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INVESTIGATION THE MICROBIAL DETERIORATION OF SANDSTONE FROM THE OSIRION'S SARCOPHAGUS CHAMBER AS AFFECTED BY RISING GROUND WATER LEVEL

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ABSTRACT

In the present study, the microbial deterioration of sandstone from the Osirion's Sarcophagus Chamber as affected by rising ground water level was investigated by means of SEM micrograph of fungal hyphae, X-ray diffraction (XRD), and Energy-dispersive X-ray spectroscopy (EDX). The following Fungi; *Cladosporium cladosporioides*, *Aspergillus terreus*, *Curvularia lunata*, and *Acremonium falciforme* were identified on the deteriorated stone surfaces and these findings were tested by SEM investigations. The hyphae penetration of the identified microfungi caused mechanical exfoliation of building stone material as well as changing in color.

KEYWORDS: Biodegradation; Osirion's Sarcophagus; Fungi; Sandstone.

1. INTRODUCTION

Deterioration of historic buildings including chemical, physical factors, and microbial growth has been reported in many works to understanding the deterioration agents (Praderio et al., 1993; Kumar and Kumar, 1999; Nuhoglu et al., 2006; Suihko et al., 2007). The colonization of stones by microbes depends on environmental factors such as water availability, pH, climatic exposure, and nutrient sources, and they can cause various damages on the stone surface, such as: formation of biofilm, chemical reactions with substrate, physical penetration into the substrate as well as pigments production (Pochon and Jaton, 1968; Bock and Sand, 1993; May et al., 1993).

A considerable number of investigations have been examined the essential role of biological agents in the deterioration of stone (Saiz-Jimenez 1997; Tomaselli et al., 2000; Warscheid and Braams, 2000; Zanardini et al., 2000; Herrera et al., 2004). For example, whole surface of monuments stone under air pollution and continental-cold climatic conditions in Erzurum, Turkey, was covered with a biofilm caused by the microorganisms (Nuhoglu et al., 2006). Discoloration and degradation of different types of stone in cultural heritage objects could be caused by the fungal ability in production of pigments and organic acids (Gupta and Sharma, 2012). Additionally, the major biodeteriogens of the historic monuments and artworks are summarized in the review article of Dakal and Cameotra (2012).

Fungal Strains of the genera of *Penicillium* and Fusarium which excreted oxalic, fumaric and succinic acids with corrosive effects on the stony materials were observed in the decayed sandstone from the church of Carrascosa del Campo (Spain) (Gómez-Alarcón et al. 1995a). From both granite and sandstone substrata, the following fungi were found; Alternaria sp. 1,2, *Cladosporium cladosporioides*, *C. sphaerospermum*, *Epicoccum purpurascens*, *Fusarium* sp., *Mycelia sterilia* (melanised), and *M. sterilia* (non-melanised) (Ljaljević-Grbić and Vukojević, 2009). Also, the formation of extracellular melanin-polysaccharide stable complexes was the main role of *Ulocladium atrum* in biodeterioration of stone (Gutiérrez et al. 1995). It was observed that *Aspergillus Scalrotium* was found as dominant in the deteriorated stones of Mahadev temple, Bastar, Chhatisgarh (Gupta and Sharma, 2012). The most representative genera found in the deteriorated stonework of the fountain of Bibatauin in Granada, Spain, were Cosmarium, Phormidium and Symploca, and the main mineral was calcite (Zurita et. al. (2005).

SEM-EDX, FT-IR and XRD have been used by almost all investigators in analytical techniques for biodeterioration studies and for all types of substrate of construction materials (Gómez-Alarcón et al. 1995a). Silicon, aluminum, calcium, potassium, titanium, magnesium, zinc, sulfur, iron, sodium, and niobium were found in the stones of the historical buildings with varying amounts through the SEM-EDS analysis (Nuhoglu et al., 2006).

The aim of this work was to evaluate the potential damage caused by fungal community on biodeteriorated stone samples from the Osirion's Sarcophagus Chamber as affected by rising ground water level by means of SEM micrograph of fungal hyphae, X-ray diffraction (XRD), and Energy-dispersive X-ray spectroscopy (EDX).

2. MATERIALS AND METHODS

2.1 Site Description

The sarcophagus chamber in the shape a huge sarcophagus or the sarcophagus of Osiris-Seti (Figure 1) is a part of the Osireion (named after and for the god Osiris), the Osireion is unfinished unique subterranean water-based nature (to represent the primeval mound of creation surrounded with the primeval waters or the waters of Nun) structure, excavated in Thebes/Esna shale formations, directly to the west and on the same axis of Seti I temple at Abydos, the sarcophagus chamber is a rectangular room (27.20 ×4.75 ×7.55 m high), located to the east of the great central hall, it corresponds and parallels to a transverse room or hall (Entrance Hall), it was built from local limestone (from Thebes and transitional Drunka formations) with Nubian sandstone saddle roof of Nubian sandstone retains on its surface a fascinating religious, funerary and astronomical inscriptions and scenes, especially on the north side where the sky goddess Nut stretched out and bended over the world while the sun is going in the heart of the sky (El-Derby, 2005).

The sarcophagus chamber has been suffering from rising ground water level which reaches about 1.15 m. above its ground level, and the lack of the ventilation openings has led to a high relative humidity to more than 90% inside it (in its microclimate), resulting in presence, growth and activity of microorganisms on the surfaces and inside the walls of the chamber. The primary source of ground water was formerly the Nile River, where a pipe was designed to provide with water from a Nile branch, which was up to the area.

The ground water source at Osirion is the high ground water level, which rose as a result of several reasons: (a) the irrigation methods in the region since has changed 1942, (b) the construction of the High Dam and the leakage of water from Lake Nasser to (c) Nubian reservoir and from there to the other subtanks, including the reservoirs located below the Osirion area, (d) the topography of the region, where the flow of groundwater from west to east changing the course from the south to the north east - after colliding Eocene limestone tongue which mediates the two bays caught west both of Nag Hammadi and Abydos and in the west of Abydos about 1 km which acts as a hydrological water barrier groundwater, inasmuch as the limestone plateau is in port, rebounding eastward and passes down Osirion leading to high water table constantly, and (e) the water flow from west to east often be the free water (a volatile water) attributed resulting from sewage, agricultural drainage water, drinking water and leaking from the soles of the irrigation and drainage channels, which linked directly or indirectly with the Nile River and affected by it and they are one of the important sources of raising the ground water level. The Influential primary reservoir of the region is the free reservoir; it is wobbling and growing steadily tank.

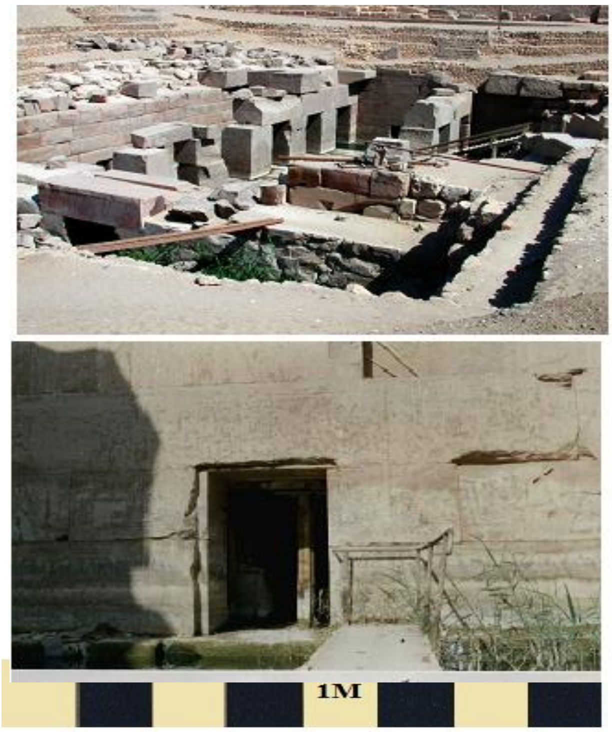

Figure 1. General view of Osirion's Sarcophagus Chamber.

Figure 2. Osirion sandstone sampling areas (A), (B), (C) and (D).

2.2 *Sampling Site*

Biological samples of Osirion sandstone (Figure 2) were collected from different spots of the temple surface that showing decay signs such as color change and typical microbial growths. Samples were taken for mycological analyses by swabbing surfaces with sterile cotton swabs. The samples were then stored at 4°C.

2.3 X-ray Diffraction Analysis of Stone

The chemical composition of Osirion stones was analyzed by X-ray diffraction (XRD) using Philips–Eindhoven Compact X-ray Diffractometer PW 1840. The identification of chemical compositions was carried out automatically using mineralogical data base.

2.4 Microscopic examination

Microscopic examination was performed using Leica Zoom 2000 microscope equipped with an Olympus e-410 digital camera. Thin sections, cut across the thickness of the stone, were used to determine the different typologies of sandstone.

2.5 Isolated microorganisms from the stone Temple

Microorganisms' isolation was performed directly in the laboratory after swabbing. The fungi were isolated by rubbing the swabs gently on different culture media. The media comprised 1-M40Y; 400g sucrose, 20g Molt extract, 5g Yeast extract, 20g Agar; 2- potato-dextrose agar (PDA); 200g Potato extract, 20g Agar and 20 g dextrose. Inoculated petri dishes were incubated at 25±2°C for fungi. Resulted cultures were purified using the hyphal tip and/or a single spore technique as described by Hildebrand (1938) and Smith and Onions (1994). Macroscopic and microscopic characteristics of the obtained isolates, as well as color, size and morphology of the vegetative and reproductive structures were examined. Fungal isolates that speculated were identified using the taxonomic keys of Ellis and Ellis (1976), and Samson *et al.*, (2010).

2.6 Colonization Test

The stone samples were cut into cubes (10 x 20 x 40 mm) using a low-speed diamond saw. The cubes' surfaces were left unpolished in order to increase surface roughness and thus facilitate microbial colonization (Guillitte, 1995). Cubes were sterilized using UV light exposure for 48 hours, after autoclaving at 121°C for 6 hours and drying in an oven at 115°C for 24 hours (Miller *et al*. 2009). For the preparation of spore suspensions, 10 ml of sterilized distilled water was added to culture plates containing PDA (7-days old), and then spores were freed by the aid of a camel brush. Afterwards spore suspensions were individually through muslin and standardized to contain 1.2×10^6 spores per ml using a haemocytometer slide. Stone cubes were deliberately inoculated by fungal and bacterial species to study the stone blocks before and after infection. The colonization was evaluated after three months' period. Degradation occurred were determined on the surface and inside the pores.

SEM model-a FEI Quanta 200 SEM FEG was used to study colonization of the stone surface by microorganisms. ESEM attached with electron dispersive X-ray spectroscopy (EDX) was used for the elemental analysis characterization of both the inoculated and non-inoculated stone sample (Danilatos and Robinson, 1979). EDX analysis was used to study changes in surface morphology and particle size of the deteriorated samples.

3. RESULTS AND DISCUSSION

3.1 X-ray diffraction analysis of stone

Result of X-ray diffraction analysis is shown in figure 3. The interpretation of the diffraction patterns indicated that the stone temple is mainly composed of silica. Also, the carbonate was found in form of Cement (Calcareous Sandstone or as biogenic carbonate precipitated as a result of dissolution of Carbonate of the adjacent walls).

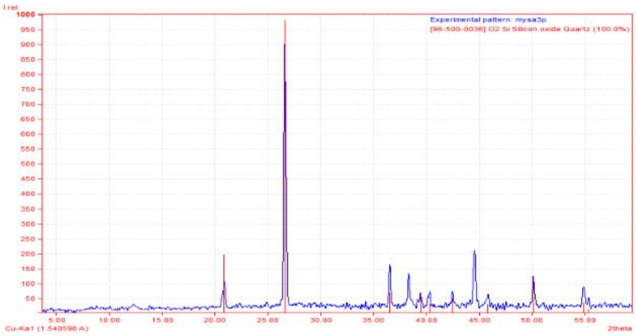

Figure 3. X-ray diffraction pattern of the sandstone

3.2 Microscopic Examination of Sandstone

Photomicrograph of sandstone thin section of quartzite (silicified sandstone) presented in figure 4, revealed the characteristic silica grains of sandstone. Due to a basalt-intrusion the interstitial space of the Oligocene sand grains is filled up with small quartz crystals precipitated from induced hydrothermal fluids.

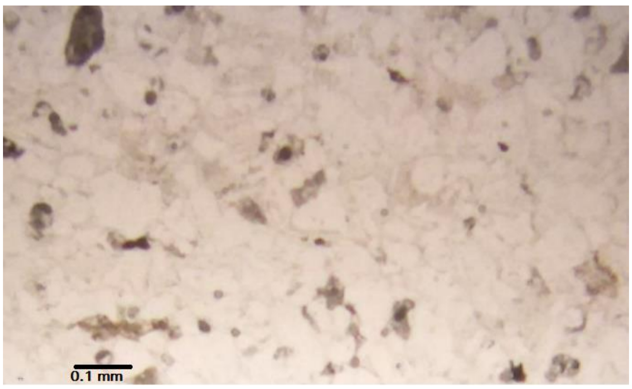

Figure 4. Photomicrograph of sandstone thin section from the site of sarcophagus of Osirion.

According to SEM examination, the original sample in Fig. 5 (SEM micrograph X 300) of Nubian Sandstone of Sarcophagus Chamber of Osirion showing existence of kaolinite either as a binding material or as a result of alteration of K-feldspar to clay minerals (kaolinite) (Kaolintization), micro-exfoliation of K-feldspar, Sodium Chloride (halite) salt, acicular radical crystals of gypsum salts. It has been apparent that fungi comprise a significant component of micro biota in a wide range of rocks including sandstone, granite, limestone, marble and gypsum (Warscheid and Braams, 2000; Burford et al., 2003a).

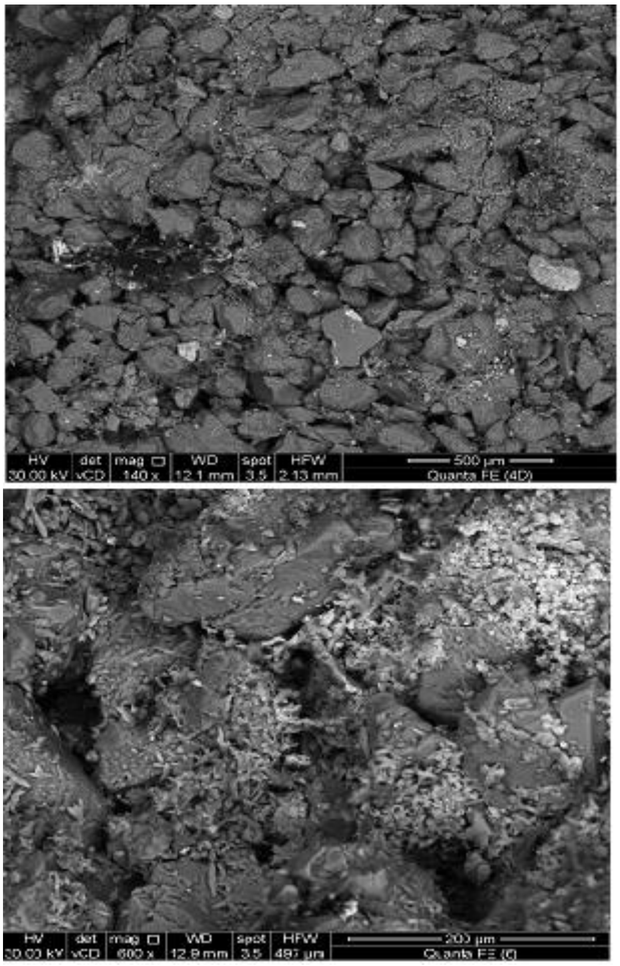

Figure 5. SEM micrograph X 1200 of Nubian Sandstone of Sarcophagus Chamber of Osirion.

Electron dispersive X-ray (Fig. 6) for check point at the same sample of weathered Nubian Sandstone surface presented high Si, O and Ca and other elemental components (Table 1) such as Cl, Fe, Al, Na, C, N, Mg and S indicating to existence salts of halite and gypsum, alteration to clay minerals and the voids between granules as a result of the loss of the binding materials (granular disintegration). Some of these elements could be used as an energy resource for the microorganisms (Nuhoglu et al., 2006). Fungi concentrated in stone crusts are able to penetrate into the rock material by hyphal growth and biocorrosive activity, due to excretion of organic acids or by oxidation of mineral-forming cations, preferably Fe and Mg (Warscheid and Braams, 2000; Ljaljević-Grbić and Vukojević, 2009). It was also reported that the large pore sandstones promote microbial contamination only temporarily, whereas, small pore stones offer more suitable conditions for bioreceptivity (Warscheid and Braams, 2000).

In the present study, the C reached 16.81 %wt and the presence of significant amounts of carbonate compounds (e.g. > 3% w/v $CaCO_3$) in calcareous sandstones results in the buffering of biogenic metabolic products, also the organic adhesives, which are present in ancient brick and mortar, increase the susceptibility of the mineral substrate to microbial attack (Warscheid and Braams, 2000). It was reported that fungi can transform and weather the mineral surface of the stone by precipitating calcium carbonate into calcium oxalate by the actions of secreted oxalic acid using the process of secondary mineral formation (Burford et al., 2003b; Adeyemi AO, Gadd, 2005).

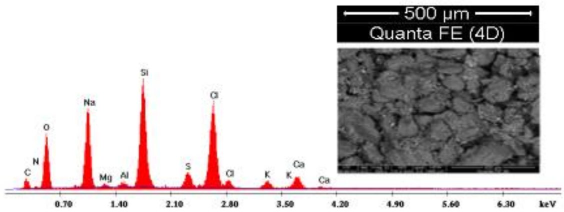

Figure 6. Electron dispersive X-ray for Elemental analysis of weathered Nubian Sandstone surface.

Table 1. Elemental composition of deteriorated sandstone

Element (Wt %)										
C	N	O	Na	Mg	Al	Si	S	Cl	K	Ca
16.81	3.62	30.17	16.95	0.68	0.80	14.33	2.18	11.42	1.16	1.89

3.3 Isolation of microorganisms from the stone temple

Isolation trials of microorganisms associated with the sandstone temple resulted in different fungal including *Cladosporium cladosporioides*, *Aspergillus terreus*, *Curvularia lunata* and *Acremonium falciforme*.

The colonization of microorganism on sandstone cubes after three month showed a growth in the cubes' surfaces. It is possible using Scanning microscopes, to identify the microorganism species through the distinctive size and shape of their spores and hyphae for fungi. SEM micrograph (Fig. 7) of Nubian Sandstone of Sarcophagus Chamber of Osirion showed gypsum crusts, fractures and microfractures in quartz grains and voids between granules as a result of the loss of the binding materials (granular disintegration).

Cladosporium cladosporioides (Fig. 8) showed small sized simple or branched loose chained spores with lemon-shape and the conidiophore forming many aerial hyphae. Gypsum crusts were recently-formed by *C. cladosporioides*, where the calcite has been converted to gypsum (from insoluble stage to soluble stage in water).

The presence of fungi on the sandstone surface of the Osirion's Sarcophagus Chamber monuments is often associated with the process of biodeterioration. Their growth on stone surface could be altered it by the excreting inorganic and organic acids as a result of their own metabolism which generates organic acids (oxalic acid and citric acid) that have chelating properties by weaken the metaloxygen bond, and increases the solubility of some metals and forms complexes with the mineral cations present on the surface matrix (Gómez-Alarcón et al., 1995a,b; Banfield et al., 1999; Harley and Gilkes, 2000). Also, the intrusion of fungal hyphae along the crystal plane by some fungi is known to destabilize the stone texture resulting in its mechanical deterioration (Gadd, 2005).

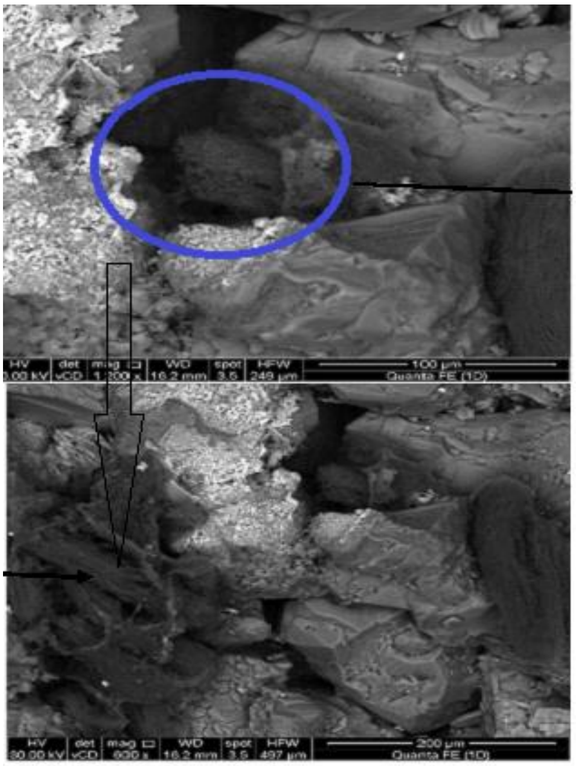

Figure 7. SEM micrograph of Nubian Sandstone of Sarcophagus Chamber of Osirion.

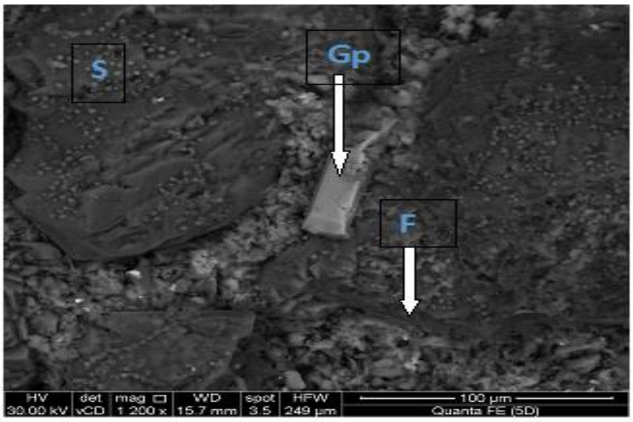

Figure 8. SEM micrograph of fungal hyphae attached to growth into sandstone after three months by Cladosporium cladosporioides (F), gypsum (Gp), and spore for C. cladosporioides (S)

SEM micrograph (Fig. 9) shows the biogenic alteration and formation of vugs filled with crystalline salts and the highly altered kaolinitic Nubian Sandstone and development of interstitial salt encrustation, vesicular, brittle sandstone and *Aspergillus terreus*.

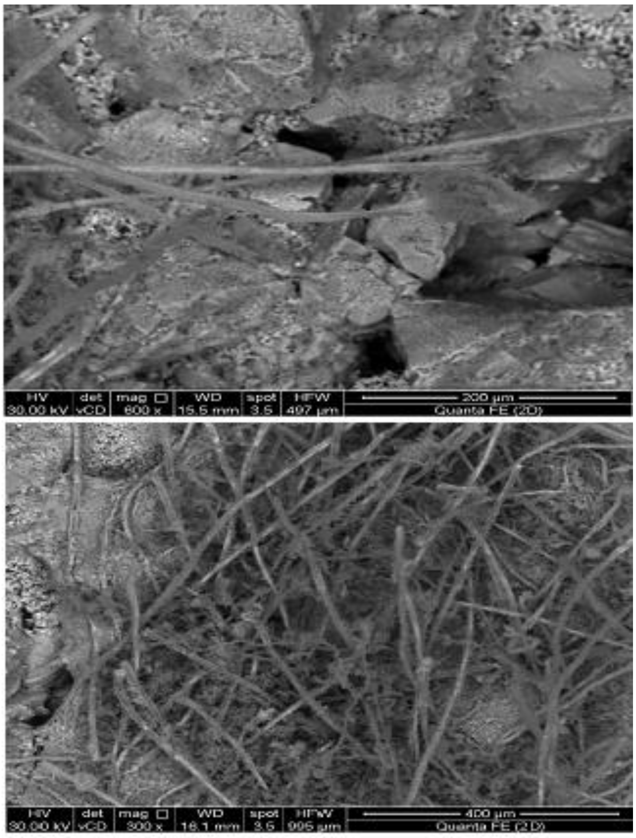

Figure 9. SEM micrograph of fungal hyphae attached to growth into sandstone after three months by Aspergillus terreus.

SEM micrograph (figure 10) shows the biogenic mamilated (nipples) texture of salts, brittle sandstone and *Curvularia lunata* matured colonies appeared dark brown to black. Conidiophores arising from surface or aerial hyphae, septate, straight or flexuous, with apices straight, curved or conidia that are relatively short 3-septate, curved and smooth branching patterns of aerial hyphae intermingled with spore-forming structures aerial hyphae, conidiophores and spores.

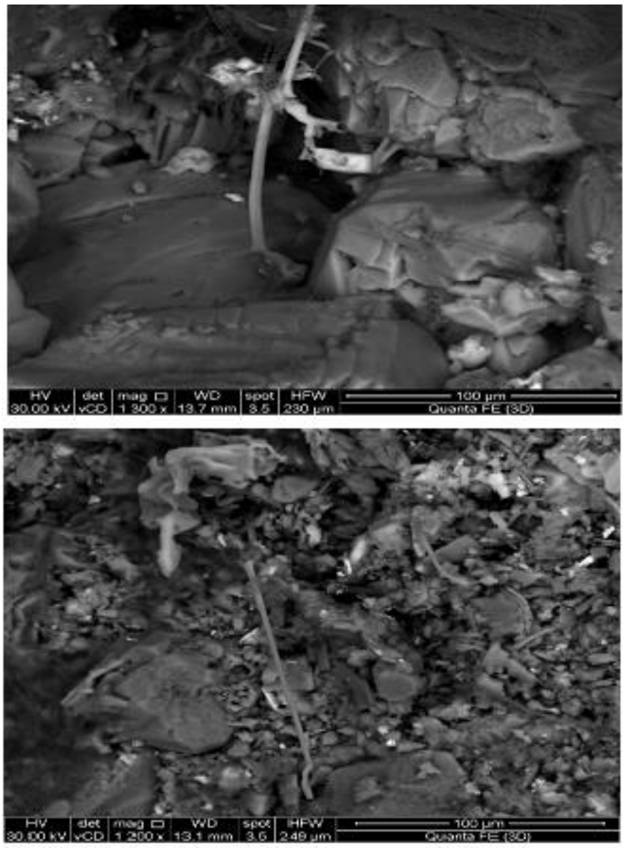

Figure 10. SEM micrograph of fungal hyphae attached to growth into sandstone after three months by Curvularia lunata.

SEM micrograph (Fig.11) shows the kaolinite structure tiny microbial accumulation Corroded Kaolin minerals as six sided crystals in right upper corner *Acremonium falciforme*. *A. falciforme* showed thin hyaline septate hyphae with phialides unbranched, solitary, formed on the hyphae, with the fusiform conidia.

Soil fungi, as well as air and plant-derived ora, are the first inhabitants on the dry inorganic or mineral surface in the in stone monuments which produce fast-spreading hyphal structures and can regenerate their presence on the stone by spore production (Gorbushina and Vlasov, 1998; Gorbushina and Krumbein, 2000). *C. cladosporioides* was previously found in the deteriorated sandstone surface biofilm of the Monument of the "Unknown Hero" (Avala Mountain near Belgrade) and "Brankov most" (Sava river, Belgrade) (Ljaljević-Grbić and Vukojević, 2009).

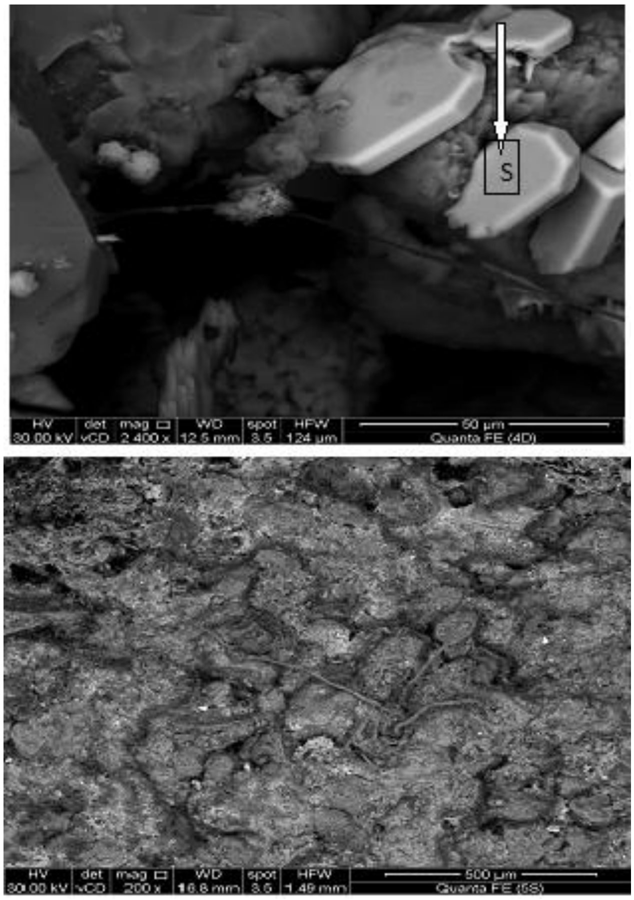

Figure 11. SEM micrograph of fungal hyphae attached to growth into sandstone after three months by Acremonium falciforme, Salt (S).

It was observed previously, the size of vegetative and generative organs (forms) of the microorganisms observed by the SEM images on stone surfaces are smaller than the cultural medium, soil and plant surfaces because the stone is a poorer enrichment media than the cultural medium for the microorganisms (Dornieden et al., 2000; van der Gast et al., 2001).

The elimination of sources appropriate for the growth of fungus environmental problem, control measures and reducing the groundwater tables of ground water in the Osirion and its surroundings, as follows:

1- Eradication, of agricultural, housing, urban abuses, encroachment and man-made destruction.

Encouragement and motivation Archaeological and cultural Awareness, whereas Osirion is an unique construction all over the ancient Egyptian civilization, this cultural value has not been corresponded and retaliated with care and conservation starting from arresting and staying the causes of failure, then the abstract and moral of the cultural value of Osirion will impel and inspire to removing neglecting before removing causes and symptoms of deterioration and inclusion in management site.

2- Lowering the high ground water tables with the following procedures:

(i) The regional insulation of presuppose context of the site (see: (in Arabic; El-Derby, 2005).

(ii) The local insulation of the structure and their close context.

(iii) Converting the unlined and uncovered channels and drains -to the covered subsurface drain system.

(iv) Converting the soaking perennial irrigation system to spray and dropping system.

3- Prevention procedures, such as continuous cleaning, compensation, repairing of cracks, joints, crevices and cavities.

4- Controlling the relative humidity (more than 90%) in the Chamber- which lacks windows and ventilation - with ventilation techniques and indoor air suction, and with using dehumidifier materials.

5- Installation an artificial lighting inside the Chamber, which should be appropriate in terms of kind of radiation, and in terms of aesthetics, so as to oust the bat and limit the appropriate environment for the growth of fungus.

6- Fungi control measures: it is suggested the chemical methods (herbicides) such as Glyphosate (commercial nom is Round Up), it is derived from Glycine acid and Fuluazifop-p-buty commercial nom is Fusilade) (El-Derby, 2005, in Arabic).

7- Consolidating the walls and ceiling with consolidate and water repellent, then fixing the surface scales plaster layers, which were expelled by hyphae.

Also conservation treatment was applied using UV light on sand-stone, where the UV light acts as sterilization against the growth of microorganisms.

4. CONCLUSIONS

Our results indicate that the microbial deterioration of sandstone from the Osirion's Sarcophagus Chamber as affected by rising ground water level was observed by the following fungi; *Cladosporium cladosporioides*, *Aspergillus terreus*, *Curvularia lunata*, and *Acremonium falcifrome*. The results included enrichment by organic substances, formation of crusts, and chemical weathering, promote the colonization of natural sandstone by fungi and thus enhances biodeterioration processes.

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