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Microbial deterioration of limestone of Sultan Hassan mosque, Cairo- Egypt and suggested treatment

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Abstract : Sultan Hassan Mosque is one of the most important mosques in Egypt and the Islamic world because of its special architectural style. However, many causes of its limestone deteriorations were found. Moreover, it is exposed to the influence of ground water caused by sewage. Therefore, the study examines the environmental factors. Chemical analyses by X-ray diffraction and X-ray fluorescence and scanning electron microscope were also conducted.
Microbial deterioration of limestone was studied on samples taken from Sultan Hassan mosque, the Islamic monument located in Cairo, Egypt. Stone samples were collected by non-destructive methods from outdoor and indoor of the mosque and were tested for inhabitance by microflora (bacteria and fungi), outdoor and indoor airospora was also investigated. Gram positive and Gram negative bacteria were isolated of which *Baillus* was dominant genus recovered from all samples, where *Aspergillus* was the most prevalent among fungi. Only *Bacillus* had shown the ability to dissolve calcium carbonate unlike other tested bacteria or fungi. Fucidic acid concidered an accurate antibiotic against most bacterial isolates where fluconazole was slightly more effective than sodium azide on fungi.
Keywords : Sultan Hassan mosque- limestone- *Bacillus*- *Aspergillus*- antimicrobial.

Introduction

Archaeological history of Sultan Hassan mosque

The reign of the Mamluks in Egypt was characterized by massive architecture to construction[1]. Mamluk sultans were interested in the construction of religious buildings with a distinctive architectural style[2]. One of them is Sultan Hassan complex (1356-1361 A.D.) that has wonderful floral decorative patterns[3] and is one of the greatest architectural installations in the Muslim world figure (1.c.) [4,5,6.] The mosque comprises four iwans[7] surrounding an abolition fountain of a perfect design [8].

Field Observations and Deterioration Causes

Limestone was extensively used in the Egyptian buildings from the pharaonic period until now [9]. It mainly consists of calcium carbonate. Because of its physical and chemical properties, it is susceptible to many weathering factors, especially heat, humidity, and atmospheric pollutants. Also, it is characterized by high porosity which allows the penetration of water, causing severe damage. Additionally, they contain many microorganisms that stimulate damage growth. Accordingly, there is a need for urgent solutions, particularly to the bacterial damage.

Weathering processes of the limestone cause many changes to the mineral, physical, and chemical components and in the size of its granules[10].Humidity, temperature, sunlight and ground water play a great role in the limestone's damage, especially salts which cause severe damage [11]. Furthermore, microbiological damage causes a change in the stones and it is probably caused by environmental reasons. Thus, conditions of air pollution and their ability to help the microbiological growth should be defined [12] .Because of their exposure, in urban environments, to many air pollutants, bacterial growth is stimulated, resulting in many acids that affect limestone buildings[13].The physical and mechanical abrasion and dissolution interact on the several stages of the deposition cycle[14]. For example, crystalline salts cause pressure that harm stone construction [15]. Therefore, damage caused by salt migration should be documented to identify its causes and indicate the most appropriate treatment methods [16]. It is noted that pore space in the damaged area of the limestone depends on the proportion of crystallized salts [17]. Microorganisms' deterioration of the archaeological stone was extensively studied [18].

Stone monuments with direct exposure to environmental conditions are affected not only by physical and chemical weathering but also by biological activities of stone dwelling microorganisms[19]. This consortium of heterogeneous microbial species causes physical alteration of the material structure by penetration of bacterial and fungal hyphae and by differential mechanical pressure as a result of shrinking and swelling cycles of the adhesive biofilms (figure 1. a & d), it also forms a pigmented biofilm that covers the sculpture and other stone structures forming of thick black crusts figure (1. e & f) [20,21]. Chemical modification of the mineral support by acidolytic and oxide or reductive corrosion processes generated by products of the microbial metabolism [22,23]reported that mixed microbial populations exacerbate physical weathering of limestone. The role of bacteria and fungi in the deterioration of stone have been studied by a considerable number of stone investigations [24,25,26].

Bacteria produce large amounts of exopolymer (EPS), the main constituents of EPS are polysaccharides in addition to lipids, pigments, and proteins. The EPS serve as a reservoir for nutrient and energy storage for bacteria. They also protect bacteria from desiccation, erosion, disinfectants and antibiotics. The EPS may also play a major role in the deterioration of stone cultural heritage materials (figure 1.b &d) [27]. Because of heterotrophic nature of fungi, they can transform inorganic metabolites to organic supports which they can utilize by excretion of several metabolites such as inorganic and organic acids[18].

The present work aimed to investigate the biological cause of biodeterioration of archeological limestone of Sultan Hassan mosque in Cairo- Egypt and suggest proper methods of treatment.

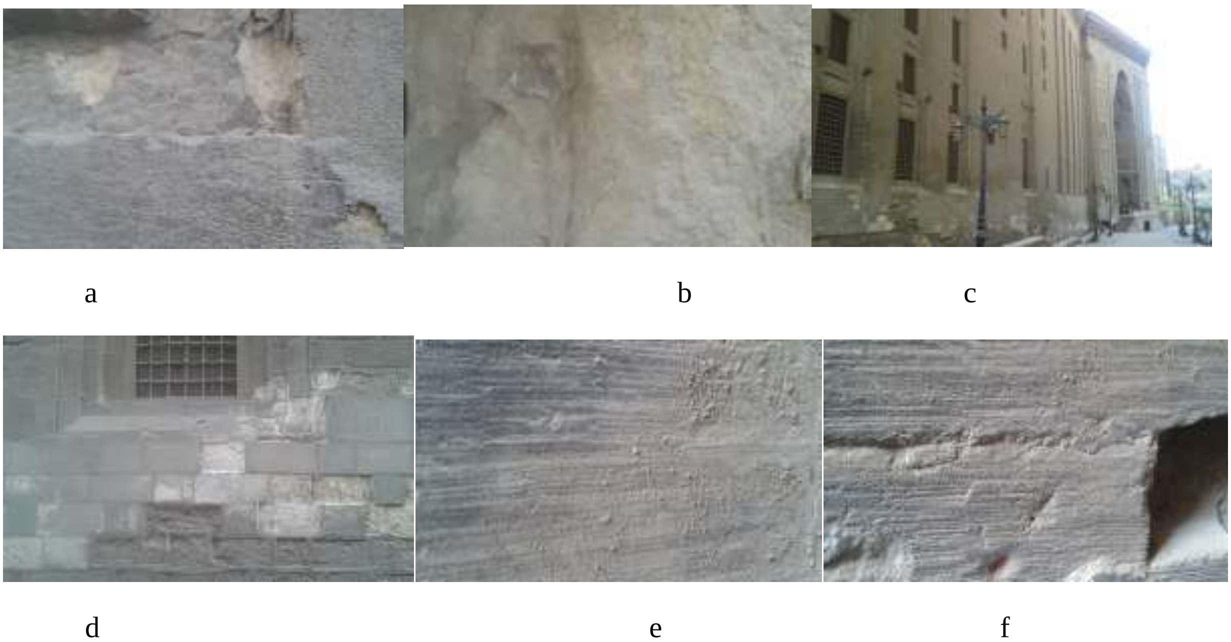

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figure (1) a) A general view of Sultan Hassan Mosque's entrance, b) Disintegration of the limestone surface, c) Exfoliation of the hard crust on limestone surface, d & e) Black pigments on the limestone surface as a result of microbial deterioration, e) Discoloring and salt efflorescence.

Materials and methods

Nondestructive of the limestone samples were collected from the archaeological site (Sultan Hassan Mosque in Cairo)

Petrographic Examination

Polarizing microscope units (LEV 100 POL) were used to the petrographic studies of the samples using Nikon polarizing microscope.

X-ray Diffraction (XRD)

XRD Unit, Assuit University, Model PW 1710 control unit Philips, Anode Material Cu, 40 K.V, 30 M.A, 2 Cita from 4 to 60.

Chemical Composition by XRF

Identifying the chemical composition of all samples was carried out by X-ray fluorescence analysis (XRF), JEOL JSX Element Analyzer with Energy Dispersive X-Ray Fluorescence system (EDXRF)- in the Central Lab, South Valley University.

Scanning Electron Microscope (SEM)

JEOL JSM- 5500 LV Scanning Electron Microscope (JEOL, Japan), Central Lab, South Valley University.

Estimation of airborne microorganisms

The exposed plate method was used to estimate the aerospora of Sultan Hassan mosque. Nutrient agar and Czapek's (CZ) agar media were used for isolation of bacteria and fungi, respectively. The plates were exposed for five minutes. Nutrient agar plates were incubated at 37 °C for 72 h while CZ plates were incubated at 28 °C for 7 d. The developed colonies were counted in plates and the average number of colonies per three plates was determined.

Isolation of micro-organisms from deteriorated limestone

A variety of non-invasive techniques were applied for isolation of micro-organisms from deteriorated parts of limestone. These techniques were represented by

i- Cotton swabs: sterile and dry cotton swabs were rubbed on the surface of the deteriorated parts over an area of $2cm^2$, under aseptic conditions, kept in sterile bag at 4^oCuntil used for inoculation as mentioned above[28].

.**ii-Small stone slices:** naturally exfoliated stone slices from the monument surface were used as samples. 1- 3 mm sized particles were sprinkled onto the agar.

iii- Adhesive tape method: [29], an adhesive tape strip was pressed firmly over the surface of compact alterations. The tape was affixed onto a sterile glass slide and was stored in the dark at 4^oC until observation. For light microscopy, sections approximately 1x1cm were cut from adhesive tape samples.

Identification of microbial isolates

The bacterial isolates were tentatively identified on the basis of classification schemes published in Bergey's Manual of Systematic Bacteriology[30]. Identification of fungal isolates was performed according to Raper[31] and Gilman[32].

Screening for calcium carbonate- dissolving microorganisms

Bacterial isolates were tested for calcium carbonate dissolution by growing colonies on Deveze- Bruni medium, the constituents (g L^{-1}): glucose, 5 g; yeast extract, 1 g; peptone, 1 g; K_2HPO_4, 0.5 g; $MgSO_4$, 0.01 g, NaCl, 5 g; $NH_4(SO_4)_2$, 0.05 g; $MgCl_2$, $CaCO_3$ 5g and 1.5% agar), incubated at 37 °C for 7 days [33]. Fungal isolates were grown on modified Czapek's (CZ) agar medium in which glucose is replaced by $CaCO_3$, incubated at 28 °C for 14 days. Bacteria and/or fungi that dissolve $CaCO_3$ can be distinguished due to the apparent halo of a clear zone around the colony.

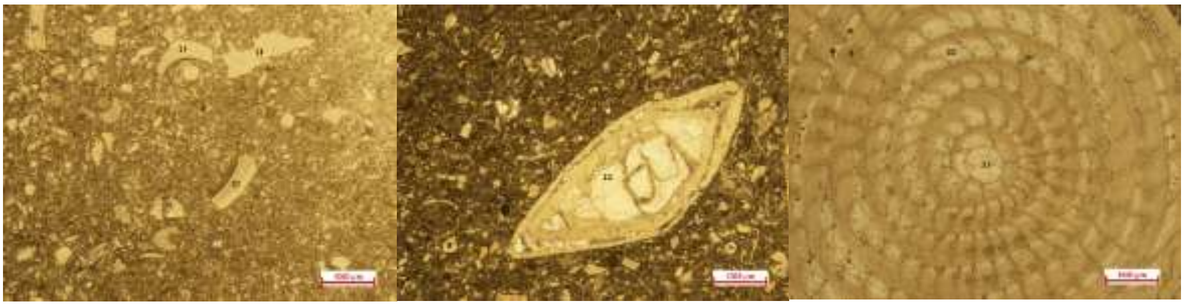
Treatment with antimicrobial agents

The most dominant bacterial isolates were treated by 10 different antibiotics (polymixin 300 UI, rifampin 5µg, amoxicillin 20/10µg, penicillin 6µg, gentamicin 10µg, tobramycin 10µg, cefoxitin 30µg, fucidic acid 10µg, chloramphenicol 30µg and nalidixic 30µg acid). Agar disk-diffusion method was used[[34]]. The most dominant fungal isolates were treated with different concentrations (25, 50, 75, 100, 150 and 200) µg of sodium azide and floconazole as fungicidal agents using the agar well diffusion method [[35]].

Results

Petrographic Investigation

Study of the thin sections under the polarizing microscope, shown in fig. (2. a, b, c & d), illustrated that limestone grains contain fossils or skeleton fragment of the fossils of foraminifera, specially Nummulites composed of calcite. The Dolomites occur as a filling for cracks or dissolved wholes with some trace of gypsum. In addition, Sparite calcite occurs in the internal voids of fossils. Micritic calcite is the main mineral composition.



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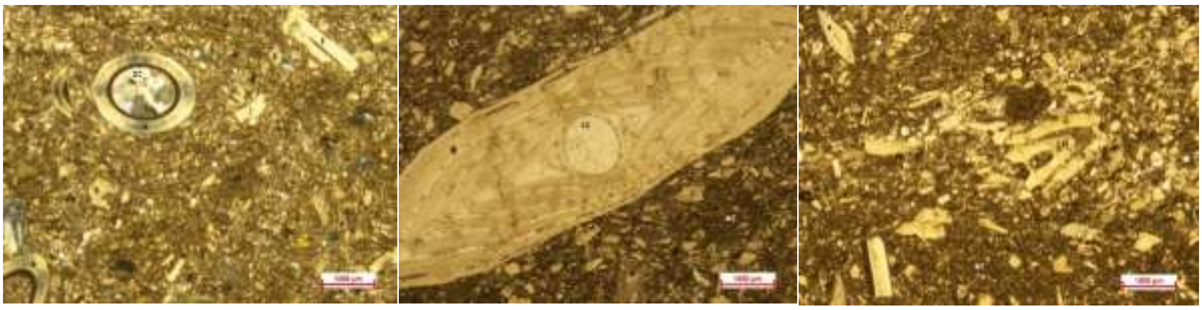

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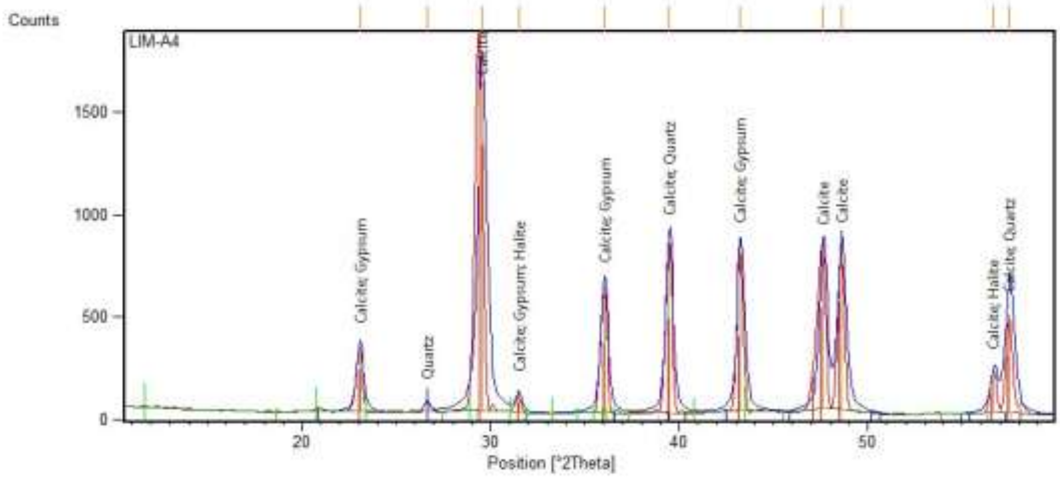
Figure (2) Sparite calcite (S C) in the internal structure after the dissolving of the original materials due to diagenesis; N = Nummulites, M C = Micertic Calcite, C= Calcite.

XRD Analysis

XRD patterns shown in figures. (3. a, b& c) of the samples illustrated that their main minerals are Calcite, quartz, gypsum, and halite. The percent of calcite is larger than quartz with some traces of gypsum and halite.



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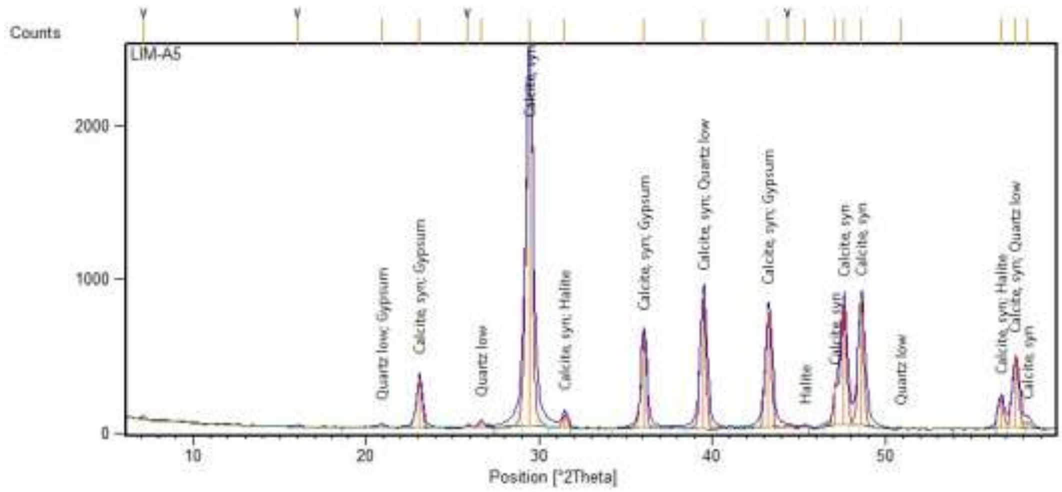

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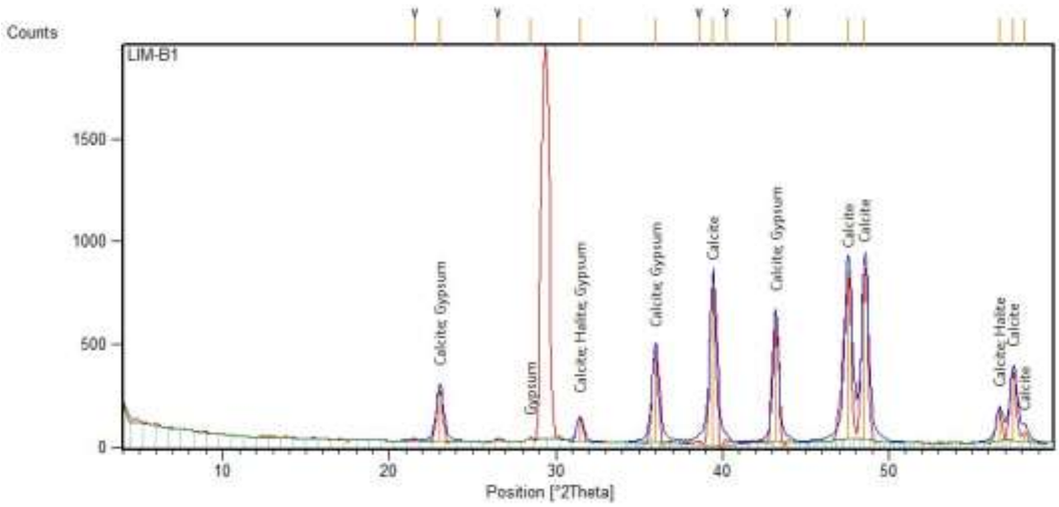

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Figure 3(a, b& c) X-ray diffraction of limestone from Sultan Hassan Mosque

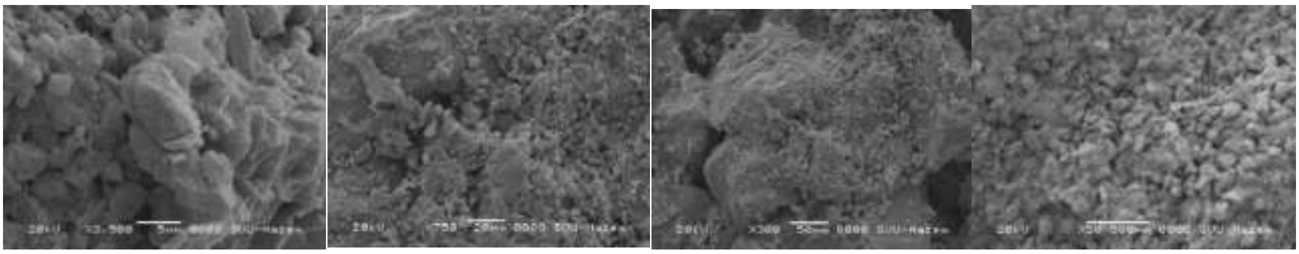
XRF Analysis

The chemical analysis of the limestone revealed that there was a decrease in the percent of SiO_2 (0.53- 0.51- 0.50), AI_2O_3 (0.14-0.06- 0.18), and MgO (0.11- 0.12- 0.11), respectively and an increase in the percent of CaO (56.46- 56.42- 56.32), SO_3 (0.14- 0.13- 0.16), P2O5 (0.07- 0.05- 0.08) and Fe_2O_3 (0.05- 0.04- 0.02) table (1), respectively. Gypsum was formed when calcium carbonate reacted with sulfur dioxide.

Table (1) XRF results of the limestone's samples

Main Constituents Wt%	Sultan Hassan Mosque limestone (a)	Sultan Hassan Mosque limestone (b)	Sultan Hassan Mosque limestone (c)
SiO_2	0.59	0.56	0.57
TiO_2	0.02	0.04	0.05
AI_2O_3	0.13	0.12	0.15
Fe_2O_3 tot.	0.06	0.05	0.08
MgO	0.12	0.13	0.10
CaO	54.28	54.33	54.41
Na_2O	0.45	0.43	0.47
K_2O	0.04	0.05	0.06
P_2O_5	0.06	0.07	0.04
SO_3	0.12	0.13	0.16
CI	0.35	0.34	0.38
LOI	43.74	43.71	43.49
MnO	0.018	0.017	0.017
NiO	0.004	0.004	0.005
SrO	0.015	0.016	0.015

Scanning Electron Microscope (SEM)



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Figures (4) a) Limestone with some micropores and phenocrystals, b) Lim-mud facies, salt cover the limestone components and micro pores, c) Fractural developed and substitution of carbonates by gypsum, d) micropores and damage in the internal structure of the limestone

Estimation of airborne microorganisms

Five airborne bacterial genera (*Bacillus, Micrococcus, Staphylococcus aureus, Streptomyces sp. and Pseudomonas aerugenosa*) were recovered from out and indoor of the Sultan Hassan mosque. Results in table (2) reveal that *Bacillus* was the most common genus comprising (25 and 28) % of total bacteria from outdoors and indoors, respectively. It was represented by four species of which *Bacillus cereus* was the most frequent comprising (10 and 18) % of total bacteria from outdoors and indoors, respectively. Gram negative bacteria were of low occurrence and were represented by *Pseudomonas aerugenosa* comprising 1% of total bacteria from both outdoors and indoors. On the other hand, thirteen airborne fungal species belonging to seven genera were isolated. *Aspergillusniger* was the most prevalent, comprising (22.29 and 39.82) % of total fungi from outdoors and indoors, respectively. *Alternariaalternata* was of moderate occurrence comprising (6.67 and 4.4)% of total fungi from outdoors and indoors, respectively. The remaining genera were less frequent than the preceding ones. Colonies without visible sporulation within 14 days of incubation were considered as sterile mycelia.

Table (2): Percentage frequency of airborne microorganisms isolated from outdoor and indoor of Sultan Hassan mosque.

Genera and species	Frequency %	
	Outdoor	Indoor
Bacteria		
Bacillus cerius	10	18
B. subtilis	5	6.5
B. megatherium	8	2.5
B. circulans	2	1
Micrococcus luteus	10	15
M. ruseus	4	1
Staphylococcus aureus	2.5	1
Streptomyces sp.	1	1.5
Pseudomonas aerugenosa	1	1
Fungi		
*Aspergillusniger*Tiegh	13.3	22.2
A. flavus Link	8.89	8.89
A. glaucus Link	0	2.2
A. nidulans (Eidam) G. Winter	0	2.2
A. sydowii (Bainier&Sartory) Thom and Church	0	2.2
*A. fumigatus*Fresen	0	2.2
Penicilliumchrysogenum Thom	2.2	0
*P. coylophilum*DiercKX	2.2	2.2
Alternariaalternata (Fr) Keissl	6.67	4.4
Cladosporiumcladosporoids (Frsen) G. A. de Vries	2.2	0
Ulocladiumcharatum (preuss) E. G. Simmons	2.2	2.2
*Drecshslera*sp.	2.2	0
Humicola sp.	0	2.2
Sterile mycellia	4.4	4.4

Isolation of microorganisms from deteriorated limestone

i- Cotton swabs: According to data in table (3), eight species belonging to four bacterial genera were recovered from samples collected from outdoor of Al- Sultan Hassan mosque. The genus *Bacillus* showed maximum frequency, it was represented by four species, *B. cereus, B. subtilis, B. megatherium and B.circulans*. Table (2) also shows that eight species belonging to four fungal genera were recovered from outdoor of which *Aspergillus* was the most common genus comprising (25.6 and 28.64) % of total fungi from outdoors and indoors, respectively. Zygomycetes represented by *Syncephalastrumrhizopi* were emerged from indoor samples comprising 3.6% of the total fungi.

Table (3): Percentage frequency of microorganisms recovered from outdoor and indoor of Sultan Hassan mosque using swab method.

Genera and species	Frequency %	
	Outdoor	Indoor
Bacteria		
Bacillus cereus	17.78	20.83
B. subtilis	2.2	4.4
B. megatherium	2.2	2.2
B. circulans	2.2	0
Micrococcus luteus	0	2.2
M. ruseus	2.2	2.2
Staphylococcus aureus	2.2	0
Pseudomonas aerugenosa	20.83	20
Fungi		
*Aspergillusniger*Tiegh	14.3	7.14
A. flavus Link	10.7	10.7
A. glaucus Link	3.6	3.6
A. terreus	0	3.6
A. sydowii (Bainier&Sartory) Thom and Church	3.6	3.6
Penicilliumchrysogenum Thom	7.14	3.6
*P. coylophilum*DiercKX	3.6	0
*P. duclauxi*Delacr	0	3.6
*Syncephalustrumrhizopi*Vuill	0	3.6
Alternariaalternata (Fr) Keissl	0	3.6
Cladosporiumcladosporoids (Frsen) G. A. de Vries	3.6	0
Ulocladiumcharatum (preuss) E. G. Simmons	0	7.14
Humicola sp.	3.6	0
Sterile mecellia	3.6	0

ii-Small stone slices: Data in figure (5a) show dominance of Gram positive bacteria represented by *Bacillus* and *Micrococcus* comprising 92.69 %of the total recovered bacteria. On the other hand, only *Aspergillus* was recovered from deteriorated stone slices of Sultan Hassan mosque. It was represented by *A. flavus*, *A.sydowii* and *A. niger*, comprising (40, 40 and 20) % of total fungi, respectively (figure 5b).

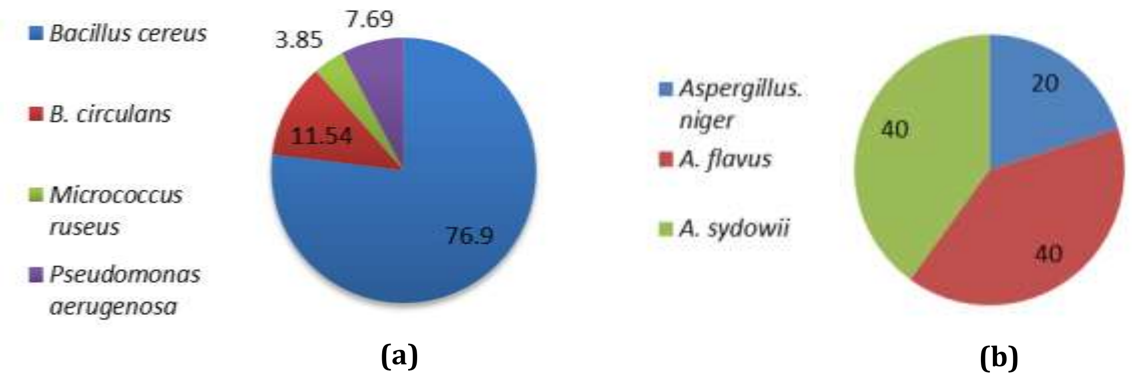

Figure (5): Percentage frequency of bacteria (a) and fungi (b) recovered from deteriorated limestone of Sultan Hassan mosque

iii- Adhesive tape: Light micrographs figure (6) shows different fungal conidia revealed from outdoor and indoor samples which were tentatively identified as *Alternaria*,*Drecshslera*, *Cladosporium*and *Ulocladium*.

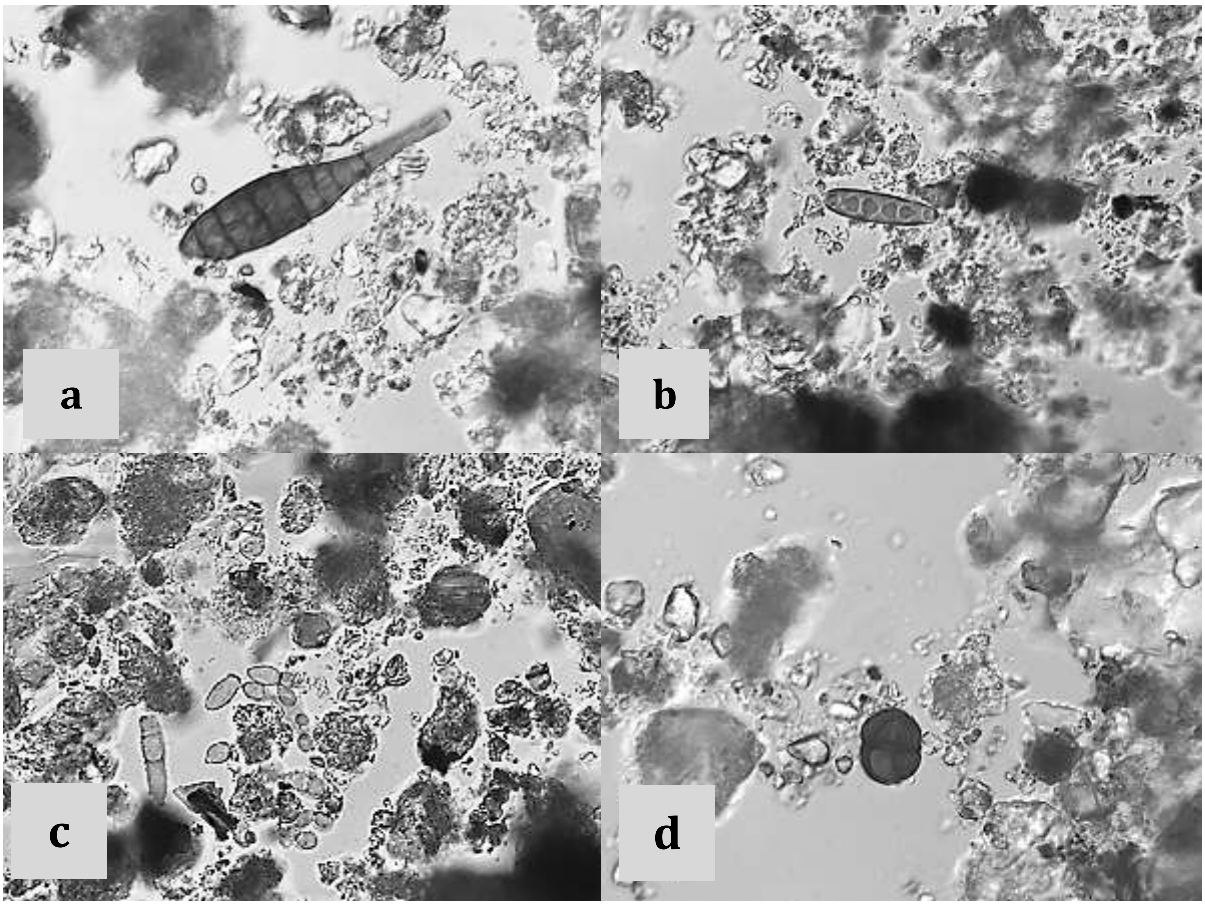

Figure (6): Fungal conidia growing on walls of Sultan Hassan mosque, Alternaria (a), *Drecshslera* (b), *Cladosporium*(c) and *Ulocladium* (d), collected using adhesive tape method.

Screening for calcium carbonate- dissolving microorganisms

Figure (7) shows of carbonate- dissolving bacteria recovered from Sultan Hassan mosque by different technique. Three strains of *Bacillus cereus* (1), (3) and (5) isolated from stone, air and walls (using swab), respectively. Two strains of *B. subtilis* (4), (6) were recovered from walls using swab method. *B. circulans* was isolated from stones. None of the fungal isolates were found to be carbonate- dissolving.

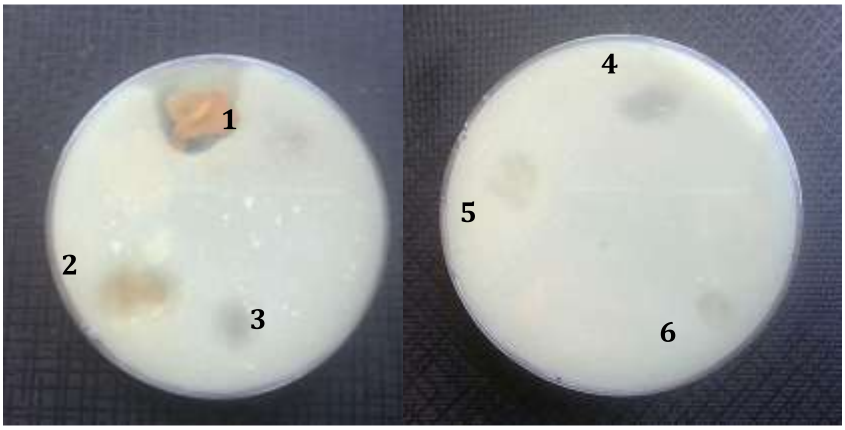

Figure (7): Zone of clearance of carbonate- dissolving *Bacillus cereus* (1), (3), (5); *B. circulans* (2) and *B. subtilis* (4), (6), on DB medium.

Treatment with antimicrobial agents:

Table (4) summarizes the results of using ten antibiotics on six bacterial strains from the infected limestone. Penicillin had no effect on any of the tested bacterial strains, where fucidic acid was the most effective. Noticeably, Gram positive bacteria were more susceptible than Gram negative bacteria.

Table (4): Bacterial susceptibility for different antibiotics:

Antibiotics	Bacterial strains					
	Diameter (mm) of zone of inhibition					
	Bacillus cereus	***Bacillus subtilis***	***Micrococcus luteus***	***Micrococcus ruseus***	***Staphylococcus aureus***	***Pseudomonas aerugenosa***
Polymixin 300 UI	10	14	-	-	12	10
Rifampin 5µg	14	18	-	15	29	24
Amoxycillin 20/10 µ	15	17	-	-	15	15
Penicillin 6 µg	-	-	-	-	-	-
Gentamicin µg	19	22	27	16	-	-
Tobramycin 10 µg	12	17	20	15	16	17
Cefoxitin 30 µg	12	6	-	-	-	-
Fucidic acid 10 µg	25	21	12	26	19	30
Chloramphenicol 30 µg	-	16	11	16	-	19
Nalidixic acid 30 µg	22	-	-	15	11	15

Results in Table (5) show the effect of two antifungal compounds: sodium azide and floconazole. At concentration of 25 µgm, sodium azide had no effect on fungal growth except for *Syncephalustrum sp.*µgm. Growth of *Penicilliumcoylophilum* was inhibited at 150 µgm. Therefore, All tested isolates were inhibited at concentration of 200 µgm. Inhibition zones ranged between (6-25) mm. Treatment with fluconazole inhibited conidial growth of all the tested fungi where mycelial growth only developed. Inhibition zones ranged between (6-20) mm. *Alternariaalternata*was susceptible to all applied concentrations (25- 200) mg.

Table (5): Effect of antifungal compounds on fungal species isolated from Sultan Hassan mosque

Genera and species	Diameter (mm) of zone of inhibition, Concentrations (µgm): Floconazole					Sodium azide				
	25	50	100	150	200	25	50	100	150	200
*Aspergillusniger*Tiegh	-	-	-	-	6	-	-	6	7	8
A. flavus Link	-	-	6	7	20	-	7	8	13	20
Penicilliumchrysogenum Thom	-	-	-	9	15	-	12	13	13	16
*P. corylophilum*DiercKX	-	-	6	8	13	-	-	-	-	6
*Syncephalustrumrhizopi*Vuill	-	-	14	15	19	9	11	16	17	25
Alternariaalternata(Fr) Keissl	6	8	9	12	15	-	6	6	7	13
Cladosporiumcladosporoids(Frsen) G. A. de Vries	-	-	8	15	20	-	6	8	12	15
Ulocladiumcharatum (preuss) E. G. Simmons	-	-	6	8	9	-	-	7	7	8

Discussion

XRD analysis and studies of the thin sections under the polarizing microscope illustrated that the limestone was subjected to several decay processes such as a dissolution of the original materials in the internal structure of fossils that were replaced with sparite calcite in the presence of dolomite due to the dolomitization process in which surface or underground water contained high concentration of Mg, causing the alteration of calcite into dolomite. However, the presences of gypsum indicated that the stone subjected to cycles of dry and wet affected stability. XRF results showed that the stone was mainly composed of $CaCO_3$ with CaO (54.28 - 54.41%). Additionally, P_2O_5 existed due to the skeleton fossils fragment and some bones. Presences of So3 indicated the presence of gypsum. Hence, the stone was susceptible to several processes of diagenesis and climate change.

Weathering in Sultan Hassan Mosque is manifested in the scaling, corrosion, color change, loss of some stone parts, and salt. Where peeling in the different limestone parts, some thin surface layers began to separate as a result of a change in the chemical or physical composition. Color change as a result of the growth of micro-organisms. Due to damage of the biodiversity, limestone’s grains lose coherence. Furthermore, dirt on the limestone surface appears as dust and clay granules working on color change. Ground water also has a significant effect on the limestone because of the absence of a good sewage network in the study area. Salt weathering is one of the most important causes of the limestone’s deterioration because of the efflorescence of salts, forming a salt crust, and color change. Examining the damaged samples illustrated the presence of high porosity and the high percentage of clay material with iron oxides and gypsum. In addition, examining the geological section showed the presence of decomposition in the surface layer which absorbs the clay minerals in limestone allowing water spillover of the crystal mud [36], because when it absorbs water, it expands resulting in internal micro-cracks. As a result of changes in temperature and humidity caused by the migration of soluble salts from ground water internal micro cracks constantly cause segmentation of stone [[37]].

SEM examination illustrated that mud covered granules calcite and that many pores appeared and separate granules in the form of fine clay granules. Also, it showed that there were some micro pores resulting from granules calcite’s damage enriched by the solvent causing loss of coherence. Salt crystallization caused the growth of granulated gypsum and limestone superficial damage. It is proved that halite spread in the Egyptian soil and that gypsum refers to the presence of ion sulfur as a result of the microbiological activity or air pollution. Additionally, damage rates increase caused by the successive cycles of drought and wetness[[38]].

Table (1) shows that there is a difference in the proportions of actress forms of damage elements, as follows (Si, Al, Fe, and S), and that they commensurate with the basic element of the construction material (i.e. Ca). Their ratios refer to the mechanical deterioration affecting the surface of the stone, caused by various factors that classify deterioration into three stages, as follows:

The elements were rated, as follows: Fe_2O_3 (0.06%), Al_2O_3 (0.13%) and SiO_2 (0.59%). They were compared to the basic element, i.e. CaO (54.28%) table (1). Such ratios were caused by the effects of dirt, dust, and a few aerosols, resulting from industry and air pollution.

(a) The ratios of elements differed, as follows: Na_2O (0.43%), SiO_2 (0.56%), SO_3 (0.13%), Cl (0.34%), K_2O (0.05%), and Fe_2O_3 (0.05%) compared to the basic element, i.e. CaO (54.33%) and others (i.e. Na, K, and Cl). However, (Al) disappears by the dirt and dust figure (). These differences may be caused by wetness and drought cycles because of the operations between air temperature and changing sources of moisture, such as ground water, sewage, and relative humidity.
(b) The observed effect of deterioration outlined the factors through the different values of the elements, reflecting the fluorescence of some salts, such as: Cl (0.38%), SO_3 (0.16%), SiO_2 (0.57%), Al_2O_3 (0.15%), Na_2O (0.47%), Fe_2O_3 (0.08%), and K_2O (0.06%) compared to the key element, i.e. CaO (54.41%) table (1).

Many XRD results were obtained that match those of XRF, in terms of outputs weathering affecting the quantity and quality of the limestone figure (3. a, b& c). It is also noted that calcite is the main component of limestone used as a building material.

Weathering of lime stones in monuments is not only a result of physic-chemical processes but also microbial deterioration is involved. The majority of microbial spores of indoor environments are derived from outdoor environments where they can be carried into monument buildings by air movement or by visitors. Slight increase was recognized in microbial counts of indoor than those of outdoor of Sultan Hassan mosque, this could be due to higher humidity. These findings come in agreement with Ammar[39] who proved that the highest numbers of fungal spores inside Khofo pyramid may be attributed to the high number of visitors. Similarly,[40] illustrated that microbial population numbers indoors can equal or exceed the numbers found outdoors in common soil. *Bacillus* followed by *Micrococcus* were the most frequent genera among airborne bacteria revealed from Sultan Hassan mosque, where Gram- negative bacteria were less frequent, the results of present study is in correspondence with Awad[41] who found that *Bacillus* and Micrococci were the major components of Gram-positive bacteria isolated from aerosols in a four-storey flourmill building located in Giza, Egypt, while Gram- negative bacteria were found in low numbers.

Maximum percentage frequency reported for*Aspergillus*among airborne fungi, while minimum percentage frequency reported for *AlternariaCladosporium, Penicillium, Ulocladium, Humicola and Drecshslera*.

Urzi[42] recorded that *Aspergillus*, *Penicillium*, *Alternaria*, *Ulocladium*,*Cladosporium*, *aureobasidium*, *Fusarium*, and *Poma*are most common isolates of terrace of Missina Museum at Sicily, Italy. Similar results were reported by Gupta[43], Kavita[44]and Shelton [45].

Airborne spores and cells may be deposited onto the wall surfaces by gravitational settling or carried by the wind or by visitors[46].Most stone inhabiting heterotrophic microorganisms need very low nutrient requirements which may be provided by remains of polluted air or animal remains and secretion [47].

High stone porosity and rough surface which are significant contributory factors in promoting microbial colonization[48]. Bacterial species of *Bacillus*, *Micrococcus, Pseudomonas* and *Staphylococcus* were recovered from deteriorated walls of Sultan Hassan mosque. Similar results were demonstrated by Ciferri[49]who isolated species of *Pseudomonas*, *Arthrobacter*, and *Streptomyces* from deteriorated wall paintings. On the other hand, Pepe[50] illustrated that two bacterial genera, *Bacillus* and *Paenibacillus*, were found in wall paintings in various churches in Italy.

Comparable investigations were established by Schabereiter-Gurtner[51];Piñar[52]; Rölleke[53].

Present study depicts that *Aspergillus* spp. was the most pridomenant throughout swab samples. Comparable results were reported by Abdelhafez[18], who isolated *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizopus, Acremonium, Stachybotrys, Cladosporium* and*Alternaria* from deteriorated archeological marble in Mohamed Ali palace, El-Ghory Mosque and Mosque of El-Kady Abdel- Baset in Cairo, Egypt. Isolated fungal genera such as *Alternaria*, *Ulocladium,Cladosporium* and *Humicola* contain melanin pigments causing color change of lime stone, they form small colonies and mycelia in the cracks and between gypsum crystals causing superficial peeling of stone. These findings come in agreement with Frank- Kamentskaya [54] who reported that *Aureobasidium pullulans*, *Cladosporium sphaerospermum* were colonizing in decaying marble and limestone monuments in Saint Petersburg, Russia, in all stages of the black crust development. On the other hand, De la Rosa-García [55] found fungi isolated from degraded stone surfaces at the ancient Mayan site of Uxmal, Yucatan, Mexico belonged to the genera: *Aureobasidium*, *Cunninghamella*, *Fusarium*, *Paecilomyces* and *Penicillium*. Comparable results were is tablished by other invistigators (Urzi [56] ; Hirsch [57]; Gaylarde [58]).

Vegetative forms of Alternaria, *Drecshslera*, *Cladosporium* and *Ulocladium* were collected using adhesive tape method. Their ability to survive prolonged and extreme dryness is characteristic of such cells which colonizing walls exposed to the sever environment. They also produce dark pigments to which provde protection against uv, which also result in wall pigmentation.Gaylarde and Gaylarde[59] &[60] reported that *Gloeocapsa, Synechocystis, Cladosporium* and *Aureobasidium* were the major microbial genera collected from biofilms on buildings of historic interest in Latin America. Similar findings were detected by Sterflinger and Krumbein [61].

Limestones are often extremely porous which provides an optimum environment for microorganisms beneath the stone surface. Endolithic bacteria as well as fungi were recovered from Sultan Hassan deteriorated limestone during this investigation.

Endolithic microorganisms have been isolated from unusual or extreme environments such as deserts, caves and the deep subsurface [62], [63]&[64].

Since $CaCO_3$ is the main mineral constituent of limestone, it was expected that the carbonate-dissolving bacteria may be keystone species in limestone weathering. In the present investigation, such species were isolated. All carbonate-dissolving recovered belonged to the genus *Bacillus*. It was remarkable that the strain with the highest ability to dissolve carbonate was endolithic which supports its role in increasing stone porosity and weathering. This agrees with Subrahmaniyam [33] who isolated carbonate-dissolving bacteria were affiliated to families (Bacillaceae and Staphylococcaceae) and Actinobacteria from 'Miliolite', a bioclastic limestone, from Gopnath, Gujarat, Western India. Li [65] and Li [66] illustrated the involvement of *Bacillus* sp. in carbonate dissolution. Fungi isolated during this work were incapable of dissolving $CaCO_3$, such result disagree with Li [67] who reported that fungi played an important role in carbonate-dissolving of limestone.

Sodium azide had mycocidal effect against all tested fungi with different concentrations (25 up to 200) µgm. Similar results were reported by Abdelhafez [18] who concluded that 100 ppm of sodium azide was the best treatment to stop the growth of fungal species belong to *Aspergillus, penicillium, Acremonium, Fusariu,Rhizopus, Cladosporium,Alternaria* and *Stachybotrys* isolated fromdeteriorated archeological marbletaken from defferent locations in Cairo, Egypt. Also, sodium azide was applied annually to pine nursery beds as an effective fungicide [68]. Moreover, Kumi [69] reported that sodium azide treatment significantly reduced both fungi and bacterial populations of plots located at the George Washington Carver Agricultural Experimental Station (GWCAES), Tuskegee University, Tuskegee, Alabama.All tested fungi were susceptible to different concentrations of fluconazole. These findings come in agreement with Patel [70] who reported high sensitivity of ocular fungi to fluconazole amphotericine B and ketoconazole.On the other hand, Mikami [71] illustrated that fluconazole, miconazole and itraconazole were effective against *Aspergillus fumigatus, Candida albicans* and *Cryptococcus neoformans* grown on different media.

Conclusions

Investigations of Sultan Hassan Mosque were conducted in the bacteria and fungi of the limestone [72]. Laser can be used to removing the surface layers of soot deposited on the surface of the stone [73]. Additionally, Acrylic polymers were used to preserve the archeological buildings made of sandstone and limestone because of their ability to form a protective layer on the surface of the stones treated [74]. Such treatment can be applied to reduce the degree of fragility of stone exhibition deteriorated by the modifying properties [75].Therefore, the design of conservation treatment should include regeneration of these properties so that the stone be able to exist environment [76]. The results obtained in this study also highlight that microorganisms (bacteria and fungi) were involved in lime stone deterioration. Damage is caused due to penetration of bacterial and fungal mycelia resulting in increasing the porosity of stone as well as forming of thick crusts. Melanin- producing fungi (*Alternaria*, *Ulocladium, Cladosporium, Humicola*) are main factors of stone pigmentation. Also, calcium carbonate- dissolving *Bacillus* strains enhance limestone weathering. Application of antimicrobial agents such as fucidic acid and fluconazole may limit the microbial growth and therefore reduce stone deterioration.

References

1. [1] Laila K. Revival of Mamluk Architecture in the 19th& 20th centuries, Islamic Art and Architecture, 2013, 11.
2. [2]Mathis S. Narratives in Mamluk architecture: Spatial and perceptual analyses of the madrassas and their mausoleums, Frontiers of Architectural Research, 2016, 5; 74–90.
3. [3] Abdullahi Y, Rashid M. Evolution of Islamic geometric patterns, Frontiers of Architectural Research, 2013, 2 (2); 243–251.
4. [4] Elkhateeb A, Refaat M. Sounds from the past the acoustics of Sultan Hassan Mosque and Madrasa, Building Acoustics, 2007, 14 (2); 109-132.
5. [5]Essawy S, Kamel B, Samir M. Sacred buildings and brain performance: The effect of Sultan Hasan Mosque on brain waves of its users, Creative Space (CS), 2014, 1 (2); 123–141
6. [6] Kareem AM. Sultan Hassan Mosque: An Islamic architectural wonder analytical study of design and its effect on Islamic Cairo, Journal of Islamic Architecture, 2010, 1 (2); 94- 105.
7. [7] Tarek M. Deterioration of the floor of the interior courtyard of Sultan Hassan Mosque in Cairo, Egypt, Mediterranean Archaeology and Archaeometry, 2009, 9 (1); 115-122.
8. [8] Radwan A. Reflection of technology on the inherited conceptual design of mosques, Master thesis, Ain Shams University, Faculty of Engineering, Department of Architecture, 2013.
9. [9]Tawfik H. Physical and mechanical characteristics of Helwan limestone: For conservation treatment of Ancient Egyptian limestone monuments, Journal of American Science, 2015, 11(2); 136-151.
10. [10]Carroll D, et al. Rock weathering, London, 1970, 6.
11. [11]Salman A, Howari F, El-Sankary M, Wali A, Saleh M. Environmental impact and natural hazards on Kharga Oasis monumental sites, Western Desert of Egypt, Journal of African Earth Sciences, 2010, 58; 341–353.
12. [12]Warscheid T, Braams J. Biodeterioration of stone*:* A review, International Biodeterioration& Biodegradation, 2000, 46; 343- 368.
13. [13]Mitchell R, Ji-Dong G. Changes in the biofilm microflora of limestone caused by atmospheric pollutants, International Biodeterioration& Biodegradation, 2000, 46; 299-303.
14. [14]Morton AC, Hallthworth C. Processes controlling the composition of heavy mineral assemblages in sandstones, Sedimentary Geology, 1999, 124; 3-29.
15. [15]Abd El-Hady M. Groundwater and the deterioration of Islamic buildings in Egypt: The restoration and conservation of Islamic monuments in Egypt, AUC, 1995; 118.
16. [16]Salman A, Howari F, El-Sankary M, Wali A, Saleh M. Environmental impact and natural hazards on Kharga Oasis monumental sites, Western Desert of Egypt, Journal of African Earth Sciences, 2010, 58; 341–353.
17. [17]Gauri KL. Decay and its prevention in natural stone: Transactions of the Kentucky Academy of Science, 1974, 35 (1-2); 29-36.
18. [18]Abdelhafez A., El-Wekeel F., Ramadan E.,Abed-Allah A., 2012, Microbial deterioration of archaeological marble: Identification and treatment, *Annals of Agricultural Sciences*, Volume 57, Issue 2, PP. 137–144.
19. [19]Khan, A. B. and Kulathuran, G. (2010): Composition of microorganisms in deterioration of stone structure of monuments. The bioscan, Vol. 1: 57-67.
20. [20]Siegesmund, S., Torok, A., Hupers, A., Muller, C. &Klemm, W. (2007): Mineralogical, geochemical and microfabric evidences of gypsum crusts: a case study from Budapest. *Environmental Geology*, 52: 385-397.
21. [21]Kramar, S., Mirtič, B. 2008, Characterization of black crusts of Robba´s fountain statues, Ljubljana (Slovenia). *RMZ – Materials and Geoenvironment*, 55 (4): 490–504.
22. [22]Warscheid, T. and Braams, J. (2000): Biodeterioration of stone: a review. IntBiodeteriorBiodegrad 46, 343–363.
23. [23]Papida S, Murphy W, and May E. (2000): Enhancement of physical weathering of building stones by microbial populations. *IntBiodeterBiodegr*46: 305–17.

24. [24]Saiz-Jimenez C. (1997): Biodeteriorationvsbiodeterioration: the role of microorganisms in the removal of pol-lutants deposited onto historic buildings. *IntBiodeteriorBiodegrad*, 40: 225-32.
25. [25]Herrera LH, Arroyave C, Guiamet P, de Saravia SG, and Videla H. (2004): Biodeterioration of peridotite and other constructional materials in a building of the Colombian Cultural Heritage. *IntBiodeterior Bio-degrad.*, 53: 135–41.
26. [26]El-Derby, A. A., Mansour, M. M. and Salem, M. Z. (2016): Investigation the microbial deterioration of sandstone from the Osirion's sarcophagus chamber as affected by rising ground water level. Mediterranean Archaeology and Archaeometry,16 (1): 273-281.
27. [27]McNamara, C. and Mitchell, R. (2005): Microbial Deterioration of Historic Stone. Frontiers in Ecology and the Environment, 3 (8): 445-451.
28. [28]Pinzari, F., Montanari, M., Michaelsen, A. and Pinar, G. (2009): Analytical protocols for the assessment of biological damage in historical documents. Coalition 19: 6–13.
29. [29]Urzì, C., Brusetti, L., Salamone,P., Sorlini, C., Stackebrandt, E. and Daffonchio, D. (2001): Biodiversity of geodermatophilaceae isolated from altered stones and monuments in the Mediterranean basin. *Environmental microbiology* 3 (7):471-479.
30. [30]Krieg, N.R., Holt, J.G., (1984): Bergey's Manual of Systematic Bacteriology, Vol. 1, eds: Williams and Wilkins. Baltimore.
31. [31]Raper, K.B., Thom, C., (1949): Manual of Penicillia. Williams and Wilkins Co. Balitimore, USA.
32. [32]Gilman, J.C., (1957): A Manual of Soil Fungi. The Iowa State University Press. Iowa USA.
33. [33]Subrahmanyam, G., Vaghela, R., Bhatt, N., P. and Archana, G. (2012): Carbonate-Dissolving Bacteria from 'Miliolite', a Bioclastic Limestone, from Gopnath, Gujarat, Western India. *Microbes Environ*. (27) 3: 334–337.
34. [34]Balouiri, M., Sadiki, M. and Ibnsouda, S. K. (2016): Methods for in vitro evaluating antimicrobial lactivity: A review. *Journal of Pharmaceutical Analysis*, 6:71–79.
35. [35]Murray P. R., Baron, E. J., Pfaller, M. A., Tenover, F. C. and Yolken, H. R. (1995): *Manual of Clinical Microbiology*, 6th Ed. ASM Press, Washington DC, 5: 15-18.
36. [36]Quayle N., 1992, Alveolar decay in stone-its possible origins, 7th international congress on deterioration and conservation of stone, Lisbona, V, 1, PP.109-118.
37. [37]Rose R., and silv O., 1992, alteration of Igneous rocks by thermo-mechanical loads, 7th international congress on deterioration and conservation of stone, *Lisbona*, V, 2, PP.651-657.
38. [38]El-Gohary M. A., 2009. Investigations on limestone weathering of El-Tuba Minaret El- Mehalla, Egypt: Acase study, *Mediterranean Archaeology, and Archaeometry*, Vol. 10, No. 1, pp. 61-79.
39. [39]Ammar and El- Deeb (1992): Air microflora inside Khofo Pyramid in the absence and presence of 1500 visitors. Egypt. *J. Microbiol*. 27: 405.
40. [40]Ogram, A. and K. Sharma (2002): Methods of soil microbial community analysis. In *Manual of nvironmental microbiology*, 2nd edition, eds. C. J. Hurst et al. Washington, D.C.: ASM Press. 554-563.
41. [41]Awad, A. A. (2007): Airborne dust, bacteria, actinomycetes and fungi at a flourmill. *Aerobiologia*, 23:59–69.
42. [42]Urzi, C. and De Leo, F. (2001): Sampling with adhesive tape strips: an easy and rapid method to monitor microbial colonization on monument surface. *Journal of microbiological methods*, 44: 1-11.
43. [43]Gupta,S., Chelak, E., Sharma, B. and Sharma, K. (2013): Chemical conservation of biodeteriorated monuments of Chhattisgarh. Trends of life sciences, 2 (1): 1-3.
44. [44]Kavita, S. (2012): Biodeterioration and microbial communities on the ancient Monuments of Chhattisgarh, India. J. Res.Microbes.1: 023-028.
45. [45]Shelton B. G., Kirkland, K. H., Flanders W. D. and Morrie G. K. (2002): Profile of airborne fungi in buildings and outdoor environments in the United States. Appl. Environ. Microbiol. 68 (4): 1743-1753.
46. [46]Lo´pez-Miras, M., Sa´nchez, I., Rodrı´guez, A. Noguera, J., Galiano, F., Ettenauer, J., Sterflinger, K. and Pinar, G. (2013): Contribution of the Microbial Communities Detected on an Oil Painting on Canvas to Its Biodeterioration. PLOS ONE, 8(11): 1-13.
47. [47]Suihko, L. M., Alakomi, L. H., Gorbushina, A. A., Fortune, I., Ma rquard and Saarela, M. (2007): Characterization of aerobic bcterial and fungal microbiota on surfaces of historic scottish monuments, Syst. Appl. Microbiol. 30: 494—508.

48. [48]Khan, A. and Kulathuran, G. (2010): Composition of microorganisms in deterioration of stone structures of monuments. The Bioscan, 1: 57- 67.
49. [49]Ciferri, O. (1999): Microbial degradation of paintings. Appl Environ. Microbiol. 65: 879–885.
50. [50]Pepe, O., Sannino, L., Palomba, S., Anastasio, M., Blaiotta, G., Villani, F. and Moschetti, G. (2010): Heterotrophic microorganisms in deteriorated medieval wall paintings in southern Italian churches. Microbiol. Res. 165: 21–32.
51. [51]Schabereiter-Gurtner, C., Piñar, G., Lubitz, W. and Rölleke, S. (2001): An advanced molecular strategy to identify bacterial communities on art objects. Journal of Microbiological Methods 45: 77-87.
52. [52]Piñar, G.; Ramos, C.; Rölleke, S.; Schabereiter-Gurtner, C.; Vybiral, D.; Lubitz, W. and Denner, E.B.M. (2001): Detection of indigenous *Halobacillus*populations in damaged ancient wall paintings and building materials: molecular monitoring and cultivation. Applied and Environmental Microbiology 67: 4891-4895.
53. [53]Rölleke, S.; Muyzer, G.; Wawer, C.; Wanner, G.; Lubitz, W. (1996): Identification of bacteria in a biodegraded wall painting by denaturing gradient gel electrophoresis of PCR-amplified gene fragments coding for 16S rRNA. Applied and EnvironmentalMicrobiology62:2059-2065.
54. [54]Frank- Kamentskaya, O., Vlasov, D. Y., Zelenskaya, M. S., Knauf, I. V. and Timasheva, M. A. (2009): Decaying of the marble and limestone monuments in the urban environment. Case studies from Saint Petersburg, Russia. Studia UBB, Geologia, 54 (2): 17 – 22.
55. [55]De la Rosa-García,S. C., Ortega-Morales, O., Christine, Gaylarde, C. C., Beltrán-García, M., Quintana-Owen, P. and Reyes-Estebanez, M. (2011): Influence of fungi in the weathering of limestone of Mayan monuments. Revistamaxicologia, 33: 43-51.
56. [56]Urzi, C., (1993): Interactions of some microbial communities in the biodeterioration of marble and limestone. In: Guerrero, R., C. Pedros-Alio (eds.), Trends in Microbiology, Society for Microbiology, Madrid, pp. 667–672.
57. [57]Hirsch, P., Eckhardt, R.J. and Palmer, Jr. (1995): Fungi active in weathering of rock and stone monuments. Candian Journal of Botany 73:S1384–S1390.
58. [58]Gaylarde, P.M., Gaylarde, C.C., Guiamet, P.S., Gomez de Saravia, S.G., Videla, H.A. (2001): Biodeterioration of Mayan Buildings at Uxmal and Tulum, Mexico. Biofouling, 17:41–45.
59. [59]Gaylarde, C. C. and Gaylarde, P. M. (2002): Biodeterioration of Historic buildings In Latin America. 9DBMC. 171: 1-9.
60. [60]Gaylarde, C., Morton, G. (2002): Biodeterioration of Mineral Materials. In: Britton, G. (ed.), Encyclopedia Environmental Microbiology, John Wiley & Sons, New York. 6: 516–528.
61. [61]Sterflinger, K. and Krumbein, W.E. (1997): Dematiaceous fungi as a major agent for biopitting on Mediterranean marbles and limestones', *Geomicrobiol J.* 14:219-225.
62. [62]Amy, P. S., Haldeman, D. L. and Ringelberg, D. (1992): Comparison of identification systems for classification of bacteria isolated from water and endolithic habitats within the deep subsurface. *ApplEnvironMicrobiol*58: 3367–73.
63. [63]Nienow, J.A. and Friedmann, E. I. (1993): Terrestrial lithophytic (rock) communities. In: Friedmann EI (Ed). Antarctic microbiology. New York, NY: Wiley-Liss Inc.
64. [64]Groth I., Schumann P. and Laiz, L. (2001): Geomicrobiological study of the GrottadeiCervi, Porto Badisco, Italy. Geomicrobiol J,18: 241–58.
65. [65]Li, W., Yu, L.Z., He, Q.F., Wu, Y., Yuan, D.X. and Cao, J.H. (2005): Effects of microbes and their carbonic anhydrase on Ca^{2+} and Mg^{2+} migration in column-built leached soil-limestone karst systems. Appl. Soil Ecol. 29:274–281.
66. [66]Li, W., Yu, L. Z., Wu, Y., Jia, L.P. and Yuan, D.X. (2007): Enhancement of Ca2+ release from limestone by microbial extracellular carbonic anhydrase. Bioresour. Technol. 98:950–953.
67. [67]Li, W., Zhou, P. P., Jia, L. P., Yu, L. J., Li, X. L. and Zhu, M. (2009): Limestone dissolution induced by fungal mycelia, acidic materials, and carbonic anhydrase from fungi. Mycopathologia, 167(1):37-46.
68. [68]Kelly, W. D. and Rodriguez-Kabana, R. (1981): Effects of annual applications of sodium azide on soil fungal populations with emphasis on Trichoderma species. Pesticide Science 12(3):235 – 244.

69. [69]Kumi, A. S., Victor Khan, Ramble, V. and Ankumah, O. (2013): Assessing the Effects of Solarization and Sodium Azide Amendments on Selected Soil Parameters, Enzyme Activities and Microbial Populations. Journal of Environmental Protection, 4: 772-778.
70. [70]Patel, M. H., Patel, A. M., Patel, S. M., Ninama, Patel, G. L. and Lavingia, B. C. (2011): Antifungal susceptibility testing to determine MIC of amphotericine B, fluconazole and ketoconazole against ocular fungal infection. National Journal of Community Medicine, 2 (2): 302- 305.
71. [71]Mikami, Y., Yazawa, K. and Matsumae, A. (2011): Evaluation of in vitro antifungal activity of amphotericin, fluconazole, flucytosine, itraconazole and miconazole on seven different antifungal assay media. Chemotherapy, 39 (8): 761- 770.
72. [72]Rebricova N., 1995, An evaluation of biocide treatments on the rock are of baical in methods of evaluating products for the conservation of porous building materials in monuments, ICCROM, Rome, P.69-74.
73. [73]Mitre G., Medina J., Calvo B., Prieto A Leal L., Perez B., Marcos F., De Frutos A., (1996) , Laser cleaning in art restoration, Applied Surface Science 96-98 474-478.
74. [74]Khallaf M., El-Midany A., El-Mofty S., 2011, Influence of acrylic coatings on the interfacial, physical, and mechanical properties of stone-based monuments, *Progress in Organic Coatings* 72 , 592– 598.
75. [75]Gauri KL, GwinnJR, Popli R. Performance criteria for stone treatment*:* Second International Symposium on the Deterioration of Building Stones, Athens, Greece, 1976, 143-151.
76. [76]Gauri, K. L., 1980, Deterioration of architectural structures and monuments: In Polluted Rain, New York: Plenum Press, p. 125-144.

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