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Strategic food reserves storage, safety and risk minimization: Problems and perspectives

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ABSTRACT

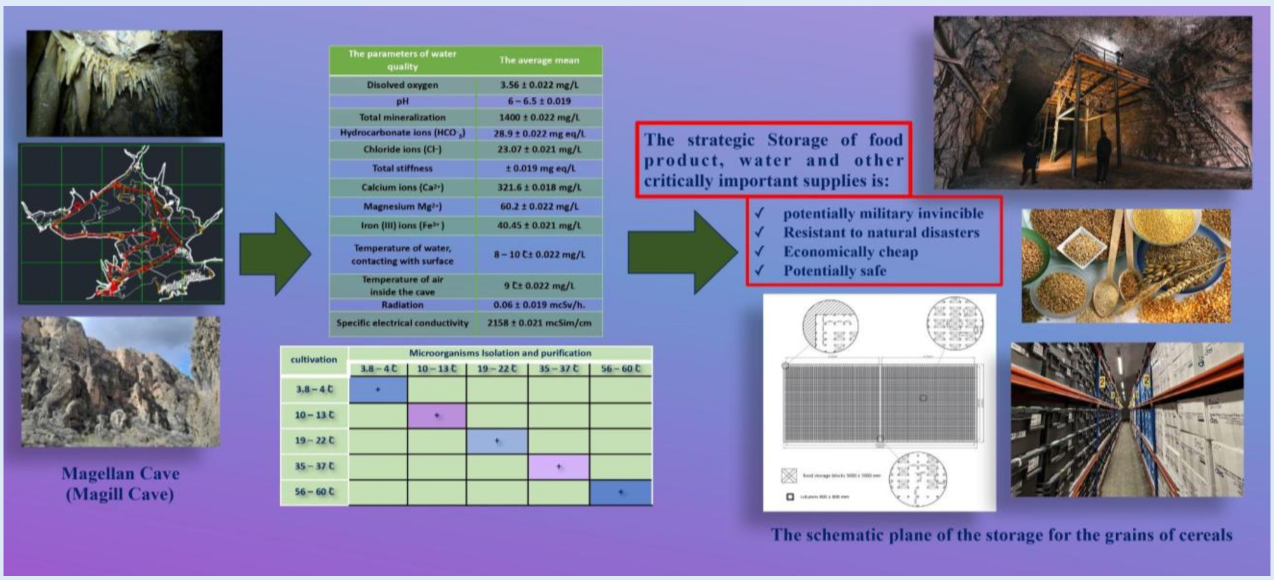
Background: The hunger problem is a significant issue arising from global challenges in the 21st century. It is a consequence of inadequate food safety and poor strategic food storage. This makes innovations and improvements in these areas particularly crucial, especially for developing countries and regions affected by military conflicts and natural disasters.

Obgectives: We aimed to study carbonate limestone caves as potentially safe locations for constructing strategic food storage facilities.

Results: The preliminary results from Magellan Cave (Magill Cave) in the Areni region of the Republic of Armenia indicate that the cave has suitable conditions for use as a potential site for strategic food storage, particularly for cereal grains. Biological studies have proved the absence of mesophilic microbes in rock minerals and in soil samples from its inner space. Additionally, psychrophilic microbes, thermophilic algae, and representatives of Cyanobionta were found in waters that meet underground hot springs. Chemical analyses revealed no toxic compounds in the rock minerals, poisonous gases, or radon accumulation. The radiation levels were also found to be safe.

Conclusions: Physical, chemical, and biological research have demonstrated the absence of risk factors for health and food safety in Magellan Cave. No spoilage microbes or human pathogenic bacteria were found. Additionally, no fungi were detected. The results suggest that this cave could be a promising location for constructing underground food storage for strategic reserves.

Keywords: Magellan Cave (Magill cave), underground constructions, food strategic reserve storage, biological safety, spoilage, psychrophilic microbes

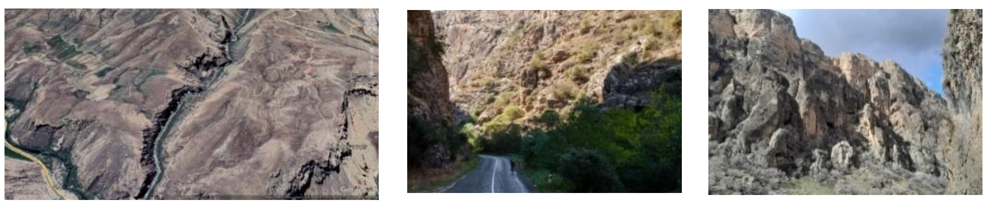


INTRODUCTION

To explore the development of underground long-term food storage solutions in 2023-2024, NUACA (National University of Architecture and Construction) organized a series of discussions. In terms of the complicated geology of Armenia, research has proved that the best way to explore long term storage solutions is to use the conglomerate keratogenic limestone layers. It is potentially possible because in both natural hypogenic caves Areni-1 and Magill human long-term presence and activity traces were identified [1,2]. That is why the main goal was to conduct parallel simultaneous research of the cave microbiome with the studies of the chemical and physical properties of minerals in the mentioned caves [3].

The choice of this type of layer is based on certain criteria. One of them is the fact that in the XX century, more than 200 km of underground cavities were dug in Armenia: Arpa-Sevan hydro-engineering complex, metal mine tunnels, such as the motorway tunnels, etc. [4]. The exploitation practice of the mentioned construction has demonstrated that all of them have a very short expiration period. Also, they have a permanent necessity for restorations. For example, in the case of the Arpa-Sevan project, the major renovations were carried out two times. In the case of the Pushkin Pass, the same procedure was used more frequently. Moreover, due to the annual planned collapse risk assessment, one more capital renovation is planned for now. The main cause of all the mentioned problems is that the constructions are placed in a zone of many different geological layers, what increases the risks of collapse. The monolete and homogenous geological environments are more stable and can exist for a very long period (Areni Cave, Magill Cave, Mozrov Cave, Archer Cave, etc.) up to billions of years. That is why for the mentioned purpose the monolete systems are appropriate. In these regards, carbonate limestone caves were chosen as the best model for the expression of the mentioned process [5].

It should be noted, that in Armenia and in the Caucasian region, there is an extensive history of architectural usage of caves. The best experiences are Kndzoresk cave village, Geghard Cave Church, Asni Cave Church, and other churches in Armenia, such as the cave city Vardzia in Georgia. The analogous experience is a well-known world-around [6-8]. In Amenia, there are other geological groups of layers with long-term warranties of stability, e.g. Geghard Cave Church which was carved in layers of volcanic settlementsDespite the continuous use of this cave church by tourists and pilgrims, the construction of Geghard has remained stable and intact for over 1,000 years and shows no signs of collapse risk [9,10]. We have decided to use the conglomerate limestone layers for the minimization of risks and the Magill cave was used as the working model for our research (Fig. 1 – 3).



A **B** **C**

Fig. 1. Magill Cave (Magellan Cave).

A: aerophotography of the area from Google Earth; B, C – the surface territory of Magill Cave.

A **B**

Fig. 2. Inner space of Magill Cave (Magellan Cave).

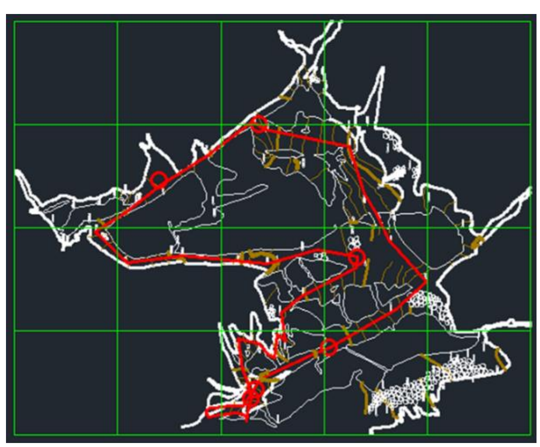


Fig. 3. The map of touristic part of Magill Cave

In these regards, it's very important to study the microbiome of the chosen cave. Cave microbiome research is significant for a better understanding of cave ecosystem biology [11]. The ecosystem of the cave is practically closed and based predominantly on producing microorganisms that are capable of chemosynthesis (chemoautotrophic or chemolithotroph organisms). Thus, the inhabitants of caves have very specific metabolism [9]. That is why, the study of cave microorganisms is very important for the discovery of new compounds like novel antibiotics, toxins, antitoxins, and other potential components for the elaboration of innovative drugs, innovative functional food creation, etc. [12,13]. Cave microbiome research also has a huge significance for fundamental science, because it can give a better understanding of extremophilic organism biology, which is important not only for the biology of planet Earth but also for the biology of space and astrobiology development [14-16].

The research on the Magill Cave microbiome has a huge significance for the risk assessment in the aspect of the presence of cave-specific microflora for further application of this cave as a potential place for the creation of food strategic reserve storages. Particularly, this research is devoted to the study of chemical, physical, and biological characteristics of the Magellan Cave (Magill Cave) and risk assessment for the construction of granaries using the internal space of this cave.

MATERIALS AND METHODS

Physical and Chemical Research of Cave: At the first step of research, the main physical parameters of cave rock and water samples of Magill cave were measured. The temperature and pH were measured using Hannah Waterproof Portable pH/Temperature Meter HI991003. The radiation intensity of Magill cave was measured using DMC 3000™ Mirion Technologies Personal Electronic Dosimeter. Chemical parameters of the speleothems were analyzed in the research laboratory of the Institute of Geological Sciences of the National Academy of Sciences of Armenia (NAS RA). Then UV fluorescence presence in rock and water samples was studied. Then, for the microbiological study, the samples of rock and water were sterilely collected. Microbiological research was conducted by the method of microscopy. In the first stage, the initial materials microscopy was done. Then UV/VIS and IR spectrometry were applied to water and mineral samples. For UV/VIS spectrometry Thermo Scientific Multiskan GO was used. For the visualization of results, Specrtragryph v1.2.16.1 software package was applied [17,18]. For FTIR and ATR Nicolet™ iS50 FTIR Spectrometer was used [19-21].

Microbiological analyses: From the collected samples some microorganisms were isolated using different cultural media for autotrophic and heterotrophic objects. Green autotrophs were cultivated in water. Heterotrophs and mixotrophs were cultivated in LB (Luria-Bertani) liquid media and in L-agar solid nutrient media. Cultivation was carried out at different temperatures: 3.8 – 4 °C, 10 – 13 °C, 17 – 19 °C, 20 – 22 °C, 30 – 35 °C, 37 – 38 °C, 50 – 55 °C and 60 – 65 °C in aerobic conditions. Then optical and fluorescent microscopy was applied to all the cultivated samples [22, 23].

Genetical analyses: Genetical analyses of the isolated bacterial strains were carried out by DNA isolation, electrophoresis, and PCR. DNA was isolated by the application of the Qiagen QIAamp DNA Mini Kit. For the electrophoretic study of DNA 0.8 – 2.5% agarose gel with ethidium bromide and ultraviolet registration by UV transilluminator were used [24-27]. PCR was carried out using the primers of antibiotic-resistance genes, which are common for the pathogenic bacteria: *catB7 (*is responsible for chloramphenicol acetyltransferase CATB7)*, aac(6')II, aph(3')IV (*responsible for enzymes of aminoglycoside antibiotics modification: aminoglycoside

N-acetyltransferase AAC and O-phosphotransferase APH, respectively), *blaOXA-10 (*responsible for β-lactamase OXA-10)*.* The following markers for determining the molecular weight of DNA fragments were used: *M-3788 SIGMAMARKER; M-3913 SIGMAMARKER; D-6293 (pUC18/ Hae III), M-103S λDNA/EcoRI/HindIII*, produced by "SIGMA" [28 -31].

Statistical analyses: The statistical assessment of the reliability of the experiments was carried out using generally accepted methods [32]. All the experiments were carried out in 5 series of 3 repeats for each probe. MS Excel was used for data analysis. SEM (Standard Error of the Mean) was ± 0.23–0.37. Significance was tested by applying the student t-test and estimated as a *p*-value less than 0.05.

RESULTS

According to the conducted research, the pH was 6 – 6.5, The radiation level was 0.06 mcSv/h, which is low and appropriate to the radiation safety standards [33]. Due to the carried-out measurements, the average temperature in the cave is 10°C. No toxic chemical compounds, including gases and minerals, were detected in Magellan Cave. The results of geological analyses of the cave are presented in Fig. 4.

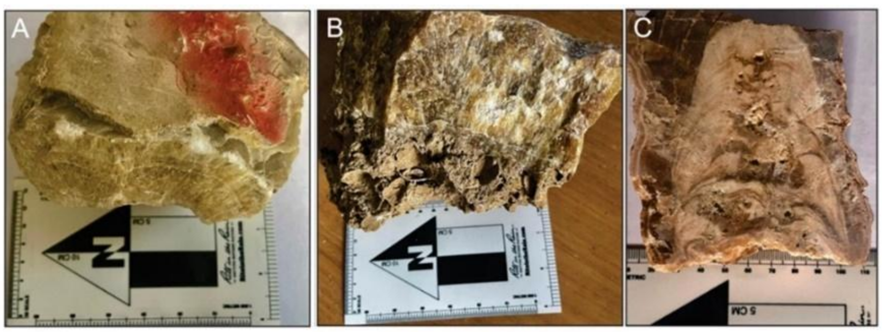


Fig. 4. The speleothems from the Magill Cave (Magellan Cave).

(A)- layered travertine, (B)-travertine developed on breccia, (C)-stalagmite.

During the conducted research it was carried out that the rock minerals of the cave have demonstrated some types of fluorescent signals in UV diapason. The fluorescence is visible as a glow of different colors (blue, yellow-brown, white) visible both to the naked eye and under a microscope (Fig. 5).

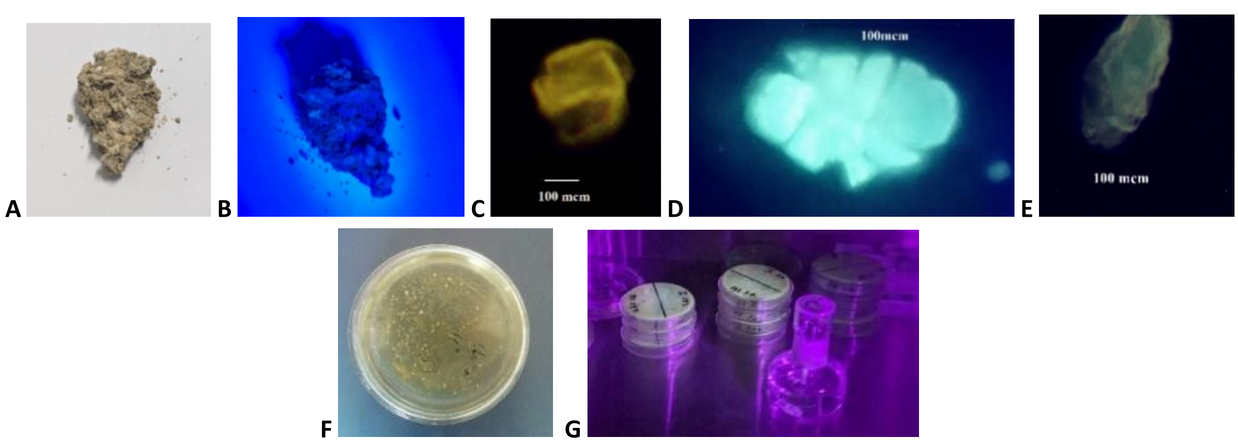
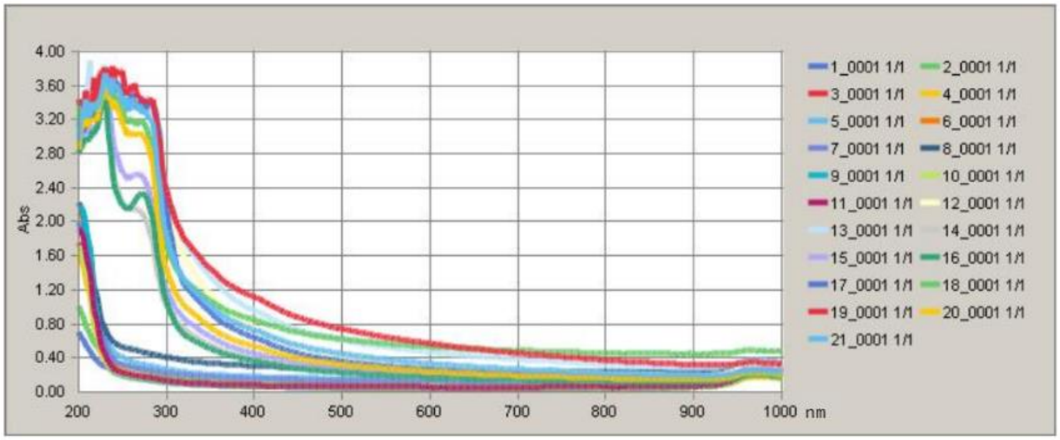


Fig. 5. Fluorescent signals, registered for the rock minerals of Magill cave and during the cultivation of microbes.

A – photograph of rock mineral of the cave, B – UV fluorescence; C, D, E – fluorescent crystals under the microscope; F – the mixed culture of microbes, G –pure cultures, which have fluorescence in UV diapason. The results of FTIR-ATR and UV/VIS spectroscopy are presented in Fig. 6 – 7



1-10 initial samples of rock minerals and water collected from the different points of cave.
11-21 samples of water and rock minerals collected from the different points of cave and cultivated at different temperatures.

A

B

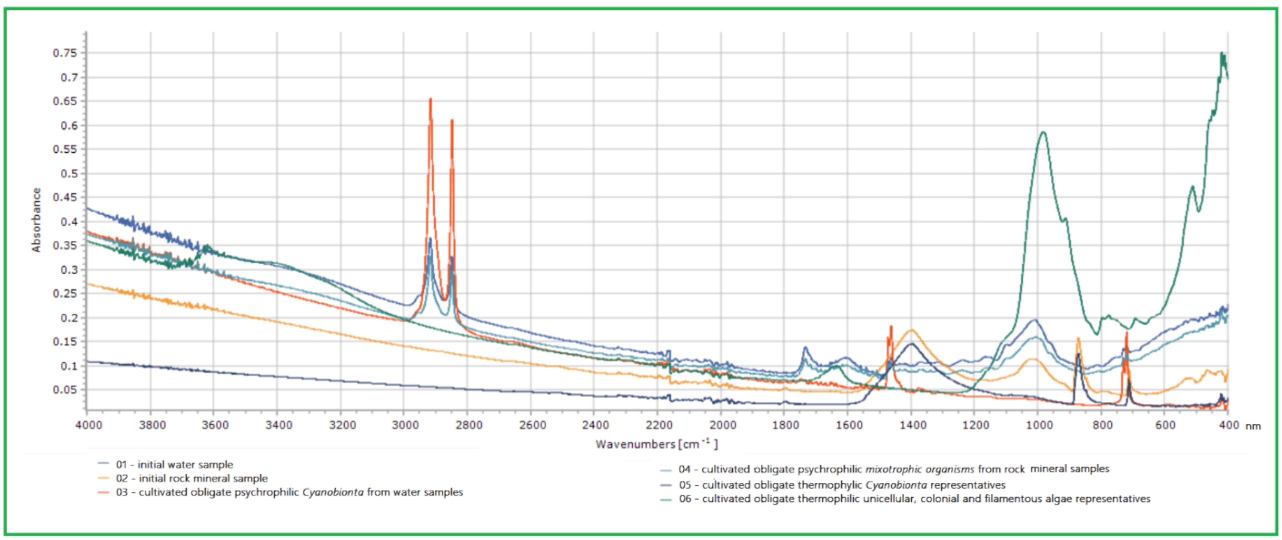
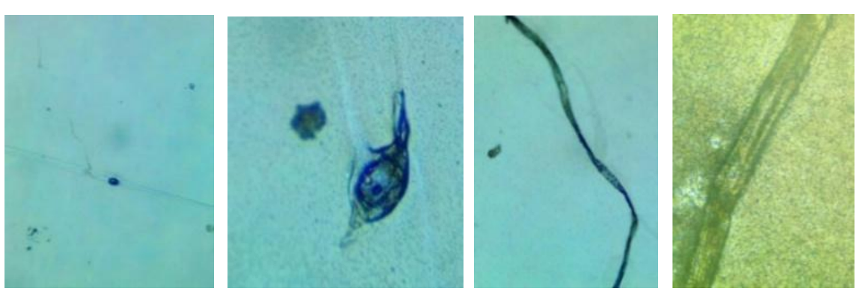


Fig. 6. UV/VIS (A) and FTIR-ATR (B) spectroscopic study of cave mud and water samples from Magill cave.

The preliminary observation of water and cave mud samples demonstrated the presence of algae, cyanobacteria, some mixotroph organisms and some detritus residues (Fig. 7).



a **b** **c** **d**

Fig. 7. Preliminary observation of cave mud, rock mineral (a: 40x magnification and b: 80x magnification) and the water (c: 40x magnification, d: 80x magnification) samples from Magellan Cave.

The observed organisms were cultivated and isolated using different temperatures and media. The results have demonstrated the presence in rock, and water samples of a wide diversity of bacteria, cyanobacteria representatives, and algae such as some mixotroph organisms, with different thermal optimums and morphological parameters (Fig. 8-9). Then the genetical research of the isolated microbes was conducted (Fig. 10).

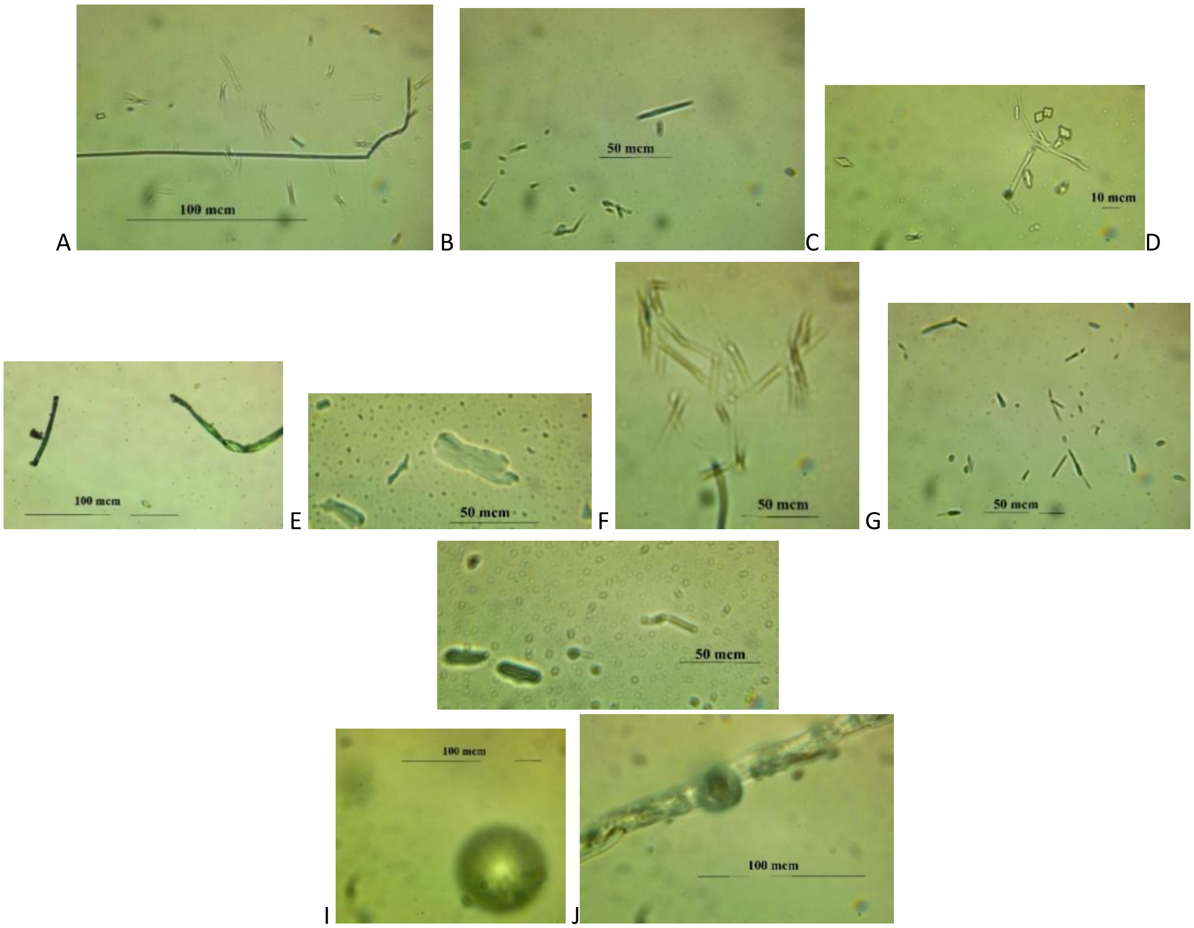


Fig. 8. Microscopy of water, rock mineral and cave mud samples, cultivated at different temperatures.

A – 56-60 ˚C water samples (800x magnification); B –56-60 ˚C rock mineral and cave mud (800x magnification); C – 56-60˚ C water samples (100x Magnification); D – 19-22˚C water samples (800x magnification); E – 19-22 ˚C cave mud and rock mineral samples (800x magnification); F – 10-13˚C water samples (800x magnification); G –10-13˚C rock mineral and cave mud (800x magnification); H – 3.8-4˚C cave mud and rock mineral samples (800x Magnification); I, J – 3.8-4˚C water samples (80x Magnification).

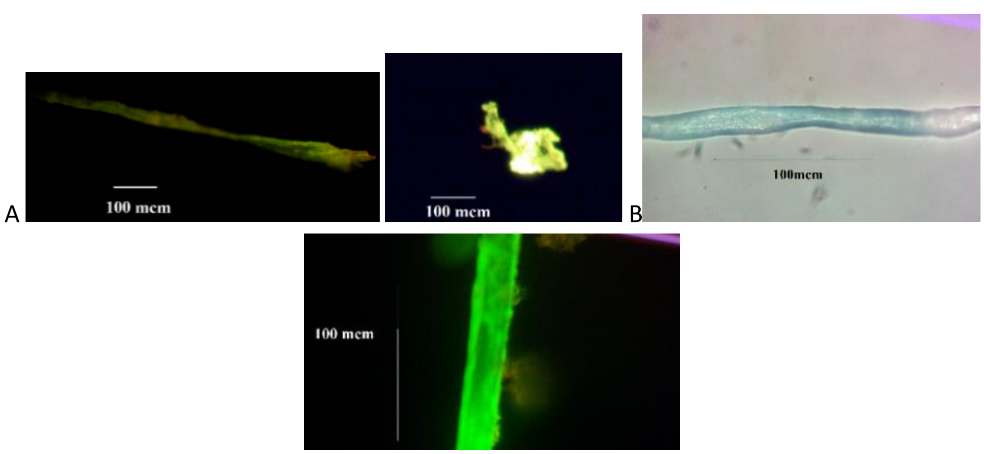


Fig. 9. Fluorescent microscopy of observed samples, isolated from water, cave mud and rock minerals.

A – 100x magnification (10-13˚C), B – 400x magnification (19-20˚C).

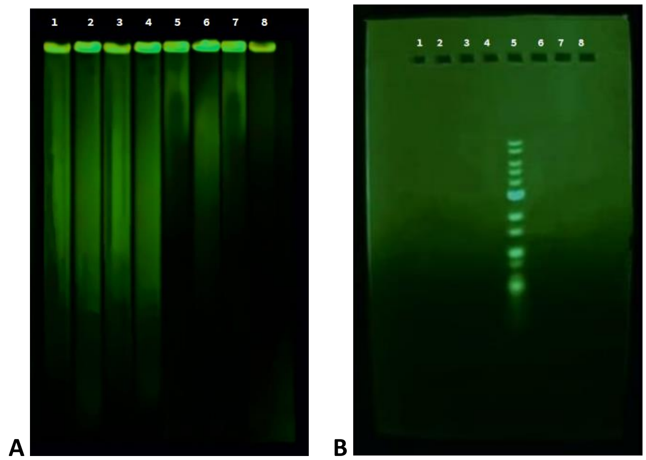


Fig. 10. Genetical analyses of isolated microbes.

A – DNA electrophoresis of four bacterial samples, isolated from water (samples 1-4) and four bacterial samples isolated from cave mud (samples 5-18) in contribution to temperatures of their isolation and cultivation: 3.8 ˚C (samples 1 and 5), 10 ˚C (samples 2 and 6), 19 ˚C (samples 3 and 7) and 50 ˚C (samples 4 and 8); B – PCR analyses of antibiotic-resistance of four bacterial samples, isolated from water (samples 1-4) and four bacterial samples isolated from cave mud (samples 5-18) in contribution to temperatures of their isolation and cultivation: 3.8 ˚C (samples 1 and 5), 10 ˚C (samples 2 and 6), 19 ˚C (samples 3 and 7) and 50 ˚C (samples 4 and 8). Then after the conducted research, the project of the creation of a granary was advised. The drawing of that is presented in Fig. 11.

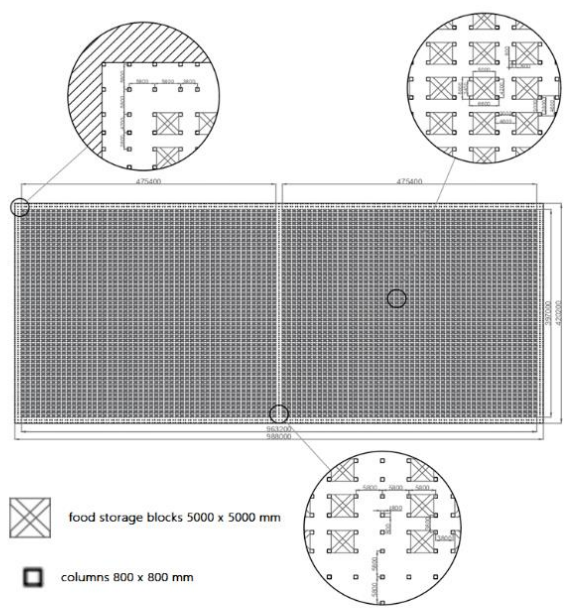


Fig. 11. The schematic drawing of a food storage (e.g. granary) project based on the internal space of Magellan Cave.

The food storage units are in accordance with the possibility to ensure that small electric vehicles (without harmful emissions that don't do ecological harm to the cave and the storing products) traffic between them. Two-way traffic is ensured through a certain number of food units, which together form one united storage module (for simplicity, 50 units are conventionally considered). In the drawing, you have two food storage modules, each of which consists of 50 x 50 units. Between the mentioned units the one-way traffic on a small electric car is ensured, while two-way traffic is ensured between the modules. All the construction activity will be carried out based on ecologically harmless materials with the minimization of harm to the Magellan Cave and the environment of its surroundings and the decreasing the risks of deterioration [34-36].

DISCUSSION

According to the obtained results, calcite and rare aragonite thin layers are deposited, creating a smooth, sheet-like flowstone covering large cave wall sections (Fig. 3: A, B). In photo Fig. 3: A, B travertine layers are deposited on top of the breccia base. Stalagmite is primarily composed of calcite, but under the microscope a few minerals of aragonite are also observed giving it a black color. Travertine and tufa deposits are also frequently associated with limestone dissolution in superficial (epigean) or deep (hypogean) hydrogeological reservoirs. Travertine in caves forms through the precipitation of calcium carbonate from mineral-rich water. Various environmental factors and results in creating diverse and intricate formations within the cave environment influence the process.

As the next step of research, microbiological experiments were conducted. Microscopy and cultivation on different media have demonstrated the various autotrophic and mixotrophic organism presence in samples, isolated at different temperatures. At 3.8 °C there were isolated small round, non-motile colonies of bacteria; small rod-shaped motile colonies; motile and non-motile bacilli and diplobacillus; cyanobacteria; white and yellow, fluorescent signals in UV light. At the temperature of 10 °C Cyanobacteria; various unicellular algae; filamentous algae; blue-green, green, and yellow, fluorescent signals. At the temperature of 19 °C Bacteria, cyanobacteria, various species of unicellular, colonial and filamentous algae; mixotroph microbes; red, blue-green, and green, fluorescent signals in UV light were identified. They also can survive at the temperature of 10 °C. It should be emphasized, that at the temperature of 35-37 °C no organism's presence was observed in samples that were collected from places that don't have contact with the surface. Also, it means that in Magill cave the conditions are not appropriate for the growth of mesophilic bacteria even in case of potential contamination during the construction process. And it significantly increases the grade of potential microbial safety in this territory. In samples, which were cultivated at 50 °C, the unicellular and filamentous algae; *Cyanobionta*; blue-green and green, fluorescent signals were found. These organisms are obligate thermophilic and don't cultivate at lower temperatures, such as the observed psychrophilic organisms are not able to be cultivated at higher temperatures.

The preliminary research of Magill (Magellan) cave, which is situated in the Areni region has demonstrated the presence of various microorganisms in its water, cave mud and rock mineral samples. During the conducted research, which was carried out by the application of all the mentioned microbiological techniques, such as UV/VIS and FTIR-ATR spectroscopic methods, the following species of organisms were identified: *Stephanodiscus astrae, Microcistis aeruginosa, Ochromonas sp., Cyanidium caldarium* in samples cultivated at 3.8-4 °C; *Navicula sp., Cyanidium caldarium, Microcistis* – in samples cultivated at 50 °C. According to the obtained results, the representatives of various taxonomic groups are presented in all the samples, but there's no human pathogenic microbe identified among them. According to the preliminary genetic study, *catB7, aac(6')II, aph(3')IV, and blaOXA-10* genes were not found in study samples. Thus, it might be concluded that the properties of potential antibiotic resistance are not related to the analogous ones that are presented in human pathogenic bacteria (Fig. 10). According to the collected data, the identified microbes differ in their morphology, type of metabolism, thermal optimum, and taxonomy, among other characteristics. There were detected psychrophilic, mesophilic, and thermophilic microbes in all the samples that were collected. The observed presence of thermophiles is evidence of the contact between Magill cave and the groundwater such as the hot springs, which are placed 10 km away from the cave. Some of the microbes have fluorescence in UV light in the form of blue-green, green, yellow, and orange signals. Further genetic study and the genotyping of the isolated microbes are planned.

Taking into consideration all the results that were collected, the plan of the potential granary was advised (fig 12). Food safety and long-term storage are some of the most important problems of food science and industry, which have dualistic applications both for a peaceful society and during wartime. The strategic storage of food carries the principal importance of all the countries. The hunger problem, which is one of the main global challenges in the XXI century, is one of the aspects of negative consequences of inappropriate quality of food safety and storage. That is why the problem of innovations and improvements construction of safe warehouses is very real, especially for developing countries such as regions of military conflicts, suspected risks of terrorist attacks, and natural disasters.

For the successful development of a project of the usage of the mentioned cave as underground granaries and food storage, the following aspects should be taken into consideration: the cost and feasibility of financial one-time investments over a long period of time; the flexibility and the structural strength of construction to the various extreme conditions; the functionality and comfort of the room (temperature, free movement of the electric cargo vehicle, etc.). Also, for the successful implementation of the project, all the construction elements should be created by the application of innovative ecologically safe materials and technologies that are being successfully used for the green building construction world around [34-36]. That will decrease both ecological risks for the environment and the potential risks for the other touristic caves which are situated near the Magill Cave. In these regards, the usage of Magill Cave offers advantages in all the mentioned aspects. In terms of durability, underground structures have a great advantage over any strategic structures and objects above ground. This might be confirmed when studying military conflicts occurring today [37]. For example, during the wars between Russia and Ukraine, Israel and Palestine, and Artsakh (known as the Nagorno Karabakh Republic) both civilians and military personnel starved or were exhausted due to lack of water and food [38]. And, due to our opinion, the proposed strategic warehouses are invulnerable both from the air and from long-range artillery and missile shells. Many can argue with our point of view with arguments that the Pushkin Pass tunnel and the Arpa-Sevan aqueduct stopped their work 2-3 times in 50-60 years and months and years of major repairs were ahead. However, the technologies that will be used for the project of the underground cave-based granary, and the types of specific excavations that are planned must be carried out in homogeneous geological strata. And it has the critical importance for avoiding the mentioned problems. In the tunnels, the accidents occurred because of the ceiling collapses in areas of tectonic cracks. The other cause of the mentioned problems was the junction of two different geological layers of rock in folded strata. In addition to the processes of water erosion and denudation, the reason for the constant penetration of groundwater is the erosion of the tunnel floor.

Thus, for the search for layers of the required volume, such as the geological and petrographic structure, we decided to study the experience of different countries, from the ancient cave cities up to the modern projects of hydrogen underground storage [39, 40]. Some examples can be found both on the territory of the RA and throughout the Armenian Highlands. Here there are many cave dwellings and cities, inhabited from prehistoric times, natural caves exploited by humans, and artificial caves, carved by humans. There are also many sacred buildings: Geghard, Kronk, Khochants (underground sacred buildings of Artsakh and Syunik), Caves with stone doors, and anthropogenic caves with evidence of the fact that man lived for a long time in them. For example, in the Stone Age, wine was produced in the Areni cave. Having the huge number of scientific materials collected from the studies of different cave-based constructions in this region, we are convinced that the settlement and sacred caves were dug into volcanic rocks. Also, we are convinced that the natural caves of Areni-1, Geghamavan were used as sacred foundations. There is a lot of historical evidence that some natural caves were constantly used by humans for different aims. For example, the 2000-year-old Magellan Cave and the 6000-year-old Areni-1 can be considered the cases of mentioned practice of cave usage [41, 42].

Summarizing the data of all the conducted research and taking into consideration the mentioned versatile examples of cave usage in some countries for different goals, it might be concluded that the potential possibility of Magill cave usage as the place for the granary and the food strategic reserves storage is very prospective. In accordance with the fact that within a period of contemporary history of the Republic of Armenia, there were no examples of cave usage as the places for storage of strategically important foods and other resources, such as water or fuel elements, the novelty of this project is very high, such as like the versatility of the potential applications of cave internal space territory. In these regards, it should be noted that the quality and functionality of food will not be decreased during the storage upon the unique conditions offered by Magill Cave. Further detailed genetic research can be interesting for the potential isolation of bioactive compounds for the elaboration of innovative products and drugs. Especially that can be potentially useful for the elaboration of innovative antimicrobial compounds and the combating of multi-drug resistance. Also, that may potentially be used in the opposite direction, particularly for the elaboration of innovative probiotic products, and other functional foods [43]. Besides, Magill Cave can be also used as a place with dualistic applications and become an important construction for the country. The experience of Armenian cave usage for mentioned purposes can also potentially be transferred to other countries with similar geoecological features. Moreover, the advised project can be potentially useful, especially for both developing countries and countries with various economic problems and the hidden risks of potential military complexities.

Abbreviations: DNA, Deoxyribonucleic Acid; PCR, Polymerase Chain Reaction; FTIR-ATR, Attenuated total reflectance-Fourier transform infrared spectroscopy; UV/VIS, ultraviolet/visual spectroscopy; NAS RA, National Academy of Sciences of the Republic of Armenia; RA, Republic of Armenia.

Author's Contributions: All authors contributed to this study.

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Appendix materials

Additional material about chemical consistency: the results of chemical and physical parameters of water are presented on Table 1.

Table 1. Chemical and physical parameters of water sample quality from the area around Magellan Cave (Magill Cave).

The parameters of water quality	The average mean
Dissolved oxygen	3.56 ± 0.022 mg/L
pH	6 – 6.5 ± 0.019
Total mineralization	1400 ± 0.022 mg/L
Hydrocarbonate ions (HCO^{-}_{3})	28.9 ± 0.022 mg eq/L
Chloride ions (Cl^{-})	23.07 ± 0.021 mg/L
Total stiffness	21 ± 0.019 mg eq/L
Calcium ions (Ca^{2+})	321.6 ± 0.018 mg/L
Magnesium Mg^{2+})	60.2 ± 0.022 mg/L
Iron (III) ions (Fe^{3+})	40.45 ± 0.021 mg/L
The temperature of water, contacting with the surface	8 – 10 ˚C± 0.022 mg/L
Temperature of air inside the cave	9 ˚C± 0.022 mg/L
Radiation	0.06 ± 0.019 mcSv/h.
Specific electrical conductivity	2158 ± 0.021 mcSim/cm