# Comparative Study on Fungal Deterioration and Ozone Conservation of El-Anfoushi and Al-Shatby Archeological Tombs- Alexandria- Egypt

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**Abstract:** Physical, chemical and biological factors playing a combined role in weathering of archeological tombs. El-Anfoushi and Al-Shatby archeological tombs is are located in Alexandria district in Egypt and suffering from biodeterioration aspects. Three xerophilic fungi (*Eurotium amstelodami, E. chevalieri, E. repens*), and six non-xerophilic strains (*Alternaria alternata, Aspergillus terrus, A. versicolor, Cladosporium herbarum, Fusarium moniliforme* and *Penicillium chrysogenium)* were isolated from Al-Shatby and El-Anfoushi archeological tombs, respectively. Analyses of the samples of the building material of the two tested tombs and were investigated by Environmental Scanning Electron Microscope (ESEM) Equipped with Energy Dispersive X-Ray Analysis (EDX). *A. versicolor* followed by *A. terrus* recorded the highest significant deterioration of the samples of limestone building material of the two tombs (3.7 and 2.5 cm halo zone, respectively). Atomic absorption was used to detect the release of calcium from the tested limestone samples after fungal degradation. The relation of fungal deterioration efficiency of alkaline limestone rock and pH sensitivity was recorded. Ozone as a powerful oxidizing disinfecting agent was applied on the isolated deteriorated fungal species. All isolated non xerophytes were most sensitive to 3 ppm of ozone after 150 min exposure time, while extending of the exposure time up to 210 min was required to stop the growth of the three isolated resistant xerophytes.
**[**Hala A. M. Afifi and Neveen S. I. Geweely **Comparative Study on Fungal Deterioration and Ozone Conservation of Al-Anfoushi and El-Shatby Archeological Tombs- Alexandria- Egypt]** Journal of American Science 2011; 7(12):776-784]. (ISSN: 1545-1003). http://www.americanscience.org.

**Key words:** Fungi, Conservation, Ozone, Alexandria tombs.

## 1. Introduction

Alexandria is considered the second largest city in Egypt and considered as the principal seaside summer resort on the Mediterranean. A soft oolitic limestone was used predominantly as building material for Alexandria city through ancient Egypt mainly during Graeco-Roman and Byzantine times. This limestone formed in the Holocene, diagnostically cemented by calcite (Dietrich et al., 2001). The historical oolitic limestone tombs in Alexandria are subjecting to the severe effects of the weathering environment as mainly changes in temperature degree and relative humidity due to the coastal atmosphere, infiltration of ground water and biodeterioration problems. These deterioration factors have a great bad impact on these tombs that consider hypogean monuments included in tourism and its outcome. Further, the microbiota contaminating these historical sites shows varying levels of harmful metabolic activities at different times. After colonization, microbes can initiate damaging and biodeterioration processes or they can continue living at a low level of metabolic activity in a biofilm (Saarela et al., 2004). Microbial cells and spores are transported to the tombs by air, rain, animals and visitors. Their fate, e.g., whether they die, remain alive but dormant, or actively grow and contribute to the biodeterioration, is determined by environmental factors such as humidity, temperature, light and the nature of the substrate they are inhabiting, its chemical and biological components, including the resident microbiota. The substrates in tombs were varied, some were built above the ground and others covered by earth, whereas others were dug into the rock (Albertano, 1995). These rock deposits are of alkaline composition (Sanchez-Moral et al., 2003). Heterotrophic microbes, has been found in most tombs samples as major colonizers and fungi being noted as part of the accompanying microbiota, especially in samples obtained near to the entrance of the tombs (Albertano and Urzi, 1999). In building materials, fungi have been recognized as being one group of pioneering and highly aggressive microbes causing biodeterioration (Warscheid and Braams, 2000). Laiz et al. (2003) stated that fungi might be overestimated and only species resistant to desiccation can be retrieved. Fungi are an important constituent of microbial endolithic assemblages in marine ecosystems. As euendoliths, they penetrate limestone, and other carbonate substrates, where they can exploit mineralized organic matter, attack their hosts. Microbial endoliths participate in many geologically significant processes, including bioerosion of limestone and other calcareous

substrates, in the production of fine grain sediment and in the modification of sediment grains by micritization (Golubic et al., 2005 ).

Xerophilic fungi (fungi which do not prefer a wet environment) such as *Aspergillus penicillioides, Aspergillus restrictus*, and *Eurotium* spp. cause foxing (the formation of brown spots in areas colonized by xerophilic fungi) on objects which cause a serious problem. The cause of foxing was suggested as a result of fungal growth more than 30 years ago (Abe, 2010). Such fungal species use organic substances as a source of nutrients and can utilize water contained in the air. The organic substances produced by fungi can cause the deterioration of such materials. Xerophilic fungi are not rare and its spores are always floating in indoor and outdoor air (Sakai et al., 2003). Xerophilic organisms are well known to be tolerant to several external stresses such as desiccation (Antony-Babu and Singleton, 2011), salt/sugar concentrations, pH (Guynot et al., 2002), osmotic pressure (Garg and Yadav, 2007) and heat (Splittstoesser et al., 1989). Xerophiles inhabit the limestone rock sub-surface as endoliths in pre-existing cracks and fissures or as crypto-endoliths in mineral pores and cavities (Buford et al., 2003). Free-living and symbiotic fungal forms are believed to be involved in the weathering of rocks by promoting mineral diagnoses (which can be generally defined as the transformation of a mineral into a different mineral) and dissolution. An important contribution to the weathering process can be the result of the excretion of metabolites (Adamo and Violante, 2000).

Increased awareness of the harmful effects caused by use of pesticides has led to interests in cleaner residue free technologies (Wilson and Otsuki, 2004). Biocides have been used to control this microbial population colonizing mineral surfaces in attempts to reduce stone erosion (Gaylarde et al., 2008). Such treatments are short-term and the surfaces are readily recolonised. Rain causes the leaching of biocides which acts as a toxic wash (Ashurst and Ashurst, 1988). Ozone is well known as a strong oxidizing disinfecting agent and can cause elevation of reactive oxygen species in living cells leading to oxidative stress in the cells. Nevertheless very little is known of the direct effect of ozone fumigation on fungal survival and development and to our knowledge a little work has examined the effect of ozone exposure on xerophilic fungi. Ozone does not contaminate the atmosphere and no fungal resistance to this substance has been reported so far (Sechi et al., 2001).

The aim of the present study is to analyze the basic building material of two archeological tombs (El-Anfoushi and Al-Shatby) in Alexandria- Egypt. The types of mould present on the building material of the light above ground Al-Shatby tombs were compared with moist underground chambers of El-Anfoushi tombs together with evidence of biocorrosive and destructive activity. The efficiency of ozone on some xerophilic and non-xerophilic fungi which isolated from the two tested tombs was carried out.

## 2. Materials and methods

### Historical background:

Tombs of El-Anfoushi are located on the Pharos Island on Alexandria's eastern side at El-Anfoushi district, Alexandria, Egypt. It contains of five main tombs from late Ptolemaic and early Roman Period. It dates back to the first half of the third century B.C. All tombs at El-Anfoushi consist of an open courtyard and were cut into the limestone rock, and suffer annually by rising water table. The main difference between the tombs is, however, the lack of kline chambers in El-Anfoushi. The cemetery consists primarily of loculus's burials, but some of the chambers are individual ones, with a central burial in the main chamber which is attached with a smaller back cult chamber (Roder, 1967). There are five tombs with moist underground chambers in El-Anfoushi date back to the Ptolemic age. They were discovered in 1901 AD, decorated with pictures of Egyptian gods and daily life, and graffiti dating back to the same period. Al-Shatby tombs above ground tombs are located in Shatby Station, in the city of Alexandria- Egypt. Al-Shatby tombs date back to the third century B.C. and were patterned after an old Greek house with an entrance, a front room and a back room. The tombs were accidentally discovered in 1893 AD. The tombs resemble a small open museum with sarcophagi and statues from the ancient Egyptians through the Roman Period surrounding the sunken tomb area (Figs. 1, 2).

### Sampling and sample site

Sampling was performed from the two tombs in Aughust 2010. The tested samples were obtained from the fifth funeral construction which is the richest in decoration and relief. Small flakes (less than 1 g) of limestone were collected from black, green, and white areas on the external surfaces of historic buildings (Gaylarde et *al.,* 2006).


Fig. (1) Al-Anfoushi tombs in Alexandria

Fig. (2) Al-Shatby tombs in Alexandria Alexandria

**Isolation of fungi**

Fungi were isolated from El-Anfoushi and Al-Shatby tombs. Swabbing with sterile cotton swabs and scalpel from markedly damaged surfaces of the limestone rock of both tombs with visible colonies of fungi was carried out. In the laboratory, swab samples were shaken mechanically for 10 min in 10 mL sterile distilled water and 1 mL aliquots of the resulting suspensions used to prepare spread plates on Czapeck's Dox agar. Plates were incubated in the dark at 27◦C for 7 days, with addition of 50% sucrose for xerophytes isolation. The microscopic fungi were identified using the diagnostic keys (Samson and Reenen-Koekstra 1988; Moubasher l993; Kern and Blevins 1997).

**Environmental Scanning Electron Microscope (ESEM) Equipped with Energy Dispersive X-Ray Analysis (EDX)**

An energy dispersive x-ray analysis was used to study the elements of El-Anfoushi and Al-Shatby rock samples qualitatively and quantitatively by Environmental scanning electron microscopy (ESEM) unit Model Phillips XL30 with accelerating Voltage 25 kV, X 420 and resolution for 50 µm in the Laboratory of Scanning Electron Microscopy and Microanalysis at the Nuclear Materials Authority, Cairo, Egypt (Hanlan, 1975).

**Fungal solubilization of limestone**

Limestone was ground to pass through a 90-um screen. The materials were sterilized by anhydrous ethyl ether and each amended at 0.25% to a Dox medium in 500 ml distilled water. The test fungi were inoculated into the center of the medium and incubated at 27 $^0$C.

Halo production around the microbial colonies indicates the weathering process; the width of the halo zones was taken as a measure of the ability of the microorganisms to solubilize the limestone (Ehrlich, 1990).

**Atomic absorption**

Displacement method as described by Henderson and Duff (1963) was used to detect the release of calcium from limestone minerals by the tested microbes after incubation for four weeks by using a flame atomic absorption in the Microanalytical Center, Cairo University. The amount of calcium released was expressed as milligrams of calcium released into 1 L of solution.

**Sensitivity of the tested fungal species to various pH values**

To investigate the relation of fungal degradation efficiency of alkaline limestone rock and pH sensitivity, the spore suspension was analyzed for its ability to grow at various pH values on Czapek- Dox's liquid medium. The following buffer solutions were used: - citrate buffer solution for pHs 4, 6; boric acid-borax buffer solution for pH 8; carbonate bicarbonate buffer solution for pH 10, each at 0.05 M concentration. Mycelial dry weight at various pH values was measured (mg/100ml).

**Effect of ozone on fungal growth**

Ozone was generated via a controlled flow of oxygen through a corona discharge in the ozone generator (OZO-2000). Pure discs (1 cm) of the isolated fungal species were grown on Dox medium plates. Plates were partially opened and were held in ozone chamber and exposed at 1, 2, 3 and 4 ppm and the exposure times were 0 (control), 30, 90, 150 and 210

min. Aliquots of about 99 ml of this medium were dispersed into sterile flasks (250 mL). Each flask was inoculated with ozone treated fungal disc (1 cm diameter) cut from the colony margin of 2-4 day old cultures growing on Czapek- Dox's medium. Triplicate flasks were inoculated with fungal discs and were incubated at 27 $^{o}$C and for 10 days. Mycelial dry weight was measured.

## 3. Results and Discussion

### Detection and identification of xerophilic and non-xerophilic fungi

Three xerophilic fungi (*Eurotium amstelodami, E. chevalieri, E. repens*), and six non-xerophilic strains (*Alternaria alternata, Aspergillus terrus, A. versicolor, Cladosporium herbarum, Fusarium moniliforme* and *Penicillium chrysogenium*) were isolated from Al-Shatby and El-Anfoushi archeological tombs, respectively. The majority of the isolated fungal species (66 colonies) were found in the underground chambers of El-Anfoushy archeological tomb may be referred to the high humidity due to the aggregated underground water in the underground chambers (Table 1). The obtained result was in accordance with Sanchez-Moral *et al*. (2005) who states that humid air of the tombs is ideally suited to more microbial growth on surfaces. Ascomycetes are known to be common inhabitants of mineral substrates and building stone, particularly in humid climates (Burford *et al.,* 2003).

The isolated non xerophytic species (*A. versicolor* followed by *A. terrus* and *A. alternate)* were among the dominant genera (24, 18 and 11 colonies, respectively) in underground humid chambers in El-Anfoushy limestone tomb. Considered the species of *Alternaria* are the main cause of brown and black stains on marble and limestone of many different monuments in Africa and it was well adapted to cold conditions over the pH 2.7–8.0 (Domsch *et al.,* 1980). *A. versicolor* and *A. terrus* are alkalophilic species that may explain the dominance of both species on alkaline rock, while *A. alternata* is therefore acidophilic, whereas the pH of the mineral substrates from which it was isolated was highly alkaline. The presence of *A. alternate* in such highly alkaline mineral substrates is therefore very interesting, since they grow most rapidly under acid environmental conditions. Filamentous fungi (*Sporotrichum, Aspergillus, Cladosporium, Penicillium*) are commonly detected in tombs (Albertano and Urzi, 1999). Godyova *et al.* (2004) stated that the commonest species isolated from the mineral substrates were *Aspergillus versicolor, Alternaria alternata, Penecillium chrysogenum* are producers of many different acid metabolites and exogenous pigments causing staining. On the other hand, only three isolated xerophytic species were captured in Al-Shatby tomb which may be refer to the over ground chambers of the tomb that directly subjected to sunlight causing drought. Fungi are able to obtain several elements that they need for their metabolism (calcium) from limestone by biosolubilization and production of various organic acids (Warscheid, 1991).

### Environmental Scanning Electron Microscope (ESEM) Equipped with Energy Dispersive X-Ray Analysis (EDX)

The petrographic investigation of the El-Anfoushi and Al-Shatby tombs samples are oolitic limestone. It is a carbonate rock made up mostly of ooliths (ooids) which are sand-sized carbonate particles that have concentric rings of $CaCO_3$. These rings are formed around grains of sand or shell fragments that were rolled around on the shallow sea floor, gathering layer after layer of limestone. The data in Fig. (3) shows that oolitic limestone is consist mainly of oolities and drusy sparite as cement (oolitic grainstone). Some oolities have quartz grains as nuclei as shown in fig. (4). The obtained results was in accordance with Folk, (1959) and Dunham, (1959). Calcium represents 63.22% in the first limestone tested sample without biodeterioration as shown in Fig. (5), this percentage in the second fungal deteriorated limestone sample was raised to 77.31% (Fig. 6). In the two samples both Sodium $Na^{+}$ and chlorine $Cl^{-}$ were recorded due to the salt crystallization of halite in marine environment accompanied with fungal hyphae in the pores, which range in diameter from several micrometers to several hundred micrometers (Gadd, 1999). The hyphae that develop in the pores of the limestone are encrusted with sharp crystalline spikes exterior to the hyphae and up to around 80% of the limestone micropores are inhabited by fungi and, in some places, pores are partly in filled with needle-fiber calcite (Verrecchia et al., 1990). The matrix around the pore being enriched in $CaCO_3$; this is sometimes recrystallized and separated from the pore by a mat of calcite crystals (Verrecchia and Dumont, 1996). The original white color of the limestone turned yellow and orange in the superficial layer associated with biological patina (Zagari, et al., 2000). The metabolites of microscopic fungi (melanins, melanoids and intracellular polymerizing products) cause pigmentation of limestone. The chemical effect of these compounds creates defects in the structure of the materials, e.g. micro- and macroscopic cracks, deformations, roughness, black stains and crusts leading to reduction of porosity and the loss of their original character (Godyova et al., 2004).

### Fungal solubilization of limestone

*A. versicolor* followed by *A. terrus* able to solublize El-Anfoushi tomb limestone significantly as indicated by halo formation on the plates (3.7 and 2.5

cm halo zone, respectively). Xerpohyte *Eurotium amstelodami* recorded the maximum degradation zone (0.4 cm) in Al-Shatby tomb limestone (Table 2). Dissolution of host-rock by fungi may clearly supply soluble nutrients critical to the survival and proliferation of microbial communities ( Aung and Ting, 2005).

**Atomic absorption**

The isolated fungal species were tested for the displacement method for their capacity to release calcium from the tested two limestone tombs and the obtaining results were in harmony with the clear zone method. *A. versicolor* followed by *A. terrus* and *F. moniliforme* release more calcium significantly (215, 178 and 112 mg/l, respectively) than other tested organisms (Table 3). Mitchell and Gu, (2000) stated that *Fusarium* species significantly increased both calcium release and weight loss from the limestone.

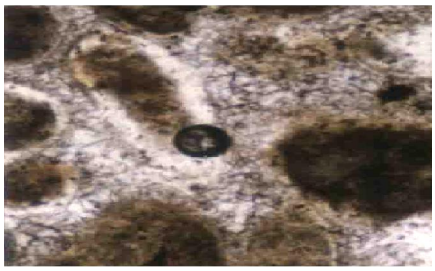

Fig. (3) Some oolities limestone have quartz grains as nuclei

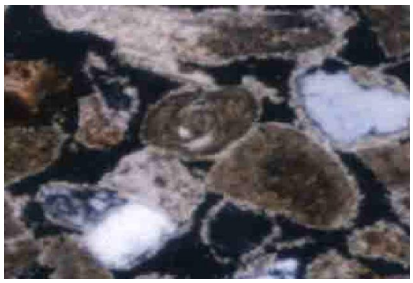

Fig. (4) Oolitic limestone that consist mainly of oolities and drusy sparite as cement

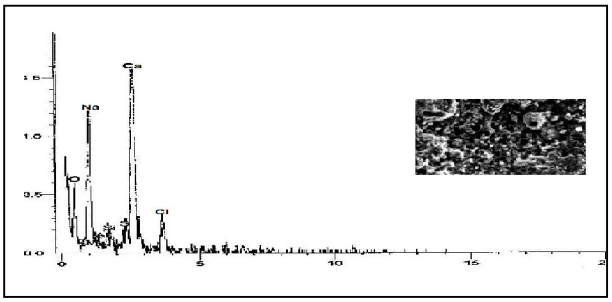

Fig.(5) EDX pattern and SEM photomicrograph of limestone sample without biodeterioration.

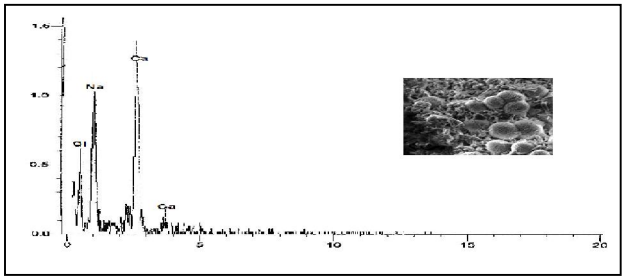

Fig.(6) EDX pattern and SEM photomicrograph of deteriorated limestone sample with fungal growth.

**Sensitivity of the tested fungal species to various pH values**

The data in Table 4 indicated that after incubation at various pH values for 10 days, the tested isolates showed different pH sensitivity on Dox medium. All xerophytes tested isolates (*Eurotium amstelodami*, *E. chevalieri* and *E. repens*) were unable to grow at highly alkaline pH (10) but they were able to grow at pH 4-8 giving the maximum growth (445, 225 and 201 mg/100 ml) at pH 8, respectively. Two of the tested non xerophytes (*A. terrus* and *A. versicolor)* were alkalophilic giving the maximum significant growth (532 and 537 mg/100ml) at pH 10, while the rest of non xerophytes (*Alternaria alternate, Penicillium chrysogenium and Cladosporium herbarum)* are acidophilic species failed to grow at highly alkaline pH, while the best growth was showed at pH6. The presence of acidophilic species in alkaline mineral substrates is paradoxical, since it grows most rapidly under acid environmental conditions. This may be referring to that their appearance could be after the production of acidic metabolite that results from biodegradation of alkaline rock by alkalophilic species. Also this may could be to the fact that the presence of these acidic species on such alkaline substrates is indicative of their ability to modify the pH of the environment by producing organic acids and other acidic metabolites

(Sonjak1 *et al*., 2006).

**Table (1) Fungal count and frequency of occurrence of fungal genera isolated from Al-Shatby and El-Anfoushy tombs (0-1 Rare, 2-3 Low, 4-5 Moderate, 6-7 High)**

| Fungal species | El-Anfoushy tomb | | Al-Shatby tomb | |
|---|---|---|---|---|
| | Count | Frequency of occurrence | Count | Frequency of occurrence |
| ***Eurotium amstelodami*** | - | - | 10 | H |
| ***E. chevalieri*** | - | - | 8 | M |
| ***E. repens*** | - | - | 2 | L |
| ***Alternaria alternata*** | 4 | L | - | - |
| ***A. terrus*** | 18 | H | - | - |
| ***A. versicolor*** | 24 | H | - | - |
| ***Cladosporium herbarum*** | 2 | R | - | - |
| ***Fusarium moniliforme*** | 11 | M | - | - |
| ***Penicillium chrysogenium*** | 5 | L | - | - |
| **Total count** | 66 | | 20 | |
| **Number of species** | 6 | | 3 | |

**Table (2) Solubilization of limestone rocks of El-Anfoushy and Al-Shatby archeological tombs as indicated by halo formation (cm)**

| Fungal species | El-Anfoushy tomb limestone degradation ability (cm) | Al-Shatby tomb Limestone degradation ability (cm) |
|---|---|---|
| ***E. amstelodami*** | - | 0.4 |
| ***E. chevalieri*** | - | 0.2 |
| ***E. repens*** | - | 0.1 |
| ***A. alternata*** | 1.5 | - |
| ***A. terrus*** | 2.5 | - |
| ***A. versicolor*** | 3.7 | - |
| ***C. herbarum*** | 0.5 | - |
| ***F. moniliforme*** | 2.2 | - |
| ***P. chrysogenium*** | 1.2 | - |
| **LSD at 5%** | 1.2 | 0.08 |

**Table (3) Calcium (mg/l) released into the culture media after incubating limestone samples with the isolated fungal species. Non inoculated El-Anfoushy and Al-Shatby limestone samples (control) = 30 and 42 mg/l**

| Fungal species | Inoculated El-Anfoushy tomb limestone | Inoculated Al-Shatby tomb Limestone |
|---|---|---|
| ***E. amstelodami*** | - | 92 |
| ***E. chevalieri*** | - | 70 |
| ***E. repens*** | - | 55 |
| ***A. alternata*** | 83 | - |
| ***A. terrus*** | 178 | - |
| ***A. versicolor*** | 215 | - |
| ***C. herbarum*** | 31 | - |
| ***F. moniliforme*** | 112 | - |
| ***P. chrysogenium*** | 34 | - |
| **LSD at 5%** | 18.0 | 16.2 |

**Table (4) Sensitivity of the tested fungal species (mg/100ml) to various pH values**

| Fungal species | pH values | | | |
|---|---|---|---|---|
| | **4** | **6** | **8** | **10** |
| ***E. amstelodami*** | 30 | 232 | 445 | 22 |
| ***E. chevalieri*** | 40 | 114 | 225 | 15 |
| ***E. repens*** | 20 | 120 | 201 | 12 |
| ***A. alternata*** | 485 | 632 | 201 | 44 |
| ***A. terrus*** | 275 | 350 | 400 | 532 |
| ***A. versicolor*** | 204 | 268 | 350 | 567 |
| ***C. herbarum*** | 115 | 432 | 120 | 86 |
| ***F. moniliforme*** | 470 | 680 | 530 | 562 |
| ***P. chrysogenium*** | 212 | 456 | 157 | 56 |
| **LSD at 5%** | 18 | 31 | 22 | 35 |

**Table (5) Effect of different concentrations of ozone gas (ppm) applied at various exposure times (minutes) on the growth (g/100 ml) of the isolated fungal species**.

| Exposure time (min.) / Fungal species | Concentration (ppm) | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | | | | | **2** | | | | **3** | | | | **4** | | | |
| | 0 | 30 | 90 | 150 | 210 | 30 | 90 | 150 | 210 | 30 | 90 | 150 | 210 | 30 | 90 | 150 | 210 |
| ***E. amstelodami*** | 0.70 | 0.52 | 0.40 | 0.33 | 0.21 | 0.45 | 0.32 | 0.28 | 0.19 | 0.31 | 0.26 | 020 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| ***E. chevalieri*** | 0.53 | 0.50 | 0.45 | 0.38 | 0.30 | 0.40 | 0.30 | 0.25 | 0.20 | 0.30 | 0.21 | 0.11 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| ***E. repens*** | 0.40 | 0.33 | 0.30 | 0.26 | 0.18 | 0.30 | 0.26 | 0.20 | 0.15 | 0.22 | 0.20 | 0.10 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| ***A. alternata*** | 1.50 | 1.21 | 0.95 | 0.64 | 0.53 | 1.00 | 0.92 | 0.52 | 0.47 | 0.85 | 0.52 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| ***A. terrus*** | 0.95 | 0.85 | 0.80 | 0.63 | 0.40 | 0.83 | 0.80 | 0.55 | 0.32 | 0.50 | 0.41 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| ***A. versicolor*** | 0.83 | 0.75 | 0.70 | 0.61 | 0.35 | 0.74 | 0.65 | 0.58 | 0.28 | 0.41 | 0.33 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| ***C. herbarum*** | 0.58 | 0.50 | 0.41 | 0.35 | 0.15 | 0.48 | 0.43 | 0.30 | 0.10 | 0.32 | 0.20 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| ***F. moniliforme*** | 1.2 | 0.92 | 0.85 | 0.78 | 0.50 | 0.96 | 0.86 | 0.70 | 0.41 | 0.80 | 0.44 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| ***P. chrysogenium*** | 0.76 | 0.50 | 0.41 | 0.32 | 0.20 | 0.65 | 0.48 | 0.39 | 0.14 | 0.40 | 0.31 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **L.S.D. at 0.05** | 0.12 | 0.20 | 0.14 | 0.11 | 0.23 | 0.15 | 0.32 | 0.21 | 0.16 | 0.25 | 0.20 | 0.10 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

**Effect of ozone on fungal growth**

The growth of the tested fungal isolates was found to be sensitive to ozone fumigation but the effects varied depending on the duration of exposure and dose of ozone. At 4 ppm of ozone dose, all exposure times were achieved 100% kill for all tested fungal species. All isolated non xerophytes were most sensitive to 3 ppm of ozone after 150 min exposure time, while extending the exposure time up to 210 min was required to stop the growth of the three tested resistant xerophytes. The obtained data was in accordance with Antony-Babu and Ian Singleton, (2011) who stated that the effects of ozone exposure on the xerophilic fungus was recorded and *Eurotium* isolate was found to be far more resistant than the strains of *Aspergillus* and *Botrytis*). Member species of the genus *Eurotium* are known to be highly resistant to heat (Yildiz and Çoksöyler, 2002). Labjar et al., (2010) stated that the corrosion inhibition efficiency increases with increasing of the inhibitor concentration. The differential activity of ozone against the test fungi might be due to the variation in their organic matter content which may accelerate or reduce the toxicity of ozone (Ali, 2006). Photolysis of ozone to oxygen atoms leads to the generation of the hydroxyl radical (OH), a key reactive species during the decomposition process (Jans and Hiogne 1998). The inhibition of fungal cell due to oxidizing action of ozone was achieved by Liew and Prange, (1994). The human safety of ozone was reported by Sechi *et al*. (2001). Ozone gas is known to possess sporicidal activity and thereby at higher concentrations could be used to reduce the initial load of contaminants and to disinfect storage and processing areas (Al-Ahmadi et al., 2009).

**Conclusion and Recommendations**

Both of El Anfoushi and Al Shatby archeological tombs are suffering from years of annual flooding resulting in biodeterioration which is getting worse each year. Our main recommendations are to save these tombs from a complete destruction, so a stationed pump is necessary to continuously clear the area from the rising ground water. Use

suitable grouting for binding the cracks, high durability reconstruction rocks, and suitable cleaning methods to remove salts from the monuments. Biodeterioration factor have a great bad effects on these tombs and it form a risk to occasional visitors and people working in the catacombs. Ozone fumigation (3 ppm after 210 min) can be used as a potential method to aid in the reduction of deterioration of limestone caused by xerophilic and non-xerophilic moulds.

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