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Microbial kaolinization of granite stones within the tomb of the King Oserkon II, Tanis, Lower Egypt

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ABSTRACT

Ochre granite stones in the tomb of the king Oserkon II at Tanis, Lower Egypt, were subjected to continuous kaolinization by microbiota composed of *Pseudomonas aeruginosa*, *Streptomyces* spp., and *Yarrowia lipolytica* (Candida). These microorganisms involved significantly in kaolinization of granite stones into clay minerals that their rosy color turned into grey color due to the acidolytic activity of attached microorganisms and clay mineralization. Grey film on the kaolinized granite stones contained a higher calcium ions and lower ferric ions. On the other hand, quartz appeared in form of separated crystals that caused roughing of granite surfaces in the investigated tomb.

Keywords: *Candida lipolytica*; Lower Egypt; Kaolinization; The King Oserkon II; quartz.

INTRODUCTION

Tanis (currently San-el-Hagar), East of Delta, Egypt, was the capital of the 14th nome of Lower Egypt after 20th Dynasty and it was mentioned as  in ancient Egyptian texts (Porter and Boss, 1968).

Furthermore, Tanis was the residence and burial place of kings of the 21st (1070-945 B.C.E.) and the 22nd (945-712 B.C.E.) dynasties, where a group of royal tombs (tombs of Oserkon II, Takelot II, Psusennes I, Psusennes II, Amenemope and Shoshenq II) still exists (Baines and Malek, 1980). The tomb of the king Oserkon II, one of the most important tombs in Tanis, was built of both red granite and limestone, where the lower layers were built by red granite blocks but the upper courses were built by limestone blocks. That may be attributed to the matter of fact that tensile strength of granite equals the fourth of its compressive strength (Sakr, 2005). This building technique was used in an ancient Egypt to resist the soil humidity in Delta because the granite has low water absorption, which ranged from 0.002 to 0.02% per volume (Smith, 1970; Sakr, 2005).

The royal tombs at Tanis suffer from increasing levels of subsurface water due to their neighborhood to El-Manzala Lake and the trenches near the tombs area were filled with water from the underground leakage (Zeid, 2005). Higher levels of relative humidity within those tombs enhanced the microbial growth and colonization where microorganisms eg. chemoorganotrophs, chemolithotrophs and phototrophs, actinomycetes, and fungi were involved significantly in kaolinization of stones' surface in addition to other deterioration symptoms such as cracks and fissures through the exertion of physical stresses of hyphae (Hendrik, 1994; Dornieden et al., 2000; Sirt, 2008). The microbial kaolinization of granite stones is attributed to the acidolytic activity of microorganisms since the produced acids reduced the pH degree of niches that involves significantly in kaolinization of granite stone via chelating metal ions (Mohammadi and Krumbein, 2008) and oxidizing minerals forming cations preferably iron and manganese (Grbič and Vukojevič, 2009).

Microorganisms are involved in biogeochemical weathering through the action of acids produced as metabolic byproducts. Under suitable conditions, the microorganisms caused kaolinization of granite stones surfaces via forming a microbial layer on their surface known as ‘biofilm. With continuous kaolinization of feldspars on the colonized granite surfaces, minerals were released and quartz crystals were separated; thus, the stones’ surface became rough facilitating the adherence of much more microorganisms (Hyun and Park, 2011). In this regard, Careli et al. (2009) documented that *Pseudomonas fluorescens*, colonizing marble and granite surfaces showed high porosity, cracks, crevices, undulations, and depressions large enough to hold and anchor high numbers of adhered cells.

In particular, microbial communities commonly isolated from deteriorated stones have been demonstrated producing both organic and inorganic acids in laboratory cultures (Cutler and Viles, 2010), which caused kaolinization through dissolving of feldspars in granite blocks transformed gradually the bright polarization colors of the micaceous phases into grey polarization colors of kaolinite (Dudoignon et al., 1988). That is affecting their aesthetic value to be preserved as a part of the cultural heritage, in addition to physical and chemical alterations (Krumbein, 2003). Additionally, the change of granite rose color into grey color was associated with the microorganisms’ abundance (Gaylarde et al., 2007) and the environmental conditions such as carbon source, salinity, and pH of colonized stone surface (Gutarowska, 2010). Finally, microbial kaolinization of rose granite was put onto the evidence; Garcia-Gimenez et al. (2013) ascribed the grey color of granite blocks within the tombs in the area around the Church of San Andrés (Avila) to microbial colonization.

Therefore, the present study was carried out to evaluate the microbial kaolinization of granite stone within the tomb of the king Oserkon II, Tanis, Lower Egypt, by metabolic activity of microorganisms and kaolinization products.

MATERIALS AND METHODS

Sampling location and the state of art

The tomb of the king Oserkon II, Tanis, Sharkia Governorate, East of Delta, Egypt (Figure 1) was selected for the current investigation due to its darkness, increasing levels of relative humidity (~85%), and bad ventilation. Visual inspection showed that granite surfaces of the tomb were abundantly covered with green biological colonization. Twenty granite and microbial samples were collected from green biofilm and peeled stone surfaces within the tomb. In addition, the granite blocks were subjected to different deterioration symptoms such as pitting and scaling on one hand (Figure 2c-2e).

Polarizing microscope investigation

To study the minerological composition of granite by polarizing microscope, thin sections of granite peels were prepared, and embedded in a low viscosity resin according to Mohammadi and Krumbein (2008). With infiltration of resin, vacuum treatment was applied. Finally, the samples were embedded in beamer capsules and polymerized. For the preparation of thin sections, the hardened blocks were cut by using a saw (Leitz model 1600) in sections vertical to the stones surface, washed in ethanol, mounted on ground (600 grit) microscope slides (46-9-27 mm), embedded in Spurr’s resin, and polymerized. The samples were polished with silicon carbide powder in a series of 320, 400, 600 and 1,000 grit. Photomicrographs of the sections were made using a Zeiss Axioscope II, Geology Department, Faculty of Science, Zagazig University, Zagazig, Egypt.

Scanning electron microscope (SEM)-EDX investigations of granite peels

The elemental composition of peels of the deteriorated granite surfaces at tomb of the king Oserkon II were investigated using SEM (QUANTA FEG 250, National Research Centre, Dokky, Giza). Aluminum (Al), calcium (Ca), iron (Fe), sodium (Na), and silicon (Si) were determined according to operating instructions.

Climatic conditions

The relative humidity and temperature were recorded inside the tomb three measurements per day from January to June 2011 using Data Logger (HOBO UX100-003).

Figure 1. Location of Tanis within Egypt where the tomb of Oserkon II is located.

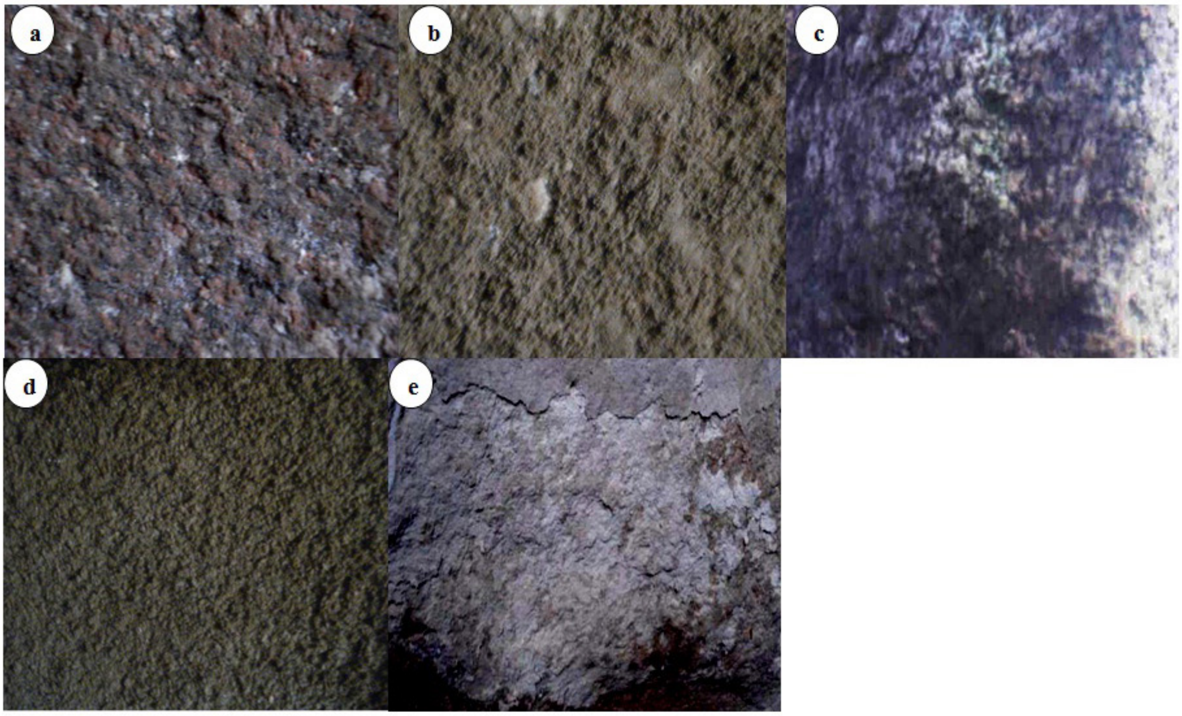

Figure 2. Microbial colonization of granite surfaces with microorganisms (a) roughing surface of granite stones after removal of micro biota, (b-d) soiling of granite stones surface with grey and olivey color and due to forming biofilm, (e) exfoliation of granite surface due to microbial colonization.

Isolation and identification of microorganisms colonizing granite stones

Microbiota colonizing granite stones within the tomb of the King Oserkon II, Tanis, Egypt, were collected using cotton swab method, which were cultured on nutrient agar medium (peptone 5, beef extract 3, NaCl 5, agar 15 g/L) for bacteria, on starch-nitrate-agar medium (SNA) (agar 20, starch 20, KH_2PO_4 1, $MgSO_4$ 0.5, NaCl 0.5, KNO_3 2, and $CaCO_3$ 3 g/L (Kuster and Williams, 1964) for actinomycetes, and on Sabarud agar medium (glucose 40, peptone 10, agar 20 g/L) for yeast. Plates were incubated for 7 days at 30 °C for actinomycetes, 3 days at 28 °C for yeast, and 24 h at 28 °C for bacteria till single colony was appeared. Bacterial isolates were identified according to Williams and Sharpe (1989), and yeast isolates were identified according to Barnett et al. (2000). *Streptomyces* were identified according to identification keys of Kämpfer (2006).

Acidolytic activity of isolated microorganisms

To determine the organic acids produced, Erlenmeyer flasks 250 ml, each one contains 50 ml of nutrient broth for *Pseudomonas aeruginosa*, Sabarud broth for *Candida lipolytica* and SNA broth for *Streptomyces* spp., incubated for 1, 2, 3, 4, and 5 days at 28 °C for both *P. aeruginosa* and *C. lipolytica* and for 1, 2, and 3 weeks at 30 °C for *Streptomyces* spp. The pH values of media were determined using a pH meter, Faculty of Science, Zagazig University, Zagazig, Egypt.

Furthermore, to determine the produced organic acids quantatively, gas chromatography/mass spectrsocopy (GC/MS; JEOL. JMS-AX500 Mass spectra, National Research Centre, Dokky, Giza, Egypt), was used according to Jones et al. (2000). A 1.0 µl of broth medium was introduced to the GC and a heating cycle of 100 °C for 5.0 mins; 60 °C/min to 290 °C for 1.0 min was carried out prior to introduction to Mass spectra. The mass spectra of broth medium were compared to the spectra of standard organic acids of oxalic and citric acids to confirm the presence of these acids in the broth medium.

White crust investigations

To investigate the white crust over the deteriorated granite stones, micro samples were investigate SEM-EDX investigations according to the previous method.

RESULTS AND DISCUSSION

Polarizing microscope of granite samples

Polarizing micrographs of granite samples indicated to the presence of twins crystals of plagioclase (Figure

3a) and phenocrystals of potash feldspars embeded in phenoground of quartz (Figure 3b). These micrographs pointed out the presence of minor elements of amphebol, iron oxide and zircon surrounded by biotite (Figure 3 c,d).

From those micrographs, it could be concluded that granite samples in the investigated tomb were porphyric K-feldspar grains and the fabric of the "rose" granites varies from amorphous to almost gneissic, but always preserving its porphyric character (Klemm and Klemm 2001), the type chemical composition of granite stones mentioned as  in ancient Egyptian texts; that means " Granite stone extracted from Aswan quarries" (Sethe, 1933), transported from Aswan quarries by boats to be used in royal buildings in Egyptian Delta (Ossian, 2001).

Scanning Electron Microscope (SEM)

SEM micrographs of sound granite samples pointed out the mica plans in the form of foils (Figure 4a) and the elemental composition by EDX of the cleaned granite surface (Figure 4b) indicated that quartz was the most presented (Si represent 90% of the elemental composition; Figure 5).

SEM-EDX micrographs of weathered granite peels showed the presence of granitic rock-forming minerals Si (45.82%), Ca (6.99%), Fe (10.95%), but SEM-EDX pattern of grey layer on the granite showed the presence of clay minerals that mainly composed of Ca (14.70%), Al (3.4%), Na (1.79%), and Fe 3.15 % (Table 1). These results are in agreement with Welch and Ullman (1993), Schiavon (2000), and Hyun and Park (2011) who

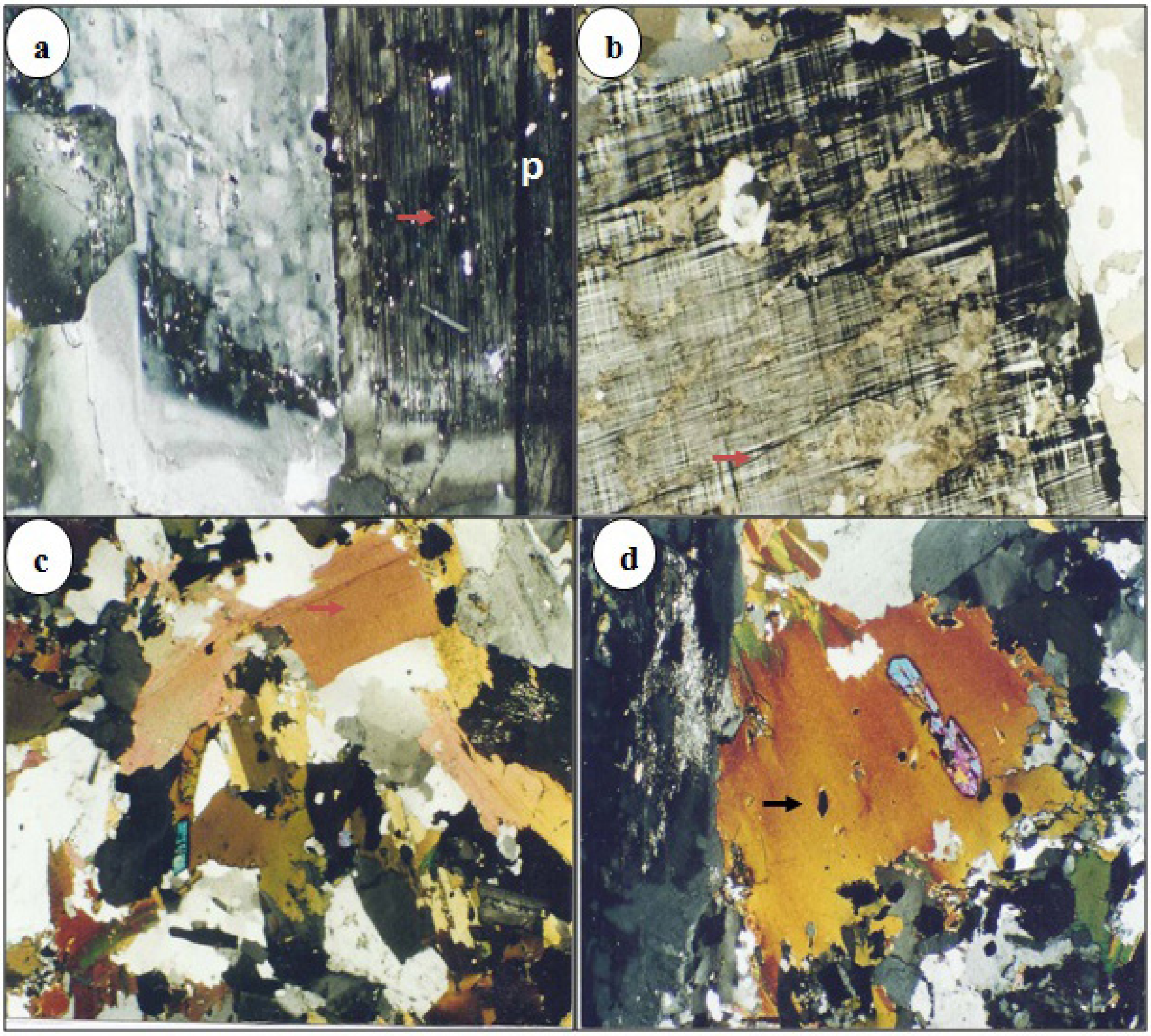

Figure 3. Polarizing micrograph of rose granite from the tomb of the King Oserkon II, (a) twins crystals of plagioclase (P), amphipole and biotite, (b) pheno crystals of potash feldspars embeded in pheno ground of quartz, (c) crystals of feldspar, biotite, amphebol, zircon and iron oxide, (d) crystals of zircon crystals surrounded by biotite.

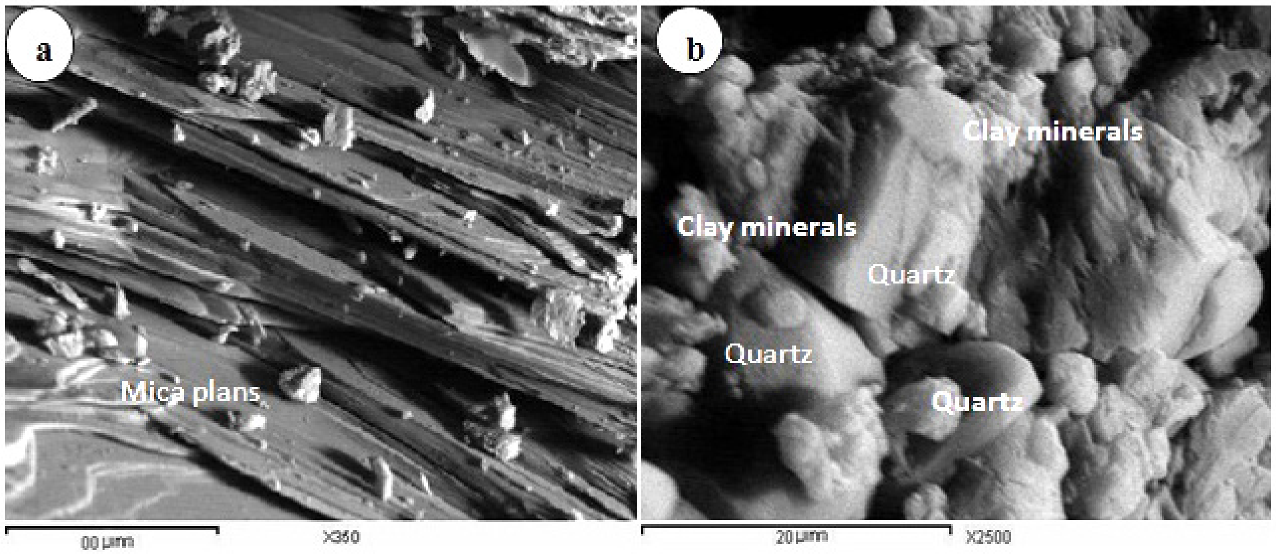

Figure 4. SEM micrograph, (a) Mica plans in the granite surfaces, X 35, (b) Deteriorated granite stones from tomb of Oserkon II, Tanis, which were composed of quartz and clay minerals.

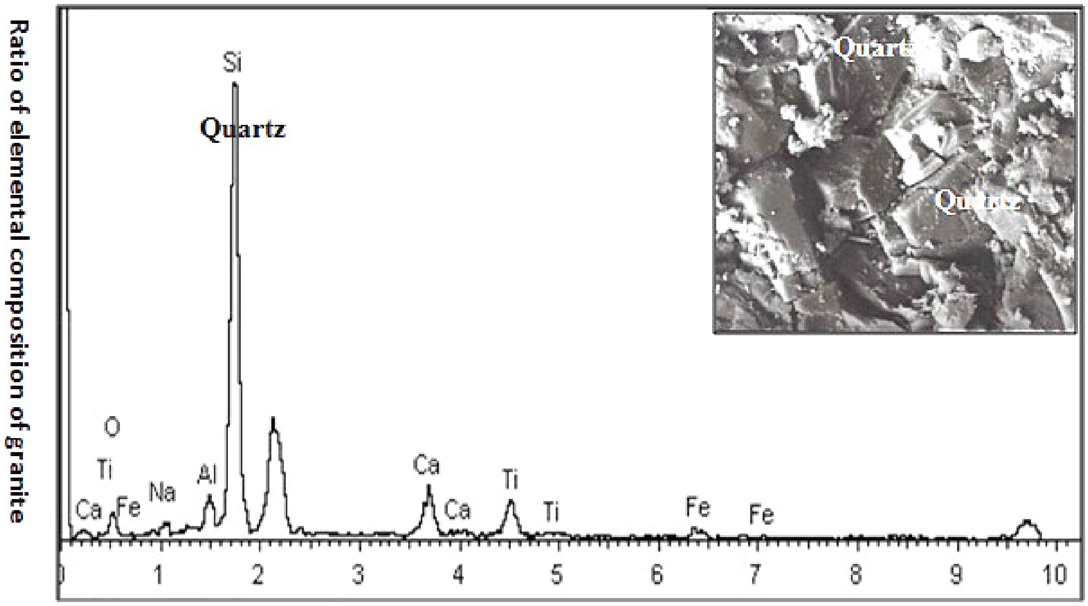

Figure 5. EDX pattern of deteriorated granite peels from tomb of Oserkon II, Tanis, where quartz was the most present.

delineated that rose granite was turned into grey patina due to the high percentage of Na, Ca, and Si which resulted from the decomposing of feldspars by acidic activity; so, the granite surface color was changed from rosy into grey color. The changed color could be considered as an indicator for the deterioration rate of granite blocks and the severity of deterioration factors (Iñigo et al., 2012). On the other hand, the acidolytic activity of microorganisms colonizing granite blocks transformed feldspars into clay minerals and quartz crystals that remain in a separated form causing the granite surfaces to be rough facilitating the successful attachment of microbial cells (Coombes et al., 2011).

Microbiological investigations

The results of biochemical tests pointed out that bacterial isolates were belonging to *P. aeruginosa* (50% of total isolates), actinomycetes (*Streptomyces* spp., 20%), and

Table 1. Elemental composition (%) of both granite stones and clay minerals resulted in microbial kaolinization.

	Fe	Si	Ca	Na	Al
Granite	10.95	45.82	6.99	-	-
Grey layer	3.15	-	14.70	1.79	3.4

yeast isolates were belonging to *C. lipolytica* (25%), and unidentified (5%) as illustrated in Figure 6. The dominance of *P. aeruginosa* is assigned into its preference to the humid niches prevailed within the investigated tomb (Sakr et al., 2012). The high humidity was due to Tanis neighborhood to El-Manzalia Lake and the subsurface water infiltration within thick fluviomarine clay layers characterizing the geology of this region (Zeid, 2005). In similar study, Prieto et al. (1999) reported that biodeterioration of Merza's Bridge (Pontevedra, Spain), Monastery of Toxosoutos (La Coruna, Spain) was enhanced due to its location over a river, which is the main reason for the high humidity. Furthermore, the obtained results revealed that *P. aeruginosa* formed a dense olivey biofilm (5×10^{-6} to $3\text{-}5\times10^{-7}$ CFU/g) on the colonized granite surfaces. This result is in agreement with Cappitelli et al. (2011) who reported that bacteria are generally the first organisms to foul surfaces exposed to different environments, through adhesion and subsequent biofilm formation.

Acidolytic activity of isolated microorganisms

The pH values of broth cultures indicated that acidolytic activity of *P. aeruginosa* was lower after 24 hours of the incubation but culminated after 4 days. Also, *Streptomyces* spp. had lower acidic activity after one week and a higher one was observed after 3 weeks of incubation. The pH values ranged from 6.0 to 6.2.

Data obtained from the GC/MS analysis delineated that all microbial isolates have acidolytic activity and the oxalic acid was the most present (unpublished data). This result is in agreement with Caneva et al. (1991) and Hutchens et al. (2010) who reported that microorganism involved in deterioration of granite stones were acid-producers under laboratory culture, and those acids played an important role in kaolinization of feldspars in colonized granite even with diluted acids. On the other hand, SEM-EDX pattern of grey layer (Figure 6) resulted in microbial colonization pointing out that ratio of Fe ions in the uppermost layer in deteriorated samples down to 3.14 % compared with the sound samples (10.95%). This result was confirmed by Winkler (1975) who reported that granite samples lost Fe with 29% and Mg with 60% due to the acidolytic activity of Aspergillus niger, because Fe is one of the most important micronutrients for the growth and colonization of microorganisms (Sequeda-Castaneda et al., 2013).

This phenomenon may be attributed to the matter of fact that Fe ions are involved in the enzymatic functions of microorganisms because Fe is the key component of cytochromes and electron carrying proteins that play a major role in the cellular respiration (Abdel-Haliem et al., 2013). In supporting these results, Frey et al. (2010) observed elevated concentrations of K, Ca, Fe, Na, and Mg in the culture solutions of *P. aeruginosa* after incubation

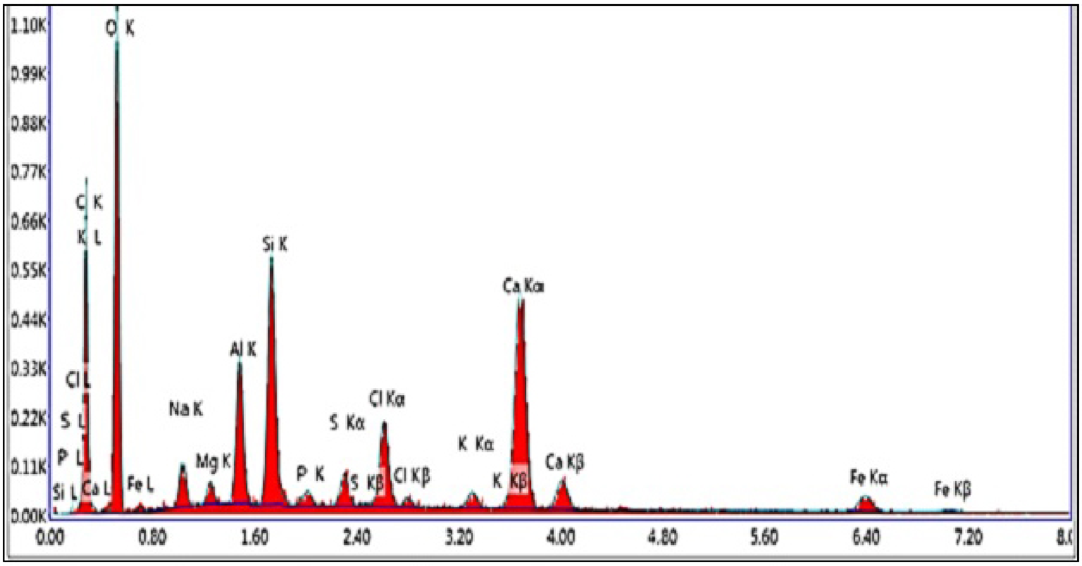

Figure 6. EDX pattern of deteriorated granite peels from tomb of Oserkon II, Tanis, illustrate composition of clay minerals that contained high ratio of Ca ions and low ratio of Fe ions.

in the presence of Rapakivi granite. Also, Abdulla (2009) reported that *Streptomyces* involved in weathering granite rocks from the Saint Katherine region and all isolates were highly acid producers caused leaching Fe ions and reprecipitating them in form of brown haloes under laboratory conditions.

Visually, the examined granite surfaces showed that colonization of microorganisms formed a white crust (Figure 7a), and SEM micrograph and EDX pattern of this white crust showed the dominance of Ca, contributed by SEM micrograph to calcite crystals with euhedral form (Figure 7b-c). This result is supported by Wierzchos and Ascaso (1998) who found a 4-fold relative increase in Ca concentration in the composition of grey colonized layer due to the microbial dissolving of plagioclase; the source of the Ca cations in clay minerals (Lamas et al., 1995). On the other hand, higher Ca percentage in the composition of clay minerals resulted in microbial kaolinization indicates to the kaolinization forming in acidic niches. This result is supported by Mannju et al. (2001) who reported that clay minerals resulted in kaolinization of granite contained a high Ca percentage that may indicates to the acidic activity of colonizing microorganisms (O'Brien et al., 1995).

Climatic conditions

With regard to climatic conditions in the investigated tomb, data logger records indicated that air temperature did not vary during the day, presenting a mean value of 15 ºC, and relative humidity (RH) was near 85%; that ratio promotes the growth and colonization of microorganisms. It was well established that phototrophic microorganisms are able to withstand the low photon flux densities

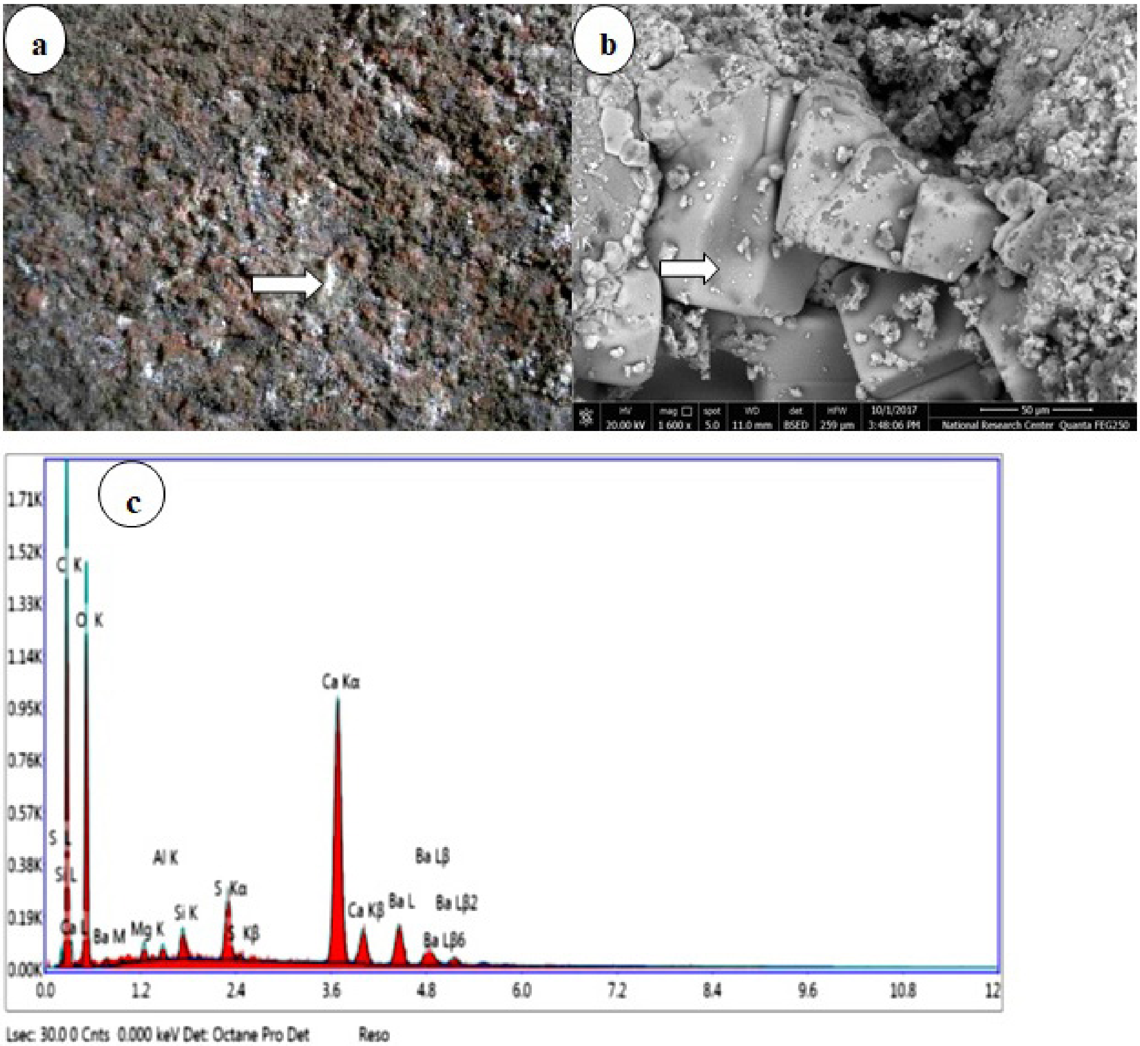

Figure 7. (a) Dissolution of Ca from granite surfaces and subsequent precipitation forms a white crust, (b) SEM micrograph of calcite crystals with euhedral from (c) EDX pattern of white crust pointed out the presence of a high Ca content.

available in the environment with dim light like caves, necropolis and catacombs.

CONCLUSION

It could be concluded from the results of the present study that the isolated *P. aeruginosa*, *Streptomyces* spp., and *C. lipolytica* were the most abundant microorganisms involved in kaolinization of granite blocks in the tomb of the king Oserkon II, Tanis, Egypt. All microbial isolates were acid producers and oxalic acid was the most present. Additionally, clay minerals were the most presented deterioration products turning the colonized stone surfaces to be rough.

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