Effects of different light wavelengths on mold growth in tomb

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Abstract: The Second Mausoleum of the Southern Tang Dynasty have a history of more than 1,000 years in China. Since its opening in 1984, the building materials and murals inside the tomb have been severely damaged by mold. Field investigation found that the mold growth on the wall illuminated by the light in the tomb was more flourishing than that in the area without the light. Lighting in the tomb is inevitable for the exhibition. Therefore, the purpose of this study is to clarify the effects of different light wavelengths on the growth of mold in the tomb, and provide the theoretical basis for the lighting design in the tomb chamber. This study is divided into two parts, including in-situ experiment and laboratory experiment. In the in-situ experiment, four kinds of light wavelengths (white, red, blue, and green) were set in the tomb chamber to observe the difference of mold growth on the mural wall. The concentration of phototrophs propagules on different auricular walls was estimated in the laboratory.

The results showed that different light sources had different effects on microbial community composition in the tomb site and the red light had better inhibition effect on fungi and actinobacteria.

Keywords: Tombs protection, illumination influence, mold growth

1. Introduction

Microbial degradation is the result of the interaction of microbial communities. The process is determined by many factors including light, humidity and nutrition supply of microorganisms. In recent years, studies have shown that different wavelengths of light have different effects on the growth of microbial communities.

Brick tomb heritage buildings, such as the Second Mausoleum of the Southern Tang Dynasty, are exposed to the earth and affected by the fluctuation of indoor and outdoor temperature and relative humidity, so the material surface is prone to high temperature, high humidity and high water content, which further aggravates the microbial reproduction and deterioration [1,2]. Originally, the tomb was in a dark state, but the artificial lighting set up for the display needs provided a suitable niche for the development of photogenic microorganisms, which made a large area of microbial diseases grow on the wall behind the lamp source. At the same time, the existence of photogenic organisms provided
sufficient nutrients for mold growth [3,4,5,6]. Under these conditions, complex biological communities are formed on the brick walls and the ground illuminated by the light source in the tomb.

In the past decades, different scholars have restrained the growth of photogenic organisms by limiting the wavelength band of light sources, so as to achieve the purpose of protecting cultural relics. Albertano P, Bruno L and others explored the influence of light on microbial reproduction in Roman catacombs, and proposed that the lighting system of underground remains could choose green light, red light or their combination, because only a few photosynthetic organisms could grow in these visible spectra; S. Sanchez-Moral and others put forward one of the urgent countermeasures to protect the underground cultural heritage from destruction, which was to establish an illumination system that could make the growth of cyanobacteria most difficult and minimize the temperature rise [7]. These studies provide a scientific basis for the idea of using light sources with different wavelengths to suppress mold reproduction in ancient tombs. However, due to the differences of biological communities in different brick tombs, whether the conclusions of these studies can be applied to the two tombs in the Second Mausoleum of the Southern Tang Dynasty in China needs further verification. In the current research, most studies only focus on which wave band of light can inhibit the growth of cyanobacteria. The influence on the whole biofilm has not been explored. However, Photoautotrophic biofilms usually adhere to and grow on solid surfaces in terrestrial or aquatic environments. The main microorganisms including cyanobacteria, green algae and diatoms and abiotic substances are embedded in the organic polymer matrix secreted by microorganisms, forming a relatively stable ecosystem [8], and its internal interspecific relationship is complex, which needs to be studied from a more macroscopic community perspective.

The case of this study is selected in the Second Mausoleum of the Southern Tang Dynasty with a history of more than 1,000 years in China. The Second Mausoleum of the Southern Tang Dynasty is divided into two mausoleums: the Shunling Mausoleum and the Qinling Mausoleum. The research site was located in the rear tomb chamber of the Shunling Mausoleum, with four ear chambers distributed on both sides of the rear tomb chamber. There was no air conditioning or other ventilation system in the tomb chamber. Ventilation was carried out through the entrance of the tomb (Figure 1). Four ear chambers with the same temperature and humidity conditions were selected, and four light sources with different wavelength bands were placed for observation in one year. By monitoring the temperature and humidity of the ear chambers, on-site observation and plate counting method, the growth state and reproduction concentration of phototrophic organisms on the wall of the ear chambers under different wavelengths of light were investigated. Mold growth is a common problem in modern buildings. This study explored the effects of different wavelengths of light on mold growth in semi-underground burial environment, which can provide a theoretical basis for preventive protection of mold growth in modern buildings.
2. Materials and methods

2.1. Measurement of temperature and RH at the ear chambers.

September 2019, light sources with different wavelength ranges were placed in the tomb, including red light (610–750nm), green light (500–560nm), blue light (450–480nm) and white light (composite light). The model of bulb was TCLYPZ 220/13-LXSRR7, and the power of bulb was 13 watts. The lights have been turned on continuously until now. The spectral distribution of the lights is shown in Figure 2. The instrument used in the monitoring process was temperature and humidity recorder (HOBO MX2301A). The temperature and relative humidity values were recorded every 30 minutes. The environmental monitoring period was from August 25, 2020, to January 14, 2021.

2.2. Field observation.

The observation equipment was a camera (Nikon/D610) and a portable microscope (DinoCapture2.0). Phototrophic biofilm on the wall of the ear chamber was recorded by photographing from September, 2020.
2.3. Sampling and estimation of propagule concentrations.
Biofilm samples were collected from the bonding layer between the bricks in the four ear chambers of the mausoleum (Figure 3). The material of the bonding layer was soil. The biofilm was gently scraped off with a sterile scalpel. Then it was immediately into a sealed bag until reaching the laboratory, and the sealed bag was put in a 4-degree refrigerator for storage. Each sample was weighed and 1g soil sample was taken to prepare bacterial suspension with three dilution gradients of 10^{-3}, 10^{-4} and 10^{-5}, and subsequently inoculated on three different cultures of bacteria (LB Medium), fungi (Potato Dextrose Agar Medium) and actinobacteria (GAUZE’s Medium) respectively. All operations were carried out on the ultra-clean working table to avoid the contamination of miscellaneous bacteria. After inoculation, GAUZE’s Medium plate and PDA Medium plate were placed in 28 ℃ constant temperature incubator for 3-5 days. The LB Medium plate was cultured in 37 ℃ incubator for 1-2 days. At the end of the culture period, the culture dish was checked and all visible colonies were counted and marked. Then, the obtained numbers of colonies were calculated according to equation (1).

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\text{CFU value} = \frac{1}{\text{Dilution factor}} \times \text{Colony count} \tag{1}
\]

Figure 3. Sampling locations.

3. Results
3.1. The results of temperature and humidity monitoring in the ear chambers.
From January 2020 to January 2021, the measurement of temperature and relative humidity in ear chamber with white light is shown in Figure 4. Due to equipment damage during the measurement period, data from September 11th to December 18th were replaced by data from the ear chamber with red light, represented by gray lines in the figure. The indoor temperature fluctuated between 6.1℃ and 21.5℃, and the relative humidity fluctuated between 54% and 100%. The annual average temperature was 15.4℃, and the annual average humidity was 95%. The relative humidity maintained above 80% for more than 10 months, and it was almost 100%, the extreme humidity environment, from April to October. The temperature and humidity monitoring results of the four ear chambers from September 2020 to January 2021 are shown in Figure 5. Since the temperature error of each sensor is ±0.2℃ and the humidity error is ±2.5%, the measured results are within the error range. Consequently, it can be considered that the temperature and humidity in the four ear chambers were basically the same.
3.2. Results of field observation.

Through on-the-spot investigation, we found that before placing light sources with different wavelengths, the distribution of photogenic biofilm on the wall as shown in the ear chamber with white light in Figure 6 and the growth state of biofilm in the four ear chambers was basically the same. After one year's exposure to different wavelengths of light, there were obvious differences in microbial community status. The morphology of biofilm in ear chamber with red light and ear chamber with white light was similar, both of which were bluish green. However, the color, morphology, and species composition of the biofilm in the ear chambers with blue and green light changed. Among them, the growth state of biofilm in the chamber with green light was quite different, showing the characteristics of multi-layer distribution. The bottom layer near the brick surface was similar to the biofilm in the chamber with red light. The second layer was dark green flocculent algae microorganism, and some of the brick surface was also attached to the second layer of algae surface with large accumulation of white microorganisms. From observation with 50 times microscope (Figure 7), we can see the white hyphae in the uppermost layer covering the surface of the algae in a network; the biofilm on the wall of ear chamber with blue light was black purple thin crust. The brick surfaces of the four ear chambers were all covered with white bacteria.

Figure 4. Temperature and relative humidity in a white ear chamber in one year.

Figure 5. Four ear chamber temperature and humidity monitoring data from August to January.

Figure 6. After one year's illumination with different wavelengths (white, red, green and blue), the algae morphology on the wall changed greatly (A. Close-up photos of ear chamber walls. B. Photo of the interior of the ear chamber).
3.3. Results of phototrophic biofilm propagules concentration.

The results of biofilm propagules concentration of samples A and B are shown in Figure 3. The values in the figure are the values of CFU numerical results of two samples in the same ear chamber. The bacteria in the ear chamber with white light were more than those in the other three ear chambers (2375000CFU/g). The number of fungi in ear chamber with red light was the least (10000CFU/g), while that in ear chamber with blue light was the most (525000CFU/g). The number of actinomycetes in ear chamber with green light was the most, which was 102500CFU/g (Figure 8).

4. Discussion

4.1. Effects of temperature and humidity in ear chamber on phototrophic biofilm.

It can be seen from the monitoring results that the internal environment of the Second Mausoleum of Southern Tang Dynasty was in high humidity for a long time. In winter (December 1st to March 1st), the temperature fluctuated in the range of 8.9°C–14.5°C, with an average temperature of 11.9°C, and humidity fluctuating in the range of 63.5%–97%. In summer (June 1st to September 1st), the temperature fluctuated in the range of 17.4°C–21.5°C, with an average temperature of 19.1°C, and humidity maintained at 100%. Compared with winter, the internal temperature and humidity of the four ear chambers in summer were more stable, and the maximum daily temperature difference was within 1°C.
The maximum daily temperature difference in winter was 3.3°C. The daily fluctuation of humidity could reach 17%. Studies have shown that the minimum relative humidity for the growth of most microorganisms is above 80%, and the high humidity environment in the tomb for ten months in a year could provide sufficient conditions for the growth and reproduction of microorganisms. However, when favorable conditions and unfavorable conditions alternate, the growth rate and elongation of mold were lower. Consequently, the stability of temperature and humidity conditions was more conducive to mold growth. In summer, the temperature and humidity inside the ear chamber were stably maintained under favorable conditions for microbial reproduction. Figure 5 shows that the temperature and humidity in the four ear chambers were basically the same, so the difference in growth of photogenerated biofilms in different ear chambers within one year can be considered not to be caused by the difference in temperature and humidity.

4.2. The effect of light on the growth of algae microorganism.
Many studies have shown that the phototrophic biofilms formed behind the exhibition lights in caves and underground burial chambers are mainly composed of cyanobacteria and algae, heterotrophic bacteria and fungi, and other eukaryotes of ESP [7,8,9].
Algae containing chloroplasts, which are photosynthetic microorganisms, are an important cause of fresco diseases. The carbon dioxide gas produced by algae respiration dissolves in water to form carbonic acid, and the chemical acid etching of carbonic acid causes corrosion of brick. The long-term illumination of the wall by the visiting lights in the mausoleum, provides sufficient external conditions for the photosynthesis of algae, which makes algae propagate in large quantities. As shown in Figure 7, under 50 times microscope, the algae in the ear chamber with red light grew more intensively, which were distributed in green dots and in good growth state. However, the growth of algae was not observed in the ear chamber with blue light. In other words, compared with red light and white light, blue light may have better inhibition effect on algae on the wall of tomb chamber.

4.3. The difference of the damage of bacteria, fungi and actinobacteria to the wall of tombs.
Algae also form symbiotic relationship with other fungi. Bacteria and fungi always exist in the same microhabitat where cyanobacteria and microalgae live. Algae use inorganic matter provided by fungi to grow, and fungi use organic matter provided by algae to multiply, which will cause mass substrate exfoliation and damage. Compared with bacteria, fungal and actinomycete diseases may cause more serious damage to brick walls [10,11]. This is because the plaque produced by fungi pollutes the wall, which seriously affects the value of cultural relics and art. Moreover, the developed hyphae will extend into the inside of the wall, causing the fall off of the floor layer and the brick wall, and secreting organic acids at the same time. These acids cause minerals to dissolve, change the material's structural configuration, erode the brick and form crystals on its surface. Actinobacteria, on the other hand, produce filamentous structures that penetrate the matrix and produce propagules and spores for reproduction. From the results of biofilm propagules concentration, it indicates that bacterial concentration in the ear chamber of white light was much higher than that in the other three ear chambers. White light can promote the growth of bacteria in the phototrophic biofilm, while red light can inhibit the growth of fungi and actinobacteria.
5. Conclusion

Through field observation in one year, it can be concluded that different wavelengths of light have obvious effects on the community structure of phototrophic biofilm. The algae in the ear chamber with red light grew well, while the blue light had a good inhibitory effect on the algae. From the results of the concentration of biofilm propagules, it can be concluded that the concentration of bacteria in the ear chamber with white light is the highest, while the inhibition effect of red light on fungi and actinomycetes is better than other lights.

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