INTERNATIONAL JOURNAL OF CONSERVATION SCIENCE

ISSN: 2067-533X Volume 7, Issue 2, April-June 2016: 381-394 www.ijcs.uaic.ro

MICROSCOPIC INVESTIGATION FOR CONDITION ASSESSMENT OF ARCHAEOLOGICAL BONES FROM DIFFERENT SITES IN EGYPT

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Abstract

Bones are found in different archaeological sites in Egypt. The bones samples examined in this study were collected from eight archaeological sites and two museum store houses. The samples were collected from different environments (dry and moist). Collected samples suffer from adverse deterioration, which was mainly due to burial environments. Many aspects of deterioration are found on the surface of the bones such as darkness, stains which may be derived from different sources, pitting etc. This study focuses on the changes that occur on the surface of bones in burial environments and in museum storehouses. Digital, optical, polarized and scanning electron microscopes were used to evaluate studied samples. (The results revealed that most environmental conditions in most locations let to many aspects of deterioration) such as pitting, erosion, change of color and etc. Humid environmental are more aggressive on the studied bone samples than dry conditions.

Keywords: *Bones; Archaeological sites; Deterioration; Microscopy.*

Introduction

Bone consists of composite material with a mineral matrix commonly thought of as carbonated calcium hydroxyapatite, precipitated in an organic collagen matrix, protein, lipids, mucopolysaccharides and carbohydrates [1-5]. Its composition varies considerably with age and type of bone. Whole cortical bone is approximately 69% inorganic, 22% organic, and 9% water [6]. Hydroxyapatite $Ca_{10}(P0_4)_6(OH)_2$ compound is considered to be a highly insoluble compound [2, 6 - 9].

After death and burial, changes begin to occur in bones which are the result of several factors [10] such as physical, biological, and cultural processes [11]. These changes in the physical properties are contemporaneous with chemical changes which occur in the organic and inorganic bone constituents [12]. Bone may be altered and may change color as a result of temperature or moisture, soil composition, sediment pH, and the changes may occur in the bone tissue in the form of ionic substitution [13]. In dry conditions, when exposed to a heat source, bones undergo important alterations affecting their color and surface texture [14]. Temperature affects the degradation of bone in several ways. Periodic freezing causes water expansion, resulting in mechanical micro fractures that can lead in turn to split-line cracking and spelling, Fluctuation in temperature also contributes to deterioration by modifying the chemical

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environment of the bone, the reactions establishing a new chemical equilibrium often resulting in degenerative changes [15]. When the bone is exposed to humid conditions, water found in the environment weakens the bonds between the organic and inorganic constituents of the matrix and the bone becomes more susceptible to further deterioration. As the bonds weaken, more water is absorbed and mechanically breaks the bonds, so that the process continues in a progressive manner [15]. Biological alteration in bone is in most cases caused by fungi, bacteria or cyanobacteria. One of the earliest types of alteration is microbial attack. Observed evidence of microbial alteration in bone shows that Haversian canals were (still) filled with bacteria 5 years post-mortem [16]. Micro-organisms will penetrate into the bone through the internal grooves [17]. Both low and high pH adversely affects bone preservation, slightly alkaline environments being the best for bone survival [18]. Acidic soils will dissolve hydroxyapatite, but the threshold of acidity required is not clear; at $pH < 5$, demineralization is promoted. The rate of dissolution will depend on the pH of the soil, the concentration of chelating agents and the degree of water percolation [17].

Bone surface modifications require the utilization of various investigation tools [19]. Different microscopy techniques have been implemented to characterize the structures of bone tissue engineering scaffolds [20], like light microscope which indicated the present evidence of certain thermal alterations and changes in the bone histology [21]. All the thin sections were examined and studied using transmitted and polarizing light microscope [22]. Morphological and micro structural analysis of the test samples was carried out using scanning electron microscope (SEM) [23].

The surface morphologies of the cortical and calcareous bone slices were observed by scanning electron microscopy (SEM). Electron microscopy is a precise tool that can image bio-nanostructures obtaining different formations and colloids [20]. This study aims to:

- Describe aspects of deterioration of naturally aged bones samples collected from different conditions;
- Use different types of microscopes to explain the changes occurred on the surface morphology of the studied bones;
- Explain the deterioration mechanisms of the samples studied;
- Take account of the dry or humid conditions of the burial.

Materials and methods

Archaeological samples

Archaeological samples were collected from the following archaeological sites and museum storehouses (Fig. 1). The description of the archaeological sites of bones is as the follow:

1. Alexandria

Alexandria was the capital of Ptolemaic Egypt, a seaport on the Mediterranean coast founded by Alexander the Great in 331 BC [24]. Historic records refer to Rhakotis or Râ-Kedet as a settlement established before the fourth century B.C, on Egypt's Mediterranean coast before Alexander the Great founded the famous Mediterranean port city of Alexandria [25, 26]. Archaeological bones (humid samples) are a part of the base of the skull and a part of the temporal bone of the skull. The samples were collected from excavations in 2012 in Sporting Square - Sedi Gaber area. This area was humid, and this may be due to its proximity tothe Mediterranean area.

2.Tanta

Archaeological bones (dry samples) were collected from museum storehouses of Tanta museum.

3.Quesna

Archaeological bones (dry samples) were collected from excavations in (Kafor Elramel) - Quesna city in 1991. The samples were taken from three deterioration skeletons. The samples dated back to the late period and the Greek-Roman period.

Fig. 1. Egypt map shows archaeological sites of bones studied.

4. Ain Shams (El Matariya) (Heliopolis)

One of the three major cities of ancient Egypt, along with Memphis and Thebes, located north-east of present day Cairo (30º05' N, 31º20' E). Today, the site is largely covered by the suburban Cairo settlements of El-Matariya and Tell Hisn. The Greek name was Heliopolis ("city of the sun") [27]. Archaeological bones (humid samples) are a part of the sternum bone (manubrium plus body) and part of the Radius. The samples were collected from excavations in 2012. The source of water in this site may be due to the underground water.

5.Giza (storehouse of Ahmed Fakhry)

The largest and most comprehensive Old Kingdom site found to date is at Giza [28], a pyramid plateau group (30º N, 31º20' E) [29] Archaeological soils were consistently found in borings between a depth of three and six meters below the modern ground surface [28]. Archaeological bones (dry samples) are Lumbar vertebra plus rib. The samples were collected from the storehouse of Ahmed Fakhry in Giza plateau.

6. Saqqara

The site of Saqqara became a royal necropolis at the beginning of Second Dynasty when royal tombs were constructed at about 1km to the south of the already existing elite necropolis in the north [30, 31], the site of the principal cemetery at Memphis (20º50-53’ N, 31º13’ E). Its name seems to derive from that of the god of the necropolis, Sokar [32]. The Saqqara necropolis was not only a burial ground for humans, it was also the important site of the burials of the sacred Apis bulls and other sacred animals [33, 34]. The chosen archaeological bones (dry samples) are rib plus a lumbar vertebra and 2 metatarsal bones (Foot bones). The samples were collected from different excavations in this area.

7. El Bahariya Oasis:

Bahariya Oasis is much closer to the Nile than Siwa, only 200km (125 miles) west of Beni Mazar. Being the smallest of the great western desert oases, Farafra, lies some 180 kilometers (115 miles) southwest of Bahariya and just over 300km (200 miles) west of the Nile Valley at Manfalut. In pharaonic times, it was termed the *Ta-iht* (land of the cows), an indication of a past richness that has since vanished [35]. The archaeological bones collected (dry samples) are 2 thoracic vertebrae plus fragmentary bones. The samples were collected from different skeletons in the site storage.

8. Asyut

A site located 410 kilometers (250 miles) south of Cairo, on the western side of the Nile (27º11’ N, 31º10' E), Asyut is renowned for an extensive ancient necropolis situated on a steep elevation (known locally as Istabl Antar) of the western hills that delimit the Nile Valley [36]. Archaeological bones collected (dry samples) are part of the fibula plus fragmentary bones. The samples were collected from different excavations in this area.

9.El Kharaga and 10. El Dakhla Oasis

The "Great southern Oasis" (*Wh*$_3$*.t rsy.t*) consists of both the Kharga Oasis and the Dakhla Oasis. Kharga, lying parallel to the Nile between Nagada and Edfu, has a maximum distance of 230km (145 miles). In it, there are many monuments and sites of Greek-Roman times [35]. In El Kharaga Oasis, archaeological bones (dry samples) are fragments of long bones. The samples were collected from different excavations. From the El Dakhla Oasis, the archaeological bones collected (dry samples) are the mandible plus 2 broken vertebrae. The samples were collected from Hole No.4.

Methods

As investigation techniques we used a digital Microscope and all thin sections were examined and studied using polarized transmitted light microscopy (polarized Nikon, Japan) attached with Nikon model AFX-IIA at the Geological Department, Faculty of Science, Cairo University, Egypt [21]. Also a Scanning Electron Microscope Laboratory from the he central Laboratory unit, Asyut University, Egypt was used.

Results and discussion

All samples suffer from deterioration. The compounds of the burial soil played an important role in the deterioration of archaeological bone. We can observe some taphonomy in the samples such as change of color (Yellow-Brown and Dark), salt crystallization, micro cracks, pitting and carbonization (Fig. 2).

Microscopy

The investigation of the surface morphology of bone using microscopes gave more details about the state of the surface [22]. Bone weathering can be described as overlapping reactions that are controlled by water, acid, oxygen, and Ca contents in the bone and soil [12].

It was clear from the control sample (Fig. 3A) that the surface is smooth. No deteriorations forms were found. It was clear from the digital microscope (Fig. 3B) that the Haversian canal system disappeared, and this is due to the accumulation of salt deposits on the surface of the sample studied. The location of the sample (excavated in Sedi-Gaber area, Sporting Square) is very near to the Mediterranean Sea, which can carry sodium chloride NaCl into the pores of the artifact during burial, leaving it behind when the water evaporates. After excavation, these salts can crystallize at or just below the surface of the artifact causing damage.

It was confirmed by Abdel-Maksoud and Abdel-Hady [21] that porous archeological material such as bone often contains soluble salts. Ground water and sea water can carry these salts into the pores of the bone during burial, leaving them behind when the water evaporates. A variety of descriptive terms are used for this damage including spelling, flaking, powdering, and sugaring. In the studied sample the damage is similar to sugaring.

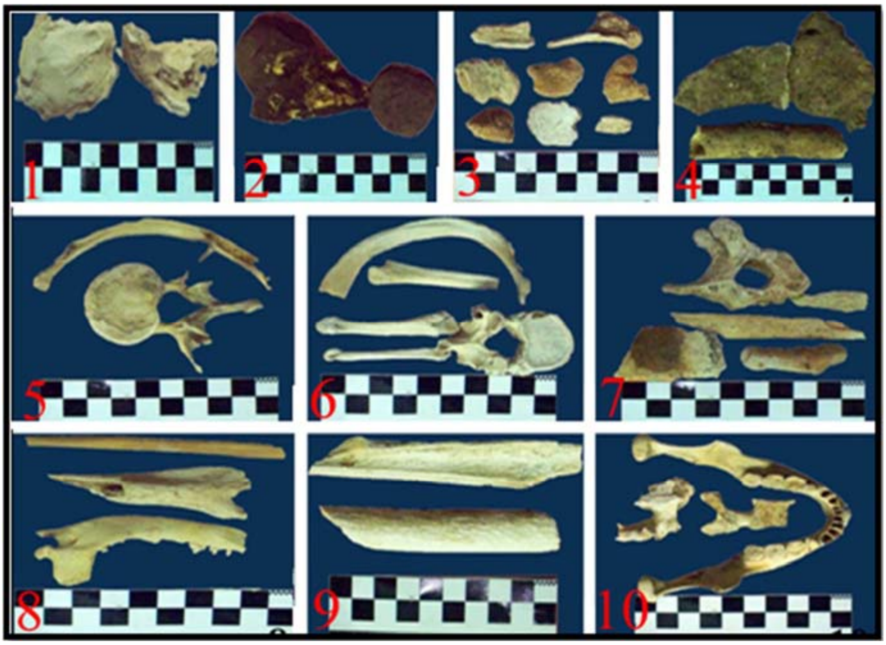

Fig. 2. Archaeological samples collected:
1 - Alexandria; 2 - Tanta; 3 - Quesna; 4 - Ain Shams (El Matariya);
5 - Giza (storehouse of Ahmed Fakhry); 6 - Saqqara; 7 - El Bahariya Oasis;
8 - Asyut; 9 - El Kharaga Oasis; 10 - El Dakhla Oasis;

It was clear that the detail of the surface was lost, and this may be due to the force of growing crystals that can break apart the surface of bone. In bad cases it can remove the entire surface of an artifact, and in the worst cases, it can destroy an artifact. The National Park Service [37] reported that soluble salts will dissolve in moisture in the air. This property is known as deliquescence. The salts can move through the porous structure of an artifact as moisture is drawn out through evaporation. As the salts reach the surface of the artifact they may crystallize as white, often furry growths on the surface. If the surface is less porous than the underlying structure they can crystallize just below the surface. These crystals exert immense pressure and may cause the surface layer to spall off. Hamilton [38] reported that bone from a salty environment will invariably absorb soluble salts that will crystallize out as the object dries. The action of salt crystallization will cause surface flaking and can, in some cases, destroy the specimen.

It was clear from the digital microscope (Fig. 3C) that the surface became coarse and the color of the sample indicated that some changes had occurred in burial environment before excavation and transportation to the storehouses of Tanta Museum. The destruction of the Haversian canal system; and pitting in different places were also noted. Abdel-Maksoud and Abdel-Hady [21] reported that pitting is the localized damage of a bone surface confined to a point or small area that takes the form of cavities. Sharp depressions were noticed in the surface of the bone that could be generally attributed to localized chemical attacks by corrosive soil. One or both of the following reasons lead to the pitting noticed on the surface of bone studied:

1. Pitting is caused if the environment contains aggressive chemical species' such as chloride.

2. Particle abrasion could easily explain the pitting if the bones were exposed to blowing silt and sand prior to burial; the impact of silt and sand grains have been observed to cause pitting on bone surfaces.

It was clear from data obtained (Fig. 3D) that different deposits from the burial environment were obtained such as soot or micro-organisms stains which were distributed in most of the surface sample. These results were confirmed by Müller et al. [39] that micro-organisms could have entered the bone. Changes that happen to bone during burial are above all the result of groundwater solutes, dissolution of soluble components, breakdown and leaching of collagen, crystallinity increase and alterations caused by micro-organisms. And De Boer et al. [40] said that the taphonomy alters because invasion of microorganisms into the microarchitecture hampers visibility of tissue architecture. The use of stain helps to differentiate between altered and unaltered parts of bone. This may be helpful in assessing the seriousness of taphonomic processes after burial. Roughness and coarseness were noticed. The Haversian canal system cannot be recognized and this may caused by the large amount of deposits that accumulated on the bone surface. It was also noted by Abdel-Maksoud [22] and Figueiredo et al [23] that the former clearly shows some Haversian systems, distinctive of cortical bone, constituted of concentric lamellae in which numerous lacunae (small dots) are also visible. The enlarged image of one of these lacunae, with a typical oval shape, clearly shows in its interior bundles of collagen fibrils as well as the entrances of the canaliculated. Outside the lacuna it is also evident that the hydroxyapatite crystals are embedded in organic material. Pitting with different forms and different colors ranging from grey to pale-yellow were also noticed.

In figure 3E we can observe that the change of color resulted from the burial environment. These results were confirmed by Abdel-Maksoud and Abdel-Hady [21], Behrensmeyer [41] and Koon et al. [42] who said that weathering processes could cause color changes on the bone surface. The Haversian canal system was very wide and some erosions were also noticed on the edges of the Haversian canal system.

It was clear from the data obtained (Fig. 3F) it was clear that the surface layer was eroded and the subsurface layer was visible. This may be due to the effect of insects.

Also, in figure 3G, different colors were noticed such as white, grey and pale-yellow colors. These colors may due to the effect of high temperatures, such as the temperature needed for cremation as explained by Abdel-Maksoud and Abdel-Hady [21]. Very small grey stains were also noticed which may due to microbiological attack. Pitting, erosion, and different size of the Haversian canal system, roughness and surface coarseness were also noticed.

In figure 3H the surface was rough, the size of the Haversian canal system varied and clear deterioration had occurred. Very small pitting, accumulated dusts and very thin cracks were noticed. Different colors were also identified. All forms of deterioration mentioned above may be due to aggressive environmental conditions such as the fluctuation in relative humidity and temperature.

It was clear from the data obtained (Fig. 3I) that the state of preservation of the sample is quite good. The surface is smooth. The size of the Haversian canal system is approximately the same. Some white remains are on the surface of the sample, which may be calcium carbonate. Different colors were also noticed.

By examining figure 3J we observed that the size of the Haversian canal system varied and clear deterioration had occurred. Different colors and clear pitting were also found. The surface is not flat. Dark deposits were noticed. This may due to the influence of the burial environment through the presence of water sources in some times and dryness in the other times. It can be added that the nature of El Kharaga Oasis environment, which is disinfected by its dryness weather.

Finally, the figure 3K presents a piece of mummy remains. The dark color in the upper left part of the photo was clear and may be due to the resinous material used in the mummification process. The second part of the photo is like a honey comb, which may be due to the effect of a biological factor (e.g., insects).

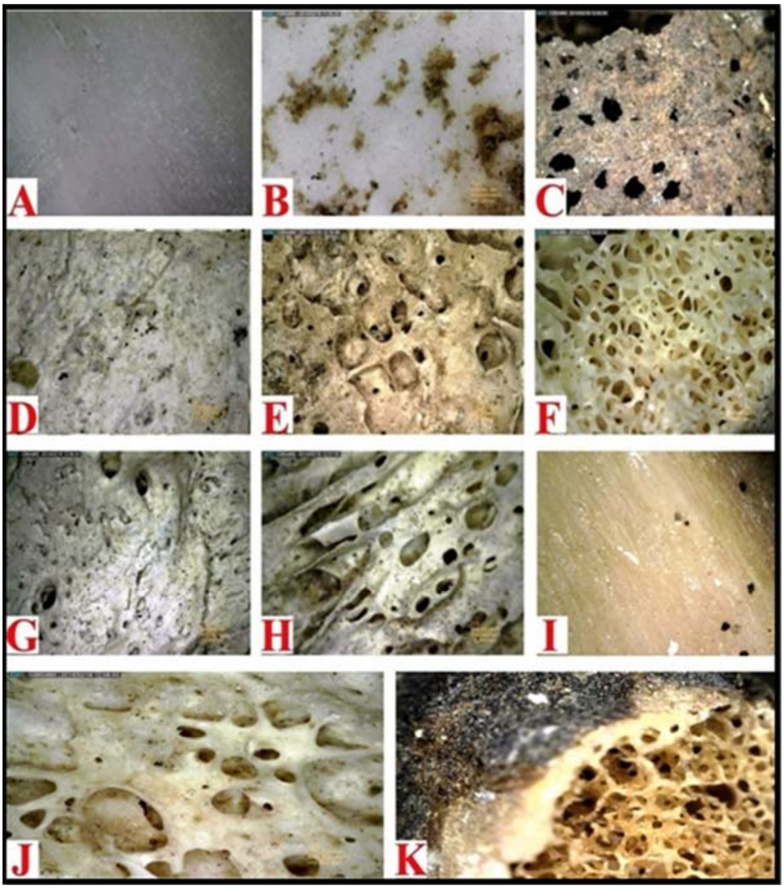

Fig. 3. Digital Microscope photos of bones samples studied with magnification 250X:
A - Modern sample (control);B – Alexandria; C – Tanta; D – Quesna;
E - Ain Shams (El Matariya); F - Giza (storehouse of Ahmed Fakhry);
G – Saqqara; H - El Bahariya Oasis; I – Asyut; J - El Kharaga Oasis; K - El Dakhla Oasis;

Polarized light microscopy

The use of a polarized light microscope became a vital tool for histological examination either under transmitted or polarized light. It also gives many details about the changes in the structure and color of bone. The interpretation for histology and structure of studied bones was established following Hammersen [43], Halluche et al [44] and Hedges et al [45].

The data obtained (Fig. 4A) revealed the structure of the modern bone sample. Abdel-Maksoud [22] demonstrated the subdivision into smaller fiber bundles by strands of loose connective tissue.

It was clear from the figure 4B that the surface appeared coarse and not flat. The Haversian systems were noticed but not with the same size. It was also noticed that some part of the sample was non-incinerated and the other parts were smoked (blackening on most of the sample), to incompletely incinerated bone (blackened and with light and dark brown in color). The blackening in color may be due to the deposits from the burial environment or from the burning of bone. Hedges et al [46] stated that a very dark grey brown color is obtained from the exposure to temperature at 285–525°C, and the neutral black color means that the bone exposed to temperature at 525–645°C.

The sample shows (Fig. 4C) neutral white, pale yellow and yellow colors. This means that the sample exposed to temperature 20 - < 285°C. The sample shows also grey color which means that the bone exposed to temperature at 285–525°C. The different colors mentioned above may also be due deposits or micro-organisms from the burial environment. The Haversian systems were recognized and varied in their size.

It was clear from the figure 4D that the Haversian system and bone matrix was unclear and most of the bone texture could not be recognized. The color ranged from tan to pale brown color.

In figure 4E the canal of the Haversian system was recognized. The destruction of the surface of the bone was noticed from the destruction of the lacunae of the Haversian system. Different colors were also noticed such as tan, grey brown and black colors. This may be due to the exposure to different temperatures, or from burial environment. It was clear from (Figs. 4 F-K) that the features of the bone histology were unclear. The color ranged from tan, pale and dark brown.

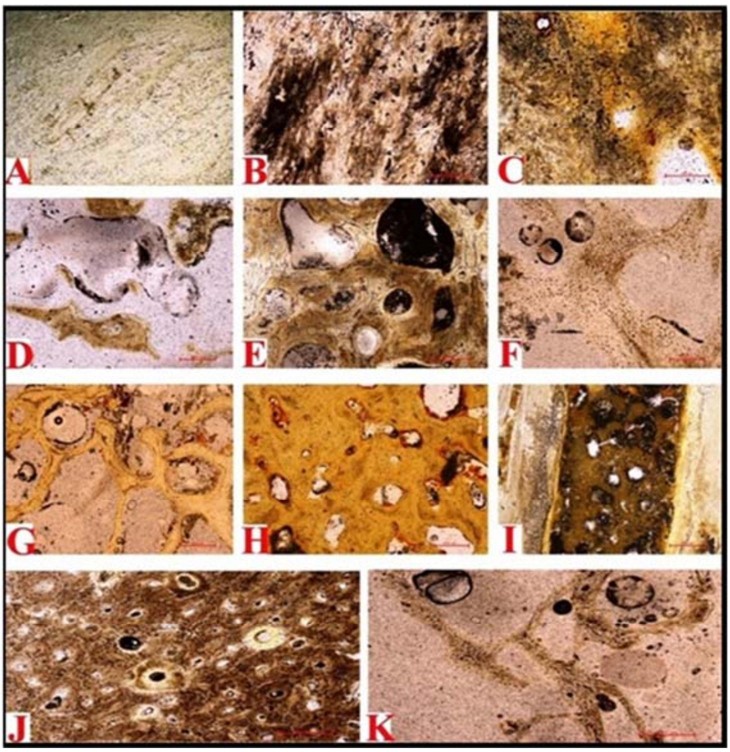

Fig. 4. Polarized light micrographs of bones samples studied with magnification 1000X:
A - Modern sample (control); B - Alexandria C – Tanta; D – Quesna;
E - Ain Shams (El Matariya); F - Giza (storehouse of Ahmed Fakhry);
G - Saqqara, (H) El Bahariya Oasis; I – Asyut; J - El Kharaga Oasis; K - El Dakhla Oasis;

It was clear from the data obtained by polarized light microscope (Figs. 4G and H) that the Haversian system is found as an individual canal system, the boundaries, which are marked by an occasional very faint line representing what has been called the cement line by Cook et al. [47]. Osteocytes, found in cavities (lacunae) within the matrix, were noticed. Dark precipitate in the form of a thick black line may indicate the presence of calcium phosphate in calcified bone.

It was clear from figures 4I and J that dark color was observed in this sample, which indicates that the sample exposed to temperature at 285-525°C. The Haversian system was recognized but with different sizes. The surface was coarse. Abdel-Maksoud and Abdel-Hady [21] said that there is a relationship between the color of bone and exposure to temperature. They also said that thermally altered bone displays surface color and texture changes, which can be a good indicator of the temperature to which the bone was heated, as well as the duration of the heating. Abdel-Maksoud and Abdel-Hady [21], Thompson [48] and Hanson and Cain [49] said that the deposition of carbon in the bone pieces throughout the matrix was noted. This is a very good indicator that the bone was exposed to high temperature. The change of bone color is an indication of the loss of organic and inorganic properties. The authors mentioned above stated that during exposure to heat, especially at high temperatures, the chemical properties of bone alter and structural integrity is impaired or lost. This results from evaporation, organic degradation, and transformation of inorganic matrix.

Scanning Electron Microscopy

The investigation of the surface morphology (Fig. 5A) of bone using a scanning electron microscope gave more details about the state of the surface [22]. According to Abdel-Maksoud [22] the modern bone has a clearly visible Haversian system (Fig. 5A), which appeared as concentric laminate or sheets around an osteonal canal or Haversian canal. It was noticed that the peripheral limits of the osteonal bone were marked by a reversal line or cement line. An osteon in its simplest form is a long cylinder with a hollow cavity, while the osteonal canal is in its center. Modern bone also showed a very smooth surface and the distribution of the Haversian systems was in many ways parts of bone.

It was clear from figures 5B and C that there are a lot of deteriorations on the surface, and all details related to the characterization of the bone have disappeared. Exfoliation is the common form of deterioration. Lawrence [50] explained that exfoliation may occur due to the migration of soluble salts through the bone, probably brushier formation. It is likely that this occurred through capillary action involving only trace surface moisture (from a humid environment) but was associated with variations in humidity permitting crystal precipitation. Silt and sand from the burial environment may help this form of deterioration to occur. This suggests that generally these deposits developed *in situ* in the tomb environment. Where deposits had precipitated beneath the bone surface, localized exfoliation mimicked pathology by creating shallow, sharp-edged, smooth-bottomed depressions in the vault – these were distinguishable from traumatic lesions by the presence of white crystals, absence of fractures or spelling and the nearby presence of ‘blisters’ of bone (similar in appearance to osteomata), where the surface was lifted as crystals grew.

Some forms of deterioration were noted in figures 5D, E, F, G and H, which are as follows:

- The Haversian system was recognized but with different sizes, which means that the degree of deterioration is not equal in all samples.
- Very thin cracks were also noticed.
- Rounding of archaeological bone samples (Fig. 5D) was seen. This may be due to the fluctuation in relative humidity and temperatures especially after excavation process.
- Erosion and missed parts were also noticed.

It was clear from figures 5I and J, that there were accumulations of dust and deposits on the remains from the burial environment. It was also clear from figure 5K that there is a coarse surface, pitting and erosion in some parts of the sample.

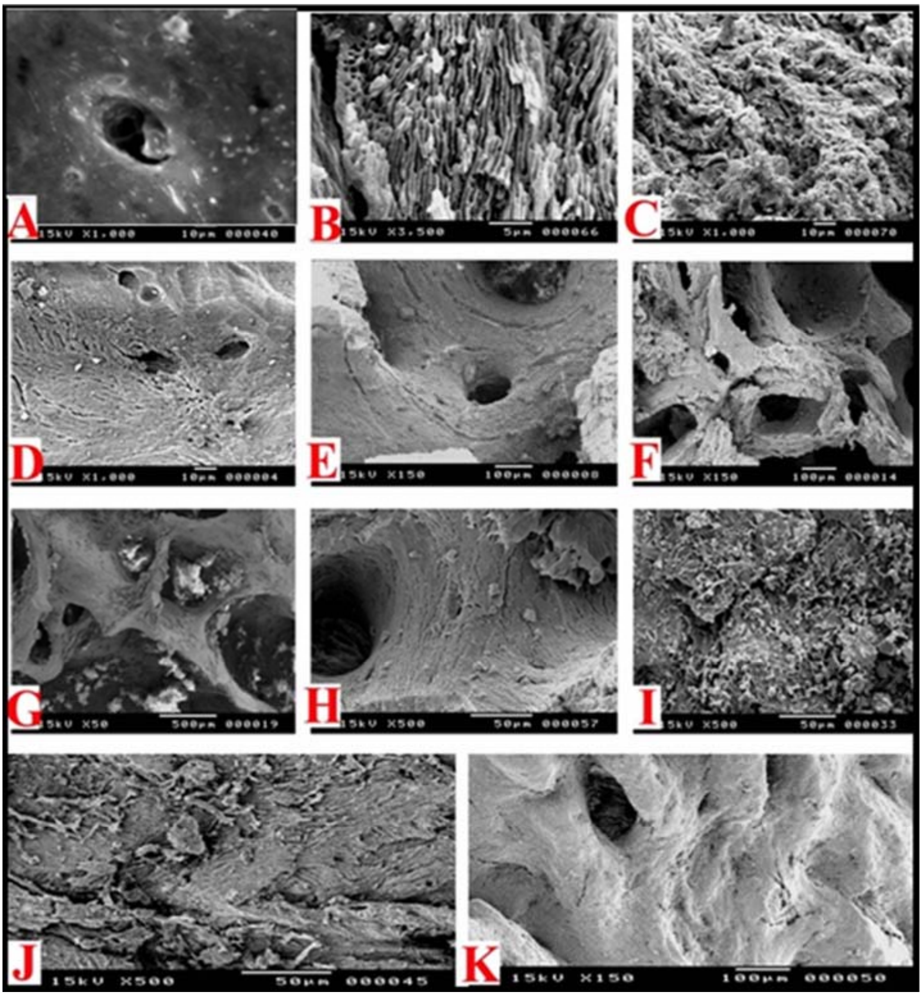

Fig. 5. Scanning Electron Microscope of bones samples studied:
A - Modern sample (control); B – Alexandria; C – Tanta; D – Quesna;
E - Ain Shams (El Matariya);F - Giza (storehouse of Ahmed Fakhry); G – Saqqara;
H- El Bahariya Oasis; I – Asyut; J - El Kharaga Oasis; K - El Dakhla Oasis;

Conclusions

The burial environment affected bones samples in different ways according to the nature of the burial conditions. It was clear that the most aggressive forms of deterioration on the surface such as salt crystallization, darkness in some parts of the sample and variation in the surface color, were the result of humid environment (such as the Alexandria bone sample) more than dry conditions (such as the Saqqara bone sample).

The microscopes used in this study (digital, light and SEM) gave a good indication of the deterioration of the bone samples collected from different locations. Through these techniques some aspects of deterioration were noted such as erosion, rounding, exfoliation, salt crystallization, cracks, surface color changes, and etc.

The results revealed that structure of bone plays an important role in the deterioration process, since investigation of spongy bone from Saqqara excavation by a SEM showed more deterioration than compact bone obtained from *El Kharaga Oasis* area.

This study revealed that the conditions of most burial environments were harmful for bone samples, since many aspects of deterioration were identified. Most samples were exposed to different temperatures. Samples obtained from dry conditions especially a desert burial environmental such as Saqqara, showed less deterioration than those obtained from humid conditions such as Alexandria.

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Received: July, 27, 2015
Accepted: March, 23, 2016