Review article

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Human Activities' Impacts on Cave Microbial Diversity: Perspectives for Cave Microbial Diversity Conservation

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ABSTRACT

Microorganisms are distributed everywhere even in extreme environments such as caves. The underground surfaces are minerals rich and the life in there is found to be related to both biotic and abiotic factors. Because the cycle of these minerals is insured by the chemolithotrophs living in there. In addition, caves are considered to be important reservoirs of bioactive compounds. However, caves are entered for different reasons. Some of the cavers are of scientific research, some of them are for recreational reasons like sports and simple cave visits. All these activities are thought to impacts the visible mat and invisible colonies of microorganisms through mechanical force or importing exogenous microorganisms. This study summarizes the different impacts which can be caused by the different human activities in caves. Finally, perspectives of the conservation of the cave microbial structure are suggested for the further uses of caves.

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Introduction

Caves are natural underground surfaces formed under geological, chemical, and biological factors. They are classified in different ways among them the type of rocks and their dissolution ways for the karst caves [1]. Characterized by extreme conditions accounting for high humidity, low stable temperature, restricted nutrients, darkness, and low oxygen, these natural environments contain numerous extreme organisms understudying [2]. Studying cave microbiological characteristics is a challenge for microbiologists because of the difficulties in the accession of the ecosystem, the living conditions which are difficult to be adapted in a laboratory environment as well as the multiple types of caves in the world. Nevertheless, they

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represent an important microbiological studying area, principally due to the adaptation of such microorganisms to the limited resources like light and others.

Caves are used for different objectives such as tourism, sport, scientific investigations, and others. Some caves are epigenic (opened to the earth's surface) and animals can have the opportunities to enter them, water from the rain or other sources can also drip on them. All these activities can impact the cave ecosystems from their abiotic and biotic factors [3]. However, it is also suggested that a limited human interference does not cause an important change in the cave structure and inhabitant populations [2].

Early before 1997, microbiologists started to study microbiology in cave environments [4]. Before the development of molecular dependent methods, these studies were multiplying slowly and only a few microorganisms were identified. After 1997, the apparition of molecular-based methods has boosted cave microbiology and numerous cave microbial importance are identified [4]. Studies found that cave ecosystems could be large reservoirs of drugs, enzymes, fertilizers as well as several human welfare compounds [5]. Therefore, the conservation of cave structure is a challenge that should be tried.

Understanding the cave activities and their impacts on the cave structure and the life in a cave is the main aim of this study. This study provides an overview of the caving impacts on cave ecosystems especially the microbial structure of caves. It also highlights some of the cave conservation protocols and gives further perspectives for cave protection. This review should be of interest mainly to scientists of different fields such as microbiologists, geologists, engineers, and ecologists. Besides, cavers, and institutions/ministries in charge of country environment protection and tourism, are also concerned.

Cave Microorganisms Inside and Outside the Cave Environments

Caves can be defined as natural openings below the earth's surface that extend beyond the twilight zone and large enough to admit a human being [1, 6]. Cave formations can be through mechanical force, differential erosion and scour, volcanic eruption, rock dissolution, or glacier melting [1]. It is reported that the most types of caves are those forming from calcareous rock dissolution and lava tubes. In caves, secondary mineral deposits formed from a primary mineral by a physicochemical reaction are known as speleothems; one speleothem

can be composed of different minerals or unconsolidated materials [6]. Behind these speleogenesis processes (natural cave formation processes), diverse microorganisms are thought to contribute to the formation and development of caves by different redox reactions even if some of the identified cave microorganisms are still under investigation for their active role in a cave. Microorganisms play an important role in dissolution and precipitation processes which result in the deposition of speleothems and other cave fabrics. They, by their metabolisms, participate in the redox reactions taken inside the caves during the formation of caves and cave structures.

Precipitation of carbonate is well observed in the formation of most caves and cave structures speleothems [6]. This process was thought to be only of abiotic origins. However, the *in vitro* studied through different karsts revealed the biogenic role played by microorganisms [7, 8]. The precipitation of carbonate is mainly depending on the pH of the environment; microorganisms can modify the pH of the living environment through their metabolic activities [8, 9]. This is due to the affinity for calcium/magnesium ions present in the involved microorganisms. Microorganisms take energy from the nitrogenated organic materials, and the adsorption of Ca^{+2} ions together with the production of CO_2 and NH_4^+ ions are involved with an increase of the pH. The supersaturation of carbonates on the cell or hyphae surface induces their precipitation [6, 8]. Bacteria identified in invitro studies from cave walls and speleothems that involve such precipitation are members of *Proteobacteria* such as *Pseudomonas sp.* and *Acinetobacter*, *Actinobacteria* such as *Streptomyces* and *Kocuria sp.*, and *Firmicutes* such as *Sporosarcina sp.* and *Bacillus sp.* [7, 8, 10, 11]. Precipitation of carbonate is observed in cave walls like in Altamira and Tito Bustillo caves in Spain, in speleothems including moonmilk deposits in Cervo Cave, Italy, and the stalactite formation in Botovskaya Cave in Siberia [8, 9, 12, 13]. Other bacteria and microorganisms were isolated or identified through metagenomic analyses from such carbonate precipitation cave formation areas are still under investigation for their role in these environments.

Cave and cave structures can also be formed or be under mineral dissolution processes. Limestone, dolomite, and gypsum rocks can be dissolved by the action of underground water or the stream dripping on rocks. The weak sulfuric acid, which is one of the most implicated acids in speleogenesis, has been reported to cause the dissolution of Limestone and

precipitation of gypsum which is more soluble in water [6]. The sulfuric acid formation is thought to be originated from sulfur-oxidizing bacteria present in the sulfur-rich cave environment [14, 15]. The mechanism of this process in the cave bacteria still under investigation but studies state that the sulfur-oxidizing bacteria like *Thiobacillus spp.* and *Thiothrix* both members of *Proteobacteria*, isolated from different caves such as the Lower Kane Cave, can oxidize H_2S completely to sulfate during their metabolic activities [6, 16]. The dominance of bacterial group belonging to *Epsilonproteobacteria* in the Lower Cave in Wyoming was observed [16]. The authors added that even these bacteria remained unculturable, other culturable bacteria belonging to this class were characterized by sulfur/sulfide oxidation. Other studies of the chemical reactions in caves that involve microorganisms, also include iron-oxides, nitrification, ammonification and, manganese oxides [6,17,18]. All these precipitation/dissolution processes involve primary the chemolithotroph bacteria, fungi, and sometimes archaea from different phylum and species, acting in a biofilm structure [12, 15, 18].

Microbiologists are most attracted by the adaptation of microorganisms in the extreme environmental conditions of the caves. Different studies revealed the application and the possibility of the application of cave microorganisms for human welfare. Antimicrobial activities are observed in bacteria and fungi isolated from different caves. The antimicrobial substances which can be isolated from these microorganisms, far from the human impacts, could be favorable in the actual drug-resistant world problem. The most antimicrobial compound producers are *Streptomyces* sp., belonging to the phylum *Actinobacteria* [5, 20]. These bacterial members are mainly studied in caves and favorable results were obtained [20, 21, 22, 23]. Peptide A12-C, cervimycins A-D, undecyprodigiosin, and xiakemycin A are some of known antimicrobial and anticancer active products isolated respectively from bacterial strains from Cueva de Los Murciélagos in Spain, Grotta dei Cervi in Italy, Miroc Mountain Cave in Serbia, and a karst cave in Chongqing city in China [21]. Besides the medicine potentials, cave microorganisms were observed with a construction capacity. As previously seen, some bacteria from cave walls and speleothems are calcifying organisms. These microorganisms were determined to be used in the construction of bio-concretes, crack healing in concretes, and soil bioremediation. In addition, diverse enzyme activities revealed

in cave isolates can serve industrial uses with low energy spending [11, 22, 24, 25]. Wastes bioremediation potentials were detected also in cave isolates [5, 25].

Cave Activities (Caving)

We can define caving as the entrance to cave environments for different reasons. Studies show that even in the Paleolithic period, humans have used these environments [21]. They were used for different uses such as housing, obtention of minerals, protection against weather phenomena, and interment [21]. Using the cave as burial chambers was a common human practice over the world. The Easter Island Anthropological Expedition revealed the uses of the caves located on the south coast of this island as human burial chambers [26]. Another study in the Adıyaman province of Turkey shown the presence of tombs as a part of the complex cave of Necropolis [27]. Authors have also found some proofs shown the use of this system of caves as a storehouse and other economic tasks. In Africa, where caves have been considered sacred places, it is found that ritual and religious practices were common in these underground surfaces. In Zanzibar island, the Kumbi limestone cave are one of the most destroyed natural environments. Human skeletons were found inside that cave and is the proof that cave was also used as a human burial chamber in this part of Tanzania [28]. On other hand, studies revealed that caves were thought to be used for treasure hiding by pirates and others [29]. Thereby, caves were the targets of treasure hunting. It is the case of the Yarımburgaz Caves in Turkey which had inhabiting by the people of 'Homo Erectus' 400 thousand years ago [29, 30]. Similarly, the Ayub Cave in the Philippines was severely damaged by illegal diggers who were treasure hunters [31].

Nowadays, caving or cave activities are improved since cavers are for different reasons. However, even those named as vocational cavers, who enter caves for their professional tasks, all cave activities can turn to recreational caving [32]. Depending on the caving distance and caving reasons, cavers can spend some hours or days inside caves. Therefore, the installation of some camps inside caves can be imperative for cavers. It is revealed that cavers started to establish camps inside caves or near the entrance of the cave in the early of 40s by US cavers the National Speleological Society, Inc. [33]. In general, as deeper, they go camping remains inevitable. Because after exhaustion of cavers, it is difficult to return

upstairs the same day. Sometimes, as the deeper they go, they should avoid handling heavy bags, so they should have a camping site to leave somethings like exceed foods and drinks [33]. However, for the conservation of cave natural quality and avoid cave diversity disturbance, cavers should be careful in front of the camping site as well as the hole cave.

The natural formations of the subterrestrial surface contribute to the history of different regions. Even if their environments are considered as magics and attractive, caves remain hardly accessible by most scientists. For mapping caves and providing more information about them, cavers enter with high curiosity to discover the inside magical world of these underground openings. The features of caves provided by cavers can facilitate to answering questions and solving problems in archaeology, geology, geomicrobiology, paleontology, hydrology, mineralogy, etc. [34].

Geochemists take advantage of cave rocks to investigate and enlighten in the geochemical structure of the regions. As previously seen, caves are formed by the combination of geological processes and other abiotic and biotic factors. Studying a cave can answer many questions, for example, the probable future geologic process in a region. In addition, the chemical aspects of a cave rock define the mineral sources founding in that cave. For the history of a region, paleontologists and archeologists can use caves where they found ancient used materials or human/animal skeletons [35]. On the other side, microbiologists and biologists are interesting in the adaptation of organisms in such extreme environments, explore to understand the different metabolisms undergoing these habitats. Therefore, the redox reactions that take place in caves, by microorganisms, are thought to contribute to the development of substances that could be used in different fields including clinical, pharmaceutical, bioremediation, food bioprocessing, and water sanitization [20, 36]. For this context, microbiological investigations in caves are multiplied over the world.

For economic uses, some caves are also opened for tourism. Cave tourism constitutes the main geological tourism in the world [37]. In many countries, the show caves are among their main economic revenues. We can enumerate the Rouffignac Caves in France opened in 1959 and visited by up to 500 visitors per day [38]. Further, more than 250,000 of visitors were reported to enter the Gongo Cave, located in the Pacitan regency in Indonesia, per year which contributes to more than 200,000 US$ per year in this regency economy [39]. Most of

the cave tourists are attracted by the natural aspect of caves. They are curious to discover new natural areas, especially geological formations. However, for further tourism attractions and facility of cave accession, humans make some improvements such as the addition of artificial light of different waves and construction of artificial walking paths. Additionally, the tourism feature of the cave can be for health rather than for recreation. It is reported that 8.5% of the Ballıca Cave (Turkey) tourists are of health tasks including asthma treatments [37].

Cave sports activities are of different levels depending on the experience of the cavers. They are generally considered extreme sports because of the dangerous aspects of some caves [49]. Cave diving is generally practiced in active caves that are filled with water. Some show caves consist of diving practices like the Dordogne valley in France [50]. However, cave diving expeditions could be for research goals: an exploration of new underground caves as well as studying these environments in different scientific fields. Divers sampled visible colonies of *Thiothrix spp*, identified in six of eight underwater caves in Florida for microbiological studies of these caves which revealed that this sulfide oxidizing mixotrophic bacteria play a role in cave formation [40].

It is reported that industrial uses in caves even if they are occasionally [41]. However, this field, which is not developed, consists of specialized forms of agriculture including mushroom farming, fish breeding, and cheese production.

Are Cave Activities Impact Cave Microbial Diversity?

Caves and Earth's surface lands are quite different in their physicochemical characteristics. The impacts of caving on the cave ecosystem depend on the type of cave. As previously seen, speleogenesis is from different processes therefore some caves are constituted with strong rocks and typically dried. Some others are fragile, and present rivers or stream waters can drip on there. Further, the diversity of microorganisms depends on the abiotic factor of their environments [4, 42]. Here, we adopt the hypothesis which stands that each cave is unique, and speleology should be specific for a cave. Although the impacts of caving cannot be generalized, the probable cave damages especially cave microbial diversity threatening are illustrated by taking all the perspective of human activities in caves and cave types.

Limit of organic matter, stable temperature, high humidity, and low or total darkness are the best environmental conditions for the biodiversity of these ecosystems. However, diverse external factors can affect these sensitive conditions thereby impacts on the life of the biogenic part of caves as it demonstrated by [38]. Microorganisms are among the main cave biodiversity and they can appear as visible mats as well as invisible biofilms on cave walls, sediments, and pools [4]. Humans during their cave visits can bring to the cave nonnative microorganisms that can compete for the indigenous ones in such oligotrophic environments. They can introduce these microorganisms through their dresses, shoes, or by touching cave structures with their body or septic materials. Draws and human finger marks were reported respectively on Azorean lava tubes and Yarımburgaz Cave [30, 43]. The authors stand that a limited microbial development was observed in these areas in two decades [43]. This might be caused to the mechanical movement which leads to the removal of the native colonized microbes while the imported microorganisms did not survive under the extreme conditions of the cave.

It is suggested that the *ex-situ* microorganisms could not persist in such an environment. It is also shown that the endemic species can mitigate the introduced organic matters through bioremediation [44]. However, cavers can leave organic matters like hair, nails, the rest of their foods, or their excreta inside caves. These organic inputs can only be favorable to exotic microorganisms. The study of human contamination in Lechuguilla Cave was run, and authors found that *Staphylococcus aureus,* which is a human skin flora, *E. coli* which is a normal human intestinal flora, and high temperature *Bacillus* which are found in soils heated by sunlight, are increased during highly visit levels and reduced when the same areas are at rest from visit [45].

Besides that, lamps used by cavers during cave explorations or other activities are unfavorable for the cave living organisms, since caves are divided into four zones where the light is limited or completely absent. At the cave entrance, still there is light since the surface and underground environments meet on there. The light progressively diminishes to zero at the twilight zones and it is absent on the transition zones. The last zone consists of a completely dark zone where the relative humidity is high, and the temperature is nearly stable continuously [41]. Light providing by cavers together with the high humidity of the cave

environment will promote the development of phototrophs like algae which are generally grow at the limit of light penetration [41]. The development of such organisms affects not only the cave microorganisms but also the cave and speleothems that are forming by the interaction of native microbes and cave minerals.

The uncontrolled visit of humans in a cave or a long camp period inside caves can induce an increment of carbon dioxide level inside these environments. It is suggested that the high level of CO_2 detected in show caves, where the bat population is limited, is due to the huge number of visitors that receive those caves [39]. It is stated that the high concentration of CO_2 can inhibit mineral deposition in caves [46]. In this fact, possible lowering of cave chemoautotrophs can be noted. The disappearance of such primary producers can directly impact the other organisms of the same ecosystem. Furthermore, human explorations of caves can lead a temperature variability inside these underground surfaces and most changes are observed in show caves [39, 47]. In such a known thermostable environment, the direct impacts of a thermal change on the cave species are not yet determined. However, an adaptation of exogenous thermotolerant soil bacteria is probable if the air temperature of these environments did not return stable after few hours [45].

Even it is difficult to study the microbial structure of the cave ecosystems, its disturbance appeared to be easier because of their minuscule and invisible sizes. While walking, climbing, or other mechanical force can compress the stones and further reduce the oxygen for the organisms living in the sediments or the cave surfaces. The decrease of humidity in Altamira Cave opened to tourists was reported to be from 90% to 75% [47]. Such decrease contributes to the drying of the cave sediments and surfaces: that is one other factor that induces the threat of cave microbial diversity.

Perspectives for Cave Microbial Diversity Conservation

Although without human investigations the positive and negative impacts of Nature remain in the dark, the conservation of these natural resources should be studied before any activity. For the life of caves as well as the continuity of industrial and biotechnological natural resources, we should elaborate a protocol of cave using. Some countries through their caver

associations have already established laws and conditions necessary for the conservation of such environments. for the protection of the microbial diversity of the cave, we suggest that:

- Show caves should be limited to a few numbers of visitors per day and per hour.
- Cave managers should avoid artificial improvement inside the caves.
- Tourism in the cave should be for the nature of caves since every cave is unique, every cave has the own characteristics, so no need for artificial improvement of caves.
- Education about the importance of caves and cave microbial diversity should be exposed to people through conferences or other education systems.
- Collaboration between cave managers, cavers, and scientists, especially microbiologists: if any new formation in a cave is observed or a new cave part is expedited, new microbial diversity should probably be discovered.
- Cavers or anyone enter the cave environment should be conscient to never leave any waste inside the cave. Wastes should be put in bags and bring out of the cave.
- Show caves as well as those that are opened for only scientific research should have a rest period in which cave microbial diversity can process into the bioremediation of the cave.
- Every cave should be used as a specific fortune and avoiding bad habits, like film capture especially historical and cultural films, which contribute to the further destruction of caves since further constructions inside the caves are processed (e.g. the 'YOR' in the 1980s inside the Yarımburgaz Caves) [30].
- Pools, rivers, and other water sources founding inside the caves should not be accessible for cave visitors, despite sampling for scientific studies. For further sampling protocols, you can visit and read more in [48]
- Camps installed by scientific groups should take small places as much as possible and new technics used for the research should be improved in a manner to spent as much as possible a few times inside the caves.
- During caving, cavers should as much as possible change or wash their caving clothes and boots frequently, especially when they move from a cave chamber to another one.
- We should be careful and conscient that every touch or walk step is susceptible to disturb the invisible microbial cave structure.

- Uses of fire for warm-up or using cigarettes inside the cave should be banned since they are a source of the CO_2 increase, organic matter input as well as toxic matter input.
- Encouraging the microbiology studies of caves since few of them are explored until now. This can be by the multiplication of caver associations in countries which should collaborate and dividing the research through the different caves present in their countries.

Conclusion

Caves are extreme environments where the life of microorganisms is important for the formation of cave structures and the continuity of life in a cave. These microorganisms are found to be sources of the most important industrial and biotechnological products. Caving is essential for the expedition of such natural environments. However, cave activities have impacts on the living organisms present in such ecosystems. For protecting and conservation of the natural feature of caves, visits to caves should be limited to a specific number of visitors per year and artificial improvement of caves should be avoided. We can say that where the human being has not been yet, remain obscure but unfortunately the disturbance is more likely to happen where he is.

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