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Study on microbial diseases and control in Yuhuangdong Grottoes

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Abstract: Due to the influence of man-made and natural factors, the microbial diseases and potential risks in Yuhuangdong Grottoes have gradually attracted the attention of researchers. In this paper, the microbial samples on the surface of the statues in Yuhuangdong grottoes and the surrounding rock mass were detected and analyzed, and then the causes were discussed in combination with environmental and other factors. Finally, the method of 3A+isothiazolinone (0.3%)+WD-10 (15%) was used for cleaning and sealing. In this paper, the microbial samples on the surface of cave statues and surrounding rock mass were collected for systematic detection, and the formation mechanism was discussed by integrating environmental and other factors. At the same time, the relevant research can provide a reference for the prevention and control of biological diseases of the same type of stone relics in Hunan.

1. Introduction

Located at the southern foot of Makong mountain in fengxianggang Township, Yongding District, Zhangjiajie City, Yuhuangdong grottoes were donated and repaired solely by the local Qing Dynasty squire lijingkai. [1] The whole cave of Yuhuang cave is more than 300 meters long from east to west. There are 19 stone statues and more than 50 stone carvings. The cave faces south, with a height of about 15 meters, a width of about 1000 meters and a depth of about 1000 meters. [2]It is divided into three parts. In 1983, the Hunan provincial government officially listed the Yuhuangdong Grottoes as a provincial key cultural relics protection unit.

Affected by many factors, such as man-made and natural factors, the microbial diseases and potential risks in Yuhuangdong Grottoes have gradually attracted the attention of researchers. Under the joint action of natural conditions and human activities, the phenomenon of biological erosion and its potential threat in the Yuhuangdong grottoes are increasingly concerned by the academic community. In this paper, the microbial samples on the surface of cave statues and surrounding rock mass were collected for systematic detection, and the formation mechanism was discussed by integrating environmental and other factors. At the same time, the relevant research can provide a reference for the prevention and control of biological diseases of the same type of stone relics in Hunan.

2. Detection and analysis of microbial samples

Adhering to the principle of reducing the interference of miscellaneous bacteria outside the samples as much as possible under the premise of limited field conditions, the working group set the sampling area as the surface of the statue of Yuhuangdong grottoes and the surrounding rock mass, and used the scalpel burned by the external flame of the alcohol lamp for 5 seconds to collect a total of 9 samples. In order to ensure the activity of microorganisms in the samples and the stability of community structure, the samples were taken back to the laboratory for follow-up observation immediately after sampling.

Comprehensive analysis of microscopic imaging and molecular biological detection results shows that the microorganisms in the samples of Yuhuangdong Grottoes include lichens, cyanobacteria, mosses and fungi. As shown in Figure 1, no dominant genera or species were found in the microbial community in most samples. Lepraria, blue-green algae and Andreaea accounted for a relatively large proportion in the samples. Among them, the occurrence frequency of Lepidoptera lichens is relatively high, accounting for about 30% of the lichen samples. Samples 1, 3 and 4 are Lepidoptera lichens, and sample 5 is Lepidoptera lichens, accounting for about 30%. Cyanobacteria accounted for about 30% of the samples, No. 8 and No. 9 were detected as cyanobacteria, and No. 5 was detected as cyanobacteria, accounting for about 70% of the samples. Litmus lichens accounted for 10% of lichens, mainly in sample 2. Most of the eukaryotic detection results were fungi, and the dominant group in the community was Engyodontium. The dominant species of fungi in sample No. 6 was Rhizoctonia, accounting for 89%.

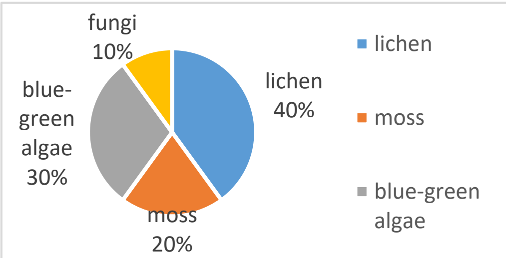


Figure 1: Proportion of microbial species in Yuhuang cave

3. Cause analysis of microbial diseases

The factors affecting the occurrence and development of microbial diseases are complex and diverse. The chemical composition and properties of materials, the exposure degree of climate and cultural relics, and even the protection methods and frequency are closely related to microbial diseases. [3]

3.1 Rock properties

According to the field survey, the rock types of Yuhuangdong grottoes are mainly divided into hematite rock and limestone. In order to clarify the characteristics of the rock body of Yuhuangdong grottoes, the working group collected rock samples and their surface precipitates around the stone carvings in different areas for detection. The stone samples were analyzed by modern characterization methods to understand the material and weathering status. In order to obtain the information of the composition, structure and properties of the samples, scanning electron microscope energy dispersive X-ray spectrometer (SEM-EDX), X-ray fluorescence spectrometer (XRF), X-ray diffraction (XRD), polarizing microscope, comprehensive thermal analyzer and other analytical instruments were used to analyze the lithofacies, composition and microstructure of the stone samples, as well as the main chemical composition of the weathering products. The specific analysis and test methods include: SEM-EDX observation of the microstructure of the sample and preliminary analysis of the chemical composition; The phase composition and chemical composition of the samples were analyzed qualitatively and semi quantitatively by XRD and XRF; The mineral composition and microstructure of stone samples were analyzed and identified by polarizing microscope, and the types of stone were identified; Comprehensive thermal analysis comprehensively analyzes the composition of the material.

According to the current investigation results of Yuhuangdong grottoes, microscopic identification shows that the rocks are mainly limestone, some dolomite metasomatism, and the debris is mainly argillaceous and bright calcite.

Microscope observation: the model of the microscope used is lecia dm4000m, which is observed with the corresponding multiple objective lens according to the sample morphology. The observation results are shown in Figure 2.

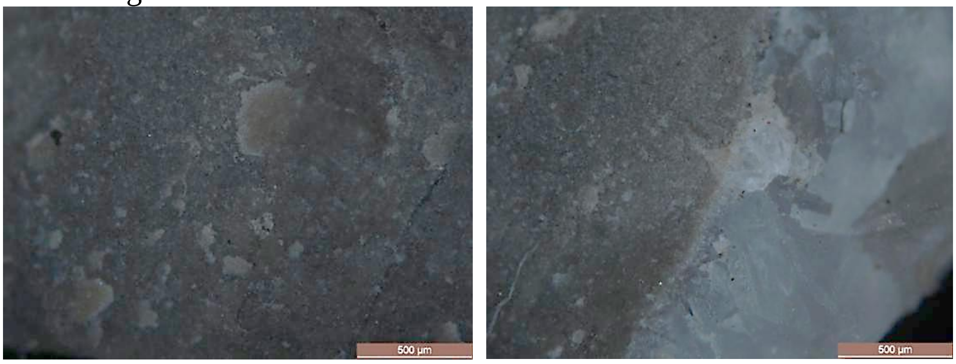


Figure 2: Micrograph

Hmadzu edx-800hs X-ray fluorescence spectrometer (XRF) was used, the working voltage was 20kV, the working current was 20mA, the fragment sample was ground into powder, and the powder was tested in powder VAC mode. It can be seen from table 2 that the mineral composition of limestone is mainly calcite and dolomite, the content of dolomite and clay minerals is high, and the content of quartz is small; Pyrite and hematite exist in some yellow samples. The observation results are shown in Table 1.

Table 1 main mineral composition of rock

Sample name	**Spectrum resolution results**					**Total clay minerals (%)**
YS6	component	quartz	calcite	dolomite	Hematite	**4.7**
	content (%)	0.9	87.2	2.4	4.8	

During the EDX test, in order to ensure the accuracy of the test, five different points were randomly selected for energy spectrum analysis of each sample, and the average value is shown in Table 2 below:

Table 2 Mass percentage of main elements in EDX test of stones (%)

Sample name	**C**	**O**	**Ca**	**Si**	**Mg**
Rock weathering layer	20.07	44.67	22.28	00.57	12.32
Rock interior	11.34	36.20	52.46	——	——

According to EDX results, the main components of rocks are Ca, O and C. The scanning electron microscope image shows that the mineral composition of the rock is diverse and uneven, the internal structure of the rock is relatively dense, and no weathered granular material is found. However, loose weathering products can be seen in the weathered layer of rock, which has been weathered to a certain extent. According to EDX data, the weathered layer contains a small amount of Mg and a small amount of Si in addition to Ca, O and C.

Japanese D/max TTR III X-ray powder diffractometer was used. Some precipitates in the rock interior, rock surface and surface layer were selected as the analysis samples. Based on the determination of the chemical composition of the sample by XRF and SEM-EDX, the phase of the sample was analyzed by XRD.

According to XRD results, the main body of the rock is mainly composed of calcite and dolomite. There is also a small amount of quartz on the rock surface. The composition of the precipitates on the rock surface is different, and the composition of precipitate 1 is close to the main composition of the rock. The precipitate 2 is mainly composed of calcium oxalate and calcium carbonate, which may be caused by biomineralization. The specific situation needs further study. At the same time, the XRD results of white precipitate 2 showed that its crystallinity was not good.

According to the above analysis of the material composition, structure, morphology and physical properties of the sample, the rock is mainly composed of calcite, dolomite and opaque minerals (trace). Generally speaking, the rock hardness is moderate, the rock is relatively dense, and the porosity is low, but the water resistance and solubility of the rock are poor, and it is easy to have microorganisms and corrosion on the surface, so hydrophobic measures should be taken to prevent.

3.2 Environmental factors

Yuhuangdong grottoes are located at the southern foot of Makong mountain(as shown in Figure 3). Limestone is widely distributed in Makong mountain. There are many karst caves and underground rivers in the mountain. As an underground water body, the existence of underground rivers helps to increase the humidity of the mountain. Because the water level and flow of the underground river are relatively stable (although there are seasonal changes), it provides a continuous water source for the mountains and caves. These water sources increase the humidity of cave walls and air through infiltration and evaporation. Taking the cause and effect cave with the lowest horizontal position in the Yuhuang cave as an example, the cause and effect cave is a natural karst cave, and there is an underground river in the deepest part of the cause and effect cave, which makes the air humidity inside the cause and effect cave relatively high. In addition, the cave is deep, and the interior of the cave is not exposed to sunlight all the year round, so the water is not easy to evaporate. Therefore, the vegetation and microorganisms in the cave can more easily obtain water, so as to promote their growth and reproduction. In addition, there are many rivers near the mountain, the water system is developed, and the moisture content in the air is high. With the coming of night, the temperature in the mountain gradually decreases, especially in the peak area. This temperature difference causes water vapor in the air to condense easily. If the water vapor content in the air is high and the wind force caused by mountainous terrain is relatively small, it is conducive to the accumulation of water vapor. When water vapor reaches saturation, it will condense into fog. Moreover, the mountains are steep and the terrain is complex. The airflow exchange between valleys and hillsides may form an updraft, which will bring moisture to high places. This makes the caves in the mountains always in a humid environment, providing sufficient water for the growth and reproduction of microorganisms.

Zhangjiajie has a subtropical monsoon climate and abundant precipitation. The average temperature of the whole region over the years is 16.8 °C and the average frost free period over the years is 270 days. Due to the large altitude difference, the vertical temperature difference is also large. The extreme maximum temperature in the region over the years occurred from July to August, and the extreme minimum temperature over the years occurred in January. In the summer and autumn with heavy rainfall, atmospheric precipitation will carry organic matter attached to the pores of rock mass, and the water vapor in the air will form a large amount of condensate on the surface of cliff statues. All these provide nutrients and suitable environment for the growth of microorganisms. [4]The average sunshine over the years is 1450 hours, and the appropriate light intensity and wavelength promote the photosynthesis of algae and the growth rhythm of microorganisms.

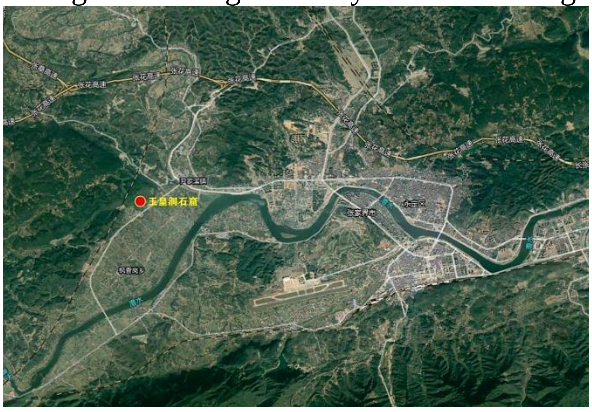


Figure 3: Topographic map of the geographical location of Yuhuangdong Grottoes

4. Prevention measures and effect evaluation

4.1 Field test

Test materials: isothiazolinone (0.3%), WD-10 (15%), 3A solution, syringe, paper towel, preservative film, brush, tweezers, absorbent cotton, sponge, scalpel.

Delimitation of test area: the test area is selected to be delimited in the middle of the east wall of Mochi Cave (where microorganisms grow most vigorously, mainly lichens of the genus Lepidoptera). The test area is about 10cm ×10cm per block. See Table 3 and Figure 4 for details.

Table 3 Design of microbial cleaning and sealing test area

Test area for cleaning and sealing traces of inactivated microorganisms	Blank control area
	3A test area
	3a+isothiazolinone (0.3%)
	3A+ WD-10(15%)
	3A+ isothiazolinone (0.3%)+ WD-10(15%)

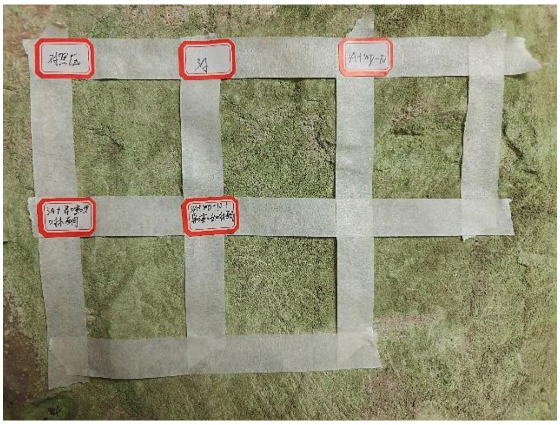


Figure 4: Overall appearance of test area

(1) 3A solution: 3A (deionized water: ethanol: acetone=1:1:1) is used as the cleaning agent, which

is dipped with absorbent cotton and brush for cleaning.

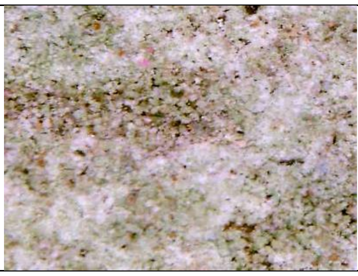
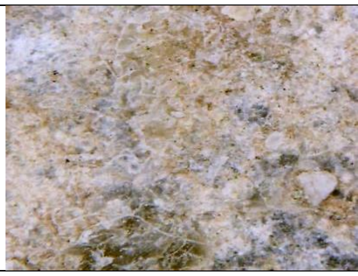
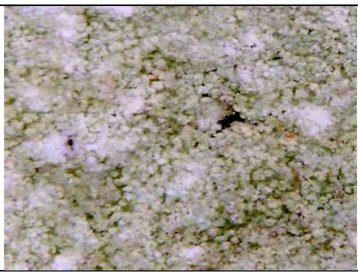
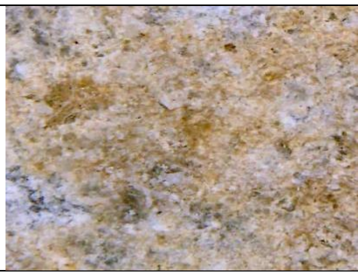
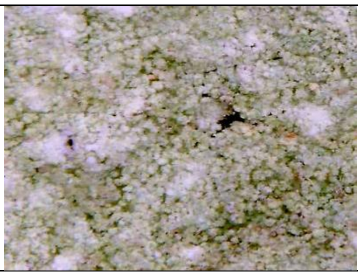
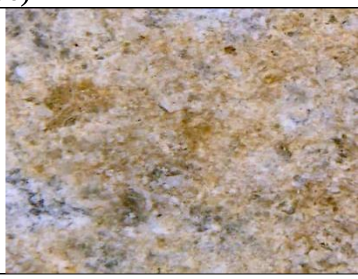
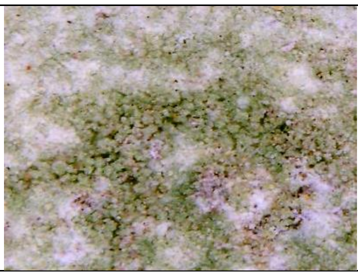
(2) Isothiazolinone: use 0.3% isothiazolinone solution as bacteriostatic agent, dip absorbent cotton and evenly smear it on the cleaned stone wall.

(3)WD-10: Use 15% WD-10 solution (WD-10: anhydrous ethanol=3:17) as the sealing agent, dip the absorbent cotton and evenly smear it on the cleaned stone wall.

(4) Evaluation of cleaning effect: two hours after the completion of cleaning test, check the status of residue and rock surface, take micrographs and overall photos after the test, and make records.

4.2 Comparison before and after test

Table 4 Micrographs before and after the test

before	after
	
3A test area	
	
3a+isothiazolinone (0.3%)	
	
3A+ WD-10(15%)	
	
3A+ isothiazolinone (0.3%)+ WD-10(15%)	

The observation results are shown in Table 4. Experiments have proved that using 3A to clean microorganisms can achieve considerable effect, and at the same time, with isothiazolinone and WD-10 can better achieve the role of bacteriostasis and sealing. In combination with the previous analysis and detection of microbial samples in Yuhuang cave grottoes, after the discussion of the working group, it was decided to take the traditional 3A solution to clean the microorganisms in Yuhuang cave grottoes, use isothiazolinone solution (0.3%) for sterilization, and finally use WD-10 solution (15%) for sealing.

5. Conclusion and Prospect

In this paper, the microorganisms in the Yuhuangdong Grottoes in Zhangjiajie, Hunan Province were sampled and analyzed. The results showed that the microorganisms in the Yuhuangdong Grottoes included lichens, cyanobacteria, mosses and fungi. No dominant genera or species were found in the microbial community in most samples. Lepraria sp., blue green algae and andreaea accounted for a relatively large proportion in the samples. Finally, after the field test, the working group chose to use 3A+isothiazolinone solution (0.3%)+WD-10 solution (15%) for cleaning and sealing.

Hunan has a unique subtropical monsoon humid climate, and its high temperature and humidity environment provides an ideal growth substrate for microbial communities. With the intervention of external pollution sources (including force majeure factors or improper human protection measures), microbial erosion tends to deteriorate exponentially, which will threaten the physical and chemical stability of stone relics and pose a major challenge to their preservation in the long run.

The prevention and control of microbial diseases in Grottoes is a process that attaches great importance to early intervention and takes preventive measures as the core. [5]The breeding and reproduction of microorganisms are profoundly affected by environmental conditions all the time, which may be accelerated due to the appropriate environment or restricted due to adverse environment. Therefore, the key to ensure that cultural relics and historic sites are protected from microbial diseases is to scientifically and reasonably regulate the preservation environment of cultural relics, implement continuous monitoring, and take decisive measures immediately when disease signs are found. However, at present, most of the antibacterial products on the market have a limited effective period, generally about three years, up to five years. If we only rely on these chemical means and ignore the overall optimization of the preservation environment for cultural relics, such as failing to take measures to reduce the air humidity in caves, not effectively controlling the condensation on the surface of cave statues, and not actively blocking or reducing direct contact between external pollution sources and the relics, the management of microbial diseases affecting these cultural relics will become increasingly complex and difficult once the bacteriostatic agent fails.

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