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Review Article

The Astrobiological Significance of Caves on Earth and on Mars

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Abstract

Caves are geologic entities that can be frequently found around the globe. Cave-like features have been documented on Mars by satellite imagery and special detection devices. On Earth Subterranean habitats like caves might host microbial growth because of their relatively stable physicochemical conditions and mineral rich content. Moreover, caves have also been isolated from UV radiation and other present environmental conditions which actually make them ideal for searching for unique microbial life. Mars is an arid planet with thin atmosphere and quite weak magnetosphere. Therefore Mars as it is known is uninhabitable. Research shows that Mars might have been a wet planet in the past, having streams of running water. Earth like subterranean cavities on Mars might provide protection from these environmental hazards. This makes Earth caves important astrobiological sites as Mars analogues for the investigation of the possibility of life on Mars. Researching caves both on Earth and Mars will provide us insight into extreme life conditions and important astrobiological questions. In this review, we are suggesting that geobiological significance of Earth caves plays an important role in searching for life on Mars and defining Mars analogues on Earth.

Keywords: Astrobiology, Mars analogues, Cave, Microbiology

Introduction

A careful observer can see a red beam of light in a clear sky with reduced light pollution. This light has been a source of wonder throughout human history, and a recurring figure in mythology, theology and astronomy. Most people call it Mars, a planet named after the Roman god of war. Considered as the god of war in Roman mythology, Mars is known as the planet of war in the mythology of Turks who have monotheistic beliefs. Although Mars is the most similar planet in many ways to Earth in our Solar System, Mars seems like uninhabitable and arid environment (Fairén et al., 2010; Catling et al., 2010). Mars atmosphere mixing ratios calculated results are carbondioxide CO_2 (0.951), nitrogen N_2 (0.0259), argon Ar (0.0194), oxygen O_2 (1.61 x 10^{-3}), carbon monoxide CO (5.8 x 10^{-4}) (Trainer et al., 2019; Almatroushi et al., 2021).

Caves have also been a recurring figure throughout human history. They have been used as homes and shelters, as well as burial grounds. Towards the end of the 19th century they became locations of interest in speleology. This interest grows in to the 20th century and speleology becomes an academic discipline (Mattes, 2015).

The exploration of caves as Mars analogues falls under the field of astrobiology. Astrobiology is an interdisciplinary study that questions the beginning and development of life, the possibility of life elsewhere and what the future life will look like on Earth and beyond. (Cockell, 2002). While the UV radiation and extreme conditions on the surface of Mars can be considered as too extreme for life, existence of possible subsurface features on Mars hint that there might be stable physicochemical conditions to host microbial life (Margulis et al., 1979). The caves on Earth are also accepted as extreme environments, and thus have been common research sites for the disciplines of microbiology and astrobiology (Northup and Lavoie, 2001; Blank et al., 2018).

Borders of Life and Astrobiology

What is life, how did it come to be and where did it come from? These questions have occupied the human mind for a long time and each academic discipline has different answer to them. These questions are also the backbones of biology. Although scientists haven't been able to agree on one definitive answer, scientists are trying to make a broad definition of living things.

If it's life that researchers looking they need a good answer to question 'What is alive and what is not? One of the definitions of life is made by Luisi(1998) to quote exactly 'A system which is self-sustaining by utilizing external energy/nutrients owing to its internal process of component production and coupled to the medium via adaptive changes which persist during the time history of the system'(Luisi, 1998).

In biology, it can be stated that if something is alive it shows 7 characteristic properties, which are: 1-

homeostasis (to be able to maintain stability), 2-organization (hierarchy in structure, like cells to tissues), 3-metabolism (chemical reactions that produce or use energy), 4-growth (increase in mass), 5-adaptation to changing environment, 6-reaction to stimulus (reaction to external or internal actions), 7-reproduction (production of offspring that are genetically similar). In order to make a more inclusive definition, Kosland (2002) suggests the 7 pillars of life as P.I.C.E.R.A.S principles. These principles are Program, Improvisation, Compartmentalization, Energy, Regeneration, Adaptability, Seclusion (Koshland, 2002).

While the definition of life changes, the environments where we look for life change with it. This perspective, discovering alternative metabolic pathways for energy and learning more about the biochemistry of cells have shown us that microbial life can sustain itself in extreme conditions. These revelation forces researchers to investigate extreme environments otherwise deemed unworthy to explore for microbial life (Nealson, 1997).

Life on other planets and possibly of human-like intelligent life has always fed our imagination. These tropes have been the main topic of many sci-fi books and movies. Life on Earth might be of extra-terrestrial origin. This is called the Panspermia theory, which suggests that life could have originated from extra-terrestrial sources. (Raulin-Cerceau, 2004).

In astrobiology, one of the first terms that come forward is habitable zone (HZ) (Lingam, 2021). For a carbon based life form to emerge, the formation of a habitable zone is directly related to the distance of the planet to its respective star. Distance is directly related to average surface temperature, which entails looking for H_2O in liquid form (Kasting et al., 1993; Franck et al., 2002; Méndez et al., 2020).

Other stellar systems might have planets in their habitable zones just like Mars and Earth do in our solar system. These planets play a major role in astrobiology. Even though it is impossible to travel to exoplanets to search for life, their surface temperatures can be measured with effective stellar flux formulas. This can be calculated by the incoming stellar radiation from the planet surface and the outgoing radiation from the planet, using the formula is $S_{EFF}=F_{IR}/F_s=S/S_o$ (Ramirez, 2018).

Mars analogues on Earth

Other celestial bodies might have surface formations caused by external forces such as meteors or internal forces such as geological processes. Data from Mars orbiter and landers suggests Mars is geochemically active because of mineral compositions that have been investigated (Gendrin et al. 2005; Bibring et al. 2006).

There are lots of openings in Earth's crust because of its dynamic nature. These cave candidates can be the result of tectonic shifts, atmospheric changes, or dissolution in a solvent. Limestone and karst caves are good examples for caves created by dissolution (Davies and Morgan, 1980; Palmer, 1991; Palmer and Hill, 2019). Wind, water and friction play an important role in the formation of caves by erosion. These physical interactions could be happening on other planets, resulting in similar surface formations. For example, just like the limestone, karst and dolomite caves on Earth, other planets may have structures formed by a solute dissolving in a solvent (Liberman et. al., 2018).

Earth has a strong magnetosphere, which protects Earth from solar radiation and atmospheric erosion by solar winds. Mars lies in a habitable zone but it lacks a strong magnetosphere, and without a magnetosphere it's subjected to atmospheric loss (Dehant et al., 2007). Life on Mars surface seems impossible. There could be other planets like Mars that have extremely uninhabitable surfaces. In this case, underground structures which are isolated from the surface become important zones to conduct astrobiological research on bio signals. Furthermore, studying analogues on Earth give us insight without traveling long distances.

Caves are known to be extreme environments as they have high humidity, low temperature and no or low levels of light. The static nature, micro climate, rich mineral content and geochemical processes of caves may provide stable conditions for life (Tomczyk-Żak and Zielenkiewicz, 2016). Conducting research on caves on Earth and Mars would give us important theoretical and practical data in microbiology, geology as well as astrobiology (Boston et al., 2001; Barton, 2006).

UV radiation, high energy particles can easily reach the surface of Mars without the resistance from a thick atmosphere and strong magnetosphere. Dust storms and harsh environments further reduce the possibility of the survival of life (Levin and Straat, 1977; Cockell et al., 2000; Patell et al., 2002; Yoshikawa et al. 20014). Away from these environmental elements, caves might have stable conditions for biomarkers or even microbial life to survive. Recent data show that there are 7 possible openings on Mars that may be caves and reported as possible research zones (Cushing et al., 2007). There are suggestions that H_2O in liquid form could have been present in the last decade on the surface of Mars (Malin et al., 2006). With water, rich underground reservoirs can be formed and become a safe haven for life. For the same reason, conducting microbial studies in similar formations on Earth will most probably lead to biological and astrobiological discoveries.

Third three sites have been discussed to be analogues for extra-terrestrial locations due to their mineralogical, physical or chemical conditions (Preston et al., 2014). Some places were chosen to be Earth analogues for different time periods on Mars because of their special characteristics. The Pilbara region (West Australia) (Walter et al., 1980; Allwood et al., 2006; Brown et al., 2006; Westall, 2008), Yellowstone National park (USA) (Parenteau and Cady, 2010; Ruff et al., 2011), and Rio Tinto (Spain) (Gónzález-Toril et al., 2003; Fernández et al., 2005; Amils et al., 2007) are considered similar to the early time periods of Mars. The Beacon Valley (Antarctica) (Horneck, 2000; Heldmann, 2013), the subglacial volcanism (Iceland) (Boston et al., 1992; Gudmundsson et al., 1997, Preston et al., 2008; Souness and Abramov, 2012), and Kilimanjaro (Africa) (Ponce et al., 2011a, b) are similar to the middle stages of Mars. The Atacama Desert (USA) (Navarro-González et al., 2003; Valdivia-Silva et al., 2003), Mojave Desert (USA) (Greeley et al., 2002; Bryant and Rech, 2008), and the Antarctic dry valleys (Antarctica) (Friedmann, 1980; Wyn-Williams and Edwards, 2000) as for present day Mars.

The Pilbara region may be an early Mars analogue in terms of its hydrothermal activity, water-induced exchange of minerals and flood basalts. The Yellowstone national park with its silica-rich soil that hosts thermophiles has been accepted as an analogue based on its hydrothermal activities, while the Rio Tinto is considered an analogue due to having river channels, iron oxide and sulphate (Preston et al., 2014). In the Rio Tinto region, iron-oxidizing and acid-tolerant bacteria predominate the microbial community, Leptothrix sp. Similar bacterial chemolithotrophic communities were observed as well (Preston et al., 2011).

The Beacon Valley has been accepted as an analogue for the glaciers in the Martian polar region, and fossil psychrophilic microbial communities living in Beacon valley glaciers have been reported (Horneck, 2000; Lee et al., 2012; Heldmann, 2013). Subglacial volcanic formations in Iceland were chosen as analogues of habitable areas on Mars due to their geothermal activities. Psychrotolerant and chemotrophic bacteria have been reported in these Icelandic glaciers. Kilimanjaro displays similarities to the surface of Mars because of its high UV flux, low atmospheric pressure, little water and nutrients. It has been chosen as a Mars analogue to study habitability in oligotrophic volcanic mineral soils and ice (Preston et al., 2014).

The Atacama Desert is characterized by extreme drought, high levels of UV, and perchlorate-rich volcanic soils which make it an analogue of Mars (Wierzchos et al., 2013), volcanic geology and arid environment of the Mojave Desert and the high UV values of Antarctic Dry valleys make these places dangerous for habitation. These conditions are similar to the extremely dry and cold deserts of Mars. The arid valleys of Antarctica especially have been accepted as the closest analogue to today's Mars due to their extreme conditions (Preston et al., 2014).

The existence of underground formations called lava tubes on Mars can be studied with methods such as high-resolution surface investigation methods, termal imaging, and radar measurements penetrating deep into the surface (Hodges and Moore, 1994; Wyrick et al., 2004; Cushing et al., 2007). Examples of lava tube analogues selected by NASA are the National Lava Deposits Monument in California and the El Mapais national monument in Death Valley, New Mexico unmanned robotic research methods have been proposed to investigate these formations (Blank et al., 2018).

The density of Haematite in the form of iron oxide (Fe2O3) is found in some regions of Mars suggests that Mars may once have been a wet planet like Earth in the past (Christensen et al., 2000; Christensen et al., 2001; Catling and Moore, 2003 Baldridge and Calvin, 2004). The presence of iron oxide, also called Haematite, can mean that water was present for oxidation to occur in the early time periods. The basis of this idea is that iron oxide is formed as a result of its interaction with the water or aqueous suspentions (Kato et al, 2009). The presence of round haematites in the Utah region of America suggests that it could be analogous to the haematite structures found in the crater Meridiani on Mars, which are round and 22 mm in diameter. (Catling, 2004). In the report of the conference titled ‘Mars Extant Life: What's Next?’, the argument of why caves are such important places to search for life has been summarized as: 1-There is evidence of the existence of caves, mostly in the form of lava caves, on the Martian surface. 2-Caves on Earth are known for their special life forms that have adapted to these environments. 3- There are multiple ways to explore caves. For example, remotely controlled vehicles and the study of the ways in which the airflow changes (Schneegurt, 2020).

Martian Caves and the Possibility of Life

Lava tunnels and cave-like structures have been observed with the use of Mars satellite imaging and special measuring tools. These have been put forward to be suitable environments to search for microbial life and bio-remnants (Léveillé and Datta, 2010). The discovery of 7 possible caves with surface openings has been discussed in the article of Cushing and colleagues (Cushing et al., 2007). Mars having above and underground structures similar to Earth and recent research about the discovery of bodies of liquid water on its surface are being discussed. This model suggests that early Mars may have had an active water cycle (Barker and Bhattacharya, 2018).

In light of this information, the landing of the Perseverance craft on the Jezero crater on Mars on February 12th, 2021 is a vital stepping stone for astrobiology. One of the studies this craft will conduct is to look for possible biosignatures and evidence of microbial life in this crater (Mangold et al., 2020). NASA’s astrobiology roadmap defines the data that will be accepted as a biosignature as; “cellular and extracellular morphologies, biogenic fabrics in rocks, bio-organic molecular structures, chirality, biogenic minerals, biogenic stable isotope patterns in minerals and organic compounds, atmospheric gases, remotely detectable features on planetary surfaces (photosynthetic pigments, etc.), and characteristic temporal changes in global planetary properties (Des Marais et al., 2008). The observations on the existence of riverbeds and potential floodplains in the Arabia Terra region of Mars have become supporting data for the theory of the presence of liquid water on the surface of Mars in the past.

These observations have strengthened the idea that there may have been life on Mars at one point in the past and this life may have been sustained even in the extreme conditions of Mars. In addition, the possibility of this potential life on Mars originating from Earth is also being discussed. Organisms from Earth may have travelled to Mars and Moon on dispersing rocks from a meteor collision with Earth vice versa, or on crafts that have been sent to Mars (Herring et al., 1974; Brockett et al., 1978; Mileikowsky et al., 2000). Organisms that can survive the sterilization of these crafts and that can endure high levels of UV have been detected. (Nicholson et al., 2005). Despite not proving the existence of prokaryotes, lichen and fungi on Mars, it has been put forward that methane gas of unknown geological origin may be a reason for the survival of some organisms on Mars-like environments (Joseph et al., 2019).

Earth Caves and their microbial diversity

In general terms, caves are defined as natural openings through which humans can fit. Different disciplines display various approaches to defining caves. In biology, caves can be defined as openings that can host organisms that have adapted to their environment (White and Culver, 2019). These openings are extreme environments that can be rich in minerals. This richness in minerals may support life by providing a suitable environment for chemotropism. General properties of caves include having a dark zone unreachable by daylight, low temperatures, and high humidity. Being away from external factors, having their own cycle, and their long-term sustained stability make each cave a unique ecosystem in itself (Poulson and White, 1969). It has been the subject of many studies that microbial life can use various chemolithotrophic metabolisms as an energy source in the absence of daylight. Iron oxidation, sulphide oxidation, sulphide reduction, hydrogen oxidation, and methanogens can be examples of chemolithotrophic metabolism. It is by these means that caves can leave morphological and molecular biosignatures that can host biomasses (Bendia et al., 2021).

The Moville Cave in Romania is an example of such an extreme environment. Cut off from the surface completely, the caves' only energy source is the hydrogen sulphide and methane that emanate from hydrothermal liquids. Chemosynthetic primary producers make up the basis of the ecosystem that occurs in this environment (Kumaresan et al., 2014).

Offering a unique chance to investigate the chemotrophs in karst environments, The Cueva de Villa Luz Cave (Mexico/Tabasco) can also be an example of an extreme environment. The water and atmosphere inside the cave are reported to quite rich in hydrogen sulphide (Hose et al., 2000). Additionally, the average measurement of the SO_2 gas in its atmosphere is over 35 ppm. These gases were found to helping the formation of sulphuric acid condensed on the cave walls by interacting with the oxygen in the air of the cave. The pH value of the drops from the cave walls have been measured to be 1.4 while this value has been observed to drop down to a minimum of 0.1. 19 clones of bacteria that are affiliated with these drops have been procured as a result of the analysis of 16S ribosomal RNA configurations. Similar bacteria clones to *Thiobacillus spp*. and *Acidimicrobium ferrooxidans* that make sulphur oxidation as a source of energy have been bred (Hose et al., 2000).

Prokaryotes make up the majority of the microbial population in caves. Proteobacteria (α, β, γ, δ, ϵ), Nitrospirae, Actinobacter, Acidobacter, Bacteroidetes, Firmicutes, Verrucomicrobia, Planctomycetes, Chloroflexi phylum are examples of bacteria that are commonly observed in caves. Evidence that bacteria play a role in cave formation through events such as precipitation have been discovered (Tomczyk-Żak and Zielenkiewicz, 2016).

It has been observed that some bacteria cause precipitation in calcium carbonate ($CaCO_3$) structures. This precipitation process occurs due to the carbonic anhydrase enzyme (Zheng and Quian, 2020). The investigation of the mechanism of this process may ensure the understanding of the role that bacteria play during the carbon cycle and geological processes.

Calcium carbonate, which is precipitated by bacteria, is formed as a result of the process of biomineralization (Barabesi et al., 2007; Dhami et al., 2013). The process occurs through the reaction of Ca^{2+} and water soluble CO_2 through carbonic anhydrase (Zhuang et al., 2018; Zheng and Quian, 2020). The process of the occurring mechanism is as follows; 1- the CO_2 in gaseous state is dissolved in water, 2- the dissolved CO_2 creates the carbonic acid (H_2CO_3) by reacting with the water molecule, 3- it separates into H^+ and HCO_3^- ions, 4- in alkaline conditions the HCO_3 reionizes to make CO_3^{2-} and H_2O, 5- if there is Ca^{2+} in the environment, it interacts with the CO_3^{2-} to precipitate $CaCO_3$ (Zheng and Quian, 2020). This geological process may occur slowly and naturally but it is considerably accelerated by the carbonic anhydrase enzyme (Lindskog et al., 1973).

This role that bacteria play in the carbon cycle has triggered an interest in discovering their impact on the calcium carbonate formations in caves. One of the studies conducted on this subject is the analysis of samples gathered from the structures known as 'moonmilk' and cave precipitations from the Altamira cave (Sanchez-Moral et al., 2012). This study is based on the stable isotopes, RNA-DNA ratio, and respirometric measurements. Through the results of this study, it is concluded that bacteria create carbonate precipitations, and that as the precipitation levels rise, bacteria activity decreases (Sanchez-Moral et al., 2012).

Other studies conducted in the Cevro Cave in Italy and Dupnisa Cave in Turkey have shown that bacteria isolated from the surface of karstic cave structures create calcium carbonate precipitates when they are planted in a medium of calcium acetate containing B4. It has been put forward that bacteria play an active role in carbonate precipitation and crystallization (Cacchio et al., 2004; Turkgenci and Doğruoz Güngör, 2021).

The microbial diversity obtained from such ‘alien’ environments has led to the emergence of new approaches to life and its limitations. The identification of the microbial diversity of such environments and the procurance of their various enzymes have shown that processes such as the carbon cycle are geobiological ones. Additionally, the discovery of potential regions for enzyme and gene collection have also brought to consideration that caves have significant value in the field of biotechnology.

Conclusion

Initiated by their curiosity about the red twinkle in the sky, humankind’s journey of space exploration has reached the point of debating the existence of life in the caves of Mars and the successful transportation of unmanned research vehicles to Mars. With the discovery of extremophiles Humankind’s journey of discovering life and itself has brought together microcosmos and macrocosmos under the roof of astrobiology; meaning first life that researchers come across might be microbial life contrary to human like multicellular organisms.

Its proximity to Earth, geological similarity, and the fact that there used to be liquid water on its surface makes Mars ideal for astrobiological studies. It is because of these similarities that researchers are able to conduct studies and research on the analogues of Mars. The proximity of Mars and Earth has made it possible to collect samples via unmanned vehicles. It can be predicted that it will be possible to send manned crafts to Mars with current space technologies. Although this journey may present humankind with a number of difficulties; the new research fields that will bring about and the answers that will provide us can be thought of as prizes that make it worthwhile.

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