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Study of Archaeological Limestone Cleaning by using Bacteria Applied on a Selected Model

A Thesis submitted for the degree of Doctor in Conservation of Antiquities.

Submitted by

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2021

Summary

This thesis entitled on "**Study of Archaeological Limestone Cleaning by using Bacteria Applied on a Selected Model** ", consists of three chapters, discussion of results, some important recommendations and a list of references as follows:

Chapter One

Study the use of bacteria in bio-cleaning of archaeological limestone surfaces

This chapter deals with study the mechanism of the black layers formation on archaeological limestone surface, and the role of wet and dry deposition in deterioration . Damage layers and surface crusts are the most common forms of weathering on the facades of archaeological buildings in Cairo. These crusts are classified according to the colors of black and white crusts. Black crusts are the most frequent forms of soiling on archaeological buildings, divided into framboidal and laminar black crusts, which develop in sheltered areas from direct rain wash. Framboidal black crust develops on sheltered ashlars , while laminar black crust can cover entire walls and facades of limestone buildings and develops most commonly on vertical areas .

It also deals with the study of bacteria . The composition of the bacterial cell , its forms and the conditions suitable for the growth of bacteria , as well as the study of the bio-cleaning of the black crusts by studying the pathways of sulphate reduction, assimilatory and dissimilatory sulfate reduction by bacteria, biocleaning applications by using sulfate reducing

bacteria in cleaning the black crusts, and types of delivery systems of bacterial cells in bio-cleaning .

Chapter Two

Examinations and Analyses of some archaeological limestone

This chapter deals with the methods of examination and analysis conducted on the black layers that formed on the outer walls of the mosques of Sultan Hassan, Al-Rifai and the wall of Salah al-Din Ayyubid Castle. The black crusts were examined using stereo microscope , polarizing microscope as well as the examining and analysis using SEM-EDX , analysis by X-ray diffraction , infrared and Raman spectrometer. The results of examination and analysis were as follows:

-The results of the examination by the stereo microscope of the black layers in the archaeological buildings under study showed that thick, irregular black layers were formed on the surface.

-The polarizing microscope examination showed that the limestone contained fossils and that some geological processes have taken place with limestone, such as cementation and Silicification at the Sultan Hassan and Al-Rifai mosques, and the process of Recrystalization of limestone in the castle wall.

- The results of SEM showed that the black crusts are mainly composed of gypsum crystals which appears in diffrent morphological forms. Besides, stable gypsum crystals habits rosette and acicular were identified in black crusts , aggregates of gypsum granules related to early stages of gypsum formation were also found. The examination by SEM also showed the occurrence of some geological processes with limestones in the archaeological buildings under study, as confirmed by the study with the polarizing microscope.

- The results of analysis by X-ray diffraction and infrared showed that the composition of the black layers is heterogeneous and consists of gypsum, calcite and halite minerals, and the EDX analysis showed that the black layers also contain iron oxides , clay minerals and quartz . Analysis by raman spectrometer also confirmed the presence of carbon particles in the composition of the black layer of Al-Rifai Mosque and the Citadel wall.

Chapter Three

Experimental applied study to clean the chosen model of archaeological limestone using bacteria

This chapter deals with an experimental study to clean the black layers formed on the surface of limestone samples using different bacterial species, to assess the efficiency of these species in cleaning the black layers, which consist mainly of sulfates. Bacteria have been isolated from the soil and the molecular definition of these isolated species. Bacterial species representing the two sulphate reduction pathways by bacteria, assimilatory and dissimilatory sulphate reduction, were used in this study,where the following bacterial species were tested *Desulfovibrio sp., Desulfobotulus sapovorans , Desulfocella sp, E. cloacae, B. subtilis and P.aeruginosa* .

It also deals with the stages of preparation bio-cleaning compresses, after the growth of bacterial species in the appropriate media and the passage of the incubation period, the centrifuge was carried out for each type of bacteria to separate the biomass from medium . The biomass is suspended in phosphate buffer solution and the addition of arbocell as a delivery system for bacterial cells and the components of the compress are well blended by mechanical mixing . The compress must be solid enough to ensure that it stays on the stone surface. Bio-cleaning compress is applied

on the surface by moisturizing the surface with phosphate buffer solution and applying japanese paper to the surface, which facilitates the removal of the compress after treatment, it is applied to the surface as a regular continuous layer with a thickness of of 5.0 to 1cm ,and cover the biosystem using plastic film to provide anaerobic conditions to ensure the activity of bacteria and good water retention, the compress is applied to the surface for 24 hours and remove the biosystem after treatment.

The efficiency of bacterial species was assessed by studying the effect of biocleaning on the overall appearance and using different methods of examination and analysis. The results of the visual evaluation after treatment showed that the following bacterial species *Desulfobotulus sapovorans, Desulfocella sp., B.subtilis and P.aeruginosa* did not give anypositive results in cleaning and did not affect the black layers, While *Desulfovibrio sp.* bacteria gave the best results in cleaning the black layers and restoring the limestone to its original color, but it did not affect the rust stains on the surface. *Enterobacter cloacae* bacteria gave good results in cleaning the black layers and iron rust stains, but some sediments remained on the surface.

The overall appearance was also assessed using the Chromatic change measurment. *Desulfovibrio sp.* achieved the best results in cleaning the black layers, but it did not affect the rust stains on the surface and gave values of 0.42, 13.38 respectively, *Enterobacter cloacae* also achieved good results in cleaning the black layers and rust stains on the surface and gave values of 4.60, 5.11 respectively, while other bacterial species did not give any positive results in cleaning the black layers.

The surface was also examined using stereo microscope, where *desulfovibrio sp.* succeeded in cleaning the black layers, but it didn't affect the rust stains on the surface. *Enterobacter cloacae* bacteria also succeeded in cleaning the black layers, but some gypsum deposits

remained on the surface, and succeeded in affecting the iron rust stains on the surface, while other bacterial species did not affect the cleaning of the black layers.

An examination using SEM before bio-cleaning showed the spread of gypsum crystals, covering the surface completely. The evaluation was also carried out after the application of bio-cleaning compresses to study the effect of different bacterial species in cleaning the black layers. *Desulfovibrio sp.* succeeded in cleaning the black layers and depositing calcite on the surface,but it did not affect the rust stains on the surface, which consist of magnetite mineral. *Enterobacter cloacae* also succeeded in cleaning the black layers and some sediment remained on the surface by reducing sulphate to cysteine acid, Where cysteine acid succeeded in cleaning up rust stains on the surface through examination using SEM. While other bacterial species did not affect the cleaning of the black layers .

The analysis was carried out using various methods of analysis by X-ray diffraction , infrared and the EDX unit attached to SEM, these methods confirmed that the composition of the black layers is heterogeneous and consists of gypsum, calcite, dolomites, quartz, hematite and magnetite. The results of the analysis after treatment showed that *desulfovibrio sp.* succeeded in cleaning the black layers and remained rust stains on the surface, as well as *Enterobacter cloacae* succeeded in cleaning the black layers and some sediment remained on the surface because of the difficulty of the bacteria reaching them, and also succeeded in affecting rust stains on the surface .

This chapter also deals with the practical application of cleaning the black layers in some areas in the wall of Salah al-Din Ayyubid Castle using bacterial species that gave the best results in the experimental study. *Desulfovibrio sp.* bacteria was used to clean the black layers and deposit

calcite,which consolidates the surface, the surface was completely cleaned after four applications of bio-cleaning compresses loaded with bacteria. The black layers and rust stains were also cleaned on the surface using *Enterobacter cloacae* bacteria, which succeeded in cleaning and gave good results after four applications of bio-cleaning compresses.