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Published: 29 August 2022
doi: 10.3389/frym.2022.739199

JOURNEY INTO DARKNESS: MICROBES LIVING IN CAVES AND MINES

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YOUNG REVIEWERS

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Microbes live just about everywhere on Earth—on and inside animals, on plants, in soils, and in water. They also thrive in underground environments. Caves and mines are underground ecosystems often visited by humans. Many interesting microbes have evolved to survive in these harsh ecosystems where there often are not many nutrients available. To compete with other microbes for nutrients, some microbes produce antibiotics, which are substances that can kill certain types of bacteria. Antibiotics are commonly used to cure infections and keep people and animals healthy, but we are in need of new antibiotics because some bacteria are becoming resistant to the usual ones. Keeping caves and mines healthy and protecting them from human damage is important so that we can continue to study the microbes that live in these fascinating ecosystems—some of these microbes might make yet-undiscovered antibiotics that can cure human diseases!

CAVES AND MINES: A FASCINATING WORLD

In the book Alice's Adventures in Wonderland, Lewis Carroll writes, "Alice opened the door and found that it led into a small passage, not much larger than a rat-hole: she knelt down and looked along the passage into the loveliest garden you ever saw." Caves and mines could be Alice's loveliest garden. Visiting a cave or mine gives us access to a fascinating world, filled with colorful and strange **mineral formations** hanging from the ceiling (stalactites) or on the ground (stalagmites) (Figures 1A–C). These mineral formations are formed when water seeps through the ceiling of a cave and evaporates very slowly, over thousands of years. The stalactites and stalagmites have the colors of the minerals they are formed from, when the dripping water evaporates.

MINERAL FORMATIONS

Minerals form by sedimentation when lava from volcanoes cools slowly, or by evaporation from water solutions like the stalactites and stalagmites in caves.

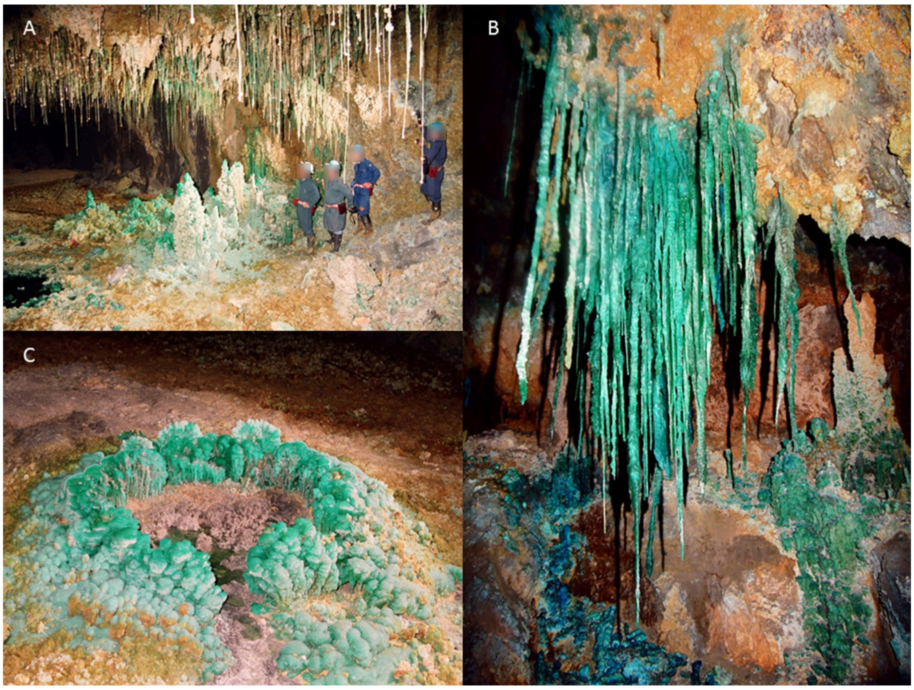

Figure 1

Figure 1

Pozo Alfredo mine, in Riotinto, Spain, is in a mining region that extends into Portugal and is one of the most important sources of iron in the world. Lousal Mine is also in this mining region. Extensive mining in the region dates back from the Roman period. **(A)** Stalactites (ceiling) and stalagmites (floor). **(B)** Green stalactites made of a mineral called hydrated iron sulfate. **(C)** Mineral formations on the floor of the mine. (Photo credits: Manuel Aragon).

Early Stone Age humans used cave walls as canvases for many colorful paintings, illustrating animals like bisons, bulls, and horses (Figure 2). Pablo Picasso visited Altamira Cave, in Spain (Figures 2A,B). He said, "After Altamira, all (art) is decadence." We do not know for sure that this story is true, but it illustrates how amazing Paleolithic art is. The panels decorating Lascaux Cave, in France, are no less impressive (Figures 2C,D). However, there is another aspect of caves and mines that people often do not think about: A microscopic world, filled with **microbes**, which are microscopic organisms that exist everywhere. These cave microbes cover the minerals, the cave paintings, and the walls (Figure 3).

MICROBES/ MICROORGANISMS

Life forms not visible with the naked eye, like bacteria and some species of fungi and algae.

Figure 2

Figure 2

Paleolithic art in caves. **(A,B)** Details of the ceiling in Polychrome Hall, in Altamira Cave, Spain. **(C)** Panel of the Bulls, Lascaux Cave, France. **(D)** Panel of the Black Cow, Lascaux Cave, France. The walls and ceilings of these caves contain a great biodiversity of bacteria, fungi, and microalgae. In the past, dangerous microbial outbreaks affected the paintings [1, 2][1] .

[1] Virtual tours of the caves and paintings can be seen here: `https://www.culturaydeporte.gob.es/mnaltamira/en/cueva-altamira/recorrido-virtual.html` and here: `https://archeologie.culture.fr/lascaux/fr`.

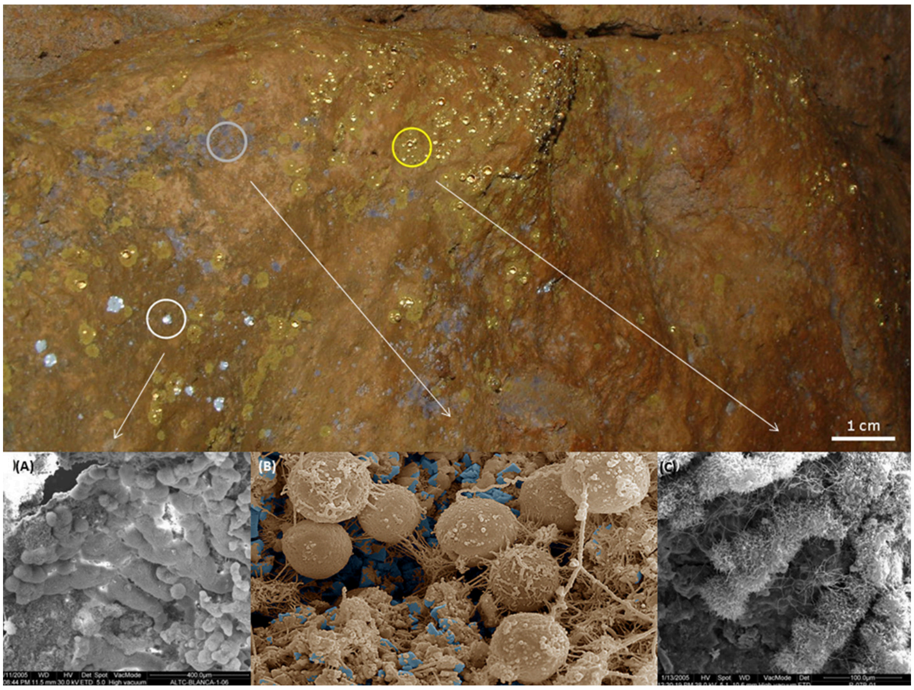

Figure 3

Figure 3

Diverse microbial communities are found on the walls of Altamira Cave, Spain. White, gray, and yellow biofilms are marked by circles. **(A)** Scanning electron microscope (SEM) image of a white biofilm with microbes and spheres made of a mineral called calcium carbonate. **(B)** SEM image (with artificial color) of a gray biofilm composed of bacteria (filaments) and mineral deposits of spherical calcium carbonate and small crystals of calcium carbonate (blue). **(C)** SEM image of a yellow biofilm composed of many different types of bacteria, mainly shaped like filaments.

MICROBIAL COMMUNITIES IN CAVES AND MINES

Microbes are everywhere on Earth. They can be found in all land and water environments, from within hot geysers to below thick Antarctic ice. How can microbial communities—live and even thrive—in caves and mines? In many cases, microbes can share nutrients by growing together in a **biofilm**, which is a complex, multilayered structure formed by various organisms that colonize and attach to cave surfaces (Figure 3). Cave and mine ecosystems have very low amounts of **organic carbon**, which is the usual energy source used by microbes. Most cave microbes that have been studied use a wide range of organic substrates as energy sources, including carbohydrates, amino acids (the building blocks of proteins), and other carbon-containing compounds dissolved in the water that infiltrates the cave through the soil. This dissolved organic carbon can support the growth of microbes.

BIOFILM

A heterogeneous layer of microorganisms that are tightly bound to inert surfaces and grow by using surrounding nutrients.

ORGANIC CARBON

Carbon present in organisms.

Caves that have not been accessed by humans are pristine environments. The input of nutrients into these ecosystems is low and depends, as we just mentioned, on the limited amounts of organic compounds dissolved in the dripping waters. Some of the microbes that live in these caves can also use **inorganic compounds**, like hydrogen, methane, sulfide, ammonia, or iron.

INORGANIC COMPOUNDS

Some microbes obtain energy from inorganic compounds, like hydrogen sulfide, ammonia or hydrogen gas, to produce carbohydrates from carbon dioxide.

On the other hand, caves that are visited by humans (which we will call show caves) are densely populated by microbes that use organic substances for energy. Where do the microbes get these organic substances? First, show caves are connected to the outside world *via* the cave entrance. Microbes from outside the cave can enter through that opening—some are airborne, and some are transported in water. In addition, animals can find shelter inside these open caves. Insects, rodents, and bats, for example, are sources of hair, waste products (pee and poop), and corpses, all of which contribute to a cave's organic carbon. Also, show caves are impacted by human visitors. Visitors entering the cave bring in lots of organic matter (skin cells, hair, clothing fibers, dirt, etc.). This organic material can profoundly alter the food web in the cave ecosystem and promote the growth of those microbes that eat organic matter.

VISITORS AND LIGHT CAN DAMAGE CAVES

Altamira and Lascaux caves, two of the most famous show caves, had to be closed due to the increasing deterioration observed in the cave paintings. The deterioration was a consequence of the large numbers of visitors and the artificial lighting. Light stimulates the growth of biofilms made of certain types of bacteria and algae [1, 2]. Artificial lighting creates a well-known problem in show caves. Extensive areas of the walls, ground, and cave formations become stained green from a green pigment called **chlorophyll** that is made by microbes and algae that use light for energy (like plants do). This green staining is quite different from the green color observed in mines due to the green minerals seen in Figure 1.

CHLOROPHYLL

A green pigment present in plants, algae, and some bacteria that captures the energy of the sun for photosynthesis.

If you look closely at the walls of a show cave, you can see tiny spots of different colors (Figure 3). The spots are biofilms with several kinds of microbes living together and interacting in various ways.

HELPFUL AND DANGEROUS MICROBES

ANTIBIOTICS

A drug that specifically kills bacteria and is used to treat bacterial infections.

According to the World Health Organization, there is a severe lack of new **antibiotics**, which are needed to combat the growing threat of bacteria that are resistant to the usual antibiotics. Therefore, the search for new antibiotics, which can be produced by certain bacteria to kill off their competition, is extremely important [3]. The bacterial genus *Streptomyces* has more than 800 species. From 1940 to 1980, *Streptomyces* species have been a significant source of the antibiotics used by doctors. In fact, about two-thirds of all known antibiotics (streptomycin and tetracycline, for example) were mainly produced by species of *Streptomyces* isolated from soils. But, unfortunately, no new antibiotics have been identified in soil bacteria recently [3].

Researchers are exploring ecosystems other than soils to find novel antibiotics. Scientists are researching little-explored places on Earth with very high biodiversity: the caves and mines below the Earth's surface. There are many unknown bacteria and fungi to be found in these ecosystems, and some of them could potentially produce new antibiotics [1–4]. These bacteria might include unstudied species of *Streptomyces*, which are abundant in caves and mines, as well as other rare microbes [5].

Hundreds of bacteria have been isolated from caves and grown in the research lab to see if they produce antibiotics. Bacteria obtained from mines in Southern Portugal and Spain were also studied. Altamira Cave and volcanic caves provided the highest number of bacteria that could produce antibiotics, and the mines also had high percentages of antibiotic-producing bacteria. On the other hand, the bacteria isolated from marine caves in Algarve, Portugal, were almost inactive. All caves and mines can be different in terms of the microbes they harbor, so we need to explore as many of these underground ecosystems as possible. Once new antibiotic-producing bacteria have been isolated, the next step is for scientists to decipher the chemical structure of the antibiotics and study the genes that allow the bacteria to produce these compounds.

Unfortunately, not only helpful, antibiotic-producing microbes inhabit caves and mines. Two novel bacterial species discovered in Altamira Cave can infect people and cause disease [5]. So, sometimes, visiting underground environments can be risky for our health.

WHY SHOULD WE CARE ABOUT CAVES AND MINES?

Caves and mines are reservoirs of both beneficial and dangerous microbes. However, nature provides us with beneficial, antibiotic-producing bacteria to combat dangerous, disease-causing bacteria. Yes, caves and mines are fascinating, subterranean worlds that many people like to visit. But we should be aware that entering these environments can pose a risk both to the microbes living there and to human health. The hard work and creativity of scientists can help us to protect these underground ecosystems and the antibiotic-producing microbes that live there. We must continually try to strike a balance between visiting and exploring caves and mines and keeping those unique ecosystems safe from human-caused damage, so that we can continue to find new types of beneficial, antibiotic-producing bacteria that will help keep people healthy into the future.

FUNDING

Financial support for research in caves and mines was obtained through project 0483_PROBIOMA_5_E, co-financed by the European Regional Development Fund within the framework of the Interreg V-A Spain-Portugal program (POCTEP) 2014–2020.

ACKNOWLEDGMENTS

This article is dedicated to Paula Saiz Esnaola, a curious mind whose interest in biology and geology motivated this paper. Data from Irene Dominguez-Moãino and Jose Luis Gonzalez-Pimentel are acknowledged. I thank Diana E. Northup, Jennifer J. Marshall Hathaway, and Sonia Balasch and her students for helpful comments on an earlier version of the manuscript, and Manuel Aragón, Nerva, Spain, for the photos of Figure 1 and the permission to use it in a scientific article. The author acknowledges CSIC Interdisciplinary Thematic Platform Open Heritage: Research and Society (PTI-PAIS) for the professional support.

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SUBMITTED: 10 July 2021; **ACCEPTED:** 15 July 2022;
PUBLISHED ONLINE: 29 August 2022.

EDITOR: John T. Van Stan, Cleveland State University, United States

SCIENCE MENTORS: Dalaq Aiysha and David Hiller

CITATION: Saiz-Jimenez C (2022) Journey Into Darkness: Microbes Living in Caves and Mines. Front. Young Minds 10:739199. doi: 10.3389/frym.2022.739199

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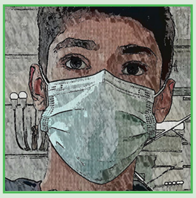
YOUNG REVIEWERS

JOHNSON ELEMENTARY SCHOOL, AGES: 9–10
We are three fourth grade classes, excited about all things science. We live in Natick, Massachusetts and enjoyed being a part of the scientific review process. We learned about how a research paper develops and that science is more than experiments. Science involves research, review, and reflection, too.



MUHAMMAD, AGE: 13
Hi, I am Muhammad and my curiosity for science originated when I secured first place in my grade 3 science project. It was about photosynthesis, which I chose after knowing the fact that plants are universal food makers. The science textbook of every grade always familiarized me about the magical wonders behind my daily life's surroundings.

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