Bacterial Deterioration in the Limestone Minaret of Prince Muhammad and Suggested Treatment Methods, Akhmim, Egypt

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Abstract

El-Amir Muhammad's minaret in Akhmim, Sohag, Egypt, is constructed of limestone and has been exposed to many factors of damage as a result of the high levels of ground water. Limestone is strongly affected by ground water, especially when being impure. The current work discusses the results of analytical techniques including chemical testing to determine the types of soluble salts through optical microscopy, electronic scanning electron microscope with an X-ray energy dispersion system (ESEM) to study and determine the causes of rapid degradation. Microbial weathering phenomena toward limestone were also studied. Different bacteria and fungi were isolated from outdoors and indoors of air and limestone of the building of which *Bacillus cereus* OK447647, *B. subtilis* OK447648, *Serratia marcescens* OK447650, *Pseudomonasoryzihabitans* OK447649, *Aspergillus flavus*, *A. niger*, *Penicillium chrysogenum* and *Cladosporium cladosporoids* were the most representative. *B. cereus* OK447647 and *B. subtilis* OK447648 have shown ability for calcium carbonate dissolving. The minimal inhibitory concentrations (MICs) of sodium azide were investigated against the growth of microbial isolates. Sodium azide at 100 ppm was found to be the best treatment for bacterial isolates although it had no significant effect against fungi.

1. Introduction

The mosque and minaret of El-Amir Muhammad dates to the Ottoman tech-

nology and is known as the Mosque of the Market. It was constructed by the aid of El-Amir Muhammad, El-Amir Hassan's father. This minaret is the most effective one left in the mosque. It is located at the western aspect of Caesarea Street (Figure 1).

The minaret consists of three floors. The first floor has a rectangular projection, the second is an octagonal projection, and is cylindrical in shape, crowned with the aid of the pinnacle of the minaret, that's punctuated with the aid of using six knotted holes and the third is cylindrical in shape and is topped by the top of the minaret punctuated by six knotted slots. Many researchers have discussed the damage to archaeological Islamic minarets [1] [2] [3] [4]. Limestone has centuries-lengthy and global culture as a constructing material [5] [6] [7]. Solution weathering is continual but generally unthreatening to the structure of a building. One such mechanism is salt weathering. Salts occur naturally within the atmosphere e.g. at the coast, but in polluted environments, their concentrations and variety are increased because of the chemical reactions between limestone and acid pollutants [8]. The most ordinarily salt produced by this reaction is calcium sulphate (gypsum) which is the most often salt related to weathered limestone [9] [10]. On drought, the salt crystals precipitate either on the surface, or within the pores of the stone. This can cause dislodgement of individual grains (granular disintegration) or the event of scales and flakes of stone [11] [12]. Salt weathering is most cases natural stone decay, and as a consequence, there is a significant problem with the conservation of cultural heritage [13]. Dissolved salts such as, sulfate, chloride, sodium nitrate, potassium, magnesium, ammonium and calcium are major factors in the damage of porous materials such as limestone. The rate of spoilage is also attributed to the behavior of salt with limestone, leading to deep crushing, partial and surface cracking, grain damage and pits as well as physical stress as a result of crystallization, hydration and differential thermal expansion.

The biodeterioration of monumental heritage is a worldwide phenomenon; it represents a significant loss of cultural heritage [14]. Microbial biodeterioration

Figure 1. Site area of El-Amir Muhammad's minaret, Sohag, Google 2021.

is one of the main causitive of archeological rocks deterioration especially in museums and mosques [15] [16].

Stone surfaces and paintings of monuments are exposed to continuous biodegradation and biodeterioration agents, such as microbial communities colonizing stone surface and paintings consisting of a great number and diversity of microorganisms, such as bacteria, action bacteria, fungi, and yeast [17].

The biodeterioration of archeological stones works occurs as a consequence of biofilm production, secretion and deposition of organic and inorganic compounds and physical penetration of microbes [18] [19] Figure 2(e) and Figure 2(f). The growth and activity of the microorganisms on stone surface results in major alterations such as surface alterations (etching, pitting, stratification, etc), staining or color alteration, bio-weathering (stone dissolution), bio corrosion and transformation of crystal into small size one [20]. The monitoring of microbial contamination represents the basis for a proper conservation strategy [21].

The present work aimed to investigate and identify the biological cause of archeological limestone biodeterioration from El-Amir Muhammad's minaret in Akhmim-Egypt and suggest methods of treatment.
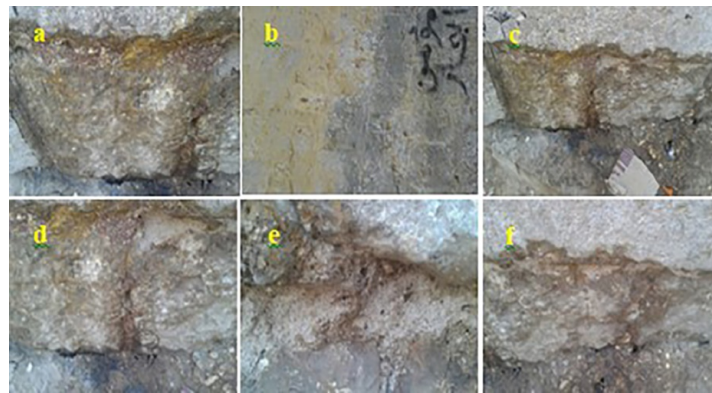

Figure 2. Effect of moisture on the lower walls (a), discoloration of limestone (b), the presence of separation and loss of some stone blocks ((c), (d)) and the effect of microbiological damage on the walls ((e), (f)) of El-Amir Muhammad's minaret.

2. Materials and Methods

2.1. Collection of Limestone Samples

Limestone samples were collected from the study site (El-Amir Muhammad's Minaret, Akhmim, Sohag, Egypt) by non-destructive methods.

2.2. Petrographic Examination

Nikon polarizing microscope (JEOL JSM5500LV) was used in the petrographic study of limestone samples.

2.3. XRay Diffraction (XRD)

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

2.4. Chemical Study by XRF

Identification of the chemical composition of limestone samples was carried out using (Axios Advanced, Sequential wd_ XRF Spectrometer, PANalytical 2005) in the Analysis Unit of the National Center for Building Materials Research in Cairo.

2.5. Scanning Electron Microscope (SEM)

SEM was used to study and understand the fine structure, decomposition, and different properties of the limestone under study. It was carried out in the Central Lab, South Valley Univ. using JEOLJSM-5500 LV SEM (JEOL, Japan).

2.6. Isolation of Airborne Microorganisms

The settle plate method [22] was used to estimate the airborne spores in of El-Amir Muhammad's minaret. 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 days. The developed colonies were counted in plates and the average number of colonies per three plates was determined.

2.7. Isolation of Microorganisms from Deteriorated Limestone

Dry cotton swabs were rubbed on the surface of the deteriorated parts of the building over an area of 4 cm^2, under aseptic conditions, stored at 4˚C until used for inoculation as mentioned previously.

2.8. Identification of Bacterial Isolates

The bacterial isolates were tentatively identified on the basis of classification schemes published in Bergey's Manual of Systematic Bacteriology [23].

2.9. Molecular Identification of the Common Bacterial Isolates

The Bacterial isolates were cultured in sterile test tubes containing 10 ml of nutrient broth medium [24]. The cultures was incubated at 28˚C for 48 hours, then sent to the molecular Biology Research Unit, Assiut University for DNA extraction using Patho-gene-spin DNA/RNA extraction kit provided by Intron Biotechnology Company, Korea. From each sample the DNA was sent to SolGent Company, Daejeon South Korea for polymerase chain reaction (PCR) and gene sequencing. PCR was performed using two universal primers where 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') were used. The purified PCR products (amplicons) were reconfirmed using a size nucleotide marker (100 base pairs) by electrophoreses on 1% agarose gel. The amplicons were sequenced with the incorporation of dideoxynucleotides (dd NTPs) in the reaction mixture. Bacterial amplicons were sequenced in the sense and antisense directions using 27F and 1492R primers [25]. Sequences were further analyzed using Basic Local Align-

ment Search Tool (BLAST) from the National Center of Biotechnology Information (NCBI) website. Phylogenetic analysis of sequences was done with the help of MegAlign (DNA Star) software version 5.05. Sequences were aligned with others retrieved from Gen Bank using ClustalX [26] and optimized manually. The positions where one or more species contained a length mutation and ambiguously aligned regions were not included in the subsequent phylogenetic analysis. Maximum parsimony and maximum likelihood analyses were made in PAUP 4 [27]. Maximum-parsimony (MP) trees were obtained by 100 random addition heuristic search replicates, and 1000 bootstrap replicates were performed employing 5 random addition heuristic searches. Maximum-likelihood (ML) analysis [28] was performed using heuristic searches with the random stepwise addition of 100 replicates and tree bisection-reconnection (TBR) rearrangements. The optimal model of nucleotide substitution for the ML analyses was determined using hierarchical likelihood ratio tests (hLRTs) Model test 3.7 [29]. The model selected as the best fit for 16s rDNA dataset was TrN. Phylogenetic trees were visualized using Njplot [30] and edited in Adobe Illustrator CS6.

2.10. Identification of Fungal Isolates

Identification of fungal isolates was performed according to Raper and Thom (1949) [31]; Gilman (1957) [32]; Domsch (1980) [33].

2.11. Screening for Calcium Carbonate-Dissolving G Microorganisms

Bacterial and fungal isolates were tested for calcium carbonate dissolution by growing colonies on Deveze-Bruni (DB) medium, consists of ($g \cdot L^{-1}$): 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; $MgCl_2$, $CaCO_3$ 5 g and 1.5% agar), bacteria were incubated at 37°C for 7 days, fungi were incubated at 8°C for 14 days [34]. Bacteria and fungi that dissolve $CaCO_3$ can be distinguished due to the apparent halo of a clear zone around the colony.

2.12. Determination of the Minimal Inhibitory Concentration (MIC) of (Sodium Azide) against Isolated Microorganisms

The microcide solution was prepared by dissolving sodium azide ethyl alcohol 95% to give concentrations ranging from 50 up to 150 µg/l (ppm). The most dominant bacterial and fungal isolates were treated by different concentrations (25, 50, 100 and 150) ppm of sodium azide using the agar well diffusion method [35]. Control without microcide was done using ethyl alcohol. Inhibition zones were measured to determine the minimum inhibition concentration

3. Results and Discussion

3.1. Weathering of Limestone

In the present case, limestone in general is quite homogeneous in its chemical

characteristics, being dominated by $CaCO_3$, but is highly variable in terms of physical characteristics such as hardness, fossil content and porosity [36] [37]. Limestone, which is often used in stone construction, where the effects of sulfate attack appear very quickly, especially in cases of stone damage, the formation of gypsum crusts, results in stone cracking [38] (**Figure 2(b)** and **Figure 2(c)**). Humidity increases the chances of damage due to wet and dry thawing and freezing. Where the soluble salts move in and out of the components of the porous stones. Seasonal changes in soil and temperature also control, which leads to the appearance of salt flowers that are soluble (**Figure 2(a)**). Weathering is also related to mechanical strength, water absorption and permeability of the stone and treatment work must include the estimation and improvement of these properties. The pore space in the weathering zone of limestone becomes clogged when the process of recrystallization of the salts takes place as a result of damage to its components and cause discoloration of limestone [39] (**Figure 2(b)**). Water has an important role in the damage of limestone because it is highly polar [40]. It also helps in the rate of deterioration of limestone, the high content of clay minerals, which are expandable minerals. The extent of the ability of limestone to absorb moisture is expressed in estimating the clay minerals content, as water retention is a result of the presence of clay minerals in the pores. The presence of clay minerals in the formation of limestone in addition to the characteristics of limestone such as its high porosity causes severe damage to limestone caused separation and loss of some stone blocks (**Figure 2(c)** and **Figure 2(d)**). Many cracks were observed due to swelling and contraction of the components of the clay minerals due to changes in relative humidity, and the expansion and contraction of the degraded limestone components as a result of the changing temperature cycles. The degree and depth of damage also indicates that the components of the minaret have been damaged by water due to previous treatments, as they appear as visible white sediments caused by microbiological damage on the stone surface **Figure 2(e)** and **Figure 2(f)**.

Optical microscopy revealed some details such as porosity, fine cracks and grain morphology, the grains formed a fragmented matrix of heterogeneous, non-solid powders, with a large amount of fine cracks. The study showed that the limestone grains contain skeletal and fossil parts of fossils of the foraminifera, especially the nemolite, which consist of calcium carbonate $CaCO_3$ and dolomite, were found to fill the cracks and dissolve with some traces of gypsum $CaSO_4 \cdot 2H_2O$. Note calcite sparite in the inner spaces of the fossils, and the main mineral is micritic calcite as shown in **Figure 3**.

XRD results showed that calcite ($CaCO_3$) is the main component of limestone samples, and the presence of $CuFeNaS_2$ compound is an indicator of the presence of gypsum and halite with gypsum ($CaSO_4 \cdot 2H_2O$) (**Figure 4**).

Unexpectedly, XRD analyzes failed to determine the presence of aluminum silicate or clay minerals, as this was inconsistent with the results obtained from ESEM-EDS analyzes, where the latter was able to detect aluminum silicate within the polished samples and this may be due to the presence of compounds Clay
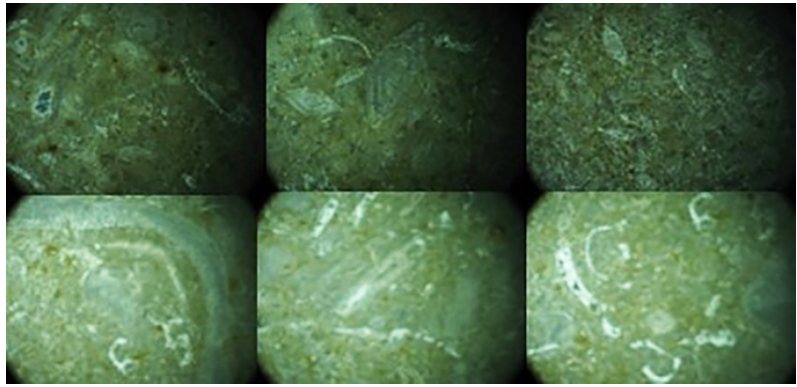
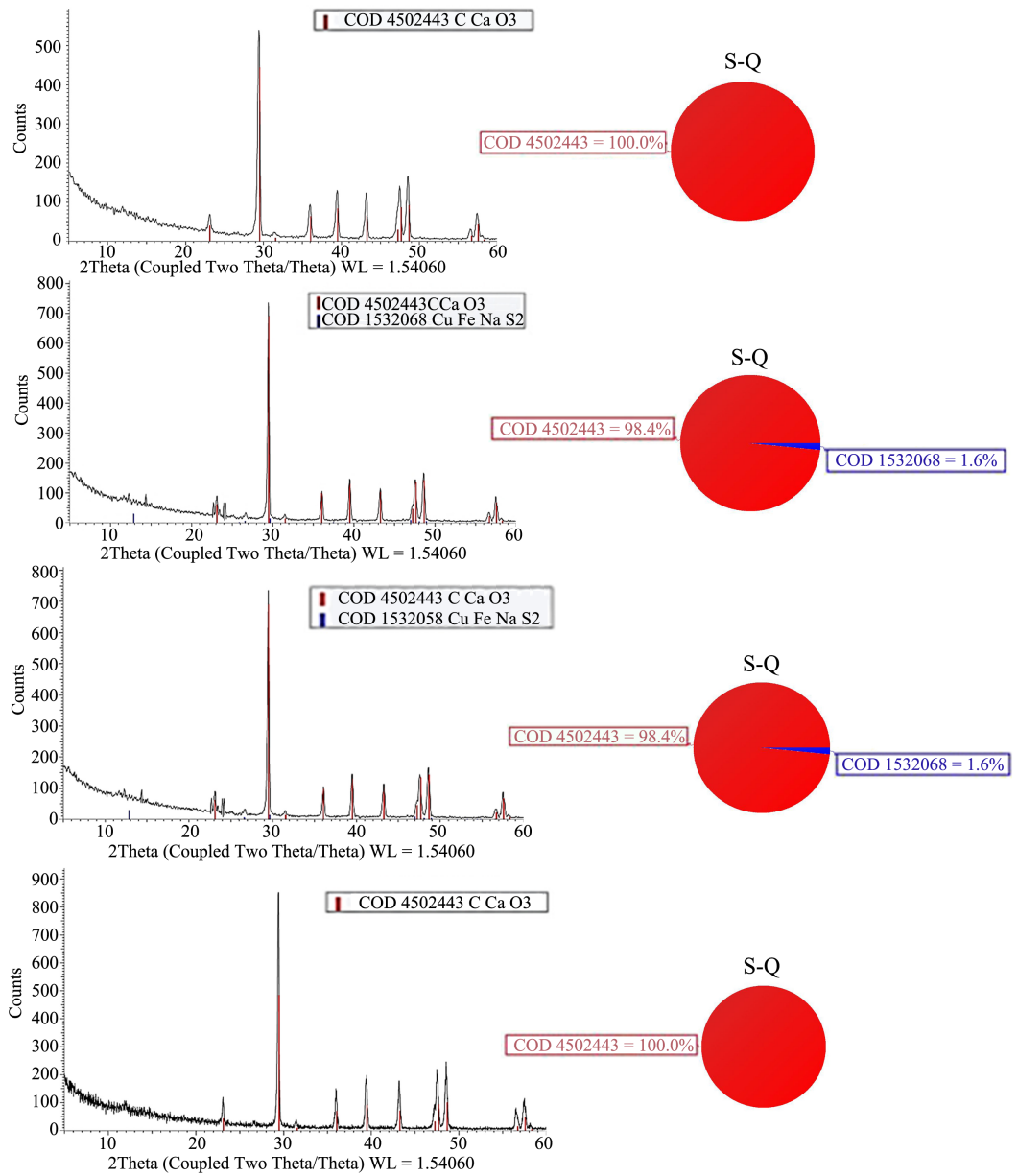**Figure 3.** Limestone with fossils of nemolite.

Figure 4. XRD showing the mineral species forming the limestone.

minerals in amounts below the detection level of XRD analyzes in the analyzed samples (about 5%).

Scanning electron microscope (SEM) in **Figure 5** clearly show that there are details in the morphology of the stone surface, especially the appearance of small and round white particles, and the preliminary analysis showed the presence of calcium (Ca), carbon (C) and oxygen (O) in most of the samples, which may indicate that these particles calcium carbonate. The grain distribution appeared inhomogeneous as silicon (Si) and aluminum (Al) **Figure 6**, which indicates the presence of aluminum silicate minerals (clay minerals), where they are found in the thin regions between the white particles as revealed by SEM images. The presence of fossil mollusks distributed throughout the matrix, and this indicates the origins of the limestone under study.
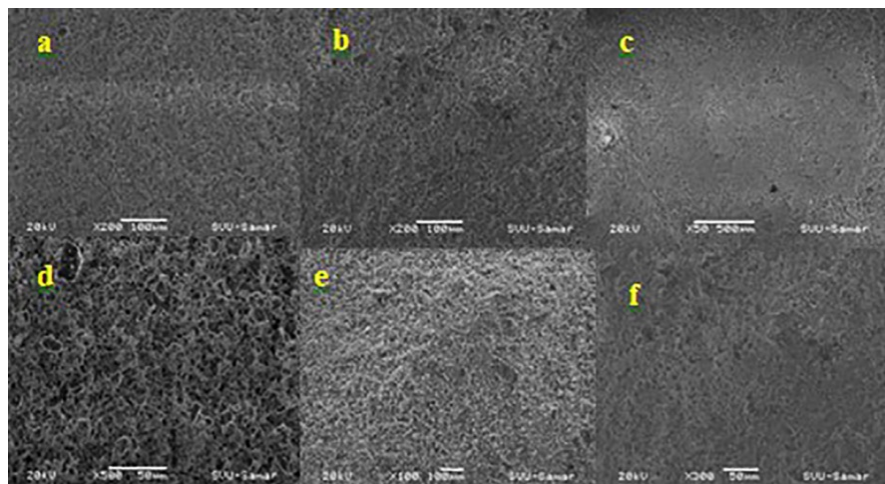

Figure 5. Contrasting pores of limestone ((a), (b)), clay facies (c), salt covering the pores (d), some limestone components (e) and the presence of fossil mollusks (f).
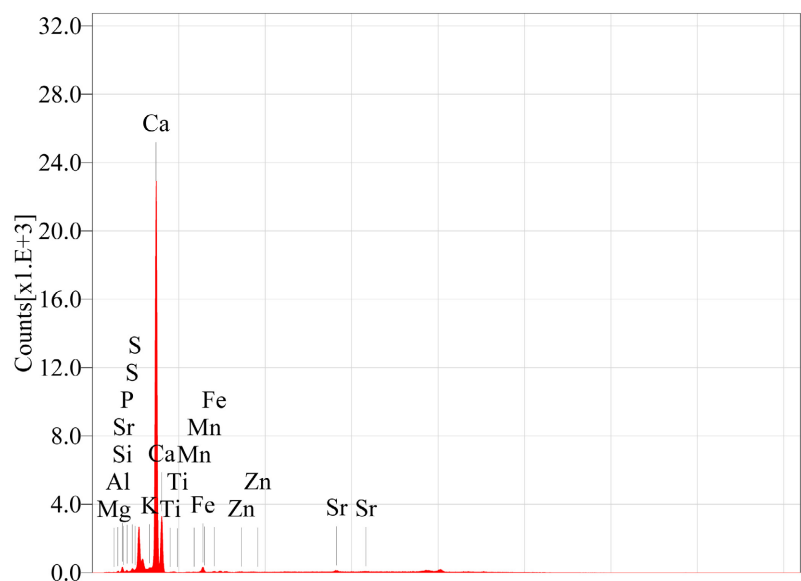

Figure 6. EDS analysis of limestone.

XRF study that Aluminum oxide Al_2O_3 shows an average percentage of 0.8102% which could be indicate to presence of clay minerals, and magnesium oxide MgO shows an average percentage of 0.5043% and ferric oxide Fe_2O_3 show an average percentage of 1.1726% and SO_3 shows an average percentage of 0.5672% which indicates to the presence of gypsum where Gypsum is formed when calcium carbonates $CaCO_3$ react with sulfur dioxide SO_2 Table 1.

3.2. Identification of the Bacterial Isolates

Based on morphological, physicochemical and physiological characterization (Table 2), isolated bacteria were tentatively identified as *Bacillus cereus*, *Bacillus subtilis*, *Micrococcus ruseus*, *Micrococcus luteus*, *Staphylococcus aureus*, *Streptomyces* sp. *Pseudomonas oryzihabitans* and *Serratia marcescens*.

3.3. Phylogenetic Analyses of Common Bacterial Strains

The 16S rDNA dataset included 20 sequences: 10 belong to the family Bacillaceae, 5 Pseudomonadaceae and 5 Yersiniaceae. The maximum parsimony dataset consisted of a total of 350 characters, of which 257 were constant, 9 variables and parsimony-uninformative and 84 were counted as parsimony-informative. Maximum parsimony produced 15 most parsimonious trees all had a length of 135 steps, a consistency index of 0.8889, a retention index of 0.9716, and a rescaled consistency index of 0.8637 and of which one is shown in Figure 7. Maximum likelihood analysis yielded a single most likely tree (–ln likelihood = 1135.93). Bayesian analysis yielded two trees similar in topology to the ML phylogenetic tree shown in Figure 7. The four bacterial strains nested within the genera: *Bacillus* (two species), *Pseudomonas* and *Serratia*. The bacterial strain

Table 1. Chemical analysis of limestone by XRF.

Main Constituents Wt%	(a)	(b)	(c)
SiO_2	1.9916	1.9817	1.9923
TiO_2	0.0282	0.0281	0.0278
Al_2O_3	0.8101	0.8103	0.8104
Fe_2O_3 tot.	1.1727	1.1725	1.1728
MgO	0.5041	0.5046	0.5043
CaO	93.7570	93.7560	93.7579
K_2O	0.4864	0.4867	0.4860
P_2O_5	0.3787	0.3782	0.3785
SO_3	0.5676	0.5671	0.5670
MnO	0.0621	0.0623	0.0628
SrO	0.2126	0.2123	0.2129
ZnO	0.0289	0.0284	0.0287

Table 2. Morphological, physicochemical and biochemical characteristics of bacterial isolates recovered from outdoors and indoors of El-Amir Muhammad's minaret.

Characteristics	*Bacillus cereus* OK447647	*Bacillussubtilis* OK447648	*Micrococcus ruseus*	*Micrococcus luteus*	*Staphylococcus aureus*	*Streptomyces sp.*	*Pseudomonas oryzihabitans* OK447649	*Serratiamarcescens* OK447650
Gram stain	+ve	+ve	+ve	+ve	+ve	+ve	−ve	−ve
Shape	bacilli	bacilli	Cocci (tetrads)	Cocci (tetrads)	Cocci (tetrads)	filamentous	Bacilli (short)	Bacilli (short)
Major pigment	−ve	−ve	rose	Yellow	orange	white	yellow	red
Nitrate reduction	+ve	+ve	+ve	−ve	+ve	−ve	+ve	ND
Acid from glucose	+ve	−ve	+ve	−ve	−ve	ve	+ve	+ve
Catalase test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
V-P test	+ve	+ve	−ve	−ve	+ve	−ve	−ve	−ve
O-F test	ND	ND	O	O	F	ND	ND	ND
Starch hydrolysis	+ve	+ve	−ve	−ve	−ve	+ve	−ve	ND
Gelatin hydrolysis	+ve	+ve	−ve	+ve	−ve	+ve	−ve	ND
Casein hydrolysis	+ve	+ve	+ve	+ve	−ve	−ve	ND	ND
Urease test	+ve	ND	+ve	+ve	−ve	+ve	+ve	ND
Citrate test	−ve	+ve	−ve	−ve	−ve	+ve	+ve	+ve
Growth on 7% NaCl	+ve	+ve	+ve	+ve	+ve	−ve	+ve	+ve
Acid and gas from glucose	−ve	−ve	+ve	−ve	−ve	−ve	+ve	ND
Growth at 65°C	ND	−ve	ND	ND	ND	ND	ND	ND
Growth at 50°C	−ve	−ve	ND	ND	ND	ND	ND	ND
Growth at 41°C	ND	ND	ND	ND	ND	ND	+ve	+ve
Growth at 4°C	ND	ND	ND	ND	ND	ND	ND	−ve
Arginine hydrolysis	−ve	ND	−ve	−ve	ND	+ve	−ve	−ve
Lipase production	+ve	+ve	−ve	−ve	+ve	ND	−ve	ND
Growth on King A	ND	ND	ND	ND	ND	ND	−ve	ND
Growth on King B	ND	ND	ND	ND	ND	ND	−ve	ND

+ve = Positive, −ve = Negative, ND = Not Detected, O = Oxidative, F = Fermentative.

RM1 was grouped with *Bacillusproteolyticus* and *B. albus* in all the phylogenetic analyses perfor/med with high statistical support (93/89/100 for MP/ML/BYPP, respectively), while the bacterial strain RM2 was grouped with *Bacillus subtilis* and *B. pizizenii* with high statistical support (91/97/100 for MP/ML/BYPP, respectively). The third strain RM3 was grouped with *Pseudomonas* sp. (KP720613)
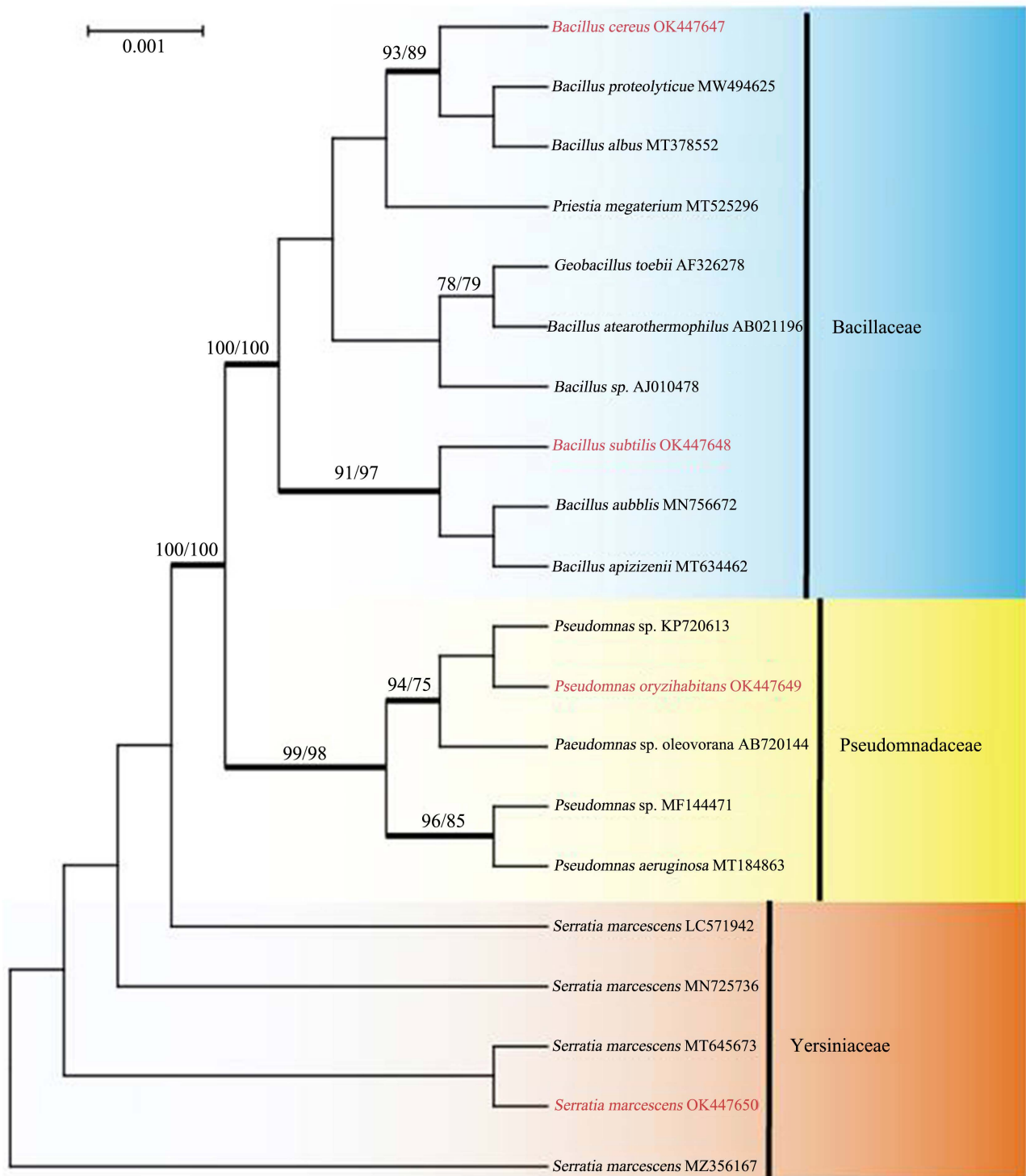
Figure 7. Maximum parsimony (MP) phylogenetic tree based on 16S rDNA of selected Bacterial isolates cultures during the present study along with phylogenetic related genera and species. Bootstrap support on the nodes represents MP and ML ≥ 50%. Branches with a BYPP (Bayesian phylogeny) of ≥ 95% are in bold. Sequences of the bacterial strains isolated in this study in red.

and *P. oleovorans* with high statistical support (93/89/100 for MP/ML/BYPP, respectively) and the fourth strain RM was grouped with several strains of *Serratia marcescens* (Figure 7).

3.4. Estimation of Airborne Microorganisms

Six airborne bacterial genera: *Bacillus*, *Staphylococcus*, *Micrococcus*, *Streptomyces Pseudomonas* and *Serratia* were recovered from out and indoor of El-Amir Muhammad's minaret. The data in Table 3 show what *Bacillus cereus* OK447647 and *B. subtilis* OK447648 were the most common and the most frequent as well comprising (53.9%; 43.3% and 30.5%; 33.9%) of total counts from outdoor sand indoors, respectively. Gram negative bacteria were identified as *Pseudomonas oryzihabitans* OK447649 and *Serratia marcescens* OK447650 comprising (0.4%; 0.3% and 0.9%; 0.5%) of total counts from outdoor and indoor bacterial isolates, respectively.

Table 3. Percentage contribution of microorganisms recovered from outdoor and indoor of El-Amir Muhammad's minaret.

Isolated microorganisms	Percentage (%) counts			
	Airborne		Limestone surface	
	Outdoor	Indoor	Outdoor	Indoor
Bacillus cereus OK447647	53.9	43.3	54.5	42.9
B. subtilis OK447648	30.5	33.9	24.2	32.1
Micrococcus ruseus	3.2	3.1	3.03	0
M. luteus	0	7.9	3.03	3.6
Staphylococcus aureus	6.2	6.3	6.1	10.6
Streptomyces sp.	4.5	4.7	3.03	3.6
Serratia marcescens OK447650	0.9	0.5	4.8	5.9
Pseudomonas oryzihabitans OK447649	0.4	0.3	1.3	1.2
Aspergillus flavus Link	34.2	23.8	27.8	25.5
A. niger Tiegh	19.7	31.7	36.7	47.3
A. sydowi (Bainier & Sartory) Thom and Church	5.3	0	0	0
A. nidulans (Eidam) G. Winter	1.3	0	0	0
A. terreus	1.3	0	0	0
A. galaucus	1.3	0	11.4	3.6
Penicillium chrysogenum Thom	26.3	14.3	3.8	3.6
P. corylophyllum DiercKX DiercKX	0	0	6.3	0
P. sp.	0	3.2	3.8	3.6
Cladosporium cladosporoids (Frsen) G. A. de Vries	10.5	22.2	6.3	1.8
Alternaria alternate (Fr) Keissl	1.3	0	0	1.8
Ulocladium charatum (preuss) E. G. Simmons	0	0	0	9.1
Curvularia lanata	0	0	11.5	0
Syncephalustrum rhizopi Vuill	1.3	4.8	0	0
Mucor	0	0	1.3	3.6
Sterile mycelia	0	0	1.3	0

Data in **Figure 8(a)** also reveal that *Bacillus cereus* was dominant, it was recorded from 100% of samples collected from indoor and outdoor aerosols of El-Amir Muhammad's minaret, followed by *Staphylococcus aureus* and *Streptomyces* (60%; 100% and 60%; 60%), *Serratia marcescens* OK447650 and *Pseudomonas oryzihabitans* OK447649 were less frequent, in outdoors and indoors, respectively.
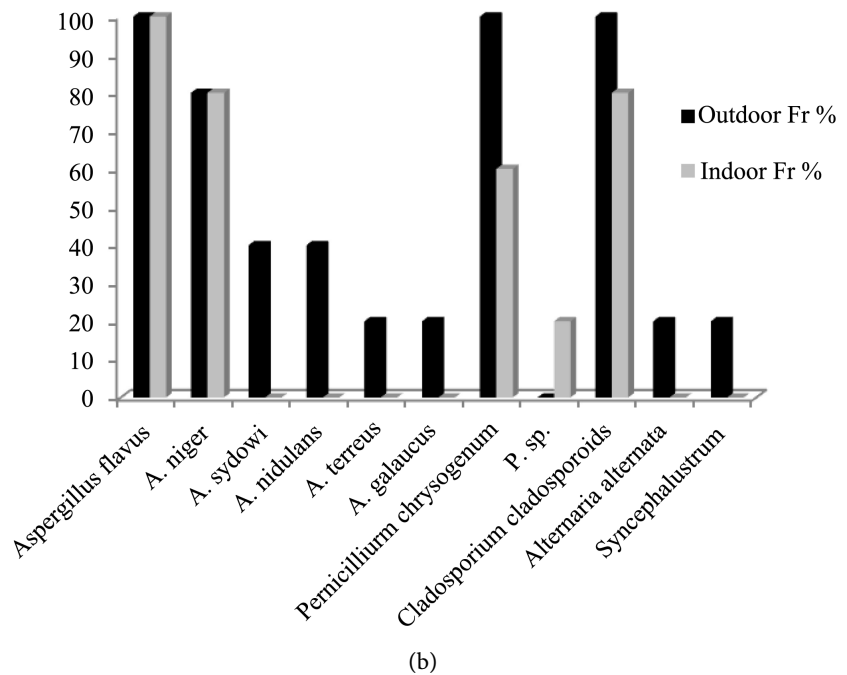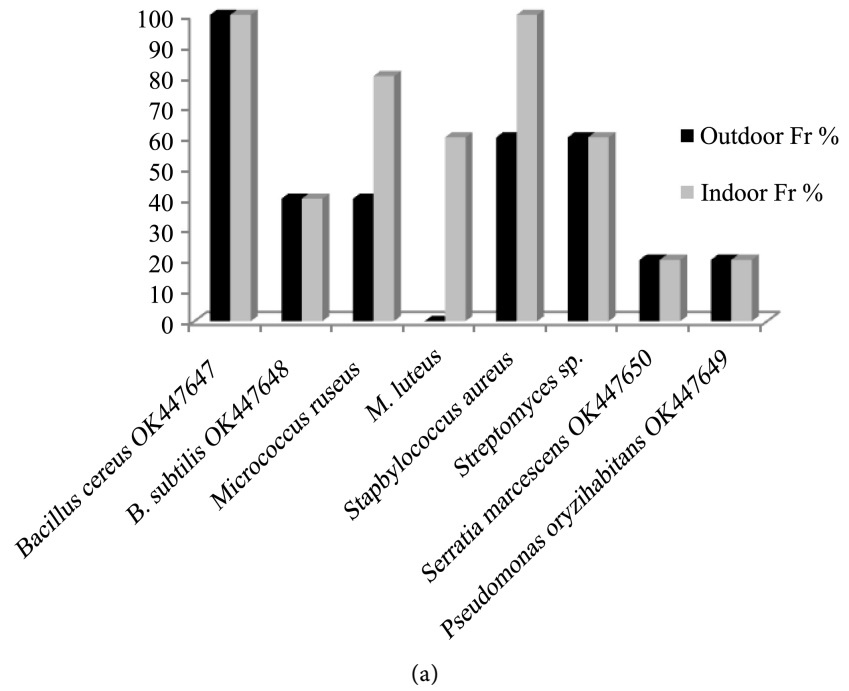

(a)

(b)

Figure 8. Frequency (Fr) of occurrence of airborne bacteria (a) and fungi (b) recorded from outdoors and indoors of El-Amir Muhammad's minaret.

These results agree with the studies presented by Carlo *et al.* (2016) [41] who isolated spore forming bacteria: *Bacillus thuringiensis* and *B. weihenstephanensis* from the *Saints Cave* environment. On the other hand, Awad (2007) [42] reported that Micrococci and *Bacillus* isolated from aerosols in a four-storey flourmill building located in Giza, Egypt were dominant where Gram-negative bacteria were found in low numbers.

The concentration and variability of the indoor airborne microbes can be affected by several factors, such as the infiltration of the outdoor air, the human presence and activities which give a good reason for the recognized increase of outdoor microbial counts compared to those of indoor counts of El-Amir Muhammad's minaret. These findings come in agreement with Katsivela *et al.* 2021 and Stelzenbach 2002 [43]. On the other hand, Rajendran and Prasad (2012) [44] concluded that the numbers outdoor aeroflora higher compared to the indoor aeroflora of Vishnu temple in India.

B. cereus and *B. subtilis* were the most dominant among airborne bacteria recovered from El-Amir Muhammad's minaret, where Gram-negative bacteria were less frequent, these results agree with the studies presented by Carlo *et al.* (2016) who isolated spore forming bacteria: *Bacillus thuringiensis* and *B. weihenstephanensis* from the *Saints Cave* environment. On the other hand, Awad (2007) reported that Micrococci and *Bacillus* isolated from aerosols in a four-storey flourmill building located in Giza, Egypt were dominant where Gram-negative bacteria were found in low numbers.

Data in Table 3 and Figure 8(b) show that eleven airborne fungal species belonging to five genera were recovered from out and indoor of El-Amir Muhammad's minaret. *Aspergillus* was the most prevalent genus represented by six species of which *Aspergillusflavus* was dominant it was recovered from 100% of samples comprising (34.2% and 23.8%) of total fungi from outdoors and indoors, respectively. *Penicilliumchrysogenum* was of moderate occurrence comprising (26.3% and 14.3%) of total fungi from outdoors and indoors, respectively, followed by *Cladosporiumcladosporoids* representing (10.5% and 22.2%) and was recorded from (100% and 80%)% of total fungi from outdoors and indoors, respectively. Lower frequencies were revealed by other genera as *Alternaria*and *Syncephalustrum.*

Among airborne fungi isolated from outdoor and indoor of El-Amir Muhammad's minaret, *Aspergillus flavus*, *A. niger* and *Penicillium chrysogenum* were the most frequent. The presence of *Alternaria alternate* and *Cladosporium cladosporoids* was pointed out. Similar results were reported by (Carlo *et al.* 2016; Abdel Hameed 2009 [45]; Gillum and Levetin 2008 [46]. Airborne spores and cells may be carried by the wind or by human activities or deposited onto the wall surfaces by gravitational settling [47]. Most microorganisms are able to successfully grow on stone surfaces covered with dust, animal remains, air contaminants and secretion or finger-marks, creating invisible layer of biofilm (Rajendran and Nisy 2012).

3.5. Microorganisms from Deteriorated Limestone

According to data in Table 3 and Figure 9(a), eight species belonging to six bacterial genera were recovered from samples collected from surfaces of deteriorated limestone of outdoors and indoors of El-Amir Muhammad's minaret. The genus *Bacillus* showed maximum frequency, it was represented by two species: *B. cereus* and *B. subtilis* comprising (54.5%; 42.9% and 24.2%; 32.1%) of total bacteria recovered from outdoors and indoors, respectively. Additionally, *Bacilluscereus* OK447647 was recorded all isolated samples. *Micrococcus, Staphylococcus, Streptomyces, Serratia* and *Pseudomonas* revealed lower counts comprising percentages ranging between (10.6% and 1.2%) of bacterial total counts. Comparable results were reported by Jroundi *et al.* (2020) [48], who
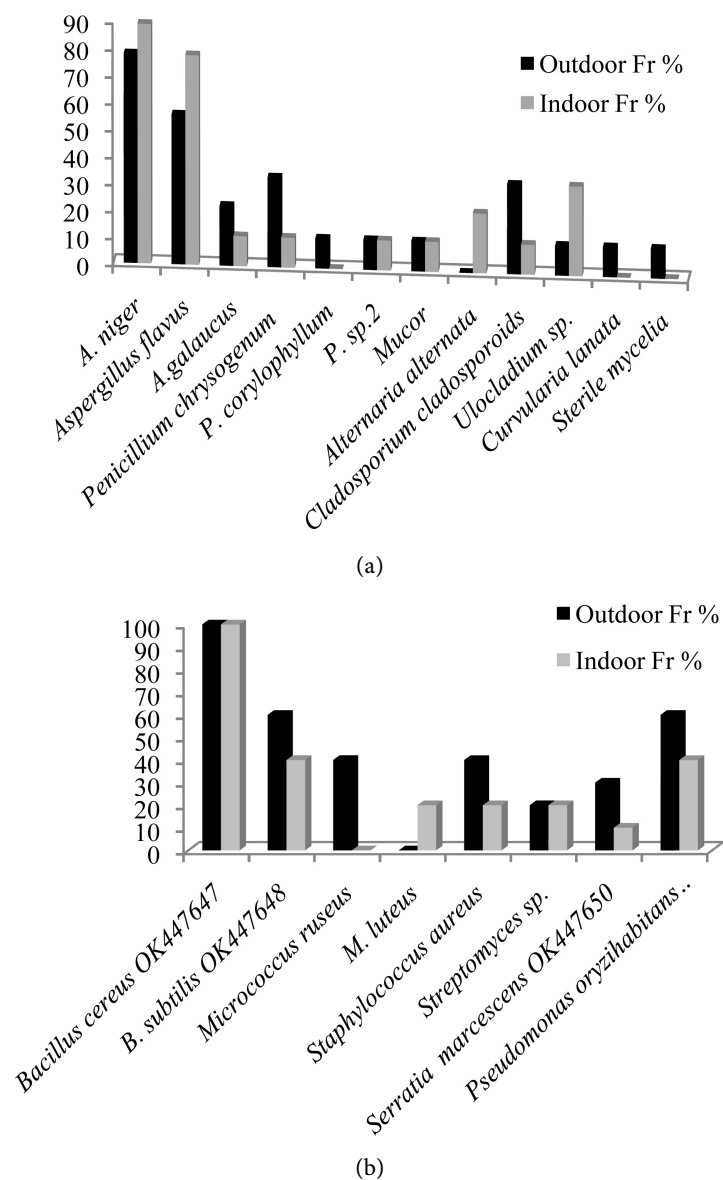
(a)

(b)

Figure 9. Frequency of occurrence of bacteria (a) and fungi (b) isolated from deteriorated limestone of outdoors and indoors of El-Amir Muhammad's minaret.

reported that *Bacillus* was detected in all samples of stones collected from Maya archeological site of Copan, Honduras. On the other hand, Banciu (2013) [49] stated that Strains of *Bacillus* spp. are some of most oftenly found bacteria identified on surface as well as inside the stone artifacts. Furthermore, *Micrococcus*, *Staphylococcus*, were recovered from pre-historic rock-paints of Kabra-pahad, India [50].

Data in Table 3 and Figure 9(b) depict that twelve species belonging to seven fungal genera were recovered from outdoor limestone of which *Aspergillusniger* was the most dominant, comprising the highest frequencies (36.7% and 47.3%) of total fungi from outdoors and indoors, respectively. *A. niger* and *A. flavus* were also reported from (77.8% and 88.9%) of samples collected from outdoor and indoor limestone, respectively. Lower frequency (6.3%) was comprised by *Penicilliumcorylophyllum* which was recovered from outdoor samples only of the total count and was recorded from 11.1% of samples. Dematiatiaceous fungi were represented by four genera (*Alternaria alternate*, *Cladosporiumcladosporoids*, *Curvularialanata* and *Ulocladium charatum*) comprising percentages of 1.3% up to 11.5% of the total fungal counts. These results corroborate with that of Biswas (2013) who isolated eighteen fungal species from Kabra-pahad rocks in India among which *Aspergillus* group were the most dominant. [51] also illustrated that within the *Aspergillus* genus, the most abundant species was *Aspergillus fumigatus* Fresen., representing up to 28% of the outdoor fungi count, while *Penicillium purpurogenum* Stoll was dominant within its genus, with a maximum representation (48%). Moreover, fungi such as *Phialophora* sp., *Cladosporium tenuissimun*, and *Aspergillus* were isolated from surfaces of stone monuments [52] [53] [54].

3.6. Calcium Carbonate-Dissolving G Microorganisms

Microbial metabolites enable some substances from rocks or minerals such as Si, Al, Fe, Mg, Mn, Ca, K, Na, Ti, to leach out from their salts, especially because of the impact of microorganisms on the dissolving rate of minerals [55].

Two bacterial isolates were positively identified as being capable of dissolving calcium carbonate, they belong to the genus *Bacillus*: *B. cereus* OK447647 and *B. subtilis* OK447648 (Figure 10). These results come in agreement with Abd-Elkareem and Mohamed (2017), who reported that calcium carbonate-dissolving *Bacillus cereus*, *B. subtilis* and *B. circulans* were recovered from deteriorated limestone of Sultan Hassan Mosque, Cairo-Egypt. On the other hand, Sonntag (2015) [56] isolated *Brevibacterium* sp. from Krast caves, this bacterium is reported their calcite dissolution ability. Moreover, three calcite dissolving bacteria (*Bacillus megaterium*, *B. aryabhattai* and *Brevibacterium*) were isolated from calcareous soil samples from Tamil Nadu, India [57]. Fungal isolates reported in this work were incapable of dissolving calcium carbonate. Morales *et al.* (2016) [58] reported that fungal isolates which were capable of solubilizing calcium by means of organic acid release, represented only 26% of fungi isolated from the

surface of Mayan buildings at Yucatan, Mexico. Palmer and Hirsch (1991) [59] as well, stated that not all fungal species or strains are able to dissolve calcium carbonate.

3.7. Determination of the Minimal Inhibition Concentration (MIC) of (Sodium Azide) against Isolated Microorganisms

Based on the results shown in Table 4, sodium azide of concentrations up to 50 ppm were ineffective against all tested bacteria and fungi. At 100 ppm, all tested

Figure 10. Zone of clearance of carbonate-dissolving *Bacillus cereus* OK447647 (1), and *B. subtilis* OK447648 (2) on DB medium.

Table 4. The minimal inhibitory concentrations (MICs) of (sodium azide) against microorganisms recovered from El-Amir Muhammad's minaret.

Tested microorganisms	Diameter (mm) of zone of inhibition	
	Concentration (ppm)	
	50	100
Bacillus cereus OK447647	0	15
Bacillus subtilis OK447648	0	11
Pseudomonas oryzihabitans OK447649	0	12
Serratia marcescens OK447650	0	9
Aspergillus flavus Link	0	8
A. niger Tiegh	0	9
A. galaucus	0	12
Penicillium chrysogenum Thom	0	17
P. corylophilum Dierc KX	0	15
Cladosporium cladosporoids (Frsen) G. A. de Vries	0	13
Alternaria alternata (Fr) Keissl	0	12

microorganisms were inhibited, the mean diameter of inhibition zone fluctuated between 8 and 15 mm. Therefore, it can be concluded that 100 ppm was the MIC of sodium azide to inhibit all the tested bacteria and fungi. Similar findings were reported by Abdelhafez *et al.* (2012), who concluded that 100 ppm of sodium azide was the best treatment to stop the growth of all microbial isolates recovered from surfaces of archeological marble located in Cairo, Egypt.

4. Conclusions

It is clear from the visual examination of the limestone samples under study that they are exposed to large levels of damage with a high percentage of different salts, as evidenced by the presence of granular disintegration and cracks and the presence of salt crystals of different sizes that can be observed with the naked eye.

This was confirmed through analyzes, as the work of microscopy, electron microscopy, X-ray fluorescence and X-ray diffraction showed the presence of soluble salts with the presence of aluminum silicate compounds, clay minerals with a high content of calcite as an essential component of the limestone under study, as was confirmed by analyzes X-ray diffraction also observed the presence of fine cracks in the limestone distributed in an unbalanced manner to the grains as a result of the impact of the destructive materials on the calcite. The calcite grains cause further acceleration of the limestone damage processes as a result of the internal disintegration that results from the activity of the salts.

Microorganisms have a destructive impact on El-Amir Muhammad's minaret limestone walls. The presence of calcium carbonate dissolving bacterial species such as *B. cereus* OK447647 and *B. subtilis* OK447648 causes severe biodeterioration of the walls. Fungal species such as *Aspergillus*, *Penicillium*, *Cladosporium* and *Alternaria* can cause many aesthetical damages to stone monuments.

More attention should be paid to salt weathering, soiling, discoloration and changing microflora. The conservation of the heritage monument is a challenging task. To ensure sustainable conservation, treatments have to be safe to the protected object, eco-friendly, derived from a renewable resource and low cost in application.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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