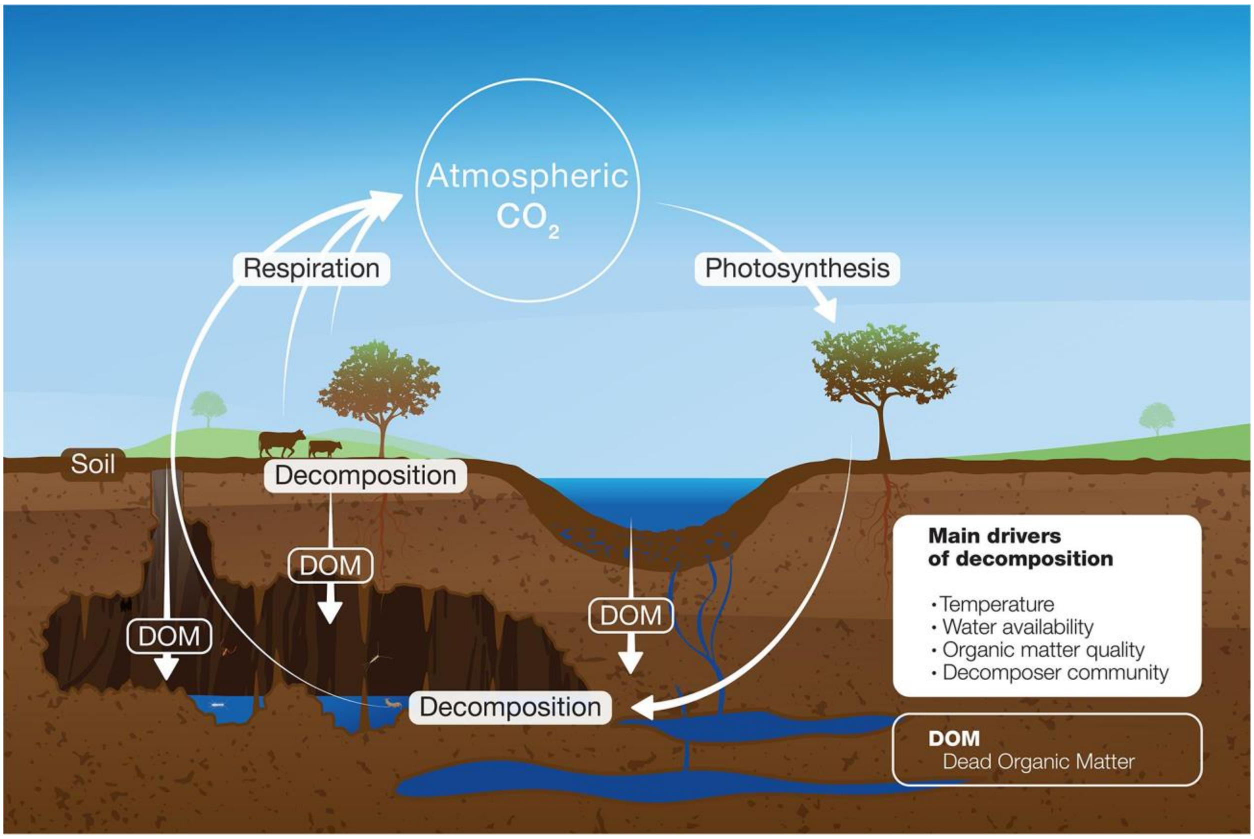


Figure - 18 Conceptual model of organic matter decomposition in ecosystems (from Ravn et al., 2020).

3.1.5. Concentration of CO_2

The decomposition of organic matter can increase the amount of CO_2 in caves to critical values (Ravn et al., 2020) as well as the presence of a human (Cigna, 1993; Pulido-Bosch et al., 1997; Calaforra et al., 2003; Song et al., 2000; Leal et al., 2009). However, most invertebrates can tolerate high concentrations of carbon dioxide for a short period (Howarth and Stone, 1990). Moreover, organisms living in soils and shallow mesocaverns can migrate to areas with lower CO_2 concentrations while in deeper caves, and deep mesocaverns migration is more limited (Howarth and Stone, 1990). There is a pattern cascade of carbon dioxide (CO2) emissions from low concentrations in the external atmosphere through significantly increased concentrations in the soil atmosphere to reduced concentrations in cave passages (Gillieson et al., 2022). Increased concentrations of carbon dioxide in the soil are the result of respiration of plant roots, microbial activity and healthy fauna of soil invertebrates. This cascade must be maintained for the effective functioning of karst dissolution processes (Gillieson et al., 2022).

3.1.6. Substrate

Underground habitats are of various sizes, from tiny voids to large caves from shallow to great depths below the surface. They are also found in many different rocks, but the most famous are caves in limestone and volcanic caves in basaltic lava. In function of void size, underground habitats can be divided into three classes depending on the environment and supporting communities: microcaverns (usually less than 5 mm wide), mesocaverns (about 5-500 mm wide), and macrocaverns (i.e., caves more than 50 cm wide) (Howarth, 1983). They can occur in any rock type where erosional or depositional processes create.

The substrate has an essential role in the distribution of biodiversity of cave organisms. Microcaverns rarely support terrestrial species because these tiny spaces are quickly blocked by debris without sufficient food access. Mesocaverns are medium-sized spaces, large enough to serve as corridors for the settlement of cavernous animals but small enough to restrict the flow of air and gas exchange. Food resources may also be limited. Nevertheless, some studies have shown that mesocaverns provide the bulk of the habitat and settlement routes between caves for many cave-adapted species (Howarth, 1993; López and Oromí, 2010). Within the mesocavernous we find the mesovoid shallow substratum (MSS) considered distinct from deeper mesocavernous profound substratum (MPS) voids (Juberthie,1983). If the MSS is contiguous to the MPS, it can harbour a subset of the cave fauna, as well as many unique species (Uéno, 1987, Eusébio et al., 2021).

Regarding to the macrocaverns include the accessible cave passages. Accessible cave passages also often harbour large colonies of vertebrates (e.g., birds and bats) that introduce large quantities of food resources into caves (Moldovan et al., 2018). More often it is a habitat for accidental and frequent inhabitants (trogloxenes and troglophiles).

3.1.7. Groundwater habitats

The precipitation that falls as rain or snow seeps underground downward through soil and rocks until it reaches the saturated zone (figure 19). Underground water is usually called groundwater, which can wholly or partially fill voids (Moldovan et al., 2018).

Water-filled voids are habitats of groundwater that come in different scales (Danielopol, 1989):

- *Macrohabitats (aquifers)* occupy from 1 to more than 100km^2. Macrohabitats are more heterogeneous and represent the main living space of most aquatic animals living in caves.
- *Mesohabitats* occupy several square meters to less than one square meter. Mesohabitats have relatively homogeneous physico-chemical characteristics and represent a part of the living space of a species used in different periods of the year or their life cycle.
- *Microhabitats* have a small size and can be measured in square centimeters. Microhabitations determine the habitat of an animal at a certain point in time. Inside microcaverns, the movement of liquid water is controlled mainly by capillary forces; however, flowing water under sufficient

pressure can keep such spaces open and interconnected, as well as transport food resources, which can provide suitable micro-habitats for tiny aquatic species.

Table 3 – Examples of aquatic cave habitats/microhabitats and connected groundwater habitats where stygobionts may be found (Howarth & Moldovan, 2018).

Cave and microcave aquatic habitats/microhabitats	Other groundwater habitats
Dripping water	Hypotelminorheic
Small pools (gours)/puddles	Springs
Big pools/lakes	Wells
Streams	Hyporheic zone = freshwater interstitial
Riffles	Littoral interstitial
Rocks in water	
Sediments next to water bodies	
Underwater/marine caves	

Unconsolidated rocks provide different microhabitats for different living communities. In consolidated rocks (limestone, dolomite, granite, basalt, and sandstone), voids can be significant to small, unlike unconsolidated rocks (from gravel to sand), where voids are usually small but can increase due to bioturbation (Howarth abd Moldovan, 2018). The groundwater habitats in unconsolidated sediments and various rocks represent one of the most extensive ecosystems (table 3). The degree of connection between cave habitats and other types of groundwater habitats depends on the permeability of rocks for animal migration and the supply of nutrients, organic carbon, and dissolved oxygen. It is predicted that groundwater-adapted animals (i.e., stygobionts) have much more extensive distribution ranges than troglobionts (Howarth and Moldovan, 2018).

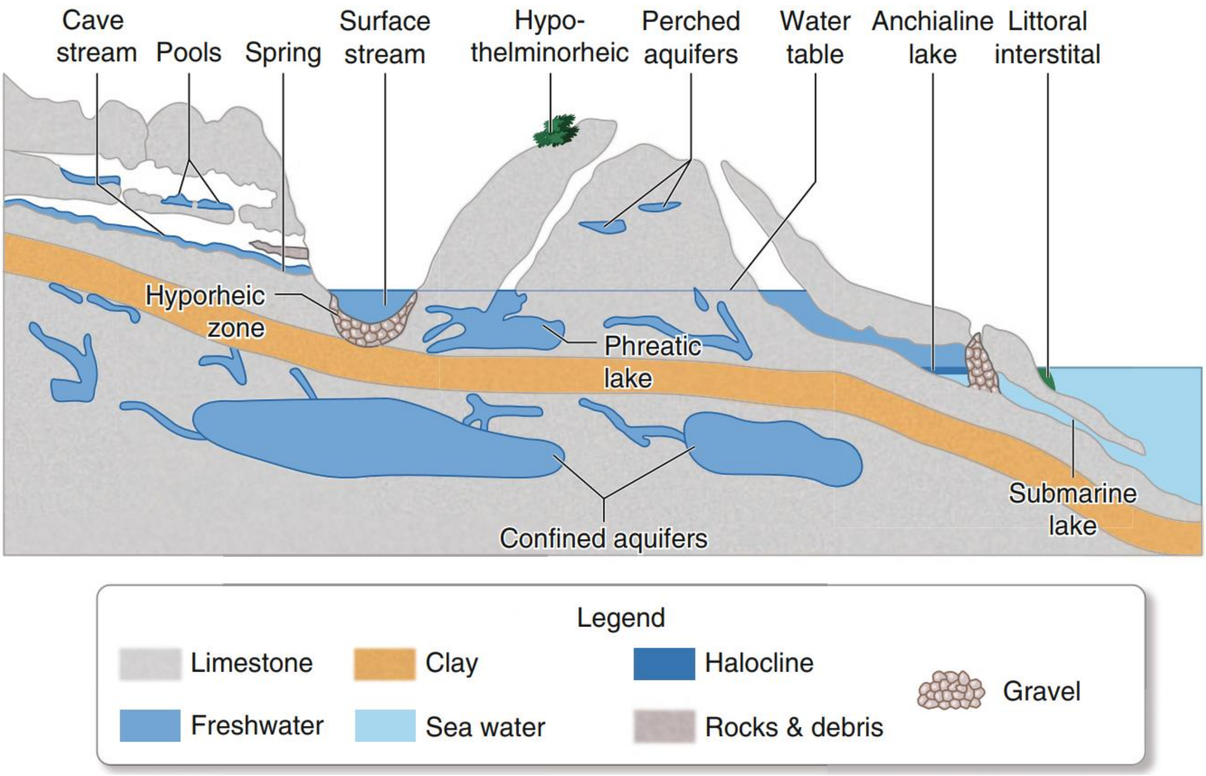


Figure - 19 The relationship between aquatic cave habitats and other subterranean aquatic habitats in karstifiable limestone (from Howarth and Moldovan, 2018).

3.2. Environmental Zones

The zonality of the terrestrial subterranean habitat is very important in the distribution of a community of organisms determined by the basis of its physical environment, such as the amount of light, humidity, airflow, gas concentrations, and the evaporative capacity of the air (table 4). Five environmental zones are recognized: the entrance, twilight, transition, deep, and stagnant air zones (Howarth, 1993). Below figure 20 shows those zone's positions, which provides a practical classification scheme to understand cave ecology. The evaporation rate depends entirely on temperature, so the boundaries between zones are more pronounced in tropical areas. However, the boundaries of these zones are pretty dynamic, and animals of one zone can make short-term migrations to neighboring zones in search of food (Howarth and Moldovan, 2018).

The entrance zone (euphotic) is the most illuminated zone where the existence of vascular plants is possible. This zone includes a combination of surface and underground communities and has good food resources and, consequently, a large variety of animals and plants.

The twilight zone has reduced light between the border of the possible existence of vascular plants and the area of complete darkness. The level of humidity and evaporation is unstable. As a result, species diversity is small, mainly consisting of randomly migrated species from neighboring zones, surface animals seeking refuge, scavengers, and predators (Howarth and Moldovan, 2018).

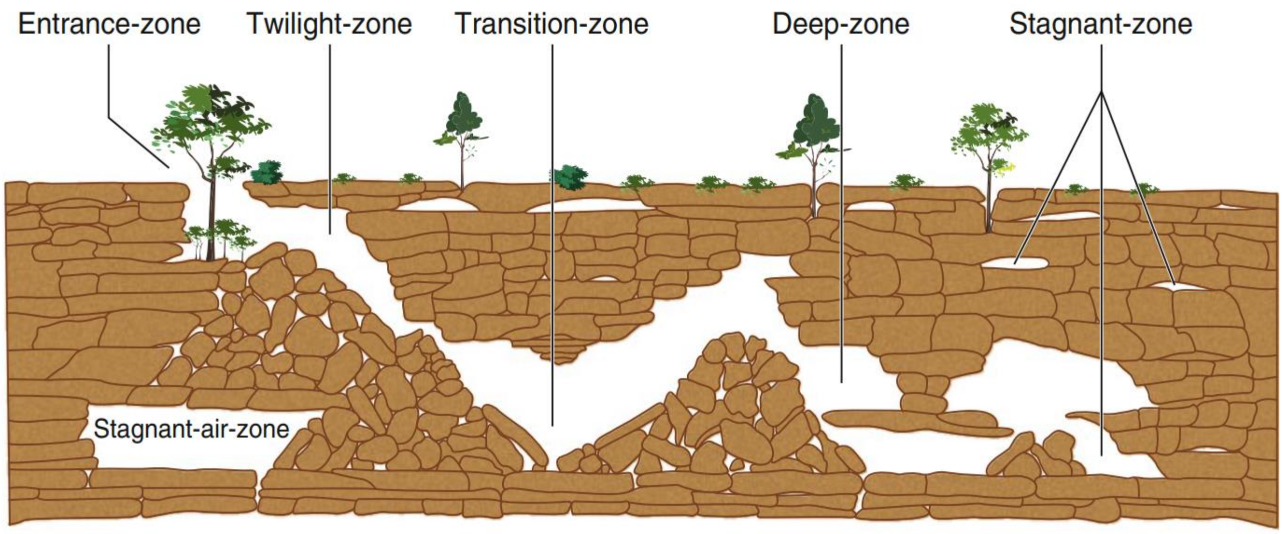


Figure - 20 Stylized profile view of a cave showing the five environmental zones. Scale for length greatly condensed relative to height. The figure modified after Howarth (1993).

The transition zone (dysphotic) is characterized by complete darkness and a changeable abiotic environment, humidity, airflow, and evaporation. Diurnal and seasonal climatic cycles and local weather events on the surface cause this variability. The habitat is usually dry, and the species diversity is usually minor and consists of trogloxenes, scavengers, predators, and strays. It can support a variety of guanophiles and guanobionts (Howarth and Moldovan, 2018).

As the name defines it, the deep cave zone (aphotic) is entirely devoid of light. In this zone, the physiological characteristics become more stable. For example, the air remains stationary and saturated, the substrate is moist, and evaporation is insignificant. Due to an obstacle (barrier) for air exchange, it is also a border with other zones in the form of a narrow passage. The barrier is a crucial component of the deep cave zone, so if the caves have several entrances or do not have such an obstacle, forming this zone is impossible. Mostly cave animals occur in this zone (Howarth and Moldovan, 2018), but several troglophiles and trogloxenes, including invertebrates and vertebrates, can be found in this zone.

The stagnant air zone occurs where air exchange is critically limited, the atmosphere periodically stagnates, and the concentration of gases, especially carbon dioxide, becomes tense (Howarth and Stone, 1990). This zone is the primary habitat of troglobitic species, and it is also a zone present in mesocavernous cracks (Howarth and Moldovan, 2018). Thus, most specialized cave species live in this condition in medium-size voids, indicating that essential habitats can significantly extend beyond the cave passages available to researchers.

Table 4 - Abiotic and biotic parameters of each of the five zones (Howarth and Moldovan, 2018).

			Dark		
Zones	Entrance	Twilight	Transition	Deep	Stagnant air
Light	Sunshine/ vascular plants	Shade to edge of darkness	Total darkness	Total darkness	Total darkness
Temperature	Ambient surface	Reduced variation	Nearly stable	Stable	Stable
Humidity	Variable/ desiccating	Reduced variation/ desiccating	Reduced variation/ desiccating	Saturated	Saturated/ condensing
CO_2 (volume %)	$\geq$0.04%	0.04–0.5%	0.04–1%	0.1–3%	3–>6%
Fauna	Surface	Trogloxenes, troglophiles, waifs	Trogloxenes, troglophiles	Troglobites, troglophiles, trogloxenes	Predominately troglobites

3.3. Ecological classification

Living in the same physical environment leads to biological adaptation, therefore the animals have a set of similar morphophysiological features. An adaptative pattern common to animals non phylogenetically related (Juan, 2010). The main adaptations are the loss or degradation of visual organs, pigment, and body and appendages elongation. Scientists have attempted to classify cave animals to study the ecology of caves. One of the earliest attempts to the classifications by Schiødte (1849), Schiner (1854) and Joseph (1882). In 1907, Romanian biologist Emil Racovitza had more precise definitions of each category and created the classification, later called Schiner-Racovitza classification (table 5), which became the basis for all subsequent classifications (Howarth and Moldovan, 2018).

Table 5 - Ecological classification of cave animals with definition by Schiner-Racovitza system.

Trogloxenes	They are lost or unintentionally visitors that do not live and reproduce in caves. The animal category rarely displays any special adaptive features for life in caves, and they usually stay near cave entrances.
Troglophiles	They can live and reproduce in subterranean environments but can also live in surface habitats. Usually, they occur in areas near the entrance. They may have a weakened visual system and some pre-adaptations to life without light.
Troglobionts	They strictly inhabit subterranean environments and usually are found only in the deepest cave areas. This animal category is significantly modified morphologically for underground environment.

Despite the usefulness and high accuracy of the classification, the placement of cave species in it is still subjective until the proper relationship of the animal with the cave is clarified by ecological and biogeographical studies. For example, it is known that some species live on the surface of soils, under stones among moss and others across other subterranean habitats, therefore, their appearance in the caves may be accidental. In this case, morphological features may not be enough to determine their habitat exclusivity. Thus, other scientists found several fundamental problems in the Schiner-Racovitza classification, such as “separation of soil organisms from randomness, use of troglomorphisms to infer troglobitic status, differences between troglophiles and trogloxenes, identification of mandatory trogloxenes.” As an alternative, Thinès and Tercafs (1972), Holsinger and Culver (1988) and others suggested another divisions and definitions, such as dividing troglophiles into subtroglophiles (permanently or temporarily inhabit an underground environment, but are closely related to the terrestrial worlds for some vital functions) and eutroglophiles (living on the surface but constantly supporting the underground population). Later, Sket (2008) re-established the subtroglophiles and eutroglophiles division. Christiansen (1965) coined the term troglomorphy to describe the adaptive traits of cave species. An animal exhibiting some troglomorphy, also known only from caves, can be considered a troglobiont. In the case of aquatic troglobionts, it has become common to define them using the term stygobionts (Howarth & Moldovan, 2018).

3.4. Overview of cave animals

The main traits exhibited by cavernicolous animals often depend on how strongly they are associated with caves, from casual visitors to highly specialized cave-adapted animals (Howarth, 1983; Trontelj et al., 2012). It is important to note that morphological, physiological, and behavioral signs of modification of underground animals are convergent, that is, the similarity between organisms of different systematic groups due to the same habitat conditions. The low or complete absence of light is the most crucial selective feature of the environment ensures the predictability and similarity of evolutionary changes (Howarth & Moldovan, 2018).

3.4.1. Adaptations to caves by terrestrial and aquatic animals

Morphological Adaptations

In addition to the previously noted loss of vision, pigmentation, and body hardness, additional morphological signs are wing degradation thinning of the cuticle of arthropods. These morphological changes were considered regressive or a long time, but later scientists concluded that this formulation is not correct from evolution and adaptation. More precisely, call it an increase and decrease in the development of certain morphology represented in balance. Because if one morphological feature degrades, some other (more suitable in these conditions) is more pronounced and develops. Thus, instead of "negative" morphological changes, there are also many "positive" changes. For example, an increase in the size and number of sensory organs (receptors); elongation of the body, legs, antennae, and other appendages. The arthropod's claws are strongly elongated and adapted for walking on bare wet rock (Moldovan et al., 2004; 2018). The well-known Morphological, Physiological and Behavioural Adaptations of terrestrial and aquatic animals are listed below (table 6,7,8).

Table 6 - Morphological Adaptations of terrestrial and aquatic animals.

Pigmentation	The lack of pigmentation of cave-adapted animals is a common biological adaptation to aphotic habitats. However, the evolutionary causes of albinism in cave organisms are not fully understood, and only a few studies focused on the molecular mechanisms underlying the loss of pigmentation. The first idea is that weakened selection by pigmented traits in the dark allows for the accumulation of mutations that ultimately eliminate pigment production. Indeed, the functions of pigments, such as protection from harmful ultraviolet radiation, camouflage, or aposematic coloring for protection from predators, attracting potential partners, and so on, are not needed in an aphotic environment (Bilandžija et al., 2017). Some of the aquatic cave vertebrates retain a pink color due to circulating blood or can be completely transparent. Albinism can make stygobionts more vulnerable to UV radiation and bring harmful consequences (Langecker, 2000 Bilandžija et al., 2017).
Ocular regression (Anophthalmia)	The general trend of morphological characteristics of stygobionts is similar to their terrestrial neighbors, which is expressed in a weak visual system. It is justified primarily by energy savings and natural selection results in conditions without light. Nevertheless, many cave species that were considered blind prove to be microphthalmic (Howarth and Moldovan, 2018).
Hypertrophy of sensorial organs	The decrease or complete degradation of the visual system is compensated by hypertrophy of other receptors such as sensorial taste, electrical perception, and chemoreceptors (Howarth and Moldovan, 2018).
Body size	The change of whole size body or its parts is associated with the size of voids (their wide variety) of subterranean microhabitats. Therefore, body size, length, and a

	number of legs can vary greatly, even in species living in the same cave (Howarth and Moldovan, 2018).

Table 7 - Physiological Adaptations of terrestrial and aquatic animals.

Dietary changes	Troglobionts are often omnivores and scavengers since food resources in caves are often scattered and difficult to extract. In addition, troglobionts can consume vast amounts of food and then go without food for a long time, which is explained by a slow metabolism (Howarth and Moldovan, 2018).
Low metabolic rate	Troglobionts with a low metabolic rate often exhibit a low level of activity and low mobility. As a result, a low level of oxygen consumption is also recorded. However, this type of energy-saving is a universal feature of many organisms, including those with a large amount of available food (Steffan 1973; Roff 1986).
Fasting	Fasting also has a close relationship with suppressed metabolism, during which troglobionts are fed by lipids, a prolonged state of glycogen and protein conservation, and low energy requirements (Howarth and Moldovan, 2018).
Osmotic regulation	An extremely humid atmosphere is stressful for organisms, so troglobionts have changed their water balance mechanisms. For example, they have lost the properties of the body that retain water and are now very sensitive to drying out (Howarth and Moldovan, 2018).
CO_2 Tolerance	Many invertebrates can tolerate temporary high concentrations of CO_2, but organisms living in not very deep areas of the cave or in soils prefer to migrate to less stressful conditions, while species living in deep parts of caves do not have such an opportunity (Howarth and Stone, 1990).
Oxygen Consumption	Oxygen consumption is low due to the reaction to hypoxia inherent in deep cave waters. This feature leads to a slowdown of the motor and respiratory systems. Recovery after periods of lack of oxygen occurs promptly due to anaerobic metabolism. (Howarth and Moldovan, 2018).

Specific cave conditions influenced the behavioral features of subterranean organizations. For example, finding food, a partner, and a haven requires special behavioral habits described below.

Table 8 - Behavioral Adaptations of terrestrial and aquatic animals.

Loss of circadian rhythm	Troglobionts have lost their attachment to daily cycles and demonstrate a continuous activity level, but this activity is irregular. It is the result of the lack of daylight (Howarth and Moldovan, 2018).
Random walks	Troglobionts move slowly along a random path, the so-called actual pattern, which involves chaotic movement or wandering (Moldovan and Paredes Bartolome, 1998/1999). This type of movement minimizes energy consumption and the chance of falling into a natural trap (pits and crevices). Flying cave organisms have adapted to hovering with their limbs extended forward to first touch the substrate with them.
Mating behavior	Visual partner identification signals for troglobionts are not possible in complete darkness. However, some have pheromone glands, which indicate that pheromones are essential for sexual communication in some species (Howarth and Moldovan, 2018).
Agonistic Behaviors	The behavior expresses low aggressive and submissive reactions. Rare fights between aquatic cave animals occur very gently can be expressed in a beating with a tail. Agonistic behavior is associated with a low level of selection, a decrease in the level of activity, and an increase in life expectancy (Howarth and Moldovan, 2018).
Feeding Behavior	Cavefish have more taste buds on the surface of the head, and some stygobionts, for example, the cave salamander *Proteus anguinus*, can feed outside the cave (Howarth and Moldovan, 2018).

3.5. The ecosystem of Pena Cave

3.5.1. Specific abiotic conditions

Temperature

As mentioned earlier, temperature in caves is very different from the ones at the surface, including the level of its temperature stability. According to the data provided by PNSAC staff, for short periods from 2000 to 2019, the average temperature in Pena Cave changed from a minimum of 11.81°C to a maximum of 13.65°C (table 9).

Table 9 – Temperature average in Pena Cave (provided by PNSAC).

Year	Date	Temperature (°C)	
		Humid air	Dry air
2000	3-24 November	13.64	13.65
	24 November to 5 December	13.66	13.66
2001	9 January to 25 April	13.45	13.51
	26 April to 24 May	13.57	13.56
2019	21-31 January	*12.83	
	1 to 28 February	*11.81	
	1 to 31 May	*11.88	
	1 to 4 April	*11.85	

**Temperature data without specifying the humidity/dryness.*

More recently, data on the temperature in the cave is not carried out regularly. From a geoconservation point of view, these data are of little value without data about visitors for tourist, educational, or scientific purposes. The Chapter 5.3.1. provides more details about the temperature and influence of visitors on Pena Cave.

CO_2 concentration

Regarding the concentration of the CO_2, there is less information available than about the temperature in Pena Cave. However, available data collected during 3–24 November 2000, and from 24 November to 5 December, provided by PNSAC, shows an average of 65 mV and 66 mV, respectively (1 mV = 20 ppm). A detailed variation over the course of days and hours can be viewed in figures 21 and 22.

A detailed analysis of the variation of CO_2 concentration caused by visitors to Pena Cave has not been done before. However, in the PNSAC report dedicated to this cave, in the chapter on the leading causes of environmental changes in the cave, human presence was put in the first place. The presence of one person in the cavity causes changes in its climate, namely an increase in temperature, water vapour, carbon dioxide, and methane. Considering that an adult emits an average of 0.33 litres of CO_2 over one minute, the greater the number of visitors and the longer the duration of the visit, the worse will be the impacts on the cave atmosphere. Thus, the presence of a large group of people leads to an increase in air temperature, a decrease in oxygen concentration, and a significant increase in the amount of carbon dioxide and water vapour.

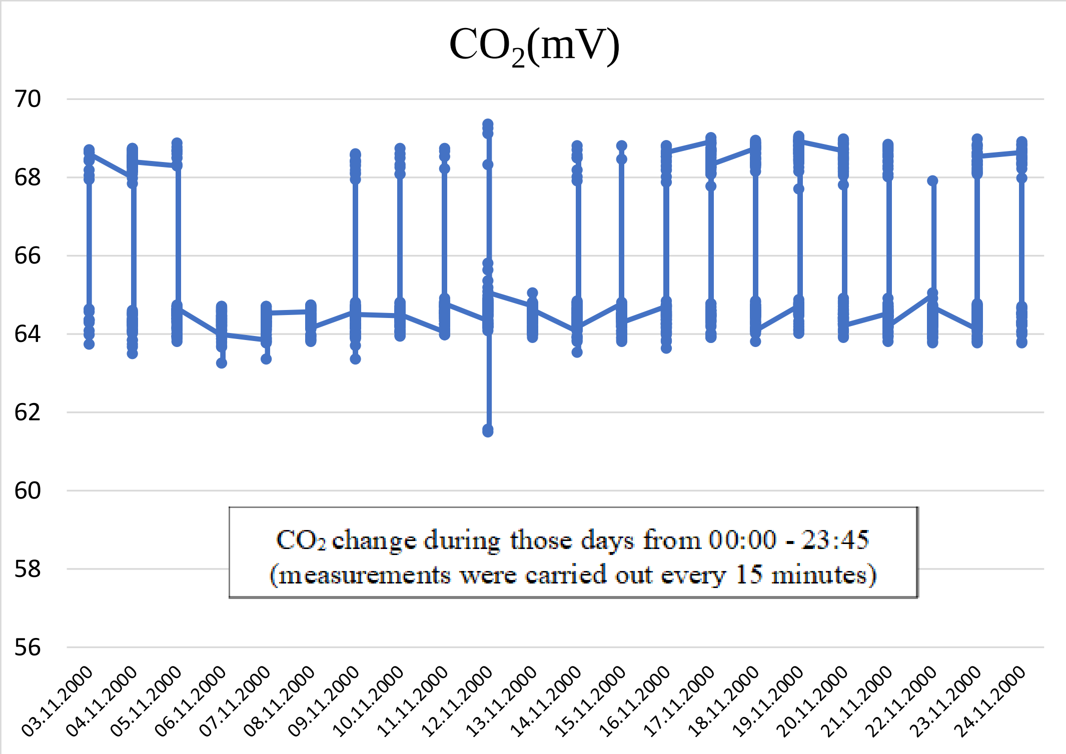


Figure 21 – Variation of CO_2 concentration in Pena Cave during 3–24 November 2000 (provided by PNSAC).

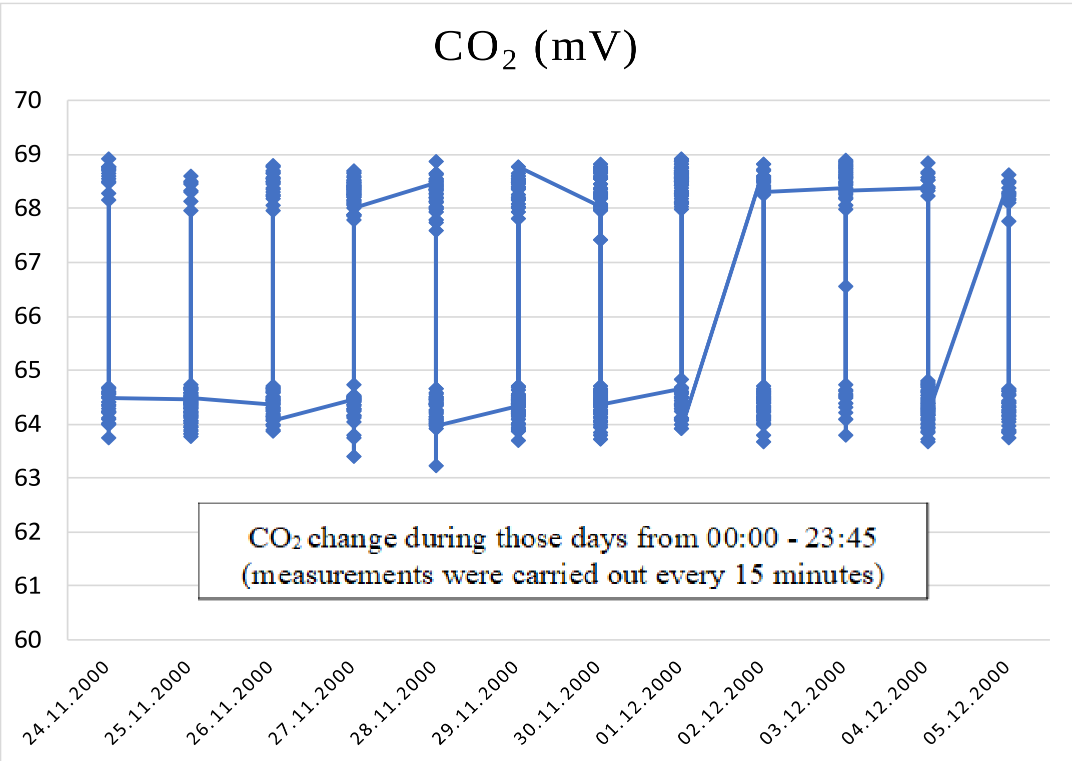


Figure 22 – Variation of CO_2 concentration in Pena Cave from 24 November to 5 December 2000 (provided by PNSAC).

Air humidity

The air has a relatively high relative humidity due to the presence of free water in an almost closed enclosure, but it is not continuously saturated, and this allows the evaporation process that keeps active the formation of stalactites and stalagmites. In Pena Cave, the relative humidity of the air is particularly close to saturation (order of magnitude of 99%), which is due on the one hand to the abundance of water and on the other to a weak air renewal. As mentioned above, the connection with the surface decreases but is not zero and, in this particular case, is carried out through small cracks in the limestone. In its natural state, this prevents the achievement of saturation but is not sufficient to significantly reduce relative humidity.

Thus, water vapor is a critical element preserving the cave since the air is almost saturated, and the chemical phenomena occurring (the formation of stalactites and stalagmites) significantly depend on the evaporation process.

Light

Pena Cave does not have natural lighting (solar) because the cave is entirely isolated. The only access to the surface is a historic shaft-shaped entrance into the cave. Therefore, artificial lighting is required for research and tourist visits. Lighting devices are heated, contributing to an increase of temperature and, consequently, a decrease in relative humidity.

Substrate

The habitat surface of Pena Cave is determined by the high humidity caused and evaporation processes that contribute to a large number of different speleothems and the overall morphology of the cave (see Chapter 1). The main rock representing the substrate is limestone with a quite cavernous surface.

3.5.2. Environmental zones of Pena Cave

There are five environmental zones in Pena Cave: the entrance, twilight, transition, deep, and stagnant air zones (figure 23). Cave-adapted fauna distributes and concentrates in each zone depending on food accessibility. Thus, practically the highest concentration of troglobionts in the Transition-zone, but the possibility of finding the troglobionts in the other ecological zones of the cave is not excluded. At least until now, it has been found out in a practical way that the cave-adapted millipede *Cylindroiulus villumi*, which is endemic from this cave, was found only in the Transition-zone of Pena Cave.

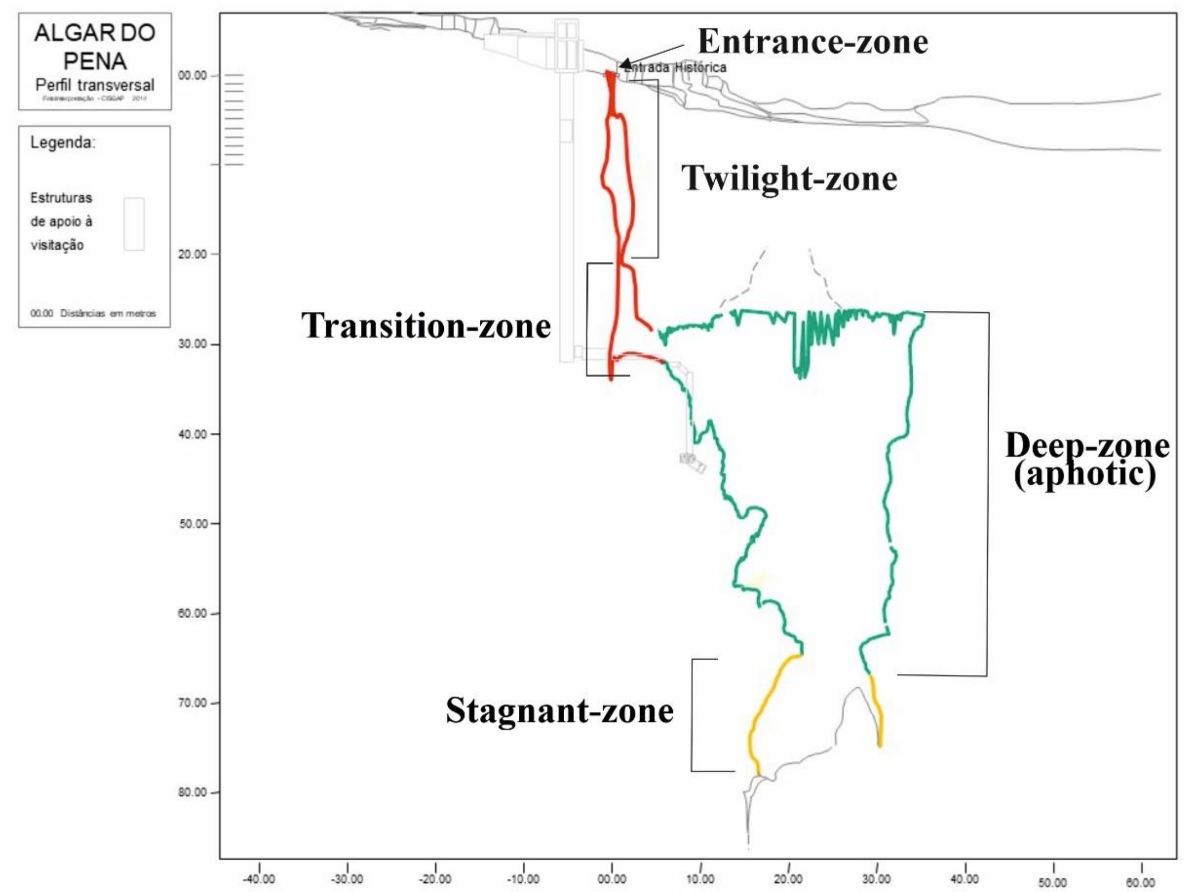


Figure 23 – The estimated distribution of ecological zones in Pena Cave on the transverse profile (implanted over cave map provided by PNSAC).

3.6. List of cave-adapted communities in Pena Cave

Pena Cave biodiversity consists of a community of six troglobionts, five troglophile species, and some birds and bats (trogloxenes) recorded by their guano. Next, a list of cave-adapted and partly adapted species is provided, as well as information on their main characteristics. In addition, a map of cave-adapted fauna provided distribution on the Estremenho Limestone Massif was made.

1. Spider *Domitius lusitanicus* (Fage, 1931) (figure 24)

General description

Domitius lusitanicus is a troglobiont spider of the family Nesticidae (Ribera, 2018). A distinctive characteristic of *D. lusitanicus* from other *Domitius* is the complete absence of eyes.

Habitat

It inhabits caves of the Estremenho Limestone Massif, where it is frequently found in cave walls (Ribera, 2018).

Figure 24 – The spider *Domitius lusitanicus* (photo: Ana Sofia Reboleira).

Ecology

D. lusitanicus has typical morphological adaptations to the underground environment: depigmentation, anophthalmia, and appendage lengthening (Ribera, 2018). Strictly cave lifestyle is confirmed by obvious adaptive signs.

2. *Cylindroiulus villumi* Reboleira and Enghoff, 2018 (figure 25).

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Arthropoda	Diplopoda	Julida	Julidae	*Cylindroiulus*

General description

Cylindroiulus villumi is a species of millipede only known from Pena Cave. The cave is the only place where this species was discovered, which makes the cave even more exclusive (Reboleira and Enghoff, 2018).

Habitat

Cylindroiulus villumi was found in Pena Cave inside a big piece of deadwood located at the base of the entrance pit to the cave.

Ecology

Cylindroiulus villumi is a blind and depigmented cave-adapted species of millipede living only in Pena Cave, it is adapted to the very constant temperature of 13 ±1 °C, and the relative humidity is close to saturation (see section 4.5).

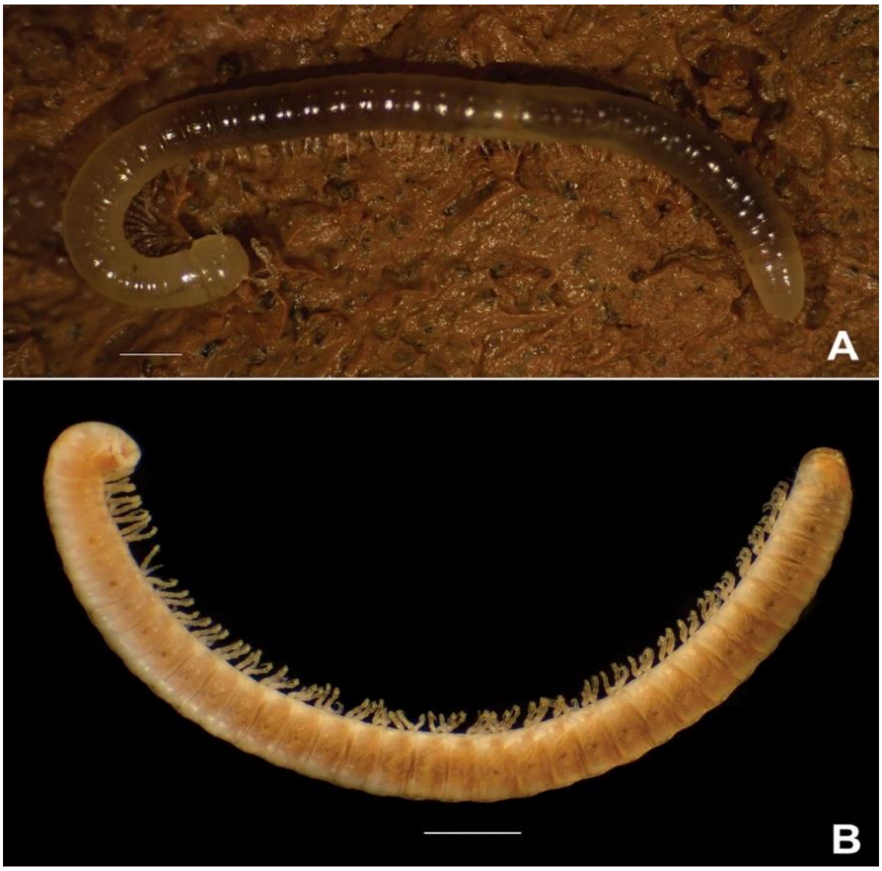


Figure 25 – *Cylindroiulus villumi* sp. n. A) habitus of live female B) habitus of subadult male. The partly darker colouration in B) is due to gut contents seen by transparency. Scale bar: 1 mm (Reboleira and Enghoff, 2018).

3. Woodlice *Trichoniscoides meridionais* (Vandel, 1946)

Taxonomy (Reboleira et al., 2022)

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Arthropoda	Malacostraca	Isopoda	Trichoniscidae	*Trichoniscoides*

General description

Trichoniscoides meridionalis is a blind, depigmented troglobiont found in caves throughout the Estremenho Limestone Massif, including Pena Cave (Reboleira et al., 2022).

Habitat

Trichoniscoides meridionalis is inhabitant terrestrial system (non-aquatic) is a troglobiont species as endemic to the Estremenho Limestone Massif (Reboleira et al., 2022).

Ecology

Trichoniscoides meridionalis is a blind, depigmented troglobiont living strictly underground, but the distribution of this species in different areas of the cave varies quite widely. It can be found from

Entrance-zone to Deep-zone, normally associated with decomposing wood. (Reboleira et al., 2022, Reboleira et al., 2015). It is one of the two troglobiotic oniscidean so far known from this large karst area (Reboleira et al., 2022).

4. Springtail *Onychiurus confugiens* Gama, 1962.

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Arthropoda	Collembola	Poduromorpha	Onychiuridae	*Onychiurus*

General description

Only a few papers have information about this species, namely Baquero et al. (2021) and Gama (1965). However, those works provide that the species *Onychiurus confugiens* was found only in caves and is known from Alcobertas Cave, in Serra dos Candeiros subunit of Estremenho Limestone Massif. In addition, the *O. confugiens* was found in Ventos do Diabo Cave, located on the Santo Antonio Plateau, which defines the troglobiont species as endemic to the Estremenho Limestone Massif.

5. Dipluran *Podocampa* cf. *fragiloides* Silvestri 1932 (figure 26)

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Arthropoda	Entognatha	Diplura	Campodeidae	*Podocampa*

General description

Diplurans, commonly known as two-pronged or bristletail double tails, are distributed in terrestrial ecosystems across the planet. Campodeidae is, the most diverse and abundant subfamily in soils in every continent except Antarctica, are widely distributed from humid areas to deserts and also tropical forests. *Podocampa cf. fragiloides* is diplurans of subsurface terrestrial habitat in the Iberian Peninsula on Estremenho Limestone Massif and has a large distribution in habitat (Sendra and Reboleira, 2020). In Pena Cave, a specific species is not defined, so the abbreviation is cf. using to show similarity with P. fragiloides.

Habitat

Podocampa cf. *fragiloides* inhabit cave habitats in karst areas from the west of the Iberian Peninsula. In Portugal it limiting in cave and soil environment on the territory of Estremenho Limestone Massif (Sendra and Reboleira, 2020).

Ecology

Most species demonstrate adaptation to cave habitats in karst and volcanic areas. *Podocampa* cf. *fragiloides* inhabit a soil environment with a preference for humid spots from the upper horizons of the soil or cave (Sendra and Reboleira, 2020).

Figure 26 – The dipluran *Podocampa* cf. *fragiloides* (photo: Ana Sofia Reboleira).

6. Beetle *Trechus gamae* Reboleira and Serrano, 2009 (figure 27)

Taxonomy

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Arthropoda	Insecta	Coleoptera	Carabidae	*Trechus*

General description

Trechus gamae is a troglobiont beetle, known from five caves and the mesovoid shallow substrate (scree slopes), all located in the Santo António Plateau subunit of the Estremenho Limestone Massif (Reboleira and Eusébio, 2021). *Trechus gamae* demonstrates a strict underground lifestyle because both adults and larvae were found in the sampled caves. Moreover, it is the only cave-adapted beetle found on the Sto António Plateau subunit of the massif.

Habitat

Trechus gamae was found in five caves of the same limestone massif, where one of them is Pena Cave. In addition, a single specimen was found in the shallow mesovoid substrate at 0.5 m depth in three slopes of Fórnea, located in the Santo António Plateau, showing that this species may also disperse through more superficial subterranean habitats (Reboleira and Eusébio, 2021). The largest population of the species is located in Pena Cave. However, all the known populations are within the Santo António Plateau.

Ecology

Trechus gamae is an inhabitant of the terrestrial system and basically it follows caves and subterranean areas (non-aquatic). It was found in the deepest parts of caves at a depth of 50 to 95m, with a humidity level of more than 98% and a temperature of 13.5°C (Reboleira and Eusébio, 2021).

Figure 27 – *Trechus gamae* from Pena Cave (photo: Ana Sofia Reboleira).

3.7. Distribution of the troglobiont species of Pena Cave

Knowledge about the distribution of species explains their rarity and importance, which implies a more precise solution to the conservation of these species, namely, which species should be preserved first.

Three main limited areas of species distribution were identified: Estremenho Limestone Massif, Santo Antonio Plateau, and Pena Cave (figure 29). The level of distribution determines the rarity of the species.

Thus, one species (*Cylindroiulus villumi*) is localized exclusively in Pena Cave, which gives it the status of the rarest and most important species of study area biodiversity. The second level of importance received *Trechus gamae*, the distribution of which is limited by the Santo António Plateau subunit of Estremenho Limestone Massif. This limitation is due to the geomorphological evolution of Massif, which resulted in the formation of isolated elevated areas (Aire Mountain, Candeiros Mountain, Santo António, and São Mamede Plateaus), which are separated by Mendiga, Alvados, and Minde depressions. The other four species described in Pena Cave were recorded in different areas of the karst massif (table 10).

In conclusion, local geology dictates the distribution of species within the boundaries of subunits, which could be confirmed by the presence of related species that migrated between the Estremenho Limestone Massif subunits, probably before the geological event uplift of the blocks.

Table 10 – Limiting the distribution of species in designated areas.

Species	**Area**
Domitius lusitanicus (Fage, 1931) *Trichoniscoides meridionais* Vandel, 1946 *Onychiurus confugiens* Gama, 1962 *Podocampa* cf. *fragiloides* Silvestri, 1932	Estremenho Limestone Massif
Trechus gamae Reboleira and Serrano, 2009	Santo António Plateau
Cylindroiulus villumi Reboleira and Enghoff, 2018	Pena Cave

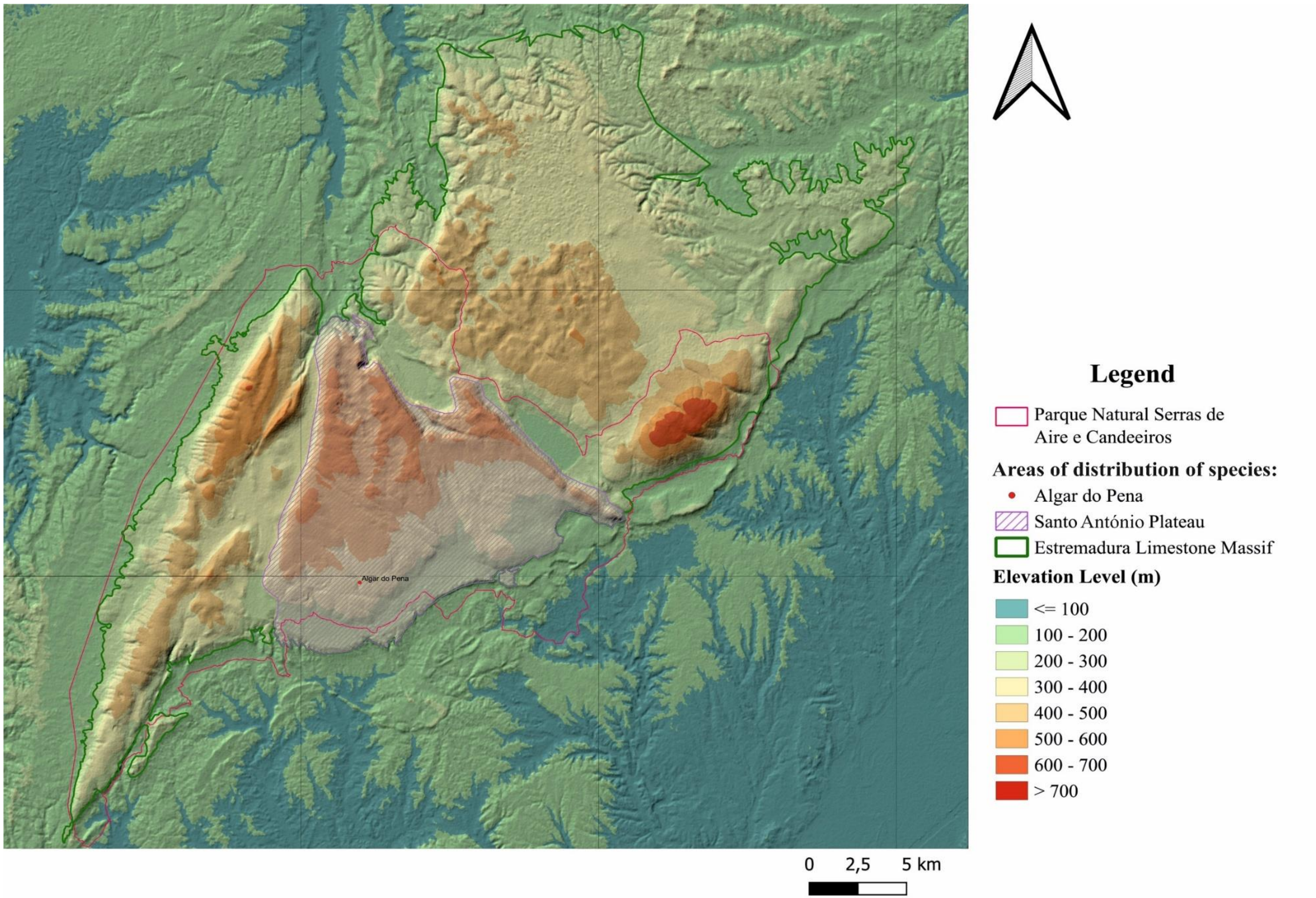


Figure 28 – Schematic representation of areas of species distribution found in Pena Cave.

3.8. List of troglophile species in Pena Cave.

1.Centipede *Lithobius pilicornis* Newport, 1844 (figure 29), 1844 - predator.

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Arthropoda	Chilopoda	Lithobiomorpha	Lithobiidae	*Lithobius*

Figure 29 – *Lithobius pilicornis*, United Kingdom of Great Britain and Northern Ireland (Publisher: iNaturalist).[3]

2. Beetle *Atheta subcavicola* Brisout de Barneville, 1863 – predator.

It is often found in bat guano.

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Arthropoda	Insecta	Coleoptera	Staphylinidae	*Atheta*

3. Beetle *Pristonychus terricola* subsp. *reichenbachii* Herbst, 1784 – predator.

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Arthropoda	Insecta	Coleoptera	Carabidae	*Laemostenus*

[3] https://www.inatu…g/photos/80358076

4. Beetle *Speonemadus vandalitiae* Heyden, 1870 – detritivore.

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Arthropoda	Insecta	Coleoptera	Leoididae	*Speonemadus*

5. Beetle *Choleva jeanneli* Britten (figure 30), 1922 – detritivore.

Kingdom	Phylum	Class	Order	Family	Genus
Animalia	Arthropoda	Insecta	Coleoptera	Leiodidae	*Choleva*

Figure 30 – *Choleva jeanneli*, Sweden (Artportalen, Swedish Species Observation System).[4]

[4] https://www.artpo....se/Image/2781648

4. Characterization of geodiversity of Pena Cave

Geodiversity is natural range (diversity) of geological (rocks, minerals, fossils), geomorphological (landforms, topography, physical processes), soil and hydrological features (Gray, 2013). However, it should be underlined that *chemical processes* are crucial to control the development of geodiversity in karst caves.

4.1. Chemical processes

The formation of speleothems is a continuous physic-chemical process that is possible only in very specific conditions, namely almost 100% humidity and low cave energy. Speleothems are mostly the result of water dripping processes due to gravity and developed by the precipitation of $CaCO_3$ according to the more or less availability of water and its CO_2 content.

According to the model developed by Dreybrodt (1980), a stalagmite results from a constant supply of water by drip on a flat surface. The water droplets are saturated with calcium bicarbonate, and slowly release CO_2 until its concentration equals the concentration of this gas in the atmosphere of the cave (figure 31). With this slow release of CO_2, calcite precipitates according to the following reaction:

$Ca^{2+} + 2HCO^{-3} \rightarrow CaCO_3 + CO_2 + H_2O$

There are four processes potentially limiting the precipitation rates of $CaCO_3$ in the model (Fairchild and Baker, 2012):

1. The diffusion of CO_2 molecules in the solution;

2. The diffusion of Ca^{2+} and CO_3^{2-} in solution;

3. The deposition of $CaCO_3$ on the solid surface;

4. The production of CO_2 on the solid surface.

It was concluded that from these four types of limiting reactions to calcite precipitation, CO_2 production is determinant for the growth rate and evolution of speleothems. In turn, this would be dependent on the concentration of Ca^{2+} in the water and the temperature. Several tests and models were created and perfected in the following years, but this was the first theoretical starting point for the empirical explanation of speleothem growth processes.

Figure 31 – Photo of the drop condensation on stalactite in Pena Cave.

4.2. Physical processes

Broken speleothems are a common feature in any cave. Sometimes, the vertical point through which the stalactite drips does not correspond to the accretion point of the corresponding stalagmite. In most cases, a portion of speleothems breaks by natural causes. For instance, stalactites may fall when their weight exceeds the capacity of support from the ceiling. Tectonic activity may also be responsible for these breaks and artificial vibration (figure 32).

Crispim (1999) inventoried the following factors involved in the speleogenetic evolution:

- Changes of slopes and strains;
- Rising of massifs;
- Proximity to neotectonic faults and earthquake epicenters.

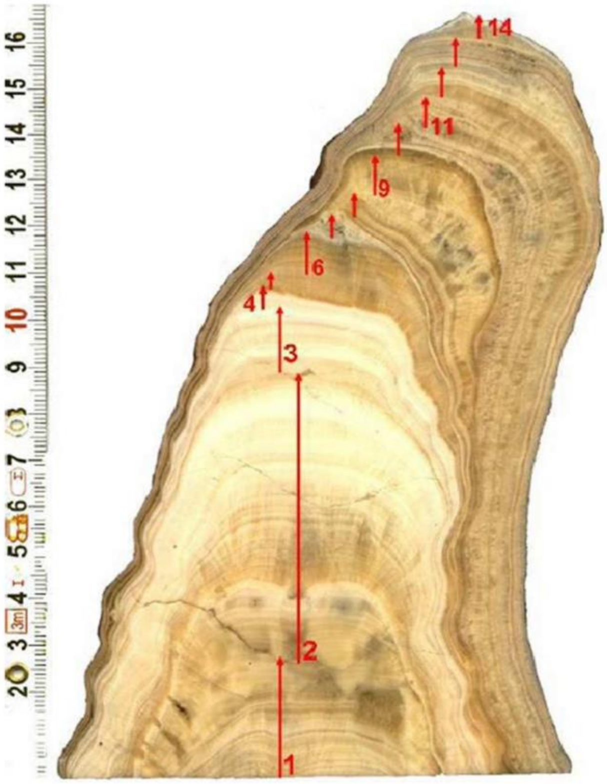


Figure 32 – Longitudinal sections of stalagmites. On the left, stalagmite-sample "Cel-1", in Cellaforza cave, with graphical reconstruction of the accretion axes at the apex of the growth blades (Lurilli, 2007). On the right, stalagmites cut (unpolished) existing in CISGAP and on display, denouncing by the various colors and shapes, the different phases of its growth, impurities and presumably, climate events.

In addition, physical processes correspond to chronological traces and are divided into two: "ancient" and "modern" traces (Crispim, 1999) (figure 33). Method limiting itself to direct observation, without using any methods of absolute dating. The remains considered "ancient" is characterised as large stalagmites or broken columns are evidenced, from which new stalagmites and stalagmitic floors are formed (second-generation forms). The "modern" traces are characterized by having open cracks that cut second-generation stalagmites, presenting free or with calcite incrustations. However, one cannot fail to call into question the hypothesis that the apparent modern of the broken forms is due to the lack of water percolation along the speleothems by old seismic events, making a new selection of water percolation paths and abandoning that specific location. On the other hand, to identify other tectonic events from broken speleothems, it would be necessary to consider cases of first-generation broken speleothems (with more than one episode of breaking) cementing or sedimentation, being able to ascertain in fact whether there would be cracks of other morphologies.

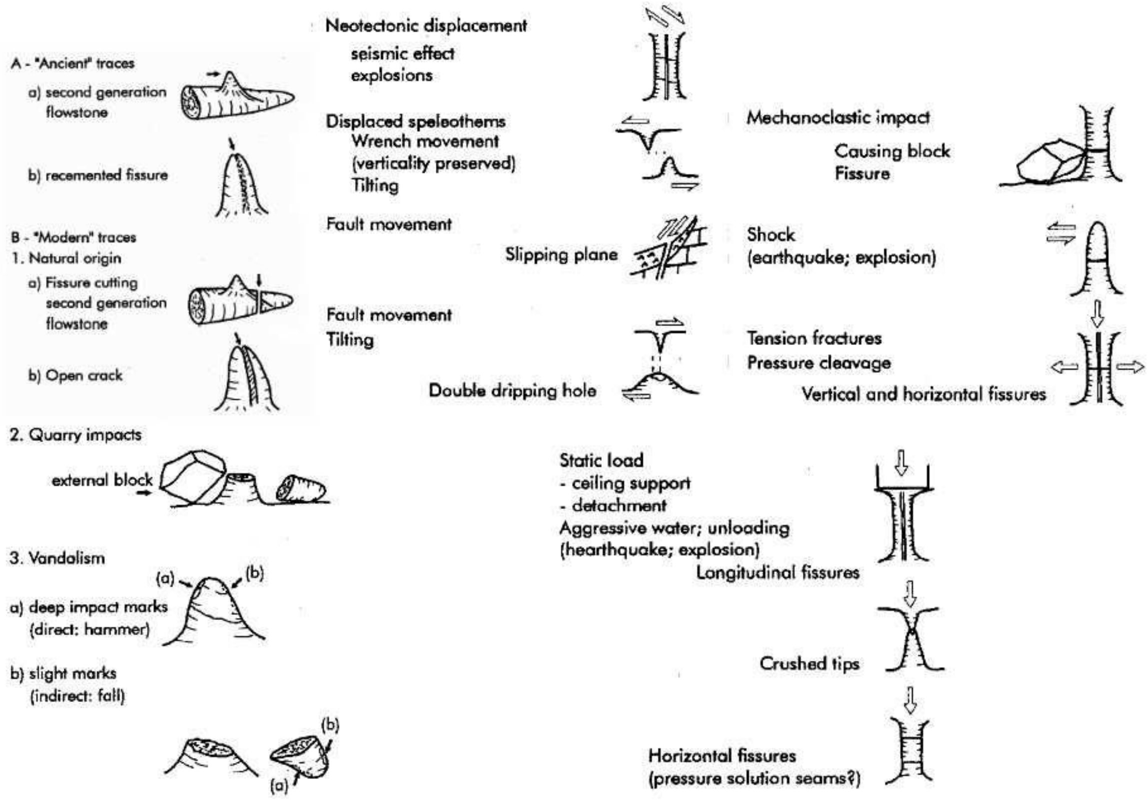


Figure 33 – Synthesis of morphologies that allow the reconstitution of a chronology of events (left column) and agents and morphologies inventoried in the Zambujal cave (Arrabida Mountain) (central and right columns) (Crispim, 1999).

4.3. Landforms

Speleothems, in addition to the scientific value as indicators of environmental changes, also have aesthetic value. When visiting a show-cave, first of all visitors admire the uniqueness of landforms, which can only be seen in a karst cave, and only after they learn about the scientific and educational importance of this environment.

As mentioned earlier, special conditions are necessary to make and develop speleothems. However, there is a lot of different types of speleothems (Crispim, 1999). There are two main groups of speleothems: *stalactites and stalagmites.* Stalactites grow from the top of the cave to the bottom, opposite to stalagmites which grow from the bottom to the top. The maximum size of stalactites is determined, theoretically, by the strength of attachment to the ceiling and the strength of the ceiling rock, so it is rare to see stalactites larger than 10 m or with a diameter of more than 1-2m (Ford and Williams, 2007). As a result of the stalactites drip, stalagmites and stalagmitic floors are formed. These correspond to a general floor covering caves, although they spread both over the floor and over the walls (Simões, 2015).

The diagram of figure 34 shows the various forms of stalactites and stalagmites, most of them can be found in Pena Cave (figures 34 and 36).

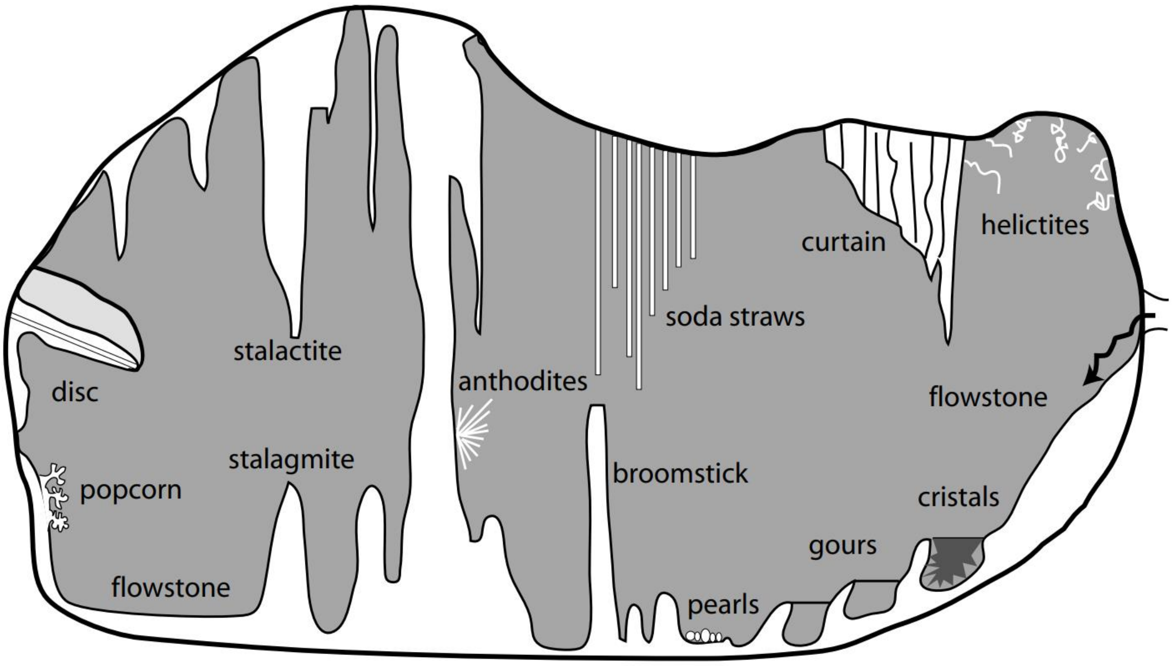


Figure 34 – Different types of speleothems in a cave (Gilli, 2015).

Stalagmitic floors are typical deposit forms of a uniform flow, which aggregate in a horizontal position (Simões, 2015).

Soda-straws stalactites requires a slow and constant water input, without organic or suspended substances blocking the thin channel. Given its extreme fragility, a minimum diameter of 5 mm is required to remain intact (Simões, 2015).

Curtains (*"bacon slices")* develop when water droplets flow along an oppositely inclined wall or along a conical stalactite growing along the outer part since water flows not only through the inner channel. However, they do not develop too much since the lower parts limit the water inlet.

More curious are the speleothems which defy the laws of gravity during their growth. These speleothems can grow both from the wall and on other speleothems or other sediments (Ford and Wiliams, 2007, apud White, 1976). Its origin may be due to microclimate influences, small temperature fluctuations in various areas of the cave. The diversity of these forms has two typologies: linear forms (helictites) and hemispherical forms. For example, in Pena Cave there are *"popcorn"* forms (figure 36) (Simões, 2015).

Helictites (linear shapes) are of different genesis: filiform or wire, with a diameter of 0.2 to 1 mm; worm-like, sinuous with a diameter of 1 to 10 mm, sometimes identified as calcite tangle; aragonite, corrugated shape, which also bends and forks. These different types of genesis are mutually common, they all have a central tube through which water is deposited at the end. Branching occurs due to swelling created by crystal growth, which occurs during drought periods, leading to deviations or bifurcations of the rarefied concentrated on the top water (Simões, 2015).

Thus, in Pena Cave almost all possible morphological forms of speleothems are represented (figure 36), which often correspond to low-energy or moderate-energy caves with a stable climate and low air hydrodynamic activity (Cigna, 1993).

4.4. Hydrological features

There are two small lakes in Pena Cave. To the south of Pena Cave, one of its internal lakes collects all the dripping water from the intermediate sector (figure 35). After several visits to the cave, it was verified that the lake's water level is variable according to the seasons, which influences the sedimentation on its floor (Simões, 2015). Another lake is located on NE in the upper sector. This lake constantly exists regardless of the season and plays an essential role in forming stalactites in this area.

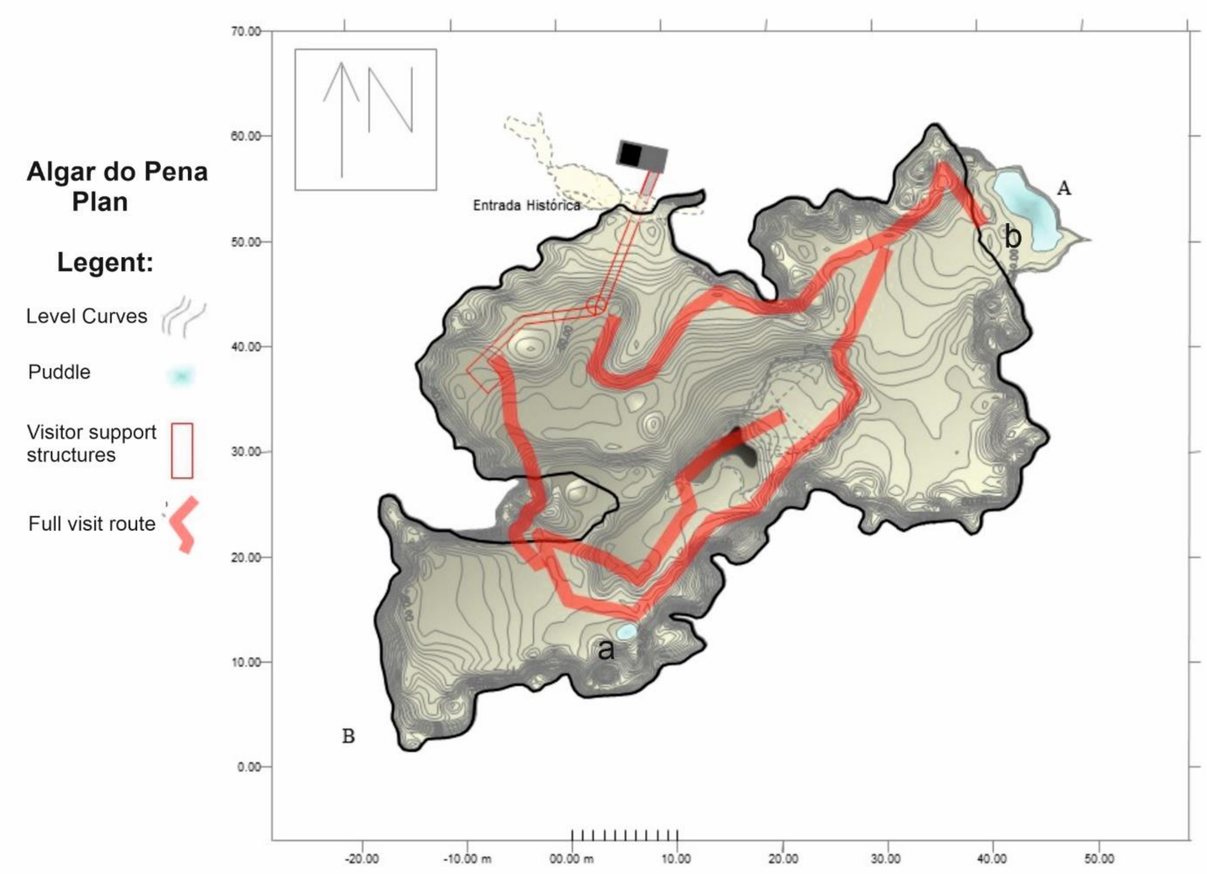


Figure 35 – Location of internal lakes in Pena Cave (a) lake from intermediate sector (b) lake from upper sector (source: CISGAP)

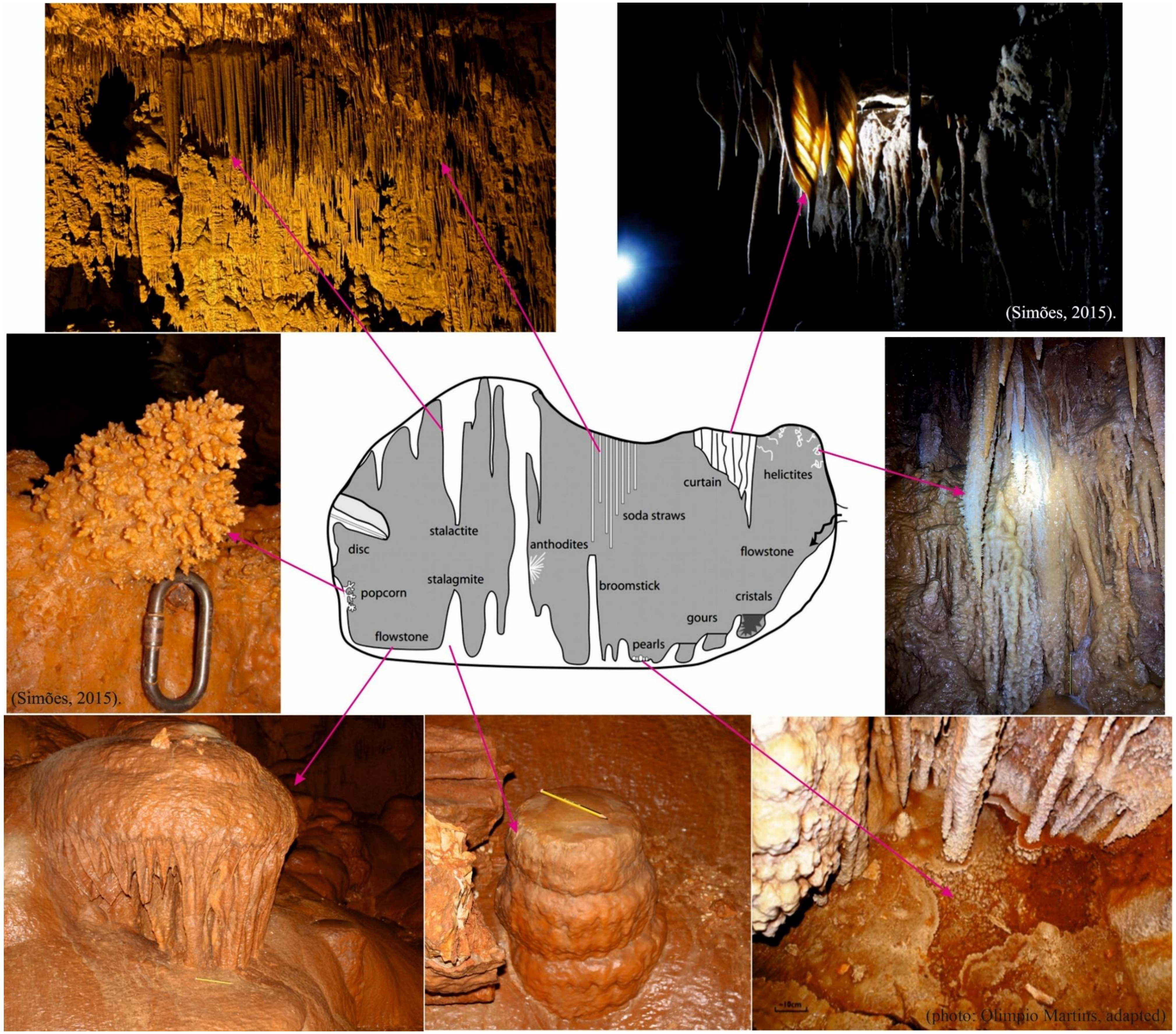


Figure 36 – Different types of speleothems in Pena Cave.

4.5. Rocks, non-consolidated deposits and soil

The only rock in Pena Cave is the Middle Jurassic limestone, already described before. The rock corresponds shallow marine environment.

The karst was developed under a humid tropical environment, presumably from late Cretaceous or early Cenozoic, ferruginous breastplates evidence this in Pena Cave (Simões, 2015). These deposits correspond to the accumulation of clays with the most diverse alterities derived from the decomposition of organic and mineral matter (Simões, 2015). Found in at least four different locations in the cave, they may indicate old piezometric levels in which stability and subsequent deposition of materials (Simões, 2015). Thus, the discovered deposits of glandular breastplates are accurate paleoecological evidence and indicators of various formation sequences of the entire cavity.

In addition, rare inclusions of river sediments and periglacial deposits could be found in the cave—fluvial deposits date from about the end of the Oligocene (Simões, 2015). Glacial deposits can reach several tens of meters, sometimes interspersed in a clay matrix or layers, becoming actual signs of the cold period (Fairchild and Baker, 2012), but the age of the deposits is unknown.

5. Possible threats affecting geo- and biodiversity in karst caves

The advent of humans in underground karst caves led to many anthropogenic threats (Cigna, 1993; Pulido-Bosch et al., 1997; Calaforra et al., 2003; Song et al., 2000; Leal et al., 2009) causing negative impacts on geodiversity and biodiversity. The origin of these threats can be defined on two groups (Reboleira et al., 2022): "from inside" which includes all kinds of anthropogenic influences from direct visits to caves and used for any purpose, and "from outside" in which, the anthropogenic activity near the cave has consequences inside the cave.

The different threats identified in past studies regarding geo- and biodiversity in karst caves and how these threats may affect the study area will be considered below.

5.1. Threats to geodiversity

5.1.1. Temperature rise

Previous studies

One of the consequences of tourism in caves is the increase of air temperature. A single person releases 80–120W of heat energy, about the same as a single incandescent light bulb. Thus, a group of 50–60 people on a cave tour can locally raise the temperature by 1–2°C (Gillieson et al., 2022). This effect has been proven in show caves in different countries (figure 37). For instance, in Marvels Cave, Spain (Pulido-Bosch et al., 1997) and in Baiyun Cave, China (Song et al., 2000). Research in Grotte di Castellana Cave, Italy, showed that just in 10 minutes, 105 visitors increased the overall cave temperature, and that recovering to natural conditions after the visit took 30 minutes (figure 38). Thus, a cumulative effect may be formed, which will require hours or even days to restore the previous equilibrium (Cigna, 1993). Researchers at Cueva del Agua Cave (Spain) also devoted their work to the issue of avoiding the cumulative effect (Calaforra et al., 2003). The maximum number of visitors was experimentally determined, with the number possible to restore the natural temperature in the cave in a few hours for preparation for a new group of visitors. The number is different for each cave since it depends on the volume of the cave and its ventilation conditions (Calaforra et al., 2003).

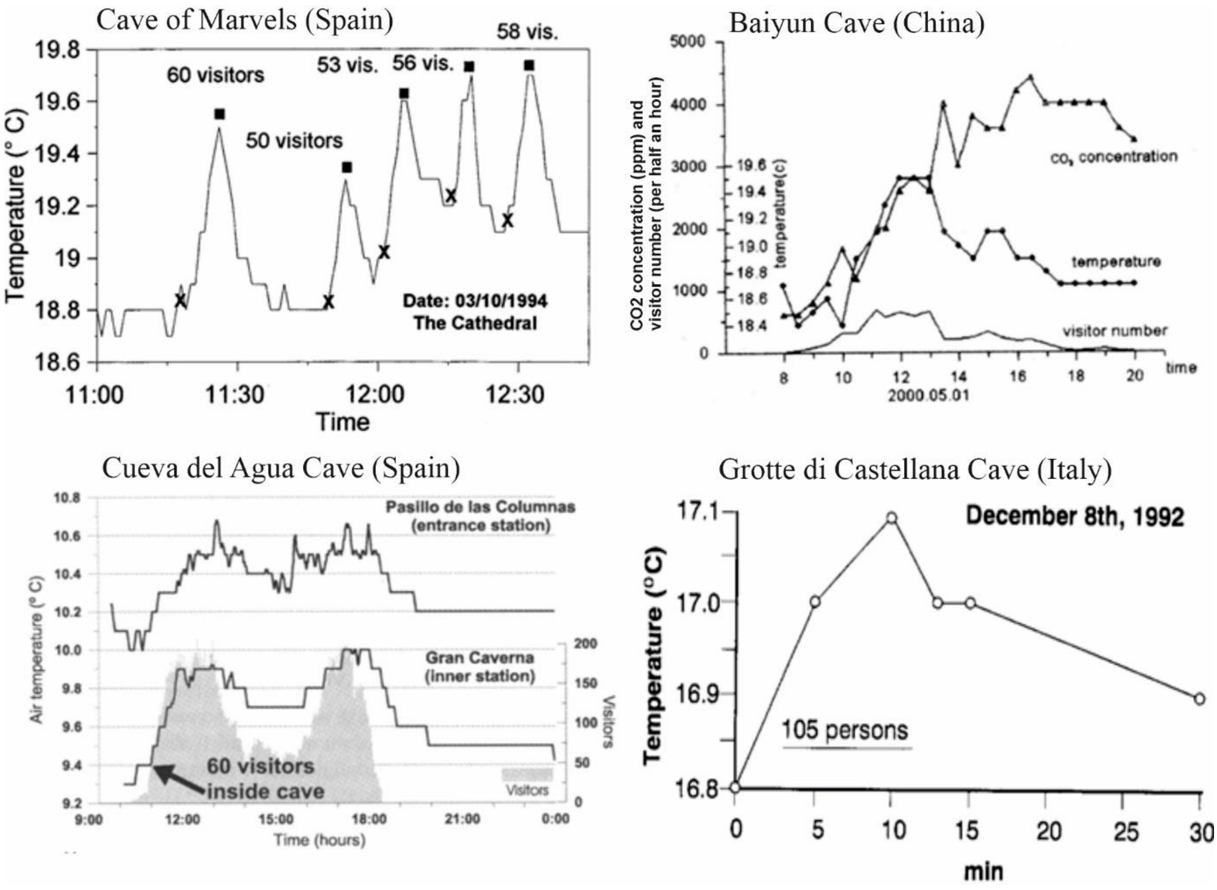


Figure 37 – Correlation of temperature, CO_2 concentration, and number of visitors in show caves in different countries; Unit of humidity measurement as a percentage (%). Marvels Cave (Pulido-Bosch et al., 1997), Castellana Cave (Cigna, 1993), Baiyun Cave (Song et al., 2000), Cueva del Agua Cave (Calaforra et al., 2003).

How temperature rise affects geodiversity?

The increase in temperature caused by the visitor's presence and by the lighting system. It is accompanied by a simultaneous decrease in relative humidity since the pressure of the saturation water vapor is directly proportional to the temperature. Thus, when the temperature rises, the steam ceases to be saturated, and the condensation process responsible for the natural growth dynamics of the speleothem is affected (Calaforra et al., 2003, Cigna, 1993).

5.1.2. Carbon dioxide rise (CO_2)

Previous studies

The natural concentration of CO_2 in caves is usually higher than at the outside, primarily due to the respiration and oxidation of organic materials (Ravn et al., 2020). Previous studies show that the average values of CO_2 closer to the exit are 500-600 ppm, and in the deep parts of the cave, 1500 ppm, whereas the room value of CO_2 is 400 ppm. CO_2 concentration due to visitor respiration can increase up to 5000 ppm (Pulido-Bosch et al., 1997; Gillieson et al., 2022).

The study in Marvels Cave (Spain) shows at least three relative CO_2 peaks per day in the period from 17 to 20 July, during the normal flow of tourist groups (figure 38) (Pulido-Bosch et al., 1997). In Baiyun Cave (China) a joint collection of temperature, CO_2 and number of visitors for three days was done, showing a direct dependence of these data (Song et al., 2000). Studies in Grotta Grande del Vento Cave (Italy) also confirm the same dependence (Cigna, 1993). It can be seen from the results of Italian researchers that in order to preserve the natural CO_2 level, the number of visitors should be below 1000 per day, which can be defined as the carrying capacity of the cave.

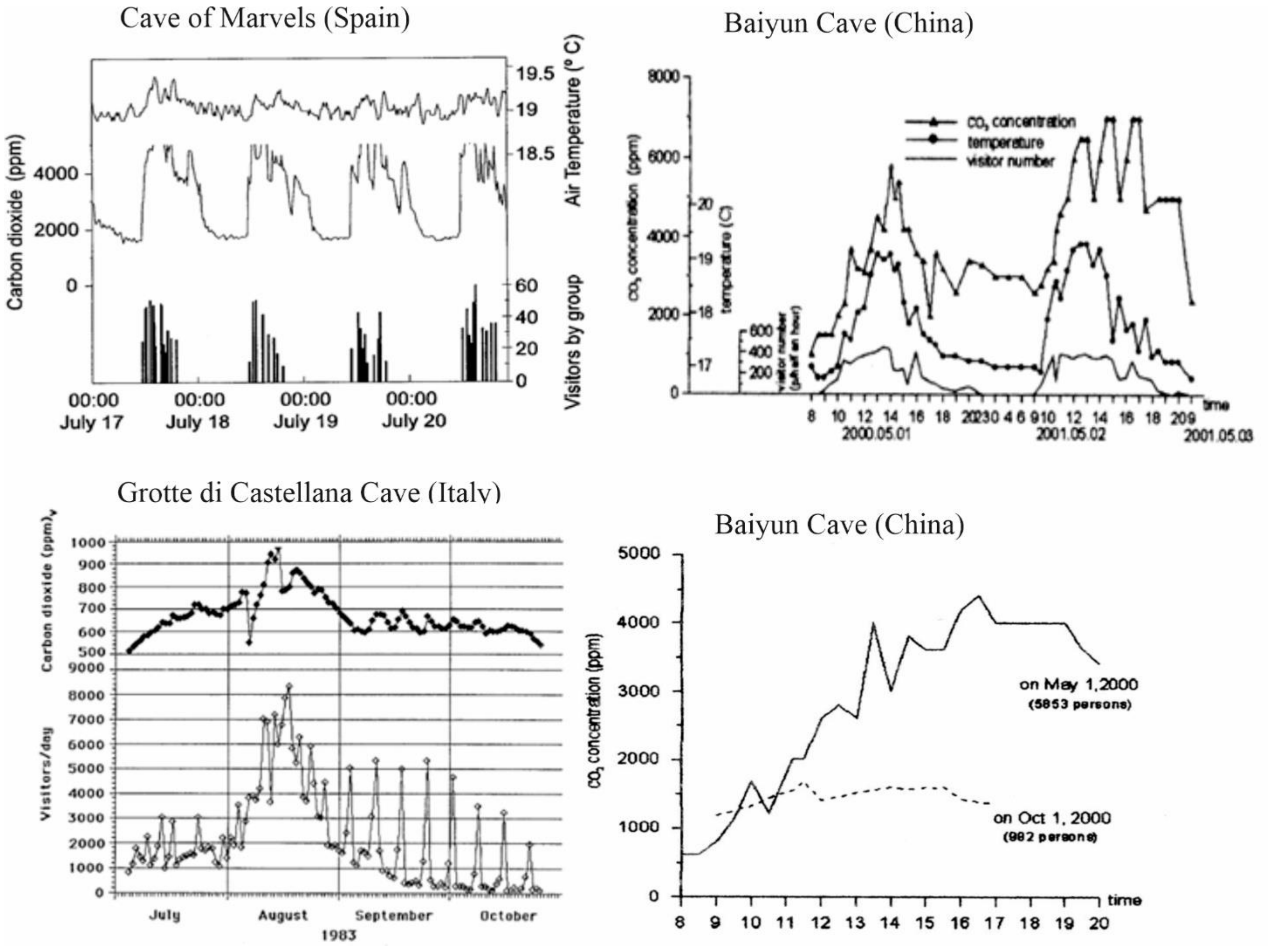


Figure 38 – Number of visitors per group, concentration of CO_2 and air temperature. Marvels Cave (Pulido-Bosch et al., 1997), Castellana Cave (Cigna, 1993), Baiyun Cave (Song et al., 2000), Cueva del Agua Cave (Calaforra et al., 2003).

The results of previous studies show that the rate and level of CO_2 accumulation depends on the volume of the cave and number of visitors, obviously, the smaller the cave is, the higher the concentration level and speed accumulation; however, even in very large rooms, the concentration of CO_2 emitted by visitors may rise quite progressively. These impacts can be mitigated by using artificial ventilation techniques.

How carbon dioxide rises affect geodiversity?

High concentration of CO_2 can seriously affect the processes of dissolution and precipitation of calcium carbonate. The three main elements of karstification system are water, rock (limestone), and carbon dioxide gas (CO_2). The latter plays an important role in the overall dissolution reactions for limestone (Gilli, 2015). Under normal conditions, $CaCO_3$ is slightly soluble: 14 mg/L^{-1} at 25°C. However, with a sharp increase of CO_2 and temperature in the environment, the level of solubility of limestone increases.

This pattern can be described by a chemical reaction (Gilli, 2015):

- Ionic dissociation: $CaCO_3 \leftrightarrow Ca^{2+} + CO_3$
- CO_2 dissolution: CO_2 (g) $\leftrightarrow$ CO_2 (l) cold-favoured reaction
- CO_2 (l) hydrolysis: CO_2 (l) + $H_2O \leftrightarrow HCO_3^- + H^+$
- Calcium hydrogen carbonate formation: $HCO_3^- + H^+ + Ca^{2+} + CO_3^{2-} \leftrightarrow (HCO_3)_2Ca_2$

Calcium bicarbonate is an unstable compound, highly soluble in water, which leads to the destruction of limestone (Gilli, 2015). On the other hand, an increase in CO_2 concentration causes aggressive condensation, which is accompanied by corrosion processes in cave sediments. This, changes in the concentration of CO_2 disrupt the chemical equilibrium about the control of dissolution and precipitation of carbonates.

CO_2 concentration in caves decreases and returns to the previous level through air interchange between the outside and inside of the cave. If it cannot return to the previous level overnight, CO_2 will accumulate during the next tourist day which may affect the equilibrium of different systems inside the cave.

5.1.3. Biological corrosion

Colonization of plants, introduced by humans into the cave in various ways, is a serious threat affecting the rock. For example, the reproduction of microflora, such as algae, lichens, and mosses, due to improper lighting and pollen transfer on shoes and clothes of visitors seriously damages carbonate deposits. Many works have been devoted to this issue (p.e. Jones, 1965; Viles, 1987; Cooks and Otto, 1990). The consequence of the colonization of plants is the progressive decomposition of the rock.

In Marvels Cave (Spain), a comparative analysis was carried out using scanning electron microscopy (SEM) and section analysis. In the sectors far away from the tourist route, no traces of accelerated dissolution were found on the speleothems, although in some cases, foreign elements were found on the surface of crystals (figure 39).

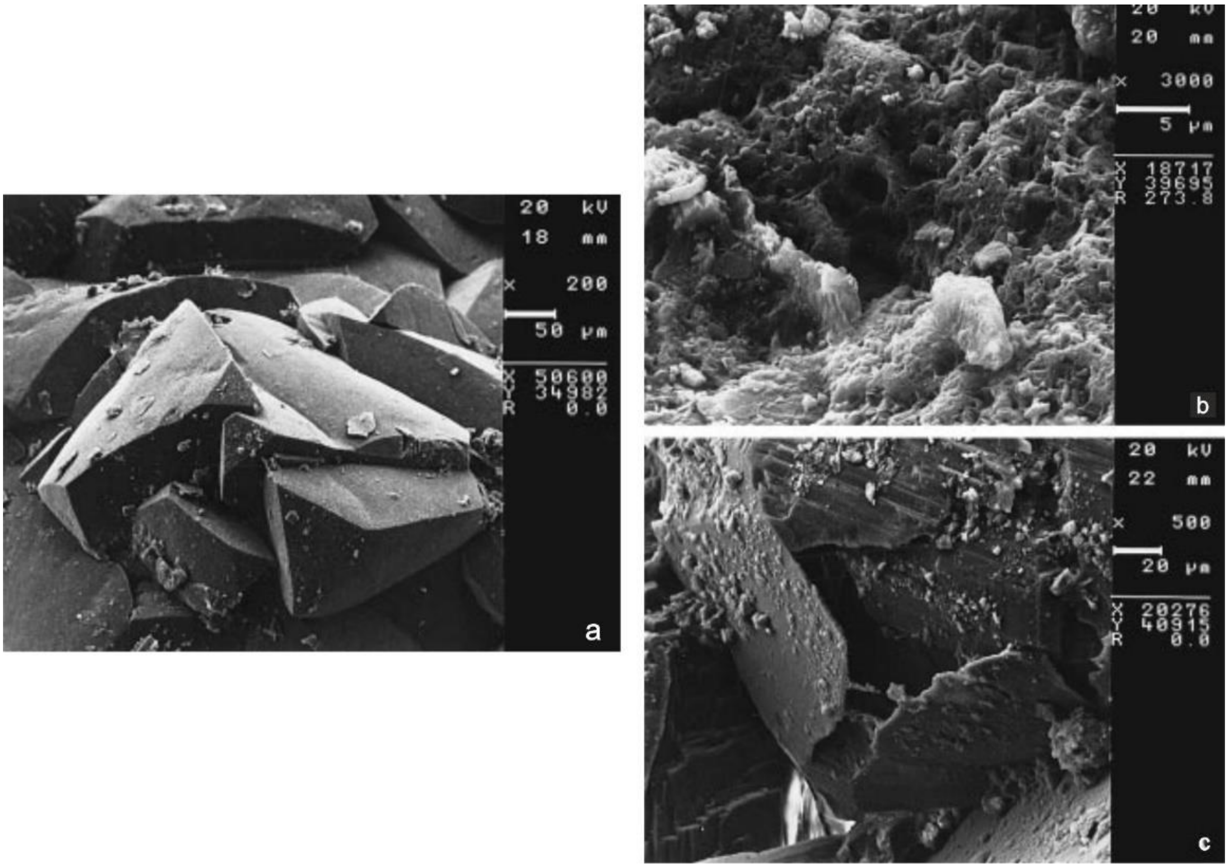


Figure 39 – a) Sample of speleothem taken in sectors far from the tourist route; b, c) speleothems affected by processes of plant colonization (Pulido-Bosch et al., 1997).

The samples affected by plant colonization showed a microtopography utterly different from the group of samples that were at a distance from the tourist route (figure 39). They showed systematic, circular engraved pits and tunnels, textures closely related to organic etching. In some samples, the change process turned out to be particularly intense, as in the case of figure 39c, which shows a clear example of crystal etching.

5.1.4. Artificial lighting

The incandescent lighting system in caves has an effect on the relative humidity and temperature, releasing both light and heat (Cigna, 1993, Pulido-Bosch et al., 1997). Both physical (thermal) and biological effects can be observed in the immediate vicinity of light sources. Incandescent lamps emit a wide spectrum of radiation characteristic of plant organisms, around which the so-called "lampenflora" often develops (figure 40) (Pulido-Bosch et al., 1997, Osborne, 2019). These include algae and mosses, which can spread quite widely away from the light source, as well as some higher plants leading (Cigna, 1993). Lampenflora is a serious problem as rootlets and hyphae micritise the calcite crystals in the speleothems making them dull and murky (Osborne, 2019; Fong, 2011). Lampenflora cause a negative aesthetic effect and also induce biochemical corrosion of the speleothems located near the light (Cigna, 1993; Fong, 2011). These effects are easily removed with chlorine bleach but this is harmful to cave invertebrates and microbes (Osborne, 2019).

Figure 40 – Lampenflora growth near an incandescent lamp in Pena Cave Interpretive Center placed just to illustrate the consequences of a wrong use of this type of lightning in caves.

5.1.5. Mechanical damage (vandalism) and specimen collecting

Vandalism is one of the most common problems in cave management. There are two types of dangerous vandalism: graffiti and mechanical impact on speleothems. Mechanical action is usually motivated by the desire to touch, get a souvenir of the speleothem, appeal to destroy or just take a photo.

Osborne (2019) presents two examples of vandalism in show caves. In the 1830s, significant damage was caused mainly by the carbide lamp, which had previously been widely used in Bendethera Cave, Australia (figure 41). Nowadays even considering the great efforts to protect speleothems and erect fences, visitors put their hands behind the fences, which seriously damaged the Furze Bush, a complex aragonite helictite (Osborne, 2019). Despite the best efforts of cave guides and management in Texas, half of the rare and fragile Butterfly in the Caverns of Sonora was deliberately broken in 2006, which forced the introduction of new cave protection laws (Osborne, 2019). Regarding to Europe, CCTV monitoring has become common practice in show caves, including those with guided tours and Pena Cave is not an exception.

In the case where this problem is still relevant, video surveillance is used in combination with an alarm system to prevent vandalism more effectively, so visitors who are going to behave badly can be warned by an audio message. However, this requires additional financial costs.

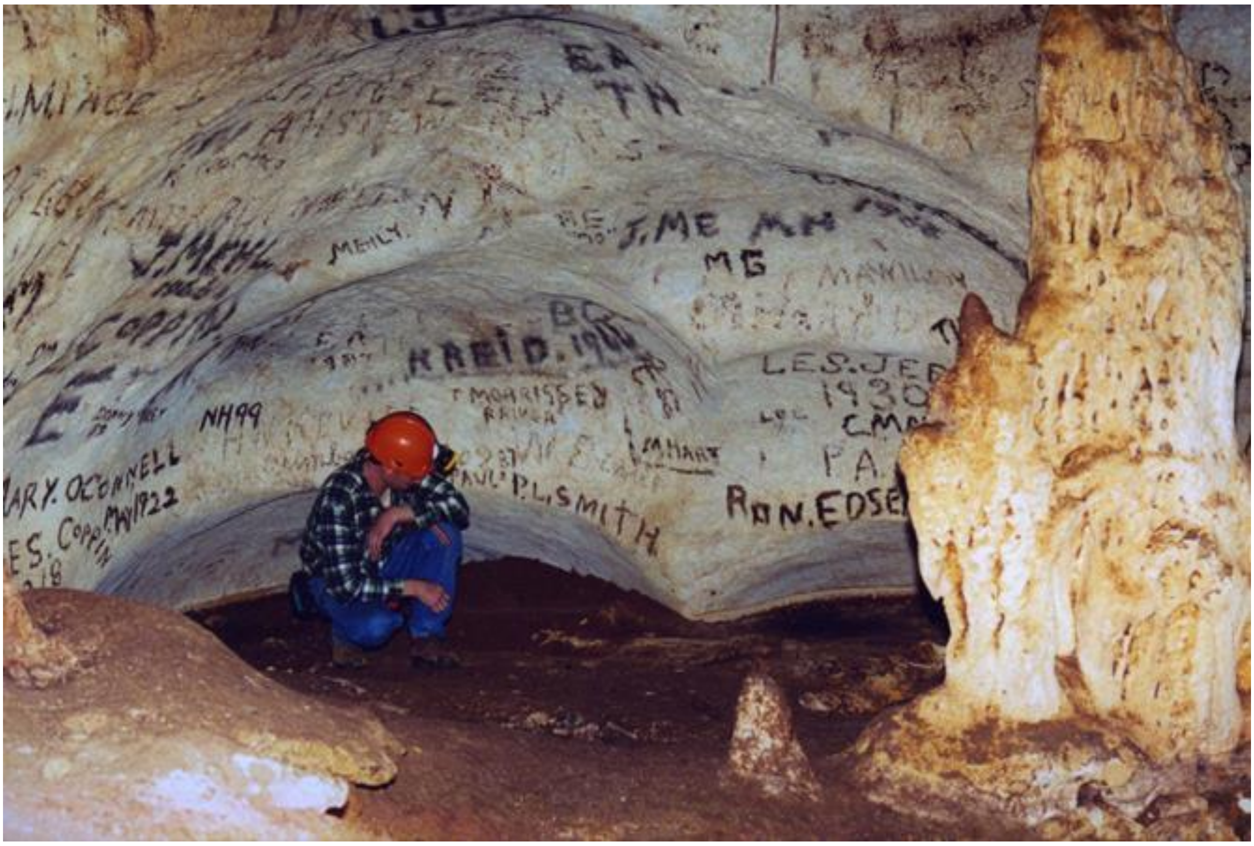


Figure 41 – Graffiti in Bendethera Cave (Australia), inscriptions are mostly composed of carbide lamp soot (Osborne, 2019).

5.1.6. Infrastructures

Infrastructures such as handrails, stairs, platforms are mandatory elements in show caves because it ensures a safe and comfortable cave use. Safety is the main priority in show caves (Gillieson et al., 2022). Well-planned infrastructure also helps to reduce direct physical contact with the cave floor and walls, which reduces the risk of damage and erosion (Osborne, 2019). On the other hand, such constructions in karst caves exert some pressure on the nature of the karst.

Taking into account the need to implement a sustainable use in caves, it is necessary to consider the features of the cave environment, such as high humidity, distribution of biodiversity concentration, location of important geodiversity elements, and how the new infrastructure will affect the aesthetics of the show cave. The task of managers is to keep the cave as pristine as possible and, at the same time, to provide a safe and pleasant visit to the cave.

5.1.7. Mining and quarrying

The most acute problems associated with preserving caves are related to mining and quarrying. Mining and large-scale engineering works are the greatest threat to saving caves as they are the only activities capable of making serious damages to caves and even its entire destruction (Osborne, 2019). The main problem is that most miners tend to mine massive limestone of high purity (Osborne, 1994).

The main topic concerning geodiversity and these type of threats in caves is related to karst groundwater. Karst groundwater is particularly susceptible to pollution from industrial work (Crofts et al., 2020). There are also examples of excessive water intake in karst caves, which leads to subsidence or even destruction of the cave (Veni et al., 2001). In addition, changes in the water flow, sediments, or carbon dioxide in a karst cave pose a potential threat to it as a result of filling with modern sediments or changes in the chemical composition of seeping water, which lead to the termination of the processes of formation of speleothems or even to their dissolution (Crofts et al., 2020).

In addition to hydrogeological problems, which focus on the context of mining and quarrying activities, it is logical to consider the mechanical effects of these works. Working with limestone is very rough, and depending on the extraction method, a completely different effect can be produced on the nearest karst systems. For example, mining by an explosive method is considered the easiest, most economical, and therefore the most popular. An example of such a mining method is known in a cave on Mount Etna, Australia (Osborne, 2019, Osborne, 1994). A large portion of the caverns area of Limestone Ridge and all the caverns area of Mount Etna has been destroyed (figure 42) (Beryl and Vavrin, 2008). Even if mining is carried out at some distance from the cave, the explosive wave or vibrations from work can cause significant damage to speleothems and hypothetically even lead to a cave collapse.

5.1.8. Agriculture

Agriculture and forestry are the most common types of human activity at the borders of karst protected areas, and both of them have a negative impact (Crofts et al., 2020). As a result of agriculture, changes in the chemical composition of seeping water occur, which lead to the cessation of the deposition of speleothems (Crofts et al., 2020). Changes in surface vegetation, for example, as a result of fire, usually lead to soil erosion and, in extreme cases, to desertification, as well as to a decrease in the concentration of carbon dioxide in the soil (Gilli, 2015).

Figure 42 – The Mt Etna Quarry, Australia (Berrill and Vavryn, 2008).

5.2. Biodiversity threats

Subterranean organisms at particular risk, since most species have a set of characteristics that increase their vulnerability to anthropogenic impacts. These characteristics include a limited geographical area (Bar and Holsinger, 1985; Christman et al., 2005), poor mobility (Verovnik et al., 2004; Haslinger, 2005) and low reproductive potential, long life expectancy and small population size (Culver et al., 1995). Thus, their characteristics are an excellent adaptation to the underground environment, but they also lead to slow population growth rates and, combined with a high degree of endemism and low ability to settle, significantly reduce the ability of these organisms to recover quickly after a population decline for any reason (Fong, 2011).

5.2.1 Temperature and CO_2 rise

Cave-adapted biota (troglo- and stygobionts) are usually not used to experiencing large temperature fluctuations since temperature changes in their natural habitat are almost invariable (Castaño-Sánchez et al., 2020). This behavior concerning constant temperature is called ectothermic (Addo-Bediako et al., 2000). However, sometimes there are stygobionts with eurythermal characteristics

(Issartel et al., 2005). In previous studies by Castaño-Sánchez et al. (2020), thermal stability tests were carried out on some species. The tests showed very different results. Some species survived from 2 to 28 °C (amphipod *Niphargus rhenorhodanensis*) (Issartel et al., 2005). Some had rigid ectothermic characteristics and could survive in a narrow thermal niche (Mermillod-Blondin et al., 2013).

Thus, the result of these tests cannot be used as a definitive conclusion for other species since there will be a different result for each species. However, we have two irrefutable facts: 1) some species did not survive with small temperature increases, and 2) temperature changes as a result of visits on average do not exceed 1-2 degrees, considering all the previous studies.

A study about troglobionts' reaction to changes in CO_2 is unknown, so we can only assume that a change in CO_2 concentration may affect the physiological processes of cave-adapted animals. However, it is known that they are very tolerant to a high level of CO_2 concentration, so slight exceedances of these values are unlikely to have a fatal effect. In addition, as the practice of previous studies shows, the average changes in carbon dioxide in caves are about 2800 ppm.

5.2.2. Infrastructure inside the cave

The introduction of observation platforms, paths, and fences into the cave for tourists' safety leads to significantly reducing the living area primarily for troglobionts and forces them to migrate to their habitual habitats (Cigna, 1993). Although in one study, it was found that this did not lead to a reduction in the population (Cigna, 1993), it can be assumed that the forced displacement of species from their habitat zones, which have rich nutrients, will entail both population reduction, and possibly to complete extinction. Nevertheless, more research on this topic is needed to draw certain conclusions.

It also can be assumed that in the case of an incorrect choice of material for infrastructure, such consequences as metal oxidation or rotting. Floor compaction, which is also a problem in exhibition caves, has a significant negative impact on cave invertebrates, so it is always advisable to install paths in a suspended or elevated position above the floor (Osborne, 2019).

5.2.3. Artificial light

The negative influence of artificial light has been mentioned in several works, such as Tercafs (1988), Reboleira (2011); Fong (2011); Langecker (2000). However, there are very few details about why artificial light negatively affects biodiversity.

Firstly, as an indirect trigger of an increase in temperature and a decrease in humidity, especially characteristic of incandescent light with all the ensuing consequences for biodiversity (Cigna, 1993, Pulido-Bosch et al., 1997). Secondly, given the sensitivities of the cave-adapted animal to light, due to the lack of pigmentation, it makes cave-adapted animals more vulnerable to ultraviolet radiation with harmful consequences (Langacker, 2000), and direct exposure to light is deadly and causes stress. Energy-saving lamps have a small dose of ultraviolet radiation, unlike incandescent lamps.

5.2.4. Mining and quarrying

There is a high degree of endemism in many karst areas, and threats to the biodiversity of karst caves, especially as a result of mining, were discussed by Tercafs (1988); Vermeulen and Whitten (1999); Reboleira (2011); Castano-Sanchez (2022), among others.

Pollutants commonly observed in underground ecosystems include metals, pesticides, emerging pollutants, and volatile organic compounds (Castaño-Sánchez et al., 2020). As point sources are mainly industrial facilities (Castaño-Sánchez et al., 2020).

As a result of limestone mining, the surface layer of the karst is destroyed, causing profound changes in how pollutants reach deep parts of the massifs (Reboleira et al., 2011). The cause of severe pollution is the discharge of industrial wastewater directly into cave systems and the lack of widespread treatment of domestic wastewater. In addition, it is a source of outbreaks of microbial infections, leading to problems, including public health (Reboleira et al., 2011).

The struggle of biologists and geologists with limestone miners is inevitable since the legalization of the development of a new deposit primarily depends on the economic importance and if it exceeds the value of the geo- and biodiversity of the karst cave, which is potentially at risk of destruction, then most likely the destruction of this cave cannot be avoided. Therefore, paragraphs (47-51) on Extractive industries were spelled out in Guidelines for Cave and Karst Protection (Gillieson et al., 2022).

5.2.5. Agriculture, domestic and urban waste water

The most significant nutrient source in groundwater is nitrogen and phosphorus fertilizers used in agriculture. These can be synthetic chemical fertilizers or fertilizers from organic waste (Khan et al., 2018). Nitrogen and phosphorus are essential macronutrients for plant growth and are crucial for primary production in the biosphere. However, when they are present at concentrations higher than natural background levels, they can cause eutrophication problems (Gruber and Galloway, 2008).

Surface soil use and hydrological disturbances can also disrupt mandatory hypogene populations. It can also have a natural cause, such as fires that cause profound changes in vegetation cover by altering the acidification of seeping water. Changes in water pH can cause profound changes in living underground communities (Watson et al., 1997). In urbanized areas where many forests were cut down, which led to a change in the flow of water and nutrients in the deep karst (Reboleira et al., 2011).

Table 11 summarizes all analysed information about possible threats. It shows which geodiversity elements can be affected by the threats and whether they affect biodiversity. The negative impact of almost all threats always affects landforms and active processes. Regarding biodiversity, almost all described threats harm cave-adapted animals, but there are no studies on how exactly carbon dioxide rise and biological corrosion affect biodiversity.

Table 11 – List of possible threats to geo- and biodiversity in karst caves.

The origin of threats	Threats	Negative impact	
		Geodiversity	Biodiversity
From inside the cave	Temperature rise	Active processes	+
		Landforms	
	Carbon dioxide rise (CO_2)	Active processes	?
		Landforms	
	Biological corrosion	Active processes	?
		Rock	
		Landforms	
	Infrastructures	Landforms	+
	Mechanical damage (vandalism) and specimen collecting	Landforms	+
	Artificial lighting	Active processes	+
		Landforms	
From outside the cave	Mining and quarrying	Landforms	+
		Hydrology	
		Active processes	
	Agriculture - Domestic and urban waste water		

* '+' marked if there is negative impact on biodiversity, '?' if there is not study of the effect.

5.3. Ongoing threats on bio- and geodiversity in Pena Cave

Pena Cave does not face many of the above-mentioned possible threats thanks to the effective management and competent staff working in the cave. For example, it will not face problems such as **vandalism** because at least two guides always accompany a group of visitors, video cameras are installed on the observation platform, and all visitors are presented with a twenty-minute briefing before the visit on how to behave inside the cave during the visit. We also will not find any problems with **lampenflora**. However, **lighting** is still one of the main triggers of temperature rise in the cave, so lighting in Pena Cave will be discussed in this chapter.

However, some other types of threats are relevant in Pena Cave and therefore they require some consideration although these threats are difficult to assess due to lack of monitoring in recent years, namely temperature, CO_2 concentration, and number of visitors.

5.3.1 Temperature rises and data analysis

The last available temperature data that can be directly related to the number of visitors were collected in January, February, March, and April 2001 (figure 43). Nevertheless, even this small amount of data helps to confirm the conclusion identified by previous researchers (Pulido-Bosch et al., 1997; Cigna, 1993; Song et al., 2000; Calaforra et al., 2003) that there is an inextricable link between the number and duration of the stay of cave visitors and the temperature increase.

In January 2001, the number of visitors is available just for one day (12th January), so it is impossible to make a comparative analysis for this timeframe. However, the main evidence is that the visit is reflected in the temperature (figure 43C).

In figure 43A, information on the number of visitors is presented for four days which allow us to make a more detailed analysis. On the 13th February, Pena Cave was visited by 19 people, which did not significantly affect the temperature. However, on the 15th and 16th February, when there were 60 and 100 visitors, respectively, with no day for the “rest” of the cave, the so-called cumulative effect led to increase in temperature by 0.05 degrees above the average values.

In the period from 13-31 March, there is almost complete information about the number of visitors (figure 43B). On the 17th March, the cave received an exceptional number of 224 visits causing no significant change in the temperature. This apparent anomaly is due to the fact these 224 visitors were divided into small groups that were just in the observation platform for less than two minutes (Martins O. pers. com., 2022). This example shows that despite the number of visitors, the time spent in the cave plays an important role in the cave temperature.

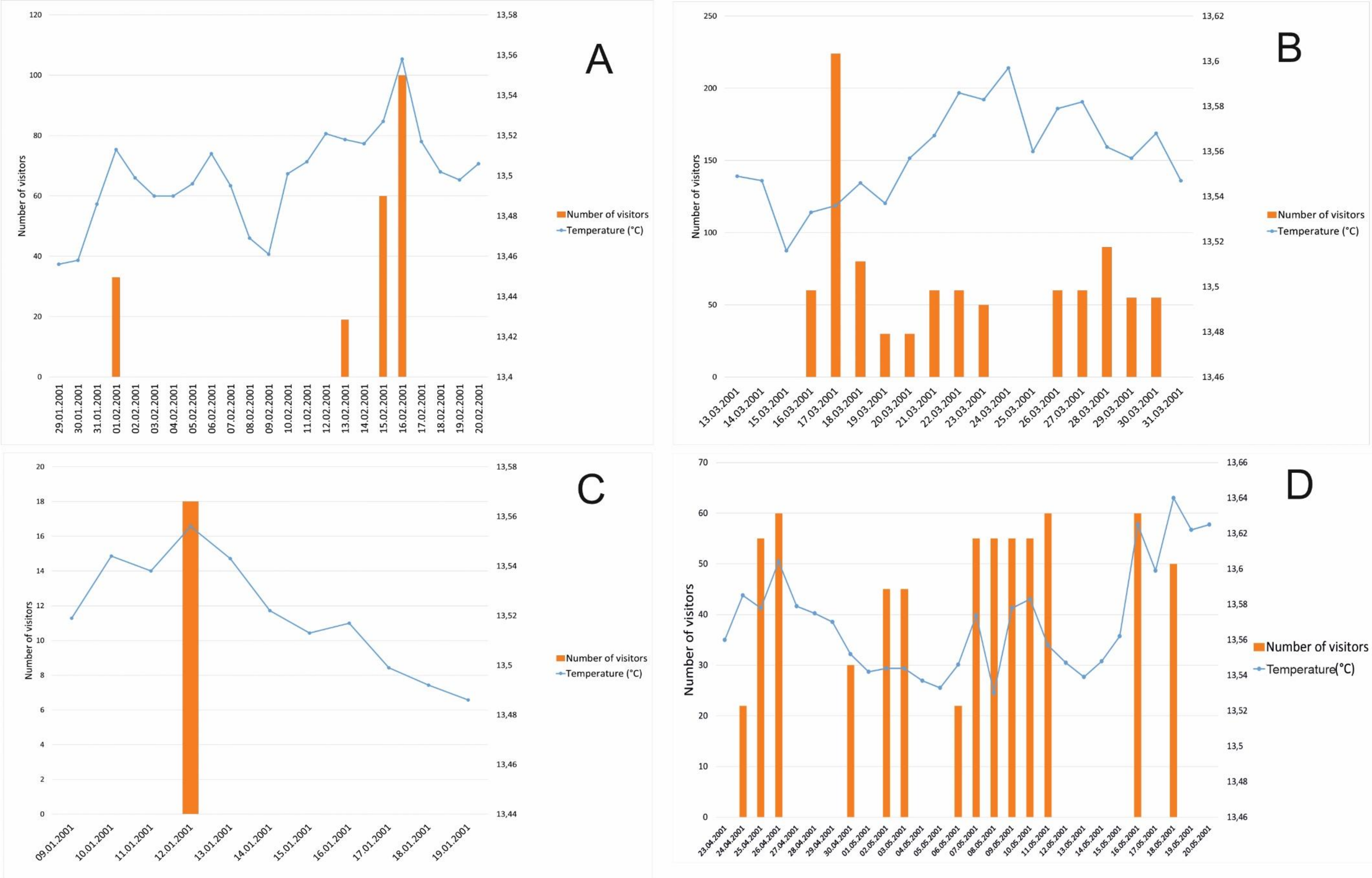


Figure 43 - Temperature variation and its relation with the number of visitors in Pena Cave (source: CISGAP).

The last period with information about visitors and temperature changes is from 23rd April to 20th May (figure 43D) when the cumulative effect is quite clear. On the 16th May, 60 people visited the cave and no visits were registered on the 17th, which mean that there was one day of rest allowing the recovery of the cave conditions. On the 18th May, there were 50 visitors but the temperature increased by 0.02°C, higher than on the day when there were 60 visitors. Based on previous studies (Pulido-Bosch et al., 1997; Cigna, 1993; Song et al., 2000), the cumulative effect happens when the interval between visits is one day since the recovery time is 24 hours on average.

The data temperature in the cave for January–April 2019, without data on visitors, are available in figure 44. Graphs show that, most often, the temperature changes during the month are less than 1°C, except for January when the temperature fluctuations from the 21st to the 23rd January were of 2.5°C. The reason for the exception is most likely a failure in the equipment since there is information that the equipment was periodically subjected to breakdowns due to high humidity or it could be result of equipment bump tests (Martins O. pers. com., 2022). Therefore, this anomaly is not considered as an important event for the study.

Thus, the minor temperature changes (about 0.1 - 0.3 °C) according to the data of 2001 and 2019 helps to summarize that such a temperature difference does not pose a significant danger to geodiversity. However, it is worth considering that we do not know the data for the last three years. Regarding to the impact of temperature variations on biodiversity, considering the temperature sensitivity of some species adapted to caves, this should be considered as a constant threat. However, for accurate conclusions, it is necessary to conduct temperature testing of species living specifically in Pena Cave. In 18 - 24 March, daily visits by groups of tourists created a cumulative effect that corresponded to an almost continuous increase in temperature until March 24. Later, the absence of visits was recorded on March 25-26, which caused a decrease in temperature by 0.03 degrees (Figure 43B).

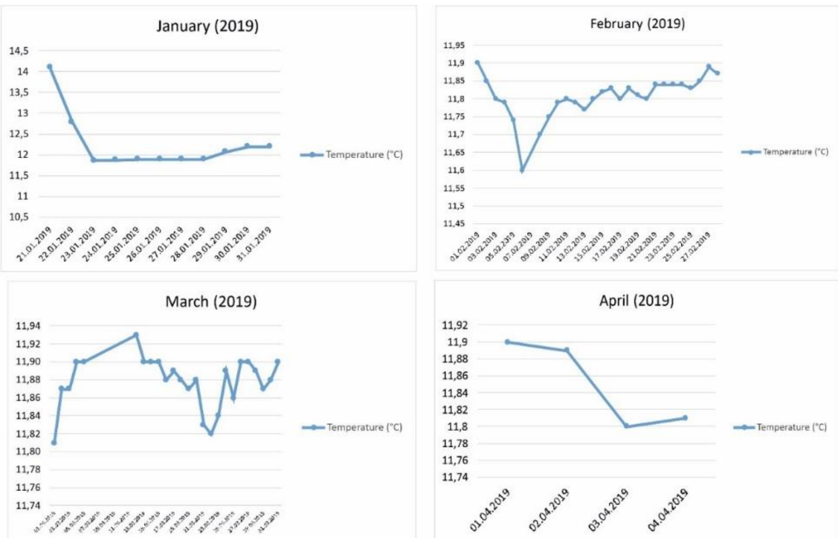


Figure 44 - Variation of temperature measured in Pena Cave (source: CISGAP).

5.3.2. Artificial light

Five sodium "cold" lights (SL) are installed in the cave which are powerful enough for the whole room. One lamp is located at the main entrance to the NW at -32m level with a power of 50 w. Four others are located in the main room of the cave, one at -40 m and three at -70 m depth, all of them with a capacity of 250 w (figure 45). Sodium "cold" lights, compared to other types of lighting, have the particularity of producing little heat and of using bands of the light spectrum whose wavelengths prevent the development of flora (Zelinka, 2002; Olson, 2006). In addition, this type of lighting does not have ultraviolet radiation, which is critically harmful to cave-adapted fauna. Light is the main source of heat in the Pena Cave, and if the lamps are located incorrectly, less than 2-3 meters from the speleothems or cave walls, it is brinks more thermal effect (Constantin, 2021).

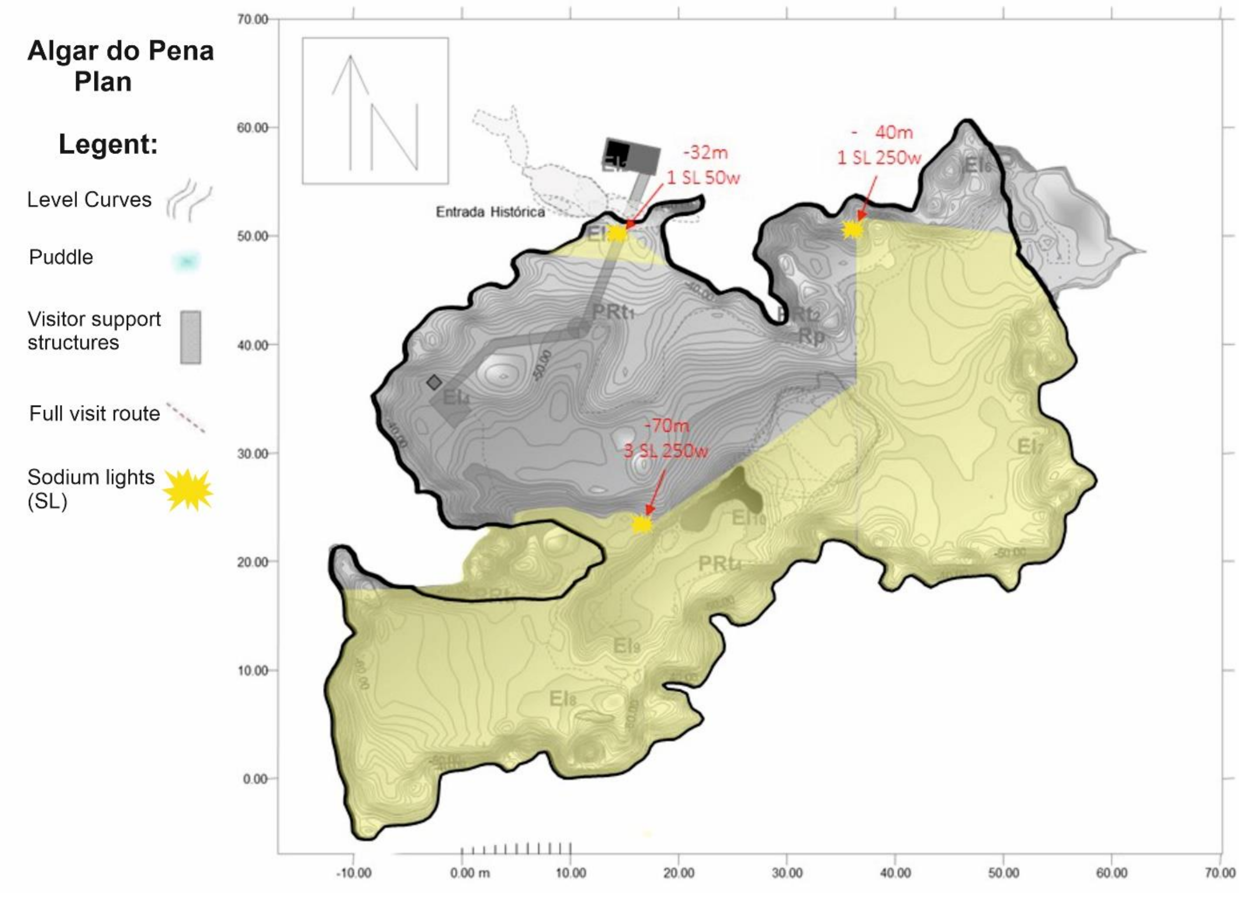


Figure 45 - Lighting distribution in Pena Cave (source: CISGAP).

5.3.3. Biological corrosion

This threat is the most uncontrollable; even the attempts of managers to ask visitors to clean their shoes with a mat soaked with a particular solution are not sufficient to accurately avoid the contamination of the cave with bacteria, pollen, and unwanted plants brought from the surface. Therefore, it is worth considering this type of threat even if there is no direct evidence of possible consequences in

Pena Cave. The integral visit is a high-risk activity in what concerns this threat. In order to determine the current situation, it is necessary to make further studies on rock samples using scanning electron microscopy.

5.3.4. infrastructures

After the installation of the observation platform, it was found that the areas with concentration of troglobionts intersect with the most visited areas in Pena Cave (figure 46b). However, it is worth saying that this is not a coincidence because the concentration of fauna is primarily due to availability of food which is higher near-surface ecological zones, namely in the Transition-zone (figure 46a). Therefore, the installation of infrastructure is usually done in the best location for visitors, most often also in the near-surface zone as it happened in Pena Cave.

It is impossible to change the infrastructure of the cave because it would require substantial financial costs and additional work, which can cause even more harm. The positive side is that the ladder and observation platform are made of stainless steel, held on columns to avoid floor compaction and minimize damage to rocks.

5.3.5. Mechanical damage

Considering that it is allowed to do rock climbing inside Pena Cave even under guides' supervision, the risk of mechanical damage increases areas where visitors do this activity. The cave staff provides clear directions to climbers with a clearly limited territory of movement, which significantly minimizes the physical interaction with the cave walls. In addition, there are also no morphologically representative speleothems along the climbing trail. From the biodiversity point of view, it can be assumed that moving along the cave surface can affect the cave-adapted fauna since it is most often more convenient to move along recesses (caverns), which are a food source, and microecozones for troglobionts (Howarth, 1983, 1993), where it is more convenient for these animals to gain a foothold. However, there are no particular studies on these effects.

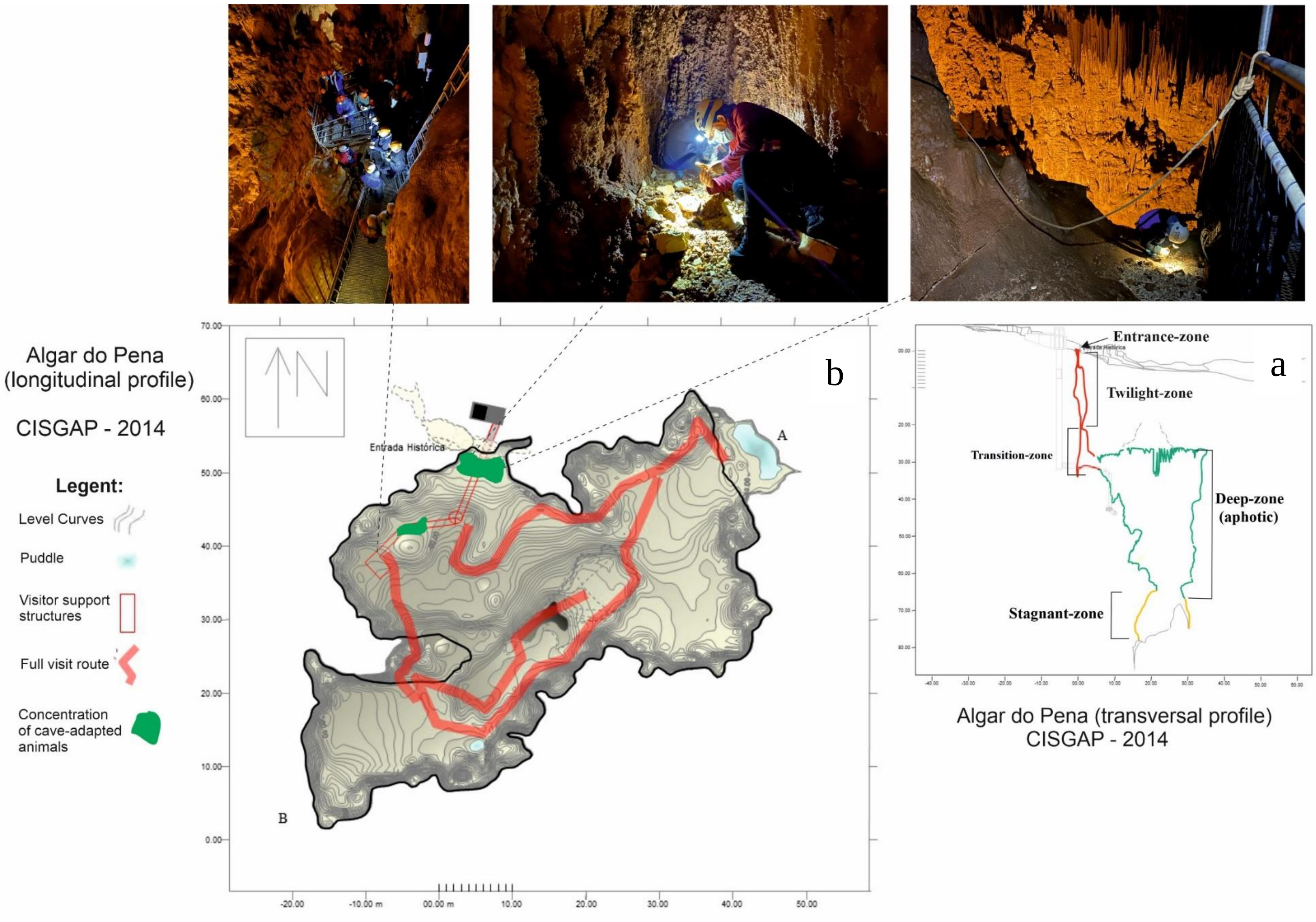


Figure 46 - Longitudinal and transversal profiles with the concentration of cave-adapted fauna and infrastructures.

5.3.6. Mining and quarrying

Quarries are considered the biggest problem for Pena Cave. The number of quarries in the region rises with increasingly intense impacts. On the southern border of Santo Antonio Plateau, the number of quarries that leave deep scars on the landscape is constant. There are not only quarries for the extraction of limestone for ornamental purposes but also quarries for sidewalks, aggregates for civil construction, and for the chemical industry (Carvalho, 2013).

Even though Pena Cave is located inside the Serra d'Aire e Candeeiros Natural Park, intensive mining is carried out in the vicinity of settlements because this extractive activity existed already before the designation of this natural park in the 1970s. Pena Cave, for instance, is located about 300m from a quarry (figure 47). The area faces severe groundwater contamination (Reboleira et al., 2013) due to the generalized lack of proper wastewater treatment (Reboleira et al., 2011). Quarrying is accompanied by explosions and vibrations caused by heavy vehicles.

The main problem for geo- and bioconservation is that quarrying is done under the permission of the PNSAC managers because this limestone mining is crucial for the local and national economy. Hence, special cooperation measures between mining companies and the natural park staff are necessary. Companies should take responsibility for the condition of the cave and reduce/change the work intensity (abandon the explosive extraction method).

Figure 47 - Map with the location of the nearest quarries to Pena Cave (source: Google Earth).

In conclusion, table 12 summarizes all analysed information about ongoing threats in Pena Cave with comments about how these threats affect geo- and biodiversity. For biological corrosion and mechanical damage cases, the possibility of a negative impact is assumed. However, there is no research on exactly how, so there is a question mark in those positions.

Table 12 - Ongoing threats on geo- and biodiversity in Pena Cave.

Threats	**Geodiversity**	**Biodiversity**
Temperature rise	The main reason is lighting, to a lesser extent, release of heat from visitors. Affects reactions directly related to the formation of speleothems.	Known temperature changes pose a possible danger to some troglobiont species. However, a temperature experiment on the species living in Pena Cave is necessary for confirmation.
Biological corrosion	There is a significant possibility that this is happening in Pena Cave but further research is necessary, mainly using SEM.	Unknown influence
Artificial lighting	Indirect effect as a trigger of temperature rise. In Pena Cave the lamps are located incorrectly, less than 2-3 meters from speleothems or cave walls.	
Infrastructures	Caused some damages to rocks and landforms at the time of installation, but does not have a permanent negative impact.	There is an intersection of frequently traversed paths with an area with a high concentration of cave-adapted animals.
Mechanical damage	Minimally affects the surface of the cave due to integral visits programme. However, the risk of damage does not affect the whole cavity.	Unknown influence
Mining and quarrying	Stress on the cave in the form of vibrations, changes in the chemical composition of groundwater, which can negatively affect speleothems, geochemical processes, as well as negatively affect the ecosystem of the cave and fatally affect the cave-adapted community.	

A general risk map on geo- and biodiversity in Pena Cave (figure 48) has been done in order to provide visual information about ongoing threats originating “from the inside". The map is presented in 2D and 3D versions with the same content, it is worth noting that the information on the map does not include the risk areas from mining and quarrying, since there is no proper inventory of geodiversity in combination with the most fragile areas to vibrations and chemical effects. Hence, at the moment it can be determined that the entire cave is in the risk zone concerning mining and quarrying. The lack of inventory also makes it impossible to specify areas where important elements of geodiversity are at risk. Therefore, this map only provides partial information that can help the Pena Cave management.

Most of the threats of showcases are dependent on each other and sometimes trigger each other. Disposing of one problem can be solved, and another, such as changing the type of lighting from an incandescent lamp to cold light, will reduce the temperature influence and the growth of unwanted fauna. Moreover, most prevention methods are effortless and do not require high financial costs, such as carrying out instructions for each visiting group of tourists to prevent mechanical actions or changing the position of lighting lamps at the correct distance to prevent thermal effects on the nearest speleothems.

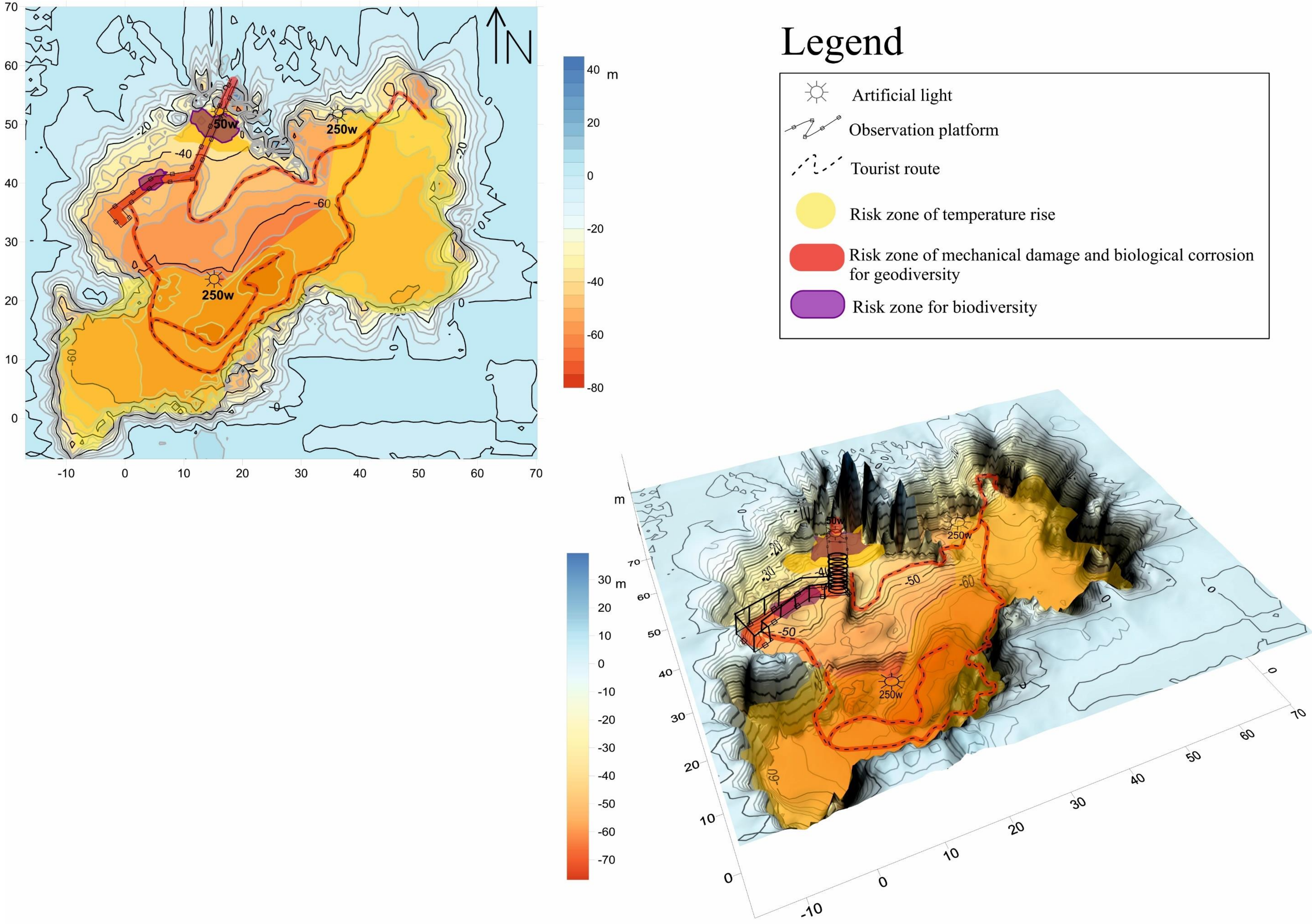


Figure 48 - Map of risk zones of threats to geo- and biodiversity (only a group of threats emanating "from the inside).

6. Developing guidelines on the management and conservation of karst caves

Karst systems, particularly karst caves, have significant geo- and biodiversity values. Therefore, it is essential to adopt a complex management approach in which all relationships between biological and geological elements of a cave are considered. However, in the actual situation, sustainable use of caves is still far from desirable management. For example, show caves require physical modification of natural passageways, lighting, paths, platforms, and related infrastructure. In addition, several chemicals, thermal, and hydrogeological changes affect the cave fauna and geodiversity. In this regard, there is a radical opinion that no conservation actions can preserve the pristine appearance of the cave and that the capacity of karst caves is zero (Osborne, 2019; Gillieson, 2011).

Thus, we are faced with the impossibility of solving problems since the decision to preserve the cave in its original form should be followed by a complete rejection of karst cave use. Moreover, this will lead to even more harmful consequences. Since the refusal to use them for scientific or tourist purposes will lead to uncontrolled caves and their vulnerability to vandalism, mining, urbanization, and agriculture, which will lead to the loss of biological and geological values and those benefits and knowledge that we could get from karst caves (Osborne, 2019). Thus, a well-managed cave usually provides cave protection and a source of income and education for the local economy (Gillieson et al., 2022; ISCA; 2014).

Cave managers should adopt new management paradigms while preserving what are essentially non-renewable resources (Gillieson, 2011) by minimizing as much as possible the damage that a person brings to a karst cave and protected by legal means from external dangers. As a rule, this complex task includes a relative assessment of the vulnerability of different cave elements, taking into account their features and the most significant values.

One of the first attempts to provide a list of recommendations was published by Raleigh Webb in July 1995 (Webb, 1995). These recommendations are called "Minimal impact code for saving" and can be discovered as the simplest axioms in using a karst cave (table 13).

Table 13 – Minimal impact code for caving (Adopted by the Australian Speleological Federation) (Webb, 1995).

1. Remember every caving trip has an impact. Is this trip into this cave necessary? If it is just for recreation, is there another cave that is less vulnerable to damage that can be visited? Make this assessment depending on the purpose of your visit, the size and experience of the proposed party, and if the trip is likely to damage the cave
2. Where possible the party leader should have visited the cave previously and hence should be aware of sensitive features of the cave, the best anchor points, and generally reduce the need for unnecessary exploration
3. Cave slowly. You will see and enjoy more, and there will be less chance of damage to the cave and to yourself. This especially applies when you are tired and exiting a cave
4. If there are beginners on a trip, make sure that they are close to an experienced caver, so that the experienced caver can help then when required, e. g. in difficult sections. Ensure that the party caves at the pace of the slowest caver
5. Keep your party size small – four is a good party size
6. Cave as a team – help each other through the cave. Don’t split up unless impact is reduced by doing so
7. Constantly watch your head placement and that of your party members. Let them know before they are likely to do any damage
8. Keep caving packs as small as possible and don’t use them in sensitive caves or extensions
9. Ensure that party members don’t wander about the cave unnecessarily
10. Stay on all marked or obvious paths. If no paths are marked or none is obvious – define one!
11. Learn to recognise cave deposits or features that may be damaged by walking or crawling on them. Examples are: Drip Holes, Stream Sediments, Paleo soils, Soil Cones, Crusts, Flowstone, Cave Pearls, Asphodilites, Bone materials, Potential Archaeological sites, Cave Fauna, Coffee and Cream, Tree Roots
12. Take care in the placement of hands and feet throughout a cave
13. Wash your caving overalls and boots regularly so that the spread of bacteria and fungi are minimized
14. If a site is obviously being degraded, examine the site carefully to determine if an alternative route is possible. Any alternative route must not cause the same or greater degradation than the currently used route. If an alternative is available suggest the alternative route to the appropriate management authority and report the degradation
15. Carry in-cave marking materials while caving and restore any missing markers. Tape off sensitive areas you believe are being damaged and report the damage to the appropriate management authority
16. If it is necessary to walk on flowstone in a cave remove any muddied boots and or clothing before proceeding or don’t proceed! Sometimes it is better to assess the situation and return at a later date with the appropriate equipment
17. Treat the cave biota with respect, watch out for them, and avoid damaging them and their “traps”, webs, etc. Also avoid directly lighting cave biota if possible

18. If bone material is found on existing or proposed trails it should be moved off the track to a safer location if at all possible. Collection should only be undertaken with appropriate permission
19. If you eat food in a cave ensure that small food fragments are not dropped as this may impact the cave biota. One way is to carry a plastic bag to eat over and catch the food fragments. This can then be folded up and removed from the cave
20. Ensure that all foreign matter is removed from caves. This includes human waste. If long trips are to be made into a cave, ensure that containers for the removal of liquid and solid waste are included on the trip inventory
21. When rigging caves with artificial anchors, e.g., traces, tapes, rope etc., ensure that minimal damage occurs to the anchor site by protecting the site. For example, protect frequently used anchors, e.g. trees, with carpets, packs, cloth, etc. Bolts should only be used where natural anchors are inappropriate
22. Cave softly!

Following Webb, in 1997, the International Union for the Conservation of Nature (IUCN) released the first Guidelines for Cave and Karst Protection to support the growing international need for guidance in cave and karst management. These Guidelines have become the main example of best practice guidelines for cave and karst conservation and has been translated into three languages: English, French and Spanish. The main aspects that this document adhered to concerned (Watson et al., 1997):

1. Introduction and general information about karst and karst caves;
2. The importance of caves and karst;
3. Threats to caves and karst;
4. Opinions in protection of karst;
5. Management;
6. International cooperation and liaison.

At the end of each chapter, the main idea included in the guidelines were summarized in several sentences, in a total of 32 points. The main achievement of this work is the consistency, conciseness, and validity of all points regarding the use, management, and conservation of karst caves.

On November 3, 2014, recommended international guidelines for the development and management of show caves were published by the International Show Caves Association (ISCA), the International Union for the Conservation of Nature (IUCN), and the Union Internationale de Spéléologie (UIS). The book's primary goal was to show how managers and operators can work towards the protection of the environment and socio-economic constraints. Therefore, it must not be a set of formulations similar to laws and regulations. Quite the opposite, it is a very feasible guide where all possible difficulties, financial, cultural, technical, etc. are taken into account. The principles are presented as goals that show that caves can work according to their circumstances and economic opportunities. These and other principles for managing show caves are included in the second edition of the Guidelines for Cave and Karst Protection, recently published and detailed below (Gillieson et al., 2022).

Another important work for developing geoconservation and managing karst caves was carried out by Crofts et al. (2020): Guidelines for geoconservation are protected and preserved areas. This book is dedicated to many different geological aspects in protected areas. Karst and cave areas are no exception. The chapter on "Geoconservation management in selected situations" contains generalized information on landforms, processes and features, threats, and management principles and guidelines. The peculiarity of these guidelines is that they emphasize the link between geo- and biodiversity conservation. Karst systems or karst caves can be a part of protected areas on a different scale from local or regional (e.g., a single karst cave in a reserve or a natural park) to national (if a karst system or cave has a significant scientific interest and is supported by the government) and to international (if those have been defined by the United Nations Educational, Scientific, and Cultural Organization (UNESCO): World Heritage Properties (WHPs), Global Geoparks, Ramsar Sites, and Biosphere Reserves) (Gunn, 2022, Crofts et al., 2020). By Gunn (2022), the situation in last years has been analysed on the assignment of significance to karst caves, where the central message is that in many cases, the level of importance may not be recognized, and there may be no geoconservation measures. Most often, this is due to a higher focus on cultural and biological interest and the lack of a look at the geological side of karst caves (Gunn, 2022). There has been ample evidence of the lack of attention to geodiversity in the works of Crofts et al. (2020), Gunn (2020), and Gunn (2022). Incredibly excellent was the result of Gunn's (2021) analysis of the database of all WHPs with carbonate karst, which showed that half of the sites described, namely 22 out of 49 WHPs, did not contain the words "karst" or "limestone," which emphasizes that this aspect was not fully recognized and that the recognition of WHPs was made on based on cultural or biological reasons (Crofts et al., 2020).

Twenty-five years after the first edition of the Guidelines for Cave and Karst Protection by IUCN, on April 13, 2022, the Union Internationale de Spéléologie (UIS) released the second edition, again in cooperation with IUCN. The new Guidelines for Cave and Karst Protection (Gillieson et al., 2022) combine the writing style and themes of the first Guidelines but added other essential aspects. There are three main chapters. The first is devoted to the Nature of the Karst System and some of its values, which is very similar to the book's first version. The second chapter is dedicated to Human Activities on Karst: Impacts and Mitigation. It is important to note that the second chapter, for the first time, consider an essential point about the management of show caves and offer 12 points of direct recommendations (Gillieson et al., 2022). The second crucial distinguishing feature from the previous version is the new chapter, "Managing karst in protected areas", which followed the example of Crofts et al. (2020) but focused only on karst areas. In total, the work has 76 guidelines, all of which are organized by topic. However, there are no chapters on international cooperation and the Complete List of Guidelines is not yet translated into more than one language, English.

Nowadays, it is the best guide from the point of view of practical application, which should form the basis for the management and preservation of all karst caves and be distributed throughout the world's speleological community.

Despite the work done on managing karst areas and karst caves described before, several difficulties and problems still require more research, dialogues within different structures, and search for compromises. Further, table 14 lists the primary management problems of different levels but are typical for all karst objects.

Table 14 – General management problems on karst caves.

1	The first problem is related to determining the boundary of any karst system due to the significant difference in the boundaries and volume of the karst on the surface and underground (Watson et al., 1997). Also, karst systems can be intermittent. Therefore, this aspect always requires prior study before starting management planning.
2	Large karst areas have high economic value (Watson et al., 1997; Crofts et al., 2020, Gillieson et al., 2022). Therefore, park managers and district administrations will always ask: "What is the priority?" To answer the question, it is necessary to carry out an inventory of karst caves and determine their value and significance, first of all, ecological and scientific, then educational and tourist. After determining the entire karst potential, it is necessary to objectively assess how much the economic benefit of mining overlaps the losses and the destruction that this mining will bring to the karst system. Unfortunately, the capitalist value system often elevates financial gain over other intangible values.
3	The following conflict of interest is that most often, the use of show caves is impossible without solid physical and chemical pressure. However, on the other side of the scale is that the priority is always the person and his safety: "The safety of the visitor and employees must be a fundamental objective of any show cave" (Gillieson et al., 2022). In turn, the provision regarding infrastructure states: "there is a need to provide visitor satisfaction and safety, but the aim should be to minimize alteration or disturbance to the cave's natural environment" (Gillieson et al., 2022). Thus, to have a comfortable and safe environment for tourists, it is necessary to keep low impacts.

	Consequently, managers immediately prepare for the consequences and directions towards minimization, even without the possibility of preventing these consequences.
4	The system of protected areas established by the IUCN includes six protected categories, where caves are mentioned under Category III. Natural monuments are "areas set aside to protect a specific natural monument, which can be a landform, sea mounts, marine cavern, geological features such as a cave, or a living feature such as an ancient grove." However, those caves and karst areas that are present in other categories may not receive the same attention, especially if they make up only a tiny part of the area, for example, one cave in a natural park, of the total protected area and the main goal is to protect objects of other interest. This problem has an international scale (Gillieson et al., 2022).

7. General principles for management optimization and impacts mitigation on karst caves

For caves with a limited set of functions or a limited extent, the entire cave is a logical control unit for most purposes. However, for longer caves, and especially for those that have noticeable internal variability in their values and sensitivity to the impact of visitors, the zoning approach is likely to be more appropriate.

Within the cave, the following approach is recommended for caves and protected areas, as a first step to write a management plan (Gillieson et al., 2022):

1. Undertake an inventory of the cave(s) and locate features of particular interest;

2. Assess the vulnerability of each feature type, i.e., cave passage morphology is generally robust, whereas speleothems and clastic sediments are more likely to be easily damaged;

3. Identify potential uses of the cave, such as for Recreational Caving, Guided Adventure Caving, Exploration and Research;

4. Based on the three previous points, identify zones within the cave that are suitable for particular uses. A simple scheme that can be adopted to suit local factors is to grade passages or cave areas as:

- Low sensitivity. Sections of the cave that are considered strong and capable of withstanding everything except deliberate destruction. They are suitable for any purpose.
- Moderate sensitivity. Areas where there are important elements that can be easily damaged if basic precautions and caution are not followed. These areas are suitable for use with an appropriately qualified supervisor and minimal exposure, but not for an initial caving adventure.
- High sensitivity. Areas with high value, easily damaged objects. The use of these zones should be kept to a minimum, and control measures should be provided to minimize the impact. Amateur cavers may be required to provide a valid reason for requesting access (for example, photographing), and they may be required to visit with a supervisor who has specific knowledge about the cave or its interesting features.
- Extremely Sensitive. A section of the cave that is very valuable, where there is a high risk of damage. These sections should be unavailable, except in exceptional circumstances, i.e., research aimed at understanding a specific feature.

The result could be presented as a table, where zones from high to low priority of management and preservation will be presented. Alternatively, being provided in the form of a map with risk zones for a more visual representation of the situation is very useful when providing data to other organizations. Thus, cave inventory is essential for documenting valuable cave features, allows comparisons between different cave areas, and helps manage classification and zoning (Gillieson, 2011). Moreover, the results can help form a competent budget and have specific requests for it.

A similar approach can be found in Crofts et al. (2020). It is worth noting that this book is not strictly dedicated to the karst area and cave and does not include concern for biodiversity, as in the previous example. This work also focuses on geoheritage = Geosites (*in-situ*) + Geoheritage elements (*ex-situ*), where principal value is scientific (Brilha, 2016). However, this approach can generally find its purpose for any geological object. A summary on management in a protected area and adapted for karst cave caves is given in table 15.

Table 15 - Stages of implementation of management in protected areas.

1. Characterisation of geo- and biodiversity	In the beginning it is necessary to describe the geodiversity including geological (rocks, minerals, fossils), geomorphological (landforms, topography, physical processes), soil, and hydrological features (Gray, 2013) and their values, such as intrinsic, cultural, aesthetic, economic, function, ecological, scientific and education (Crofts et al., 2020). Then characterise the diversity of species living in the research area and their rarity and peculiarities.

2. Inventory of important sites	An inventory and documentation of the site should be carried out in sufficient detail to catalogue and map the exact location of each object within the territory in order to show to protected areas managers what exactly is of interest and where it is located (Crofts et al., 2020). However, before starting the inventory of geological sites, it is essential to answer a few questions: What is the topic (palaeontological, geomorphological, etc.)? Which value (scientific, educational, or touristic)? What is the scale (size of the inventory area)? Moreover, what is the purpose of the inventoried sites (economic support of the area, touristic developments, local geodiversity or an educational programme, etc.) (Brilha, 2016)? For the qualitative assessment of each geosites in the list of potential geosites, based on the following four criteria (Brilha, 2016): representativeness, integrity, rarity, scientific knowledge; The qualitative assessment of each site in the list of potential geodiversity sites, based on the following criteria (Brilha, 2016): (Educational Value): didactic potential, geological diversity, accessibility, safety; (Tourism Value): scenery, interpretative potential, accessibility, safety. If the purpose is to identify sites with scientific value, then it is necessary to follow a strategy of inventory of geosites. However, if the aim is to identify sites with educational, touristic, or cultural values, then it is necessary to use another approach of inventory of geodiversity sites (Brilha, 2016). An example of the inventory implementation approach for a limited area (smaller than 3000–4000 km2) of geosites is illustrated in table 16. Then, more detailed documentation of confirmed sites is built on the initial inventory (Crofts et al., 2020). A geoheritage inventory and management process scheme in protected areas is presented in figure 49. Each site selected during the inventory should be fully characterized with the following details: 1). Name; 2). Location inside the cave (illustrating on the cave map); 3). General characterisation and values; 4). Accessibility; 5). Fragility and vulnerability; 6). An observed condition of the main geodiversity features and processes; 7). Most remarkable features justifying a site; 8). Links with ecological assets; 9). Limitations and restrictions on scientific, education and touristic access and use; 10). Limitations on visitor numbers. A prime requirement for managing and conserving cave fauna and the ecosystems in which they occur is an inventory to permit mapping of the distribution of the fauna in space and time to determine the species richness and assess their ecological status and vulnerability

	(Schneider and Culver, 2004). The inventory can be carried out at different levels of detail, depending on the available resources. However, helpful recommendations can be followed regardless of the inventory level. It can range from a one-time collection setting a minimum baseline level of species occurrence to regular monitoring of the distribution and abundance of cave fauna, which will detect minor changes and take into account the possibility of taking measures to correct adverse circumstances (Humphreys, 2011). Following a fundamental requirement of management to have an inventory of spatial information on the fauna and, as a minimum, to record "what," "where," "when," "how," and "by whom the data is gathered" (Humphreys, 2011) (table 17). The samples included in the inventory become formally described species located in the phylogeny and placed in a historical biogeographic context. Since most samples cannot be identified in the field, it is necessary to organize the collection of samples for long-term storage (Humphreys, 2011). This work can be carried out by institutions that manage and research fauna collections or state museums. Once the fauna of a particular cave is identified, conservation strategies can be supported by monitoring sampling (Venarsky et al., 2007).
3. Identification of threats	In order to determine the priority of management actions, it is necessary to analyse threats and risks associated with various types of human activity and natural changes (Brilha, 2016; Crofts et al., 2020). It is necessary to list all possible threats to both geo- and biodiversity. Some potential threats in karst cave systems were proposed by Pulido-Bosch et al. (1997), Prosser (2006), Gillieson (2011), Osborne (2019), Crofts et al. (2020), etc. These and other works will help formulate and draw attention to threats that may not have been previously identified. Then it is necessary to describe how these risks can affect the degradation of specific sites identified during the inventory. To implement this management step, a quantitative assessment of the degradation risk can be used (Brilha, 2016), which is one of the tasks for the inventory of geosites/geodiversity sites.
4. Monitoring	Monitoring protected areas is essential for establishing the condition and state of objects of interest, whether they are changing and how they are changing (Crofts et al., 2020), and whether the conservation and management tasks assigned to managers are being well done. A cave and karst monitoring programme should include abiotic resources such as water, air, and soil, geological and geomorphological features, and biotic resources such as fauna and flora. However, protected area management agencies often lack sufficient funding to support such a comprehensive assessment programme.

5. Interpretation and promotion	Interpretation is a method of communication that aims to reveal the significance of a protected area's resources rather than convey factual information (Crofts et al., 2020). Interpretation and promotion are the keys to understanding sites, which means understanding their values and ultimately understanding that it needs to be preserved. On-site interpretation can be particularly effective because it can complement the public's first-hand experience of geo- and biodiversity values (Crofts et al., 2020). The goal is to meaningfully correlate the content with the visitor's experience, provoking emotions, reflections, or further study of the subject. Interpretive planning is the initial step in planning and designing sites, and by the time they are ready to receive visitors, interpretation is used to send a message to them. Promoting site, tourism, and education is a tool to attract attention and financial support. Nowadays, there are a large number of ways to promote. For instance, creating websites, advertising, open days and to do a 3D digital visualization, augmented reality (a process that enriches discovery through digital media or provides a virtual reality with which one can engage) (Crofts et al., 2020), and so on depending on the resources.

Table 16 - Sequential tasks for geosite inventory in limited and large areas, taking only into consideration scientific value (SV) (Brilha, 2016).

GEOSITES	
Inventorying limited areas	**Inventorying large areas**
Geological literature review Consulting with experts that have worked in the area	
	Definition of geological frameworks and assignment of the respective scientific coordinators Scientific characterisation of each geological framework Identification of geosites representative of each geological framework
List of potential geosites	List of potential geosites by geological framework
Fieldwork for the identification of new geosites and for the qualitative assessment of each geosite in the list of potential geosites, based on the following four criteria: - representativeness - integrity - rarity - scientific knowledge	
Final list of geosites with complete characterization	Final list of geosites by geological framework with complete characterization
Quantitative assessment of SV	
Quantitative assessment of the degradation risk	
Final geosites list of the area sorted by the SV and degradation risk	Final geosites list of the area by geological framework, sorted by the SV and degradation risk
Eventual quantitative assessment of educational and touristic potential uses	

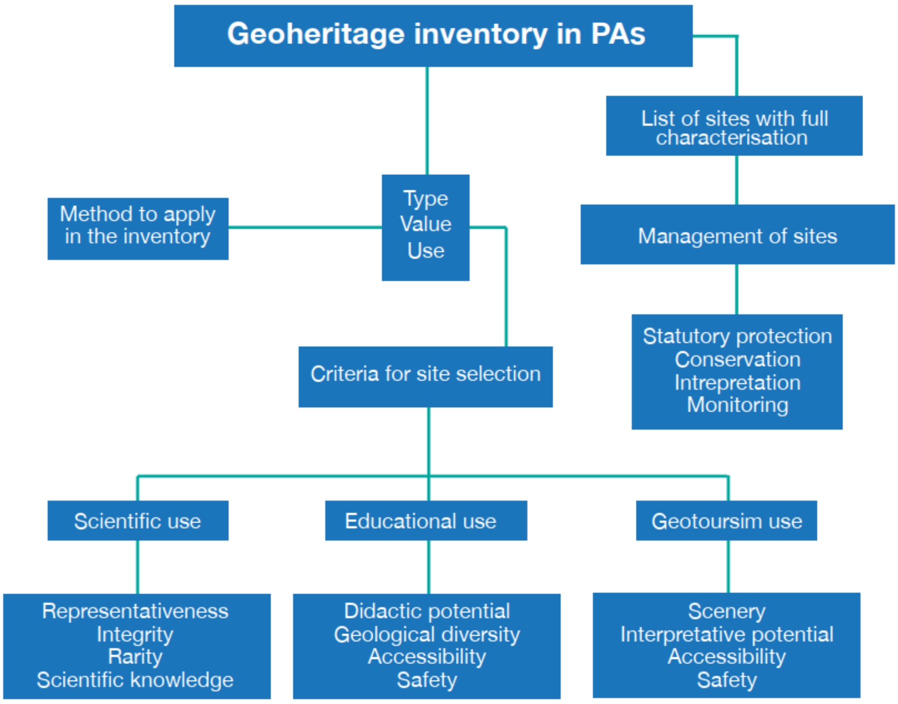


Figure 49 – Geoheritage inventory and management process in protected areas (Crofts et al., 2020) *PAs (Protected Areas).

Table 17 – What, where, when, in what and by whom – guide to make fauna inventories, from basic essential background information progressively to more sophisticated understanding of distribution and variability of fauna (Humphreys, 2011).

Level of information	Basic	Adequate	Good	Extended
What (unknown fauna)	Specimens labelled with data in this column and deposited in collection (collection method)	Named species identified by competent authority	Morphological and molecular material deposited in permanently managed collection	Phylogeny and historical biogeographic context developed
What (known fauna)	Individual identified to species by competent observer	Number of individuals	Life history data: developmental and reproductive stage and dynamics, sex	Behavioural observations
Where	Geographical location and situation	Location in cave, etc., habitat (flowing water, in sediment, gour pool, ……)	Coordinates with grid and method details (e.g., UTM zone ## using GPS on grid ##)	3-D GIS
When	Date	Time	Cross reference to previous samples	
In what	Medium	Physical parameters velocity, depth, specific conductance, pH, Eh, DO, temperature	Basic chemical water quality	Natural and induced variability, contaminants
By whom	Name of collector of specimen or data	Supplementary data deposition		

7.1. Monitoring as a central tool for managing karst caves

Monitoring can be described as a continuous process that controls change in the state of sites or their elements and the possible forecast of their development. This process will tell us if there are weaknesses in management and if some actions are needed to improve its effectiveness (Woo and Worboys, 2019). Methods and approaches of monitoring depend on the objective of management.

As already mentioned, ideally, the cave and karst monitoring programme should be comprehensive and include all geo- and biodiversity and ecosystem elements. However, most often, resources are limited. Therefore, it is necessary to develop a plan to prioritize sites based on their value or significance, vulnerability or fragility and the severity of actual or expected threats or impacts (Woo and Worboys, 2019, Gillieson et al., 2022). Cave monitoring should include the area surrounding the cave since external influences can affect the dynamics of the cave system (Gillieson et al., 2022).

After determining the priority management objects, selecting the appropriate indicators for monitoring is necessary. Monitoring indicators and measurements are selected in such a way as to provide reliable information about the current state of cave and karst resources, which can be compared with the conditions that existed at the time of the start of management, and, ideally, before any anthropogenic changes occurred (Gillieson et al., 2022).

The criteria for selecting indicators include whether they are relevant and scientifically reliable, feasible, have a low measurable impact, and are cost-effective. Indicators and monitoring methods should be selected so that they can be easily understood and performed by trained personnel, where possible, to minimize the need to involve third-party experts or specialists (Gillieson et al., 2022). As a rule, it is better to control an indicator that is simple and cheap to measure on many objects than an indicator so complex and expensive that it can only be used on one or two objects. Frequent monitoring of one key indicator is preferable to periodic monitoring of many indicators (Gillieson et al., 2022). Automated monitoring, if possible, should be a priority, as it minimizes the physical appearance of a person on the site.

7.2. Monitoring indicators in karst caves

Selecting and studying appropriate monitoring indicators are the keys to its success. Woo and Worboys (2019) provide some indicators and monitoring methods for different types of geological objects, including monitoring of caves and karst areas. Monitoring methods include different levels, from monitoring a small object to monitoring large areas. The most acceptable monitoring indicators defined by Woo and Worboys (2019) and Gillieson et al. (2022) are presented in table 18.

Table 18 - Indicators for cave monitoring.

Woo and Worboys (2019)	Gillieson et al. (2022)
General indicators for caves • Physical surface condition • Temperature • Humidity • Partial pressure of CO_2 and radon contents in cave • Lampenflora • Dust input • Vegetation cover **Indicators for karst caves** • Damage of speleothems • Water discharge and quality • Cave fauna	• Water quality and quantity • Vegetation condition • Cave atmosphere • Cave fauna • Speleothems and sediments • Climate change and extreme events

Indicator species for monitoring cave fauna may be troglobionts or stygobionts, which are often endemic and are perhaps the most vulnerable species. Furthermore, species such as bats, swiftlets, and cave crickets are considered indicator species due to their importance in bringing food into the cave that other organisms depend on (Gillieson et al., 2022). In addition, air temperature, rainfall, freshwater temperature, water discharge by rivers and springs, lake level, and groundwater elevation are priorities for responding to climate change and extreme events, and biological and ecological indicators of the last indicator. A change in the timing of phenological events such as leaf budburst and flowering in plants, as well as changes in the timing and range of migrations in animal species such as birds and bats (Gillieson et al., 2022).

7.3. Different methods of monitoring indicators in karst caves

Depending on the indicators, the monitoring method changes, and therefore the level of resources, in the form of money, equipment, physical labour and hiring of specialists, which is necessary to perform their monitoring. For some indicators, the same methods may be suitable, such as for climate change and cave atmosphere, allowing to save resources. However, the methods and approaches are often very different for different cases, and it is necessary to strive for monitoring with the possibility of a long-term perspective.

There are three main types of methods: physical monitoring, 3D scan monitoring, and geophysical monitoring (Woo and Worboys, 2019). These methods are mainly suitable for physical surface conditions, damage of speleothems, climate change, extreme events, and vegetation (lampenflora).

7.3.1. Physical monitoring

Includes all types of photomonitoring, the use of the simplest equipment for measuring changes in cracks, as well as monitoring rockfalls, for which cloth or a mat on possible rockfall sites is used (table 19).

Table 19 - Different types of physical monitoring (Woo and Worboys, 2019).

Photomonitoring	In show caves, carbonate speleothems can be effectively monitored by regular photographing at some vulnerable sites (figure 50). The frequency of photomonitoring may vary, but it is useful to photograph the outcrops after each serious physical impact, for example, a hurricane, tsunami, earthquake or major anthropogenic work.
Monitoring of cracks	Suitable for geological areas prone to cracking. There is a high-resolution sensor for measuring the distance between cracks (figure 51). However, a simple method is to stick a glass plate on the cracks, where the cracks may become wider.
Monitoring of rockfalls	Rockfalls occur in karst caves. A simple method of monitoring rockfalls in caves is to cover possible rockfall sites with a cloth or rug. This is extremely convenient in caves. However, this affects the aesthetics of the cave.

Figure 50 – Photo monitoring of a vulnerable stalactite in Baegnyong Cave, Korea. Note the stalactite damaged by visitor (Woo and Worboys, 2019).

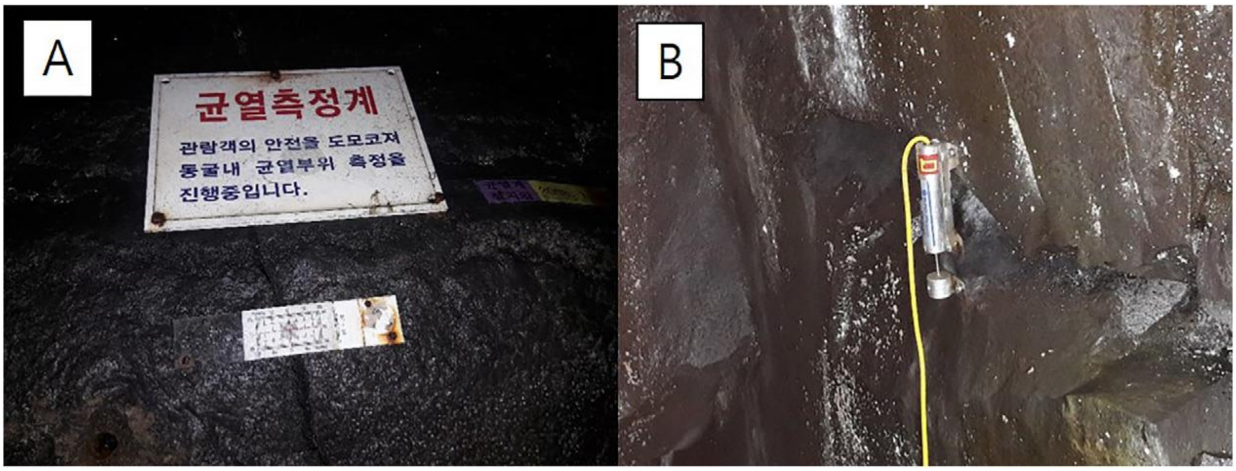


Figure 51 – Crack monitoring using glass plate (A) and monitoring gauge (B) in Manjanggul Cave (lava tube), Jejudo Island, Korea (Woo and Worboys, 2019).

Speleothems and sediments should be selected for photomonitoring based on their special scientific or aesthetic value or in a vulnerable place, for example, next to a hiking trail. Photomonitoring involves photographing selected speleothems or sediments from a fixed position and with fixed camera and lighting settings so that the photos can be accurately reproduced and compared over time to assess the impact of visitors. A one-year photomonitoring interval may be suitable for many exhibition caves (Gillieson et al., 2022).

7.3.2. 3D scan monitoring

The method is suitable for tracking subtle changes in rock outcrops that are subject to physical changes. However, before the correct approach was developed, the method was difficult to apply in caves due to the lack of light, humidity, and inaccessibility of monitoring objects. However, new software developments now allow for reduced field equipment to create accurate 3D models (Tsakiris et al., 2007). For photogrammetry to provide the necessary information, two factors must be considered: (1) installation of uniform and bright light directed at the object. Data mining is necessary to provide such lighting, and (2) compactness and lightness of equipment are necessary since space and accessibility in caves are limited. To reduce the amount of equipment brought into the cave, the authors use trekking poles and speleological lanterns to illuminate the place. Figure 52 shows how information is collected for the model and the result.

Thus, photogrammetry is a methodology developed to use photographs in order to accurately measure the size, location, and context of 3D. It is a relatively simple and inexpensive monitoring method, requiring sufficient illumination and specialists to collect and process data. The operation of this method is possible using Agisoft Meta Shape Professional©. This program has a low threshold for studying and produces relatively good results. In order to collect enough data for the models, a minimum of 30 photos are taken for small, individual objects, and 50 or more photos are taken for more significant monitoring sites (Henderek et al., 2015).

7.3.3 Geophysical monitoring

It includes air photo (satellite) image monitoring, remote sensing, and LIDAR (Light Detection and Ranging) monitoring. These types of monitoring may be expensive, are preferred for large territories, and are also helpful in the case of some places hardly accessible (Woo and Worboys, 2019). Furthermore, it is beneficial for controlling the soil layer over fire systems. How soil is essential for karst processes is described in detail in Gillieson (2021). For cave monitoring, a more suitable LIDAR is a surveying method that measures the distance to a target by illuminating the target with pulsed laser light and measuring the reflected pulses with a sensor (Woo and Worboys, 2019; Gillieson et al., 2022). The primary effect of laser scanning is the point cloud. In this way, LIDAR creates a detailed three-dimensional image of the cave, which can be used as a baseline for detecting changes in speleothems or sediments, as well as other anthropogenic changes in the cave environment (Gillieson et al., 2022). An example of the image of LIDAR results on the surface is shown in figure 53.

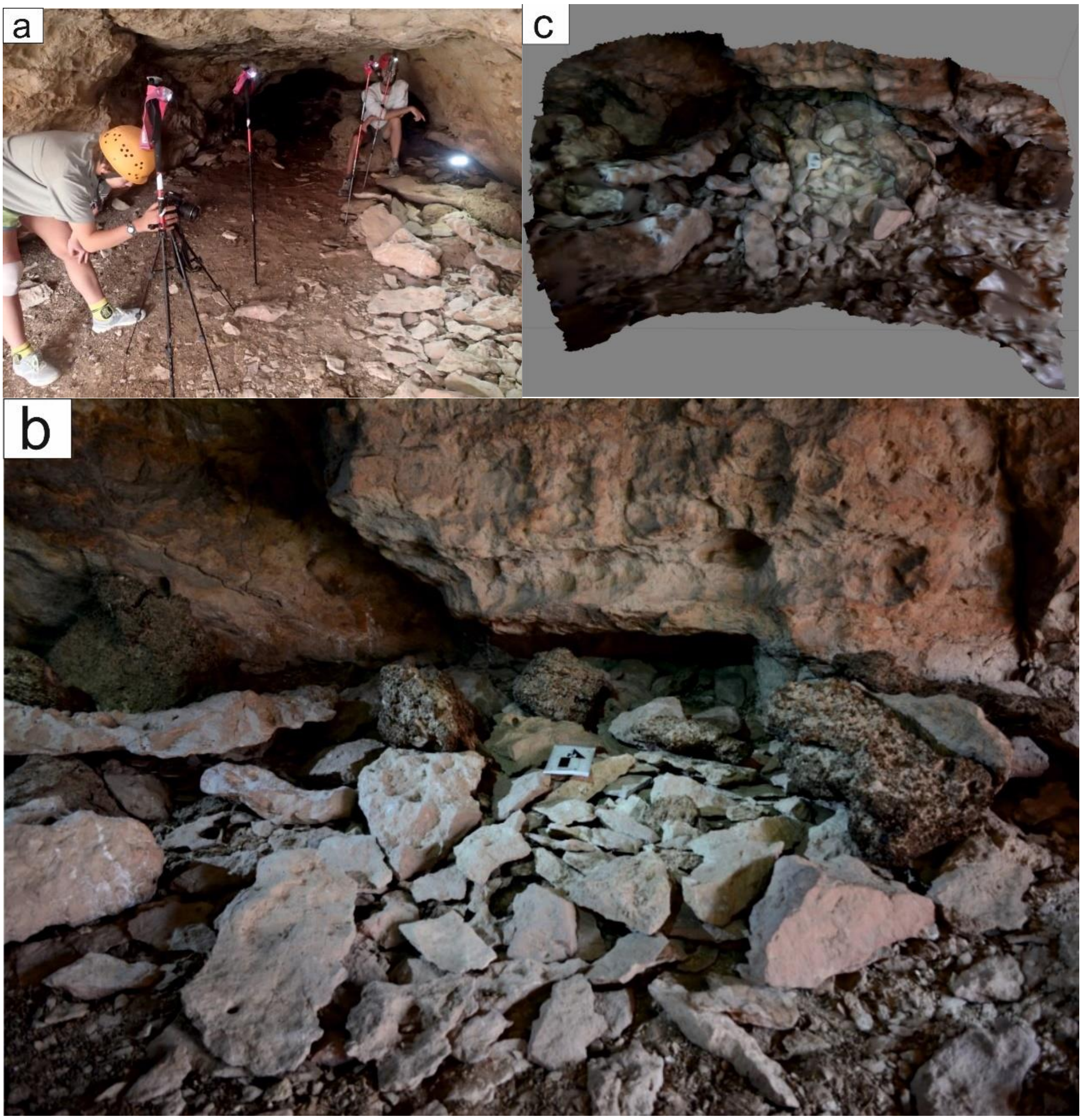


Figure 52 – (a) Image of an entrance area photogrammetry set up at Grand Canyon National Park; (b) photo of a monitoring site; (c) resulting 3D model of this monitoring site (Henderek, Wood and Tobin, 2015).

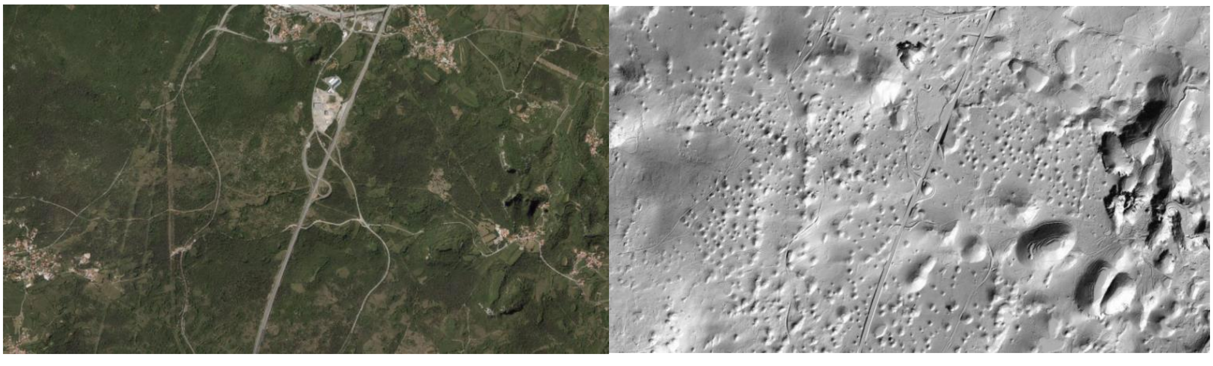


Figure 53 – Aerial (left) and LIDAR (right) images of dolines adjacent to a major highway near Divaca in Slovenia. Reproduced from the Atlas of the Environment, Environmental Agency of the Republic of Slovenia (EARS) (Gillieson, 2022).

With regard to the other monitoring indicators, the methods clearly differ, so the approaches for the remaining indicators will be considered separately for water discharge and quality, vegetation condition, cave atmosphere and cave fauna.

7.3.4. Water discharge and quality

Installation stations for monitoring water in karst caves, stream drains, valleys that provide point recharge (entry points), springs, and wells (exit points) should be used. Continuous monitoring should be carried out (Gillieson et al., 2022).

Relatively inexpensive data loggers are available for continuous measurement of critical parameters, including water depth, temperature, dissolved oxygen, electrical conductivity (a surrogate of total dissolved solids), and turbidity (a surrogate of suspended solids) (Gillieson et al., 2022).

Other parameters, such as nutrients, metals, hydrocarbons, organic pollutants, and bacteria, are more suitable for event-based monitoring since they usually require specialized laboratories to measure them and are expensive (Gillieson et al., 2022). In addition, concentrations are commonly highest during low flow periods, which may present a particular threat to aquatic organisms. However, during rainstorms and floods, the most significant load (concentration multiplied by discharge) of most pollutants and sediments is transported. Another way to obtain information about the state of cave streams and surface waters can be obtained by monitoring biological indicators, such as the number of sensitive species with a low resistance to pollution, for example, macro-invertebrates (insects, worms, snails, crustaceans) or certain fish species.

7.3.5. Vegetation condition

Maintaining and improving the condition of local vegetation is often a priority for karst protected areas because there are several interactions between limestone soils and vegetation. The free vertical drainage of most limestone soils creates unique conditions for evaporation, gas exchange, and root penetration (Gillieson, 2021). The vegetation structure (significantly projected foliage cover) is essential for the interception of rainwater, soil water infiltration, and temperature control in soil and subcutaneous zones. This directly affects the quantity and quality of water available as feed water for speleothem growth in underlying caves—finally, the penetration. Tree roots and the release of complex organic acids and phenolic compounds by them aid the enlargement of bedrock fissures in karst and ensure the high degree of secondary porosity characteristic of limestone terrains (Gillieson, 2021).

There are two main approaches to monitoring the state of on-site vegetation assessment and remote sensing methods. First, forest sizing and carbon accounting techniques on the ground can be easily applied to many sites, and local foresters and landowners can be trained in their application (Gillieson et al., 2022). Several metrics from plant ecology have remotely sensed proxy measures, such as the normalized difference red edge (NDRE) index, which measures photosynthetic activity. Shrub encroachment can also be estimated using persistent green cover measures (Gillieson et al., 2022).

7.3.6. Cave atmosphere

The leading indicators of the cave atmosphere to be monitored are barometric pressure, temperature, humidity, CO_2, airflow, evaporation, and radon (Gillieson et al., 2022). The measurement of radon concentration is commonly required as part of tourist cave health and safety regimes (Osborne, 2019; Gillieson et al., 2022). Climate and atmospheric monitoring in tourist caves are carried out using automatic weather stations with electronic sensors and dataloggers (Gillieson et al., 2022). Stations should preferably be installed in places most exposed to risks.

Temperature: Both accuracy (the degree of correspondence of data with an absolute value) and precision (the degree of agreement among repeated measurements) must be taken into account in the choice of the most suitable instrument (Cigna, 2002). For simple evaluations of the temperature distribution along a cave, a precision of at least 0.1°C can be accepted; the accuracy of the same order is generally enough because the same instrument generally records the data. A better accuracy may be required when data from different sources must be compared - 10^{-2} °C. When it is necessary to investigate rapid temperature changes, the accuracy of the order must be ensured at 10^{-3}°C (Cigna, 2002).

Humidity: Sling or whirling hygrometers used to be considered the simplest methods of measuring humidity, but they are a frequent cause of accidents when they crash (Cigna, 2002). A hygrometer Asmapt is currently used for calibration purposes or point measurements (Cigna, 2002). Humidity sensors, which can be divided into two categories, are now replacing these devices: capacitive sensors and dew point sensors. The former has a problem because when the relative humidity is close to 100% (often in caves), they give incorrect results due to condensation forming over the sensor (Cigna, 2002). Dew point sensors do not suffer from such inconvenience, but their cost is about an order of magnitude higher than the cost of capacitive devices. However, it should be emphasized that the solutions described above are acceptable only when the relative humidity values are in the range not very close to 100%. On the other hand, when it is necessary to distinguish, within 100%, condensation or evaporation conditions, the error affecting the measurements is too large and is no longer acceptable (Cigna, 2002). Therefore, if the humidity is close to 100%, it usually indicates ≈100%.

It is also essential to take this into account. This parameter is often highly dependent on temperature, so if temperature monitoring is carried out constantly and an anomaly is recorded at one moment, it is worth paying attention to the humidity readings.

Concentration of CO_2: There are different sources of CO_2 in the cave: visitors and natural sources (Bourges et al., 1998; Pulido-Bosch et al., 1997; Cigna, 1993; Song et al., 2000). The CO_2 entering the cave in the form of gas released as a result of oxidation in the humic layer above the cave was on the order of several tons per day, while the CO_2 exhaled by tourists over the same period of time was less than 200 kg (Bourges et al., 1998; Castellani, 1988). Before start monitoring CO_2, it is necessary to know its origin. If CO_2 enters the system through water seeping into the cave, part of carbon dioxide will pass from the liquid phase into the atmosphere, and water saturated with respect to $CaCO_3$ precipitates it. If, on the contrary, CO_2 enters the system through the atmosphere, for example, emitted by people in an amount greater than the normal state in a cave, then some carbon dioxide will dissolve in water, which becomes insufficiently saturated and, consequently, aggressive (Cigna, 2002).

Measurements are carried out using the infrared absorption of carbon dioxide. Each manufacturer chooses different solutions to avoid non-CO_2 interference. Sensors with measurement ranges are currently available for any cave environment (Cigna, 2002).

Radon: The measurement of radon content in caves is carried out for scientific research and radiation protection (Cigna, 2002). Point measurements are made using ionization chambers or other radiation detectors. However, other methods are preferred for long-term monitoring, such as etching trace detectors. In this case, the plastic film is exposed only to radon gas, filtering out its decay products, for a suitable time (up to several months, if the radon concentration is not too high). Then the films are extracted, and the traces released by alpha particles are counted. The combination of these two methods is the most convenient: point measurements to obtain an approximate idea of the radon concentration and etching traces to obtain a value averaged over a longer time interval.

These measurements should aim to keep atmospheric conditions as close to the natural baseline values as possible or to allow rapid recovery of conditions to baseline values after visitation.

Cave fauna: Indicator species for monitoring may be troglobionts or troglobionts or stygobionts, which are often endemic species and possibly the most vulnerable (Gillieson et al., 2022). However, "key" species such as bats, swifts, and cave crickets should also be considered as indicator species due to their importance in delivering food to the cave on which other organisms depend. Ideally, the key species selected as indicators should be abundant and widespread in the caves (Gillieson et al., 2022). Considering that periodic food intake for cave dwellers occurs during precipitation, attention should be paid to seasonal monitoring of cave fauna (Gillieson, 2011), as well as to large natural and anthropogenic events occurring on or near the surface of the karst.

There is widespread classification used for assessing the vulnerability of species. IUCN classification is based on the Red Data Book (IUCN 1986) with the following hierarchy of categories:

a. **Extinct**: are those for which there is no reasonable doubt of their extinction.
b. **Extinct in the wild:** are those which survive only in cultivation, in captivity or as a naturalized population well outside their natural range.
c. **Critically endangered**: when it is facing an extremely high risk of extinction in the wild in the immediate future, as evidenced by severe population decline over the last decade, or when its extent of occurrence is less than 100 km^2.
d. **Endangered**: is not critically endangered but is facing a very high risk of extinction in the wild in the near future, evidenced by severe population decline of 50 per cent over the last decade, or when its extent of occurrence is less than 5000km^2.
e. **Vulnerable**: faces a high risk of extinction in the wild in the medium future, evidenced by severe population decline of 50 per cent over the last twenty years, or when its extent of occurrence is less than 20 000 km^2 or its population is fewer than 1000 individuals.
f. **Conservation dependent**: must be the focus of a specific conservation programme, the cessation of which would result in its being reclassified into one of the three higher categories.
g. **Low risk**: are those that are close to qualifying for the above, are abundant or are of less concern.
h. **Data deficient**: are those for which there are inadequate data to make a meaningful evaluation, but may be listed as threatened when more data become available.
i. **Not evaluated**: species that are not evaluated.

The IUCN Red Books provide helpful information about karst fauna (table 20). It gives a good idea of the range of problems faced by cave dwellers and their current status, which can be compared during monitoring.

Thus, this classification can become the basis for the inventory and monitoring species in any cave. However, it is worth remembering that collecting species is a very long and time-consuming job that takes months and a professional approach of biologists. Since the cave fauna has a size from millimetres to the first centimetres, it is usually attached to the bottom of stones, sticks from trees, fragments of speleothems, and other cave debris, as well as in stagnant waters. Species are most often transparent and merge with the surrounding substrate.

Table 20 – Template for inventory and monitoring of cave fauna with examples (Culver 1986; IUCN 1986).

Species	Common name	Known localities	Status	Threatening processes
Myotis sodalis	Social bat	Caves in eastern USA	Vulnerable	Human disturbance
Adeloeosa anops	No-eyed, big-eyed cave spider	Koloa cave and one other lava tube, Kauai	Endangered	Groundwater pollution, withdrawal owing to tourism development

In conclusion, table 21 shows a summary of the main indicators and what management approaches are suitable specifically for them.

Table 21 - Summary of monitoring indicators and suitable monitoring methods.

Monitoring indicators by Woo and Worboys (2019)	**Monitoring indicators** by Gillieson et al. (2022)	**Monitoring methods**
Physical surface condition Damage of speleothems	Speleothems and sediments	Photomonitoring/3D scan monitoring/Geophysical monitoring
Cave fauna	Cave fauna	Indicator species for monitoring may be troglobionts or stygobionts, which are often endemic and possibly the most vulnerable species. In addition, bats, swifts, and cave crickets should also be considered indicator species due to their importance in delivering food to the cave. The IUCN classification employed in the Red Data Book employs a functional approach to assessing the vulnerability of species.
Temperature Humidity Partial pressure of CO_2 Radon	Cave atmosphere	• Each measuring factor has its own characteristics and needs its own measurement approach and equipment. • The choice of accuracy of the equipment for temperature depends on the desired duration and purpose of measurement. • Dew point sensors are the least problematic and most accurate even at 100% humidity, but their cost is an order of magnitude higher than the cost of capacitive devices. • Measurements are carried out using the infrared absorption of carbon dioxide. Sensors with measurement ranges are currently available for any cave environment. • Spot measurements of radon are made using ionization chambers or other radiation detectors.
Lampenflora	-	Photomonitoring/3D scan monitoring
Vegetation cover	Vegetation condition	The two main approaches to monitoring vegetation conditions are on-site assessment and remote sensing. Remote sensing is increasingly employed for monitoring vegetation conditions because it offers broad-scale, automated, and repeatable methods. It is well-suited to detecting changes in vegetation conditions.
-	Climate change and extreme events	Photomonitoring/3D scan monitoring
Water discharge and quality	Water quality and quantity	Better to use water entry and exit monitoring points. The use of loggers for continuous measurement of critical parameters, including water depth, temperature, dissolved oxygen, electrical conductivity, and turbidity, in a particular case laboratory study. As an additional method – the use of biological indicators.

7.4. Carrying capacity in karst caves

The visitor carrying capacity of a show cave is a planning and management tool for establishing the maximum number of visitors that the cave can accommodate on tour or over a given period (Gillieson et al., 2022). Alternatively, other authors define visitor capacity as "that flow of visitors into a defined cave that confines the changes in its main environmental parameters within the natural ranges of their fluctuation" (Cigna, 1987; Cigna and Forti, 1988). This concept helps reach a sustainable compromise between a maximum number of visitors allowed and the protection of the karst ecosystem. Identification of the visitor capacity for every single cave should ideally be derived from a specific monitoring program (lasting not less than one year) (Cigna, 1993), while results obtained in a cave should never be taken as transferable to another site since each cave has its features and characteristics, depending upon a significant number of variables, including size, depth, presence of water and air circulation (Huppert et al., 1993). Further, given the increasing variability of the climatic regime, it would be preferable to adopt monitoring programs for longer timeframes, at least 3–5 years. This will allow controlling the response of the karst system even on the occasion of particular events, from extreme rainfall to drought, to other local situations related to anthropogenic activities.

Physical and chemical parameters should be considered when determining carrying capacity (Gillieson et al., 2022). For physical parameters, factors to consider are the size of the passageways, the distance from the speleothems, whether the infrastructure holds up, and whether guests will enter and exit the cave at different locations, providing a linear flow of visitors or they will enter and exit at the exact location (Gillieson et al., 2022). Considering the chemical parameters of the environment, such as airflow, air quality, temperature, and humidity, a large number of visitors, in some cases, can significantly increase the air temperature and the concentration of carbon dioxide. One person emits thermal energy with a capacity of 80-120 watts (Gillieson et al., 2022). Thus, a group of 50 or 60 people during a cave tour can locally raise the temperature by 1-2°C (Gillieson et al., 2022). The same kind of change concerns the level of humidity and carbon dioxide. The presence of cave fauna, such as bats or cave-adapted species, should also be considered to minimize the impact on those creatures that find their home in the cave (Gillieson et al., 2022). One of the most common ways to reduce the number of visitors is economical. Increasing the cost of visiting the show cave can improve the quality of visitor service and, at the same time, reduce the impact of overcrowding on the environment (Gillieson et al., 2022). In other cases, there is a practice when cave managers contact geologists and biologists of partner universities (if any) and ask for help to calculate the capacity of caves considering all parameters.

8. Pena Cave system of management and suggestions for its optimization

Pena Cave management directly depends on Serras de Aire e Candeeiros Natural Park, where it is located. The category "Natural Park" can be considered between category III (natural monument or feature) and category IV (habitat or species management area) but probably is closer to category VI (protcted area with sustainable use of natural resources), as defined by IUCN and its World Commission on Protected Areas.

Pena Cave is also considered a show cave. The International Show Cave Association (ISCA) defines show cave as a cave that "play important nature tourism role of sustainable economic development, providing jobs, and helping the economy of their regions."[5]

Returning to the method proposed in the 2nd edition of Guidelines for Cave and Karst Protection (Gillieson et al., 2022), it is possible to trace which steps were done in the management of Pena Cave, and which are missing (table 22).

[5] https://www.i-s-c-a.org/

Table 22 - On-going management in Pena Cave.

1. Characterisation of geo- and	There are two excellent books dedicated explicitly to geology, geomorphology, tectonics, and a tiny mention of the biodiversity of Pena Cave: "Geological framework of Pena", Ferreira (2000) and "Caves and Paleoenvironments: Study of structural deformation in Pena", Simão (2015). However, the main idea of these works is not to describe geodiversity and its values. Regarding biodiversity, the most informative document about species in the cave was done by Reboleira and Eusébio (2021) and Reboleira (2022). These works can be considered as an example of how it is necessary to describe all species in the cave with their characteristics as a separate document.
2. Inventory of important sites	The inventory of essential sites should be based on geo- and biodiversity descriptions described above. A methodological approach is needed for a high-quality inventory of the cave, something that has not been done so far which could benefit the cave management plan. It is necessary to know precisely the location of essential sites in order to know where they concern the infrastructure and how often they are under the visitors' influence. A complete photo coverage is also necessary. Considering the biodiversity inventory, the active collaboration of Pena Cave staff and biologists from Lisbon University is assuring this requisite. Thanks to the scientific interest of biologists, collecting species in the cave and working in the laboratory are periodically carried out. This is a reasonable basis for a more formal inventory of the fauna in the cave. It could be reached by following the inventory method provided by Humphreys (2011), which was described in Chapter 6.
3. Identification of threats	The characterization of threats to geo- and biodiversity is also mentioned in various forms in the works of Ferreira (2000) and Simão (2015) and in more detail in the cave managers' unpublished report. In addition, Reboleira and Eusébio (2021) and Reboleira (2022) have described ongoing threats for some species in Pena Cave. However, the ongoing threats to the cave highlighted in chapter 5, such as temperature rise, biological corrosion, artificial lighting, infrastructures, and mechanical damage, were not considered in previous works. Moreover, many of the effects of the highlighted threats are unknown and need additional research and analysis, which will eventually help make a list of management priorities.

4. Monitoring	The main problem of management today is monitoring. Even the cave's most straightforward and basic indicators have not been monitored in recent years: temperature, CO_2, and physical surface condition. The last available temperature and CO_2 data were taken in 2019 and 2000, respectively, as the equipment was damaged due to almost 100% humidity conditions. In general, there is no monitoring plan in the management system because there is no inventory. Therefore, in a general sense, at the moment, it is unclear what exactly should be measured in the first step. In addition, because managers need funding to install new and more modern equipment, it is necessary to justify this by inventorying and evaluating the value of the cave elements—the same problems with monitoring specimens. However, a record is kept of the number of visitors and the time of their stay, and thanks to the help of Coimbra University, the caring capacity of Pena Cave was calculated. However, this does not help much for high-quality monitoring without one-time monitoring of temperature/CO_2/humidity with a tourist visit.

5. Interpretation and promotion	Pena Cave has an interpretation centre (see chapter 2) where high-quality information about the processes of formation of speleothems is provided while the visitor descends the stairs to the main room. In every few meters, visitors can observe the evolution of the growth of stalactites and stalagmites and how they become a column, which is a perfect approach, and this information does not go unnoticed. Also, in one case, electric lighting was installed on the stairs, causing the growth of lampenflora, to demonstrate how improper lighting can negatively affect caves. In order to show examples of the cave biodiversity, there are some transparent boxes with species living in this cave on the observation deck. There are even hand-held magnifying glasses with lamps for a more detailed study of tiny creatures. In conclusion, excellent work has been done on the interpretation of Pena Cave geo- and biodiversity equally. Nevertheless, in terms of promotion, there is work to do. The fact that the carrying capacity of Pena Cave allows to receive 120 people a day, but in reality, the visit never exceeds 60 people, is good for the cave condition. However, it also can mean that people are not interested in visiting or is not aware of the existence of the cave or the place is sparsely populated and difficult to access (which is most likely the reason). A good promotion can play a significant role to increase the number of visits to the cave. The primary source of information about Pena Cave is the website Natural.PT[6] brand where there is a concise description of the cave and a few photos, which is not a competitive promotion in today's realities. In addition, as recent studies in the field of cave tourism motivation have shown, the desire to visit the cave is indirectly influenced on ecosystem inventory (for example, radon and CO_2 levels), as well as a demonstration of safe use, which reduces psychological fears before visiting the cave (Antić et al., 2022). Thus, the attractiveness of the cave for tourists can be increased by demonstrating on the website the high-quality infrastructure installed in the Pena Cave.

[6] https://natural.pt/protected-areas/parque-natural-serras-aire-candeeiros/geosites/penas-shaft?locale=en

8.1. Suggestions for optimize Pena Cave management

Geo- and biodiversity characterisation

1. Based on the available information, describe the geo- and biodiversity of Pena Cave and determine their value in proper way.
2. In this dissertation, it is proposed to use the definition of geodiversity presented by Gray (2013) and the definition of geodiversity values presented in Crofts et al. (2020) as the most common and accepted in the Portuguese geoconservation community.

Inventory

3. Identify the main topic that can be considered in this cave (paleoenvironment, neotectonics events, karst geomorphology and so on). It is necessary to determine the main type of use, such as scientific, educational, and tourist, and the inventoried sites' purpose, for instance, economic support of the area, touristic development, local geodiversity or educational programme purposes.
4. The main values of the cave should be specified. For geology: identify the most spectacular landforms (speleothems) that characterize the process of any karst cave and what is characteristic only of Pena Cave as a low-energy cave. For example, choose the most representative "Popcorn" speleothem as the best proof of low-energy processes in the cave. Highlight areas in the cave that represented chemical (precipitation and dissolution) and physical processes (tectonic movement) and complemented by archaeology and palaeontology values (if there are some).
5. Inventory of all significant geological features, depending on the purpose of the inventory (see paragraph 4).
6. Each selected feature should be fully characterized with the following details: 1) Name and photo; 2) Location inside the cave (illustrating on the cave map); 3) General characterisation and values; 4) Access; 5) Fragility and vulnerability; 6) Integrity; 7) Links with ecological assets; 8) Limitations and restrictions on scientific, education and touristic access and use; 10) Limitations on visitor number.
7. Based on the description of biodiversity, make an inventory of the species living in the cave in the form of a list with a description of their characteristics.
8. Map the distribution of fauna in space and time to determine the species richness and assess their ecological status and vulnerability.
9. Collect the necessary data for the inventory, for each specie it is necessary to answer questions like: What? Where? When? How? Who collected these data?

Threats

10. Complement the list of ongoing threats for both geo- and biodiversity based on the own management experience of this and other caves (see chapter 5).

11. Map ongoing threats to visually provide information about risk zones in order to prioritize management.

12. Move lamps 2-3 meters away from speleothems or cave walls, which reduces the effects of thermal pollution.

13. Replace existing sodium lights (SL) lamps by new generation LEDs with a significantly reduction of heat production and power consumption.

14. Installation of sealed doors at the artificial entrances. This reduces the number of spores and bacteria entering from the outside and will not affect the natural ventilation in the cave.

15. In order to determine the current situation of biological corrosion, it is necessary to analyse rock samples near the touristic trail using scanning electron microscopy.

16. It is necessary to monitor the population trends of species. Measures should be taken to prevent the penetration of wastewater from nearby deposits into the soil and underground habitats and to minimize the negative impact of the quarry on the habitat.

17. It is necessary to take protective measures aimed at the cave fauna considering human activity, sewage infiltration and pollution.

18. Temperature changes are a possible threat for some species of troglobionts, it is necessary to understand the thermal tolerance of the Pena Cave animal community.

Monitoring

19. The basic monitoring of the cave should be carried out in accordance with the monitoring schedule: temperature, CO_2, humidity, physical surface condition, lampenflora, radon and water discharge and quality.

20. Air temperature and CO_2 concentration are the two most important variables to measure. It is necessary to collect data continuously and simultaneously with data on the number of visitors and the time and duration of their stay in the cave.

21. It is necessary to check the instrumental drift because in very humid conditions the equipment may mislead and show incorrect values.

22. After the inventory of features and threats, focus on the indicators that can be followed for each feature.

23. Photomonitoring can be easily used to monitor physical surface condition and damage of speleothems. For a more progressive approach, 3D monitoring using Agisoft Meta Shape Professional can be used. This program has a low threshold for studying and produces fairly good results. Link to a lesson on using Agisoft Meta Shape Professional by Professor Renato Henriques, University of Minho:

a) Calibration of a model - Agisoft Metashape - https://www.youtube.com/watch?v=XjNAsVoNQd4

b) Obtaining DSM and ORTO from imaging VANT Part1 - https://www.youtube.com/watch?v=qvDuMrB28lk

c) Obtaining DSM and ORTO from imaging VANT Part2 - https://www.youtube.com/watch?v=9UbTqi_gNo4

Interpretation and promotion

24. Building a 3D model of the cave will help the interpretation and dissemination (this 3D model could be uploaded to the Natural Park website instead of using ordinary still photos of Pena Cave).

25. A focus on the safety of infrastructure and a safe ecosystem (CO_2 and radon levels) when distributing information about the cave can help to increase the public motivation to visit the cave (this information could be on the website Natural.PT).

26. To save resources and attract public attention to the cave, the above suggestions for inventory and monitoring can be proposed as tasks for students' term papers and dissertations.

27. The carrying capacity of the cave allows to receive more visitors daily without harming the environment. It is worth considering other methods of attracting visitors and disseminating information about the cave to increase public interest in the cave from an educational and tourist point of view, as well as providing economic revenues for the municipality.

9. Conclusions

The different types of uses of karst caves require particular management approaches in order to consider the features and vulnerability of geo and biodiversity elements. This dissertation demonstrates the principles of threat mitigation, the main problems, and modern approaches to cave management. As a result, the knowledge gained has been applied to the case study of the Pena Cave, located at the Estremenho karst massif, Central Portugal.

The information obtained during the literature revision, the compilation of available data about the geo- and biodiversity of Pena Cave and visitation rates, as well as *in situ* observation, made possible the identification of potential and ongoing threats in Pena Cave. As it turned out during the study, some threats need more research to be fully understood. An analysis of the available data on temperature and visits was done and some patterns that have already been previously recorded in other countries were confirmed: with an increase of visitors, the temperature increases, which can lead to a cumulative effect. It was concluded that the temperature change is insignificant and does not have catastrophic consequences for geo- and biodiversity. However, more research is needed to know better about eventual impacts on fauna and flora, complemented with new data on temperature and visitation.

Moreover, a detailed study of the main approaches to manage karst caves was conducted, starting with geo- and biodiversity characteristics, inventory, identification of threats, monitoring and interpretation, and promotion. A strong emphasis in the dissertation was paid to monitoring as a central management tool. All possible methods of monitoring important indicators were given.

Based on all this, the definition of mitigation measures in the form of a list of proposals was compiled to optimize the management of Pena Cave, taking into account the identified threats, current management, and not extensive resources. The biggest problem today is the complete lack of inventory and monitoring of the most basic indicators, which are necessary for managing any geological object and biological element. Therefore, to change the current situation for the better, accessible and easy-to-understand inventory and monitoring methods suitable for this case were prescribed. In addition, the cave could receive more visitors without harming the environment, which underlines the potential of creating conditions to increase the motivation of tourists to visit the cave.

In conclusion, the central values of Pena Cave are landforms (speleothems), chemical and physical processes which are responsible for the cave landforms. The most dangerous threats are temperature rise provoked by artificial light, mining, and quarrying nearby the cave. The priority suggestions for mitigating the threats are to resume monitoring temperature and carbon dioxide simultaneously with monitoring the number of visitors, replacing existing sodium lights with new generation LEDs, and monitoring species' population trends. Finally, the priority suggestion for management optimization of the cave is based on the available approaches to implement inventory in Pena Cave, starting from the inventory of all potential geosites and geodiversity sites depending on the purpose of the inventory.

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