The Role of Inappropriate Outdoor Exhibition in the Biodeterioration of an Archaeological limestone Water Duct

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Abstract

The use of Inappropriate Outdoor Exhibition method is one of the main factors that accelerate the degradation of archaeological stone works. Here, we describe and diagnosis the biodeterioration of limestone water duct located in the courtyard of Sultan Qaitbay's mosque (1475). The used exhibition method provides the suitable environmental condition for biological grows. Several samples taken from various colors of microbial biofilm on the water duct surface were isolated and cultivated. The microorganisms were documented and analyzed using optical light microscopy (OLM), scanning electron microscopy with the energy dispersive X-ray (SEM-EDX) and high performance liquid chromatography (HPLC). The study showed that the main biofilm formers were fungi and bacteria. The fungi were identified as: *Aspergillus niger- Aspergillus terrus – Acremonium* sp. *Alternaria* sp. *Paecilomyces SP. Stemphylium.* Two kinds of bacteria are present *(Coccus – and Bacillus).* The organic acids produced by identified fungi were qualitative and quantitative assessed. The degradation caused by the identified microbial activities is discussed and the criteria for archaeological stonework outdoor exhibition were presented.

Keyword: limestone / Biodeterioration / Exhibition / water duct / SEM / HPLC.

1- Introduction

The main aim of the conservation and restoration works is to preserve the monument and to adapt it for use. This task must be comprehensive to include all the elements of the building, whether

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it is architectural, ornamental and functional elements. Because many historical stone structures are often located outdoors, they pose particular conservation challenges due to the surrounding environmental conditions. Deep scientific investigations are needed to enable the conservator to choose the suitable materials and methods for stone structures conservation and exhibition. The use of inappropriate materials and methods in conservation and exhibition of historical stone structures make them an easy target for physical, chemical, and biological degradation factors attack. Here, we present an emblematic case of the limestone biodeterioration as a result of human unstudied intervention.

The aim of this work is to study the effects of the preservation state of limestone water duct located in the courtyard of Sultan Qaitbay's mosque (1475) on its biodeterioration by means of characterization of the microorganisms formed on the limestone surface and evaluating their deteriogenic ability. Definitions of the disinfection and exhibition method were also proposed.

1.2 Description of the studied water duct.

The Qaitbay Medersa was founded by al- Malik al- Ashraf Abu an-Nasr Qaitbay in 1475. Sultan Qaitbay ruled Egypt from 1468 to 1496. The Medersa is located in Qal'at el Kabch street, beside el Sayda Zynab region, Cairo, Egypt. It has four façades and two main entrances, the western entrance and the eastern entrance. There is a minaret in its eastern corner. A basin for animal drink is subjoined to the western side of Medersa. The restoration works of Medersa were completed in 2006. In the northwestern side of Mederasa there is an open courtyard containing modern Mayda'a, which is not belong to the original construction of the Mederasa[1]. 8 m. away from the Mayda'a there is a limestone water duct. It measures 9.5 m long, 0.5 m wide and 0.35 m height [fig1].

1.3 The preservation state of the water duct

1- Hosny M. Nowaiser, studies in Circassian architectures, Cairo university, Cairo, 1992??, pp 219-223.

One of the conservation tasks is to preserve a limestone water duct, which was discovered during the restoration works, from to be buried under the accumulated dust and waste. The water duct is surrounded by modern limestone wall that is about 60 cm. height, 10 m long and 15 cm thick. This enclosure wall is covered with very thick glass (9mm thick) [fig2]. This man-mad structure which aimed to protect the archaeological element was categorized as a semienclosed environment[2]. The microclimate of this kind of environment tends to–produce many integral degradation factors that can be concluded as follow:

- Thermal excursions because of the glass cover, which restrain (keep) the day temperature in the space underneath. The high temperature is one of the acceleration factors of the chemical and physical degradation process[3].
- High humidity due to penetration and leaking of the water from the Mayda,a and the condensation of water vapor on the glass cover surface. In addition to the essential role of the humidity cycles in dissolution and crystallization of salts in stone monuments, the high humidity increase the chance of biological attack[4]. Some micro-organisms develop rapidly if the air has a relative humidity of over 65%, and they spread quickly if there is light[5].
- Bad ventilation due to the absence of airing opening in the exhibition stone case. It is well known that the bad ventilation enhance the intensity of microbial attack due to the increasing of temperature and humidity[6].

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[2]- Ada R., Sandra R., and Anna M., Semienclosed Environments, in " Plant Biology for Cultural Heritage- Biodeterioration and conservation", the Getty Conservation Institute, Los Angeles, 2008, pp.141.

[3] Camuffo D., Microclimate for cultural heritage, Elsevier, Netherlands, 1998, pp 3-14.

[4] Kumar R. and Kumar A., Biodeterioration of Stone in Tropical Environments, Paul Getty, USA, 1999, pp. 1-3.

[5] Bernard M. Feilden, Conservation of Historic Buildings, Third edition, Elsevier, Oxford, 2003, pp.134.

[6] Maekawa S. and Toledo F., controlled ventilation and heating to preserve collections in historic buildings in hot and humid regions, The Getty Conservation Institute, USA, pp 1-17.

Catherine Woolfitt [7]reported the problems that were developed due to using of protective structure containing glass cover with some archaeological sites. Lack of ventilation exacerbates the problem. Even when these materials are used over larger areas this can be a problem if there is inadequate ventilation (greenhouse effect). One problem is visual; such glass and plastic surfaces, especially in close range to building surfaces, tend to be obscured with dust, organic growth or condensation. They tend to trap moisture and create differential thermal and moisture levels (internal/external).

1.4 degradation phenomena

The water duct is in very bad preservation state and shows typical decay phenomena of a surface subjected not only to the outdoor exposure but also to the unstudied human intervention. Very dense layers of biological colonization accumulated on the surface of the water duct [fig.3]. The accumulation of black, blackish brown, green and white stains of microorganisms caused the discoloration of stone surface [fig 4A]. Some vascular plants are growing in the bottom of the water duct [fig 4B]. The edges of the water duct are eroded because of the exfoliation and detachment of the superficial layer [fig 4C]. Salt efflorescence and incrustation are seen all over the surface of the water duct [fig 4D].

3. Experimental section

3.1. Sampling, isolation, purifying and identification

Several representative samples of the microflora observed on the water duct surface were collected with a sterile scalpel and placed into sterile plastic vials. The collected samples were cultivated on PDA media plates for obtaining pure culture from the sample of monuments. After 7 days from incubation at 25-30c the microorganisms (fungi and bacteria) were developed. The developed fungi were isolated and purified using the hyphal tip.

[7] Catherine Woolfitt, Preventive conservation of ruins: reconstruction, reburial and enclosure, in "conservation of ruins", Elsevier, Oxford, 2007, pp 179.

The pure culture of each fungal isolate was identified by plant pathology unit of the National Research Center, Cairo, Egypt.

3.2 pathogenisty

Other pieces of deteriorated stone were autoclaved then the pieces of stone were sprayed using atomizer with the spore suspension of identified microorganisms. Then the plates were incubated at 25c for 8 weeks under 70% relative humidity. The inoculated stones were examined by SEM-EDX

3. 3 Estimating the organic acids secreted

The ability of each identified fungi for attacking stone was investigated by estimating the organic acids secreted by them. Small pieces of deteriorated stones were inserted in the conical containing about 100 ml of Czapek's solution salts (the level of solution was adjusted to covered approximately half length of stone) then autoclaved at 121 C for 20 min. The sterilized stones were inoculated as usual with the identified fungi and incubated at 28 C for 12 weeks. All fungi and bacteria could grow successfully and covered the surface of the inoculated stone samples. The solutions were filtered and submitted to the high-performance liquid chromatography (HPLC) analysis.

3.4 examination and analytical methods

- The optical light microscopy (OLM) (Nikon Eclipse E 800) was used to detect microorganisms.

- Environmental Scanning Electron Microscopy (ESEM) were carried out by Philips (XL30) microscopy, equipped with EDX micro-analytical system (EDX) in order to examine the morphology of the different microbial populations and the relationships between microbial growth and mineral substrate of limestone.

- X-ray powder diffractometry (XRD) was carried out using a diffractometer Philips PW 1840 (CuKα 40 kV, 30 mA) in order to characterize the products of the deterioration.

- High-performance liquid chromatography (HPLC) was carried out to detect the organic acids secreted by the identified

microorganisms. The filtered culture solution were analyzed directly for organic acids by HPLC on an Aminex HPX-87H column (BioRad, 300. 7.8 ram).

4. Results and discussions

4.1 biodeterioration of the water duct (characterization and mechanisms)

The pure culture of the developed microorganism shows the same morphology and colored stains that were seen on the limestone surface [fig.5]. The thin section examination showed the presence of six kinds of fungi; *Aspergillus niger- Aspergillus terrus – Acremonium* sp. *Alternaria* sp. *Paecilomyces SP. Stemphylium* and two kinds of bacteria *Coccus (positive-gram) and Bacillus(negative-gram)* [fig.6]. These groups of microorganisms living on inorganic substrates form more or less complex communities structured in biofilms or microbial mats. The development of specific biological species on a particular stone surface is determined by the nature and properties of the stone (mineral constituents, pH, relative percentage of various minerals, salinity, moisture content, texture). It also depends on certain environmental factors (temperature, relative humidity, light conditions, atmospheric pollution levels, wind and rainfall). In other words, the response of living organisms to a potentially colonizable surface depends on the ecological and physiological requirements of the biological species involved[8]. The ability of these organisms to cause serious damage to many kinds of stone has been well established. It was estimated that biological weathering is 100–1,000 times greater than inorganic weathering[9]. The interactions between these microorganisms and carbonate stone are very complex. The microbial growth can deteriorate the stone not only chemically or mechanically ways but also aesthetically way. In

[8] Kumar R and Kumar AV., Biodeterioration of stone in tropical environments, Getty conservation institute, Los Angeles, 1999, p. 1 –2.

[9] Aghamiri, R., & Schwartzman D. W, Weathering rates of bedrock by lichens: A mini watershed study, Chemical Geology, 188(3–4), 2002, p. 249–259.

some instances, the presence of microbial growth on stone is a purely aesthetic issue, since visible growth of pigmented microorganisms can alter the appearance of building and status[10]. According to the results received with the petri-dishes in this research five fungi (*Aspergillus niger- Aspergillus terrus – Alternaria* sp. *Paecilomyces SP. Stemphylium*) produce pigments [fig.6petri -dishes]. The pigments produced by the fungi are very often involved in the melanins[11]. They are brown to black pigment that are formed in the cell wall and free in the substrate of cultured fungi[12]. Many of fungi found on stone, especially on surfaces exposed to direct sunlight, do indeed exhibit a dark pigmentation due to the presence of melanin, which provides them with protection against physical agents (e.g., UV radiation, x- and γ-rays) and cellular lysis[13]. ESEM observation of the studied samples confirmed the data obtained by optical microscopy and verify the existence of bacterial and fungal bioflms on the stone surface and within the crystalline structure of the limestone [fig 7 and fig 8]. It was reported in many scientific researchs that the combination of bacteria and fungi, forming biofilms on the material surface, are involved in degradation of historic limestone materials.[14] Eroded crystals of calcite and poorly crystalline mineral precipitates on fungal hyphae were observed to be a common feature in some studied samples [fig7A and fig 9].

[10] Christopher J. McNamara and Ralph Mitchell, Microbial Deterioration of Historic Stone, Ecological Society of America, JSTOR ,2008, pp.445-451.

[11] W. Krumbein & E. Diakumaku, the role of fungi in the deterioration of stones, in" Environment/Protection and Conservation of the European Cultural Heritage, research report N2,Belgium, 1995, pp.154.

[12] Mary-Lou E. F., fungal facts solving fungal problems in heritage collections, Archetype Publications, London, 2004, p.65.

[13] D. Pinna and O. Salvadori, biodeterioration processes in relation to cultural heritage materials: stone and related materials, in " Plant Biology for Cultural Heritage-Biodeterioration and conservation",the Getty Conservation Institute,Los Angeles,2008,pp.134.

[14] Ralph Mitchell, The role of microorganisms in the deterioration of atmospheric pollutants of stone used in historic buildings and monuments, US Department of the Interior National Park Service Publication, USA, 2001, pp5

The results of XRD analysis of the six samples taken from the curst formed on the surface of water duct show the presence of calcite ($CaCO_3$), Quartz (SiO_2) and Halite (NaCl) in all samples. Gypsum ($CaSO_4.2H_2O$) is detected in two samples. The high-performance liquid chromatography (HPLC) analysis of the filtered culture solution shown in table (1) revealed that all the identified microorganisms are able to produce organic acids. Oxalic acid was detected in all the identified microorganisms culture solution. Acetic acid and fumaric acid were detected in the Stemphylium sp. culture. Shikimic acid was detected in the culture of bacteria (bacillus), Acremonium falciform, Stemphylium sp and Aspergillus terres. Ascorbic acid was detected in Paecilomyces sp. culture.

The ESEM, XRD and HPLC results confirmed that the identified microorganisms play an important role in the degradation of the water duct limestone by mechanical and chemical attacks. The mechanical attack of the stone mineral by fungi occurs through hyphal penetration. In calcitic stones, the hyphae penetrate the calcite crystals not only along the planes of the crystals, but also across transverse lines, and the surface of stone appears clearly corroded under SEM examination[15]. Fungal hyphae can also penetrate grain boundaries, cleavages, and cracks to gain access to mineral surfaces[16]. It could be shown that fungi can have a very hard cell structure which mechanical resistance values are comparable with the values of stone or rock parameters[17]. Shrinking and swelling of biofilms can result in mechanical pressure to the mineral unit, causing erosion or abrasion[18]. The Chemical attack of

[15] D. Pinna and O. Salvadori, biodeterioration processes in relation to cultural heritage materials, op cet, 2008, pp. 135.

[16] Geoffrey M. Gadd, Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation, mycological research 111, Elsevier, 2007, p.3-49.

[17] Thomas D. and Anna A. G., new methods to study the detrimental effects of poikilotroph microcolonial micromycetes (PMM) on building materials, 9th International Congress on Deterioration and Conservation of Stone, Venice 19-24 June, 2000, pp. 461-468.

[18] Warscheid T, Krumbein WE, Biodeterioration processes on inorganic materials and means of countermeasures, Materials and Corrosion 45, 1994, pp. 105–113.

the identified bacteria and fungi are believed to be more important in the water duct biodeterioration cycling. It was reported that the identified fungi in this study (Aspergillus *niger- Aspergillus terrus – Acremonium* sp. *Alternaria* sp. *Paecilomyces SP. Stemphylium*) and bacteria *(Coccus and Bacillus)* have the ability to grow and interact with carbonate minerals through the biochemical processes[19,20,21,22]. The microorganisms are able to obtain several elements that they need for their metabolism (e.g., calcium, aluminum, silicon, iron and potassium) from the limestone by biosolubilization. This biosolubilization process generally involves the production of various organic and inorganic acids. The release of aggressive acids is one of the best known biogeochemical destructive mechanisms at rock surfaces and it occurs through the leaching of rock-binding materials with the consequent weakening of the crystal structure. According to the result obtained by HPLC analysis(table 1), the excreted organic acids are oxalic acid, acetic acid, fumaric acid, shikimic acid and ascorbic acid. These acids are efficient for rather slowly-solving cations such as Ca, Al, Si, Fe, Mn and Mg from minerals forming stable complexes. It has been shown that biogenic organic acids are considerably more effective in mineral mobilization than inorganic acids and are considered as one of the major damaging agents biogeochemical destructive mechanisms at rock surfaces[23]. In this study, the oxalic acid is the abundant organic acid detected by HPLC analysis of the studied

[19]K.L. Garg, Kamal K. J., A.K. Mishra, Role of funfi in the deterioration of wall paintings, the science of the total environment 167, Elsevier, 1995, pp. 255-271.

[20] K.Sharma and S.Lanjewar, Biodeterioration of Ancient Monument (Devarbija) of Chhattisgarh by Fungi, J Phytology microbiology 2/11, *2010,pp. 47-49.*

[21] Geoffrey M. Gadd, 2007, op cit. pp. 3-49

[22] Euan P. B., Martin K. & Geoffrey M. Gadd, Geomycology: fungi in mineral substrata, Mycologist, Volume **17**, Part 3, Cambridge University Press, UK, 2003,p.98-107.

[23] Y. Nuhoglu, E. Oguz , H. Uslu , A. Ozbek, B. Ipekoglu , I. Ocak , I. Hasenekoglu, The accelerating effects of the microorganisms on biodeterioration of stone monuments under air pollution and continental-cold climatic conditions in Erzurum, Turkey, J. Science of the Total Environment 364, Elsevier, 2006, pp. 272– 283.

culture solutions. According to D. Pinna and O. Salvadori[24] oxalic acid can be produced through three metabolic paths. In which the glucose and other organic acids (e.g. glyoxylic acid, succinic acid and ascorbic acid) turned to oxalic acid in the Krebs cycle and its deviation. The oxalic acid can react with the $CaCO_3$. To form calcium oxalates [whewellite (Calcium Oxalate monohydrate) (CaC_2O_4. H2O)] or [weddellite(Calcium Oxalate hydrate)(CaC_2O_4. $2H_2O$)] minerals. However, oxalate salts are not the only minerals associated with fungal hyphae, secondary calcium carbonate can be reprecipitated[25]. In our study, no calcium oxalates minerals were detected in the studied samples but calcite was detected in all the collected samples. The detected calcite is corresponded mainly to the mineral composition of limestone; however the presence of calcite as a secondary reprecipitate is not eliminated, since some secondary minerals precipitated on the hyphae, which were similar in elemental composition to the original limestone, were observed by SEM examination. The non detection of calcium oxalates minerals is probably due to the transformation of oxalates, with presence of bacteria, to carbonates[26]. Further, calcium oxalate can be degraded to CaCO3 in the oxalate–carbonate cycle[27]. The identified bacteria that belonged to genera Bacillus and Micrococcus are seemed to play an important role in the deterioration of limestone. Biofilm bacteria produce large amounts of exopolymer (EPS), which consists mainly of polysaccharides as well as pigments, lipids, and proteins. The EPS serves a variety of functions, including protecting the microor-ganisms from desiccation, erosion, antibiotics, and dis- infectants,

[24]D. Pinna and O. Salvadori, biodeterioration processes in relation to cultural heritage materials, op cet, 2008, pp. ١٤١

[25] Geoffrey M. Gadd, 2007, op cit. pp. 20

[26] Eric P. Verrecchia and Jean-Louis Dumont, A Biogeochemical Model for Chalk Alteration by Fungi in Semiarid Environments, Biogeochemistry, Vol. 35, No. 3, Springer, (1996), pp. 447-470.

[27] Geoffrey M. Gadd, 2007, op cit. pp. 20

as well as serving as a reservoir for nutrient and energy storage. The EPS can increase the dissolution rate of calcium carbonate and may precipitate calcium carbonate[28]. The sulphates frequently found on stone, may be caused by the sulfur-oxidizing bacteria (among which is the genus *Thiobacillus)* which, by utilizing various reduced compounds of sulphur or elemental sulphur , produce Sulfuric acid; this, in turn, reacts with calcium carbonate of stone and gives rise to gypsum. These sulfates can be precipitated within the pores of the stone and, upon recrystallization, exert considerable stress in the pore walls causing the degradation of stone[29]. The detection of gypsum in two studied samples indicated that the identified bacteria (Bacillus) may be is the genus of *Thiobacillus* that most probably has a role in gypsum formation on the water duct surface of. In addition to the roles of the identified microorganisms in the biodeterioration of the water duct, the plants growing in the bottom of the water duct has a compatible role in the biodeterioration process. The deterioration caused by plants is both mechanical and chemical. The pressure exercised by the growth and radial thickening of roots (which can be up to fifteen atmosphere) can cause serious damage to the substrate. In addition to the production of carbonic acid through respiration process, chemical action is due to the acidity of the root tips and the acidity and chelating properties of the exudates[30]. The results of the biological studies showed that the biodeterioration process of the water duct is active and effective, so the control of biodeterioration and protective interventions are essential demanded.

[28] Christopher J. McNamara and Ralph Mitchell, 2008, op cit, pp.447.
[29] P. Fernande, Applied microbiology and biotechnology in the conservation of stone cultural heritage materials, Appl Microbiol Biotechnol 73, Springer, (2006), pp. 291–296.
[30] D. Pinna and O. Salvadori, biodeterioration processes in relation to cultural heritage materials, op cet, 2008, pp.١٤٣

4.2 control of biodeterioration and protective interventions proposal

The key issue that has to be considered when establishing a strategy for the preservation and restoration of stoneworks is the need for doing so through methods that do not cause any damage to the artifact. The control of biodeterioration processes should start with the adoption of measures that will prevent favorable growth conditions for the contaminating microflora. This objective might be achieved by the reduction of moisture within the stone material, e.g., by optimizing drainage systems, correcting faulty architectural details or by the application of stone protective treatments. In outdoor environments the possibilities of an intervention to bring the levels of humidity, temperature, and light to below the threshold values necessary for the growth of biodeteriogenic organisms are generally limited and difficult to put into practice, and in any case they are most often part of an overall conservation plan that requires an assessment of the variables concerning the work in question, of the environmental conditions surrounding it, and of the type of use and presentation that is intended for it[31]. Concerning saving of the water duct the conservation process must be based on the use of preventive and remedial methods.

Preventive methods aim at inhibiting biological attack on water duct. Control of moisture level present in the water duct is considered the essential aim to control the biological attack. The moisture's sources that are present in the water duct are various. In addition to the raising capillary action, since the water duct contact directly with soil, infiltration, percolation, because of nearness of Mayda'a, and condensation phenomena, due to the presence of glass protective cover, are the main sources of moisture. To reduce the level of moisture some steps could be taken:

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[31] - Antonella A. and Daniela P., prevention of biodeteroration; outdoor environments, in: "Plant Biology for Cultural Heritage- Biodeterioration and conservation", the Getty Conservation Institute, Los Angeles, 2008, pp.287.

- Separate the water duct away from the Mayda'a by making an elongated cavity parallel to the water duct to prevent the percolation of water resulting from using the Mayda'a and cleaning of its floor.
- Isolate the water duct from the soil by cutting the wall of water duct and insert isolating materials. Various materials and methods to provide a chemical barrier against capillary rise of water has been experimented and applied[32].The increased hydro repellence of the constituent materials through the application of protective products can contribute to slowing down new biological colorizations on conserved works, but the choice of the type of materials to be used to this end must be evaluated very carefully. Tests should be carried out both in the laboratory and in situ to verify the effectiveness and also the chemical, physical, and biological effects produced by the protective materials[33].
-Remove the glass cover that prevent air circulation and create a greenhouse effect. It was reported in the literatures that using a glass as a protective structure covering the entirety of the stoneworks accelerate the degradation process of the stone intended to be preserved[34]. In the planning of a permanent protective structure, one of the goals should be to guarantee microclimate conditions that, if not optimal, at least do not accelerate the process of degradation of the work. The coexistence and interaction of certain microenvironmental factors can trigger and encourage the development of organisms beneath the shelter. Consequently, the main objectives in the planning of a protective shelter that is efficient and that prevents biodeterioration should be[35]:
- An adequate protection from the action of rainwater.

[32]- P. Lopez-Arce, E. Doehne, J. Greenshields, D. Benavente, D. Young, Treatment of rising damp and salt decay: the historic masonry buildings of Adelaide, South Australia, j.Materials and Structures42, RILEM, 2009, pp.827–848.
[33]- Francesca C., Pamela P., Roberta P., Lucia T. and Claudia S., Bacterial and fungal deterioration of the Milan Cathedral marble treated with protective synthetic resins, j. Science of the Total Environment 385, Elsevier, 2007, pp.172–181.
[34]- Antonella A. and Daniela P., prevention of biodeteroration, 2008, op cit, pp. 289.
[35]- Antonella A. and Daniela P., prevention of biodeteroration, 2008, op cit, pp. 291

- Construction of channeling and drainage systems for rainwater.
- Protection from condensation phenomena.
- Possibility of regulating the aeration and the natural lighting of the spaces protected by the sheltering structure.
- Careful evaluation of how to enclose the site or artifact and ongoing subsequent monitoring of the enclosure boundaries.

Appling the previous criteria in planning the structure of the protective covering for the studied water duct; overhanging open structure is probably the most suitable structure. Simplicity of the covering, low construction costs and use of traditional materials that are easily available are considered the main principles. Using of simple aluminum frame covering with roof of semitransparent plastic materials could be use to make shelter that provide natural light source, good ventilation, prevent condensation and good view for visitors.

Remedial methods aim at eradicating the biological agents responsible for biodeterioration. This is mostly performed by mechanical, physical and chemical methods. The mechanical methods consist of the physical removal of the biodeteriogens with manual instruments such as scalpels, paint brushes, brushes spatulas, small scrapers, microaspirators, etc. These methods are recommended because of their simplicity and the immediacy of their results. It should be pointed out, however, that it is very difficult to achieve a complete removal of a fungal mycelium because it is never limited to a superficial phenomenon. Many physical methods make use of electromagnetic radiation (UV, gamma, or beta waves), microwaves, low-tension electric currents, or low and high temperatures. Some of these methods such as ultraviolet radiation, infrared lamps and laser were used for cleaning stone artifacts[36]. But in our case these methods are not available due to their high costs. Treatment with chemical

[36]- Stefano B., Flavia P. and Piero T., Control of biodeterioration and bioremediation techniques: Physical methods, in: "Plant Biology for Cultural Heritage- Biodeterioration and conservation", the Getty Conservation Institute, Los Angeles, 2008, pp.313-318.

substances is by far the most frequently adopted method for the elimination of biodeterioration from stone artifacts. Removing biological growth before applying biocidal agents is usually recommended. Applications of many chemical substances such as dilute ammonia, a poultice based on the chelating agent ethylenediaminetetraacetic acid (EDTA), carboxymethyl cellulose, diluted solutions of hydrogen peroxide and sodium hypochlorite to remove biological growth on stone surface have been reported[37]. Numerous biocides have been in use for the elimination of biodeteriogens from stone[38,39]. (Table 2) Tests to determine the efficacy of treatment on the biodeteriogens should be carried out both in the laboratory and in situ.

Conclusion

The study of the preservation state of the limestone water duct located in the courtyard of Sultan Qaitbay's mosque (1475) showed the importance of accurate choosiness of outdoor exhibition materials and methods used for stone artifacts. Knowledge based on the characterization of the microorganisms active on the stone and assessment the ability of these organisms to cause aesthetical, mechanical and chemical serious damage to the stone is the starting point for the choice of suitable restoration and conservation interventions of the water duct. The more urgent intervention is the reduction of moisture within the water duct through the separation of the water duct away from the Mayda'a and removing the glass cover. Some remedial methods aimed to eliminate and prevent the biological growth should be applied. The protective covering

[37]- Kumar R and Kumar AV., Biodeterioration of stone in tropical environments, Getty conservation institute, Los Angeles, 1999, pp.31-32.

[38]- Maria P. and O. Salvadori, Control of biodeterioration and bioremediation techniques: chemical methods, in: "Plant Biology for Cultural Heritage- Biodeterioration and conservation", the Getty Conservation Institute, Los Angeles, 2008, pp.326-329.

[39] - Clara U., Filomena L., Mariagrazia G. and Paola S., Efficiency of biocide in "in situ" and "in vitro" treatment study case in the "templete de mudejar", Guadalupe, Spain, in "9th international congress on deterioration and conservation of stone", Elsevier, 2000, pp. 531-539.

(shelter) for the water duct based on using an overhanging open structure that is made of suitable materials is proposed.

Fig.1 Shows the modern Mayda'a, which is 8 m. away from the studied limestone water.

Fig.3 Shows the dense layers of biological colonization accumulated on the surface of the water duct. The arrow point to the water drops condensed on the glass surface.

Fig.2 Shows the exhibition method of the studied water duct .

Fig. 4 Shows the degradation phenomena of the water duct. (A) Shows the discoloration of stone surface due to the accumulations of colored stains of microorganisms. (B) Shows the vascular plants growing in the bottom of the water duct. (C) Shows the eroded edges of the water duct due to the exfoliation and detachment of the superficial layer. (D) Show the black crust accumulated on the stone surface

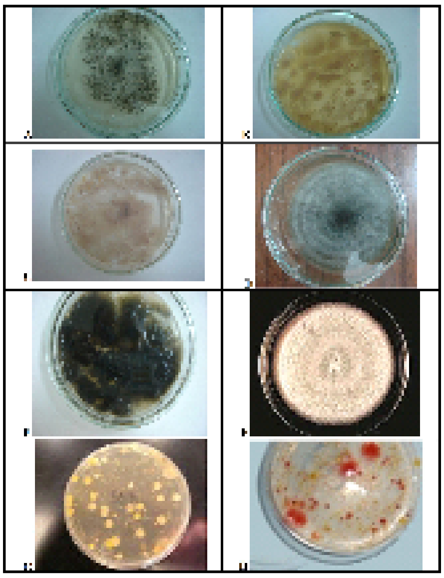

Fig.5 Shows the plates of the isolated fungi(A,B,C,D,E,F) and bacteria (G, H) from the water duct, (A) *Aspergillus niger*, (B) *Aspergillus terres* , (C)*Paecilomyces* , (D) *Alternaria* sp, (E) *Stemphylium sp*, (F) *Acremonium falciforme* , (G) *bacteria (Bacillus)*, (H) *bacteria (Coccus)*.

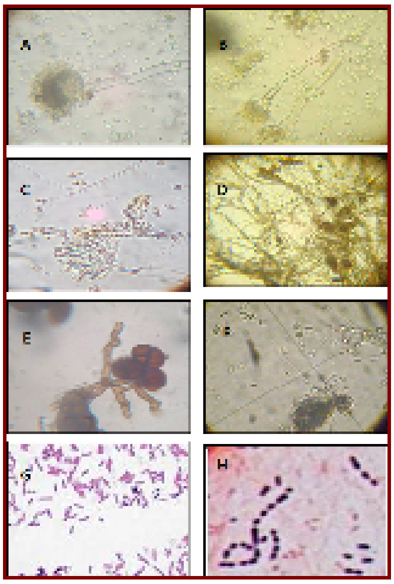

Fig. 6 Microphotographs of isolated fungi and bacteria, (A) *Aspergillus niger*, (B) *Aspergillus terres* , (C)*Paecilomyces* , (D) *Alternaria* sp, (E) *Stemphylium sp*, (F) *Acremonium falciforme* , (G) *bacteria (Bacillus)*, (H) *bacteria (Coccus)*.

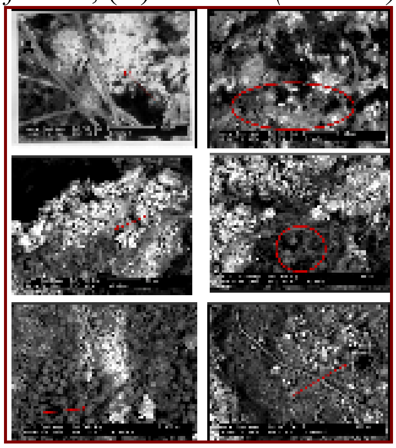

Fig. 7 shows the SEM images of the identified fungi and bacteria grows on and in the stone of water duct.(A) shows a complete body (hyphae and conidiophores) of *Aspergillus terres* penetrating the crystals spaces of calcite (the arrow point to the corroded calcite crystal).(B) *Aspergillus terres* growing within the stone. (C) Very dense growing of *Paecilomyces* (the hypha indicated by an arrow). (D) Shows the *Alternaria s*p. growing within the subsurface of stone. (E) shows very dense growing of *Stemphylium sp*.,(the spores indicated by an arrow). (F) Shows the superficial growth of *Acremonium falciforme* on the stone.

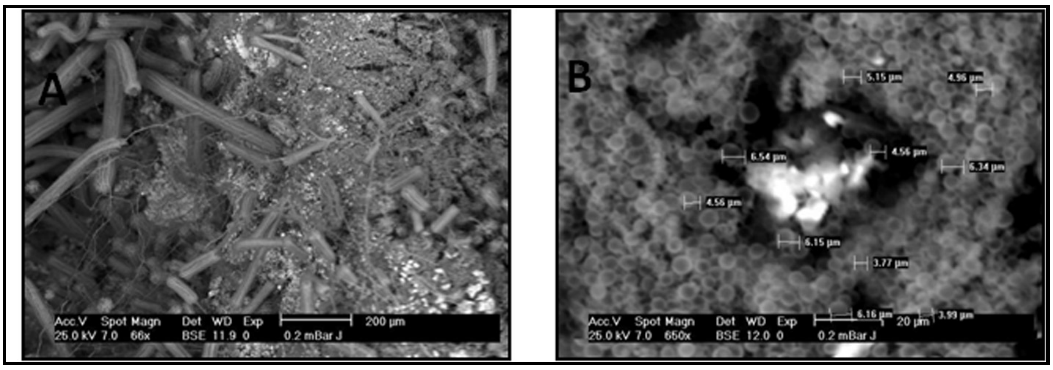

Fig. 8 SEM micrographs show (A) very dense bacteria *(Bacillus)* growth on the stone surface, (B) very dense bacteria *(Coccus)* growth on the stone surface (its dimension ranging from 3µm to 6µm).

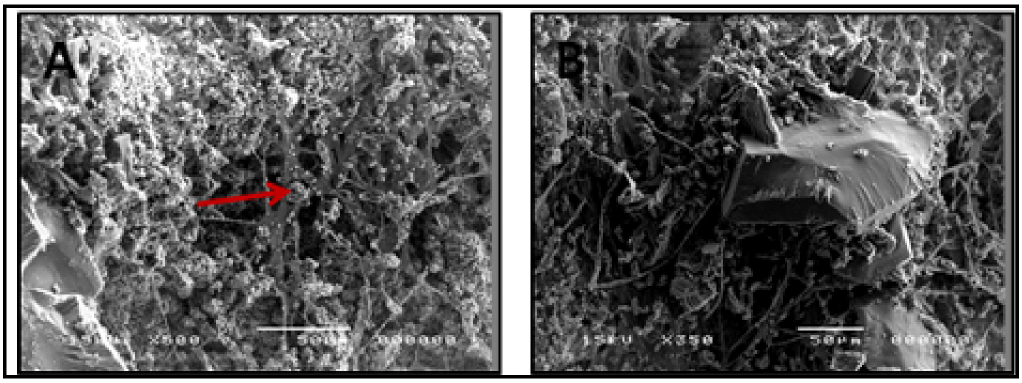

Fig. 9 SEM micrographs of (A) mineral secondary precipitates over fungal hypha, (B) shows calcite crystal embedded within the fungal growth.

Table 1:- Results of HPLC analysis of the studied culture solutions of the identified microorganisms

Sample	Bacteria (bacillus)	Acremonium falciforme	Stemphylim sp	Aspergillus terres	Paecilomyces sp	Alternaria sp	Aspergillus niger
Acetic acid			8.833 (mg/100ml)				
Citric Acid							
Formic Acid							
Propionic Acid							
Oxalic Acid	3286.0 (mg/100ml	2632.0 (mg/100ml)	2999.0 (mg/100ml)	1717.0 (mg/100ml)	1896.9 (mg/100ml)	2546.0 (mg/100ml)	2916.0 (mg/100ml)
Trataric Acid							
Succinic Acid							
Ascorbic Acid					0.814 (mg/100ml)		
Lactic Acid							
Shikimic Acid	1.257 (mg/100ml)	0.330 (mg/100ml)	0.334 (mg/100ml)	0.147 (mg/100ml)			
Maleic Acid							
Fumaric Acid			0.2049 (mg/100ml)				

Table 2. Most frequently used biocides in the Conservation of stone (modified from Caneva and Pinna 2001)

Chemical Classification	**Chemical Composition**	**Commercial Name**	**B F**	**C A**	**L**	**P**
Inorganic compounds	Sodium and porassium hypochiorire		+	+	+	
	Lithium hypochlorite				+	
	Sodium suiphite				+	
	Hydrogen peroxide		+	+	+	
	Sodium ocraborate	Polybor		+		
Phosphoorganie compounds	Glyphosate	Roundup, Spasor, Rodeo			+	
Alcohols	Ethanol		+			

Phenol derivatives	Ethanol		+			
	'Ihymol		+			
	o-phenyl phenol	Lysol	+			
	p—chloro m—cresol		+			
	Chlorinated and phenolic compounos	Panacide, Halophane, Thaltox C		+	+	
	Sodium peotachlorophenate			+	+	
Nitroorganic compounds (ureic and carbamates(	Diuron	Karmex	+	+	+	
		Diuron	+	+	+	
	Chlobromuron	Maloran			+	
	Fluometuron	Lito 3			+	
	Sodium dithiocarbsmare					+
Quaternary ammonium salts	Alkyl-benxyl-dimethyl-ammooium chloride	Preventol R50	+	+		
		Preventol R80	+	+	+	
		Neo Desogen	+	+	+	
		Hyamine 3500		+	+	
		BAC	+	+	+	
		Catamin AB		+	+	
	Benzyl-dodecyl-bis(2 hydroxyethyl)-ammonium chloride	Bradophen	+			
	)Diisobutylphenoxyethoxyerhyl)dimethyl-benzyl- ammonium chloride	Hyamine 1622		+	+	
	Dodecyl-benzyl--trimerhyl-ammonium chloride	Gloquat C		+		
	Lauryl—dimethyl-benzyl-ammonium chloride	Cequartyl		+	+	
Organic metal salts	Tn o—butyl tin oxide	TBTO	+	+	+	
		Thaltox		+	+	
	Tn-n butyl tin naphthenate	Metatin N58-10	+		+	
Pyridine	2,3,5,6 tetrachloro-4-methyl sulfonyl pyridine	Algophase		+	+	
	piclocam	Tocdon 22K, Uniran				+
Heterocychc compounds (diazines and triazines)	bromacil	Hyvar X			+	+
	hexszinone	Velpar, Velpar L		+	+	+
	terbutryn	Igran			+	
	terburylazine	Pnimarol M50				+
	secbumeton	Primarol 3588				+
Mixtures	quaternary ammonium salt + rri-n-butyl tin naphthenate	Metatin N58-10/101	+	+	+	
	quaternary smmonium salt -I- tri-n-butyl tin oxide	Thaltox Q		+	+	
		Thaltox20			+	
		Murasol 20			+	
	Dimethyl-thio sodium carbamate + 2-mercaptobenzotbiaxole	Vancide 51	+	+	+	

Key: B = bacteria; F = fungi; C = cyanobacteria; A = algae; L = lichens; P — higher plants