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ANALYSIS OF ARCHAEOLOGICAL BONES FROM DIFFERENT SITES IN EGYPT BY A MULTIPLE TECHNIQUES (XRD, EDX, FTIR)

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ABSTRACT

Archaeological bones were collected from different sites and conditions in Egypt to be analyzed by multi-techniques to determine the reaction of bones in different burial environments (dry and moist conditions). Different analytical techniques have been used to accurately recognize archaeological bones such as X-ray diffraction, energy dispersive X-ray (EDX) and Fourier transform infrared spectroscopy (FTIR). The results revealed that XRD confirms that all bone samples taken from archaeological sites have high crystallinity. EDX analysis showed different types of elements. It also proved the increasing of calcium and phosphor elements in archaeological samples compared to the control sample. FTIR indicated deterioration of amides groups compared to the control sample.

KEYWORDS: Archaeological bone, deterioration, analysis, XRD, EDX, FTIR.

1. INTRODUCTION

Bone is a natural composite of organics, minerals and water assembled in the form of a complex hierarchical structure (Barth et al., 2011). The chemical composition and the structure of the mineral phase are close to hydroxyapatite $Ca_5(PO_4)_3OH$, precipitated in an organic collagen matrix, protein, lipids, mucopolysaccharides and carbohydrates (Pate, 1994; Rogers and Daniels, 2002; Godfrey et al., 2002; Theppeang et al., 2008; Barth et al., 2011). After death and burial, changes begin to occur in bones which are the result of several factors (Ortner et al., 1972) such as physical, biological, and cultural processes (Todisco and Monchot, 2008). These changes in the physical properties are contemporaneous with chemical changes that occur in the organic and inorganic bone constituents (White and Hannus, 1983). When bones, mainly composed of collagen matrix, are exposed to a heat source around 300°C, they are rapidly carbonized; above 450°C, the remaining organic carbon is gradually removed. The mineral phase is also intensively altered from 100°C, as water is progressively removed; above 300°C, carbonate content rapidly decreases. As a result, the amount of defects in the lattice decrease and lead to crystal growth above 500°C. (Lebon et al., 2008), these impurities induce a large disorder in the lattice and thus limit the crystallite size. Bone mineral can thus be described as a poorly crystalline non stoichiometric carbonate HA. (Lebon et al., 2008), The crystal structure of bone can be seen to very slowly alter towards a more ordered composition with an associated increase in overall crystal size following death. The speed of this increase in crystal order can also be increased by weathering conditions (Wess et al., 2001). Changes in bone porosity resulting from various other diagenetic pathways have also been associated with changes to CI (Nielsen-Marsh and Hedges, 1999; Lebon et al., 2008). When 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 (Stone et al., 1990).

X-ray diffraction (XRD) is suitable and reliable technique, because of some important advantages with respect to its angular dispersive counterpart. The hardening-crystallization process is generally described by a sigmoid increase of the grain size over time (Generosi, 2010). The CI is calculated by measuring the ratio of specific spectra peaks, and changes to this ratio indicate a change in peak sharpness. Significantly these changes in CI have been shown to be the direct result of burning (Thompson et al., 2009), Figueiredo et al., (2012) reported that the size of the apatite crystals and the porous structure increased with temperature, resulting on increased crystalinity (as confirmed by X-ray diffraction) (Figueiredo, 2010) Full Width at Half Maximum (FWHM) measurements were made on the apatite d_{002} peak (Farlow and Argast, 2006).

FTIR spectroscopy constitutes an excellent tool to characterize the bone matrix because its main components (carbonated hydroxyapatite and collagen) absorb infrared radiation at distinct, almost complementary regions within the 400-4000 cm^{-1} range (Figueiredo et al., 2012). The three indexes calculated are: the Crystallinity index (CI), the Carbonate/Phosphate ratio (C/P) and the Carbonyl/Carbonate ratio (C/C) (Bonniere, 2010). The Crystallinity is a function of the extent of splitting of the two absorption bands at 605 and 565 cm^{-1} from phosphate group, The Carbonate (CO_3) gives absorption peaks at 710, 874 and 1415 cm^{-1} whereas PO_4 gives absorption peaks at 565, 605 and 1035 cm^{-1}. The carbonate absorption peak at 710 cm^{-1} is characteristic of $CaCO_3$. The C/C ratio is determined dividing the carbonyl (CO) peak (1455) and the CO_3 peak (1415) changes also. But after several investigations, this ratio is in reality CO3/CO3 ratio. Indeed the peak at 1455 cm^{-1} is a carbonate peak (Bonniere, 2010; Figueiredo et al., 2012).

It was determined that Energy Dispersive X-ray Spectroscopy (EDX) was mainly dominated by the presence of Ca, obviously accompanied by phosphorus. Simultaneously, other elements accompanying Ca, such as Fe and Sr were found at significant concentration levels (Farlow and Argast, 2006).

This study aims to:

- Explain the deterioration of bone compounds.
- Explain the behaviors of bone towards different burial environments.

2. MATERIALS AND METHODS

2. 1. Archaeological samples

Archaeological samples were collected from the following archaeological sites and museum storehouses (Fig. 1, Table. 1).

Figure 1. Egypt map shows archaeological locations of bones studied.

Table 1. Archaeological Locations

Sample Number	**Location**	**Samples conditions**
A (Control)	--	Standard
B	Alexandria region in north Egypt	Humid sample
C	Tanta museum storage in north Egypt	Dry sample
D	Quesna region in north Egypt	Dry sample
E	Elmatarie and Ain shams region in Cairo	Humid sample
F	Ahmed Fakhry storage in pyramid region in Giza (middle Egypt)	Dry sample
G	Saqqara region in Giza (middle Egypt)	Dry sample
H	Elwahat Elbaharya region in west Egypt	Humid sample
I	Mountain of West bank in Asyut region in south Egypt	Dry sample
J	Elkharga region in south Egypt	Dry sample
K	Mute region in New valley in south Egypt	Dry sample

2.2. X Ray Diffraction

This measurement has proven useful as a measure of apatite crystallinity by Compact X-ray Diffractometer System PW1840 - Analytical Equipment - Philips – Eindhoven – the Netherlands (CuK _ radiation with Ni-filter), at the Conservation Department, Faculty of Archaeology, Cairo University.

2. 3. Dispersive X-ray analysis

Samples were carried out using Energy Dispersive X-ray analysis Laboratory, The central Laboratory unit, Assiut University, Egypt. All the samples examined have been prepared by coating in gold.

2.4. Fourier Transform Infrared Spectroscopy

The FTIR instrument used for this analysis is JASCO FT/IR-6100 FT-IR Spectrometer made in Japan, in the range of 4000 - 400 cm^{-1}, in transmission mode. The analysis was performed at the Infrared Spectroscopy Laboratory, National Research Center (NRC) in Cairo, Egypt. About 3 mg bone was hand-grinded and mixed with KBr in the weight ratio 1:100 respectively, to make pellets suitable for beam irradiation. It should be considered that bands of the infrared spectrum of recent and fossil bones are relevant to obtain molecular information concerning the phosphate/carbonate group ratio. Additional bands may also be evaluable due to minerals other than those related to the apatite-like structure (Piga et al., 2011).

3. RESULTS

3. 1. X Ray Diffraction

It was clear from data in Fig. 2 that all samples analyzed generally present characteristic X-ray diffraction patterns typical of poorly crystalline hydroxyapatite. The results showed that there is difference between the control sample and the archaeological samples. The crystallinity index of the modern sample (control) was 0.81 mm. The crystallinity index of the peak (002) reflection that the high crystallintty for the peak (002) was obtained from Gabel El-Bar El-gharby in Asuyt. The crystallinity index recorded 0.2 mm, as the condition was in dry area. The archaeological samples taken from Gabel El-Bar El-gharby in Asuyt may have been exposed to high temperatures, as indicated by Hiller (2003); Wang et al. (2010) and Figueiredo (2010) who found that when bones were exposed to temperatures in excess of 600°C, inorganic compounds became damaged, dissolved and there was an occurrence increase in the crystallinity index.

It was clear from the data obtained Fig. 2-E that the humid sample collected from Ain Shams (El Matariya) area recorded that 0.25 mm. This confirms that a humid environment plays an important role in the damage of the archaeological bones. It was reported by Stone et al. (1990) who confirmed that when bones are 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.

It was clear from the data from the dry samples collected from El-Kharga and El-Dakhla area recorded 0.3mm (Fig. 2- J, K).

It was clear from data from the humid samples collected from Elwahat - Elbaharya region and dry samples collected from Saqqara region, Quesna region and Ahmed Fakhry storage in Pyramid region recorded 0.4mm (Fig. 2- D, F, G, H).

It was clear from data from the humid samples collected from the Alexandria region and dry samples collected from Tanta museum storage recorded 0.6mm (Fig. 2- B, C)

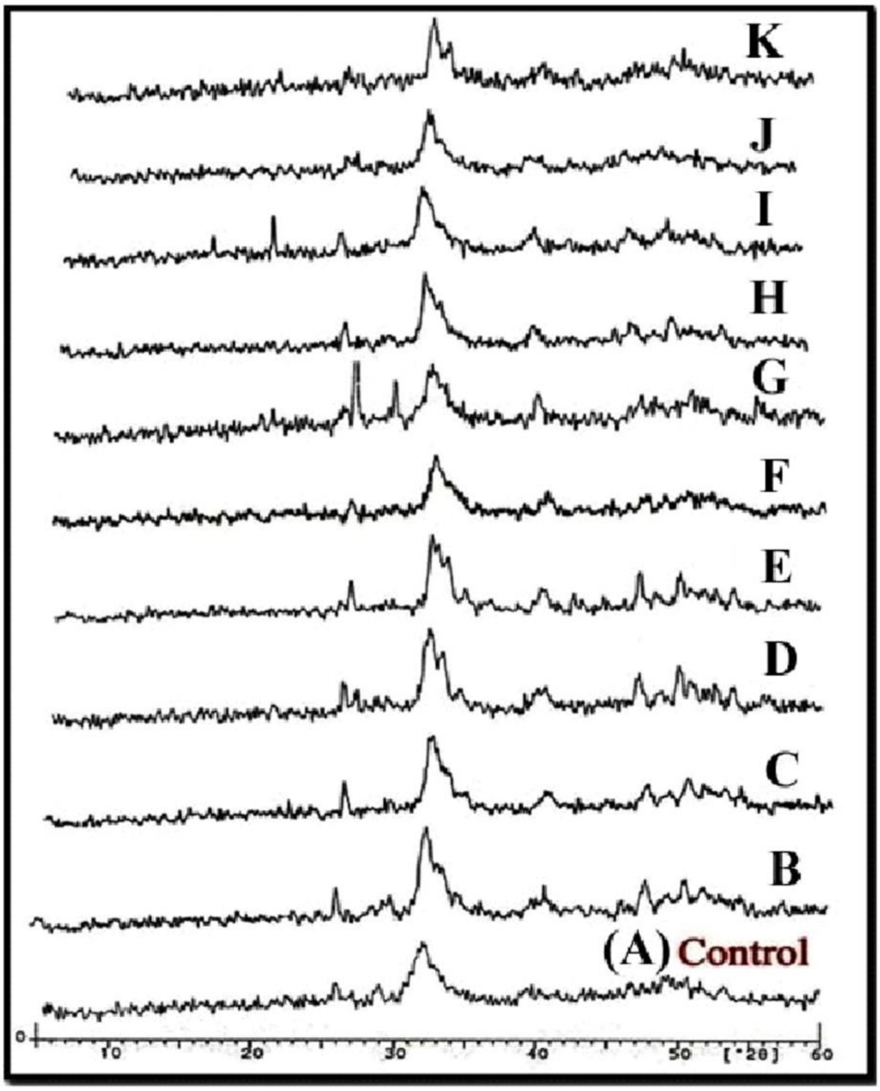

Figure 2. X Ray Diffraction patterns of archaeological bones samples studied (A) Modern sample (control), (B) Alexandria, (C) Tanta, (D) Quesna, (E) Ain Shams (El Matariya), (F) Giza (storehouse of Ahmed Fakhry), (G) Saqqara, (H) El Bahariya Oasis, (I) Asyut, (J) El Kharaga Oasis, (K) El Dakhla Oasis.

3. 2. Dispersive X-ray analysis

Bone hydroxyapatite contains about 18.5% P and 39.9% Ca. If bone is 70% mineral, un weathered bone should contain 12.95% P and 27.9% Ca (White and Hannus, 1983). It was clear from the data obtained (Fig. 3, Table 2) that all samples analyzed by EDX show many of the elements concentrated in archaeological bone, including Ca, P, Sr, Ba, Pb, Al, S, Fe, Cu, Si, Zn, K. Calcium (Ca) and phosphorus (P) are the main parts of the mineral component in the bones. The bone's mineral part consists of a mostly stable mineral hydroxyapatite. This was confirmed by Allmae et al. (2012) who reported that the measurement of the calcium content in micro analyses is mainly made with the purpose of assessing the quality of the material. In the case of the fully preserved collagen, the Ca content varies to the degree of 26%–38% in archaeological bones (Allmae et al., 2012).

The contents of copper (Cu) and zinc (Zn) in bones are considered to be the indicators of meat in the diet. For example, the organism, that consumes more animal food (fish, crustaceans, mollusks), always contains more zinc (Zn). It is confirmed by Couoh and Ruvalcaba (2007) ; Darrah (2009); Generosi et al. (2010); Swanston et al. (2012) and Allmae et al. (2012) from the comparative studies of the zinc content in the bones of carnivores and herbivores. Here the only exception from plant food is nuts, containing also noteworthy amounts of zinc. Also, the people, in whose diet marine food has had a big role, may have a higher content of Zn in their bones than the people consuming the food. The main food stuffs containing copper (Cu) are liver, red meat, fish meat, beans, peas, products of full cereals, and nuts. In the organisms of carnivores there is always more copper than in herbivores. Also, crustaceans and mollusks contain much copper. The indicators of plant food in bones are considered to be the contents of manganese (Mn), barium (Ba), and strontium (Sr). The best sources of manganese (Mn) are from plants. The content of manganese in them depends on the soil they have grown in. Manganese almost cannot be found in meat, chicken, fish, milk and milk products but it is found in big quantities in legumes, green vegetables, cereals (especially in wheat sprouts and whole-meal bread), and nuts. The manganese content in bones is considered to be a good indicator in assessing the proportions of plant food but contrary conclusions have also been made. The strontium (Sr) content in bones is considered to be the indicator of plants consumed. Plants get strontium from the soil. Regarding Pb, in the course of time the content of Pb in bones accumulates if the organism is exposed to the source of pollution. Biologically Pb behaves in the organism similar to Sr and Ba replacing calcium in the mineral part of the bone.

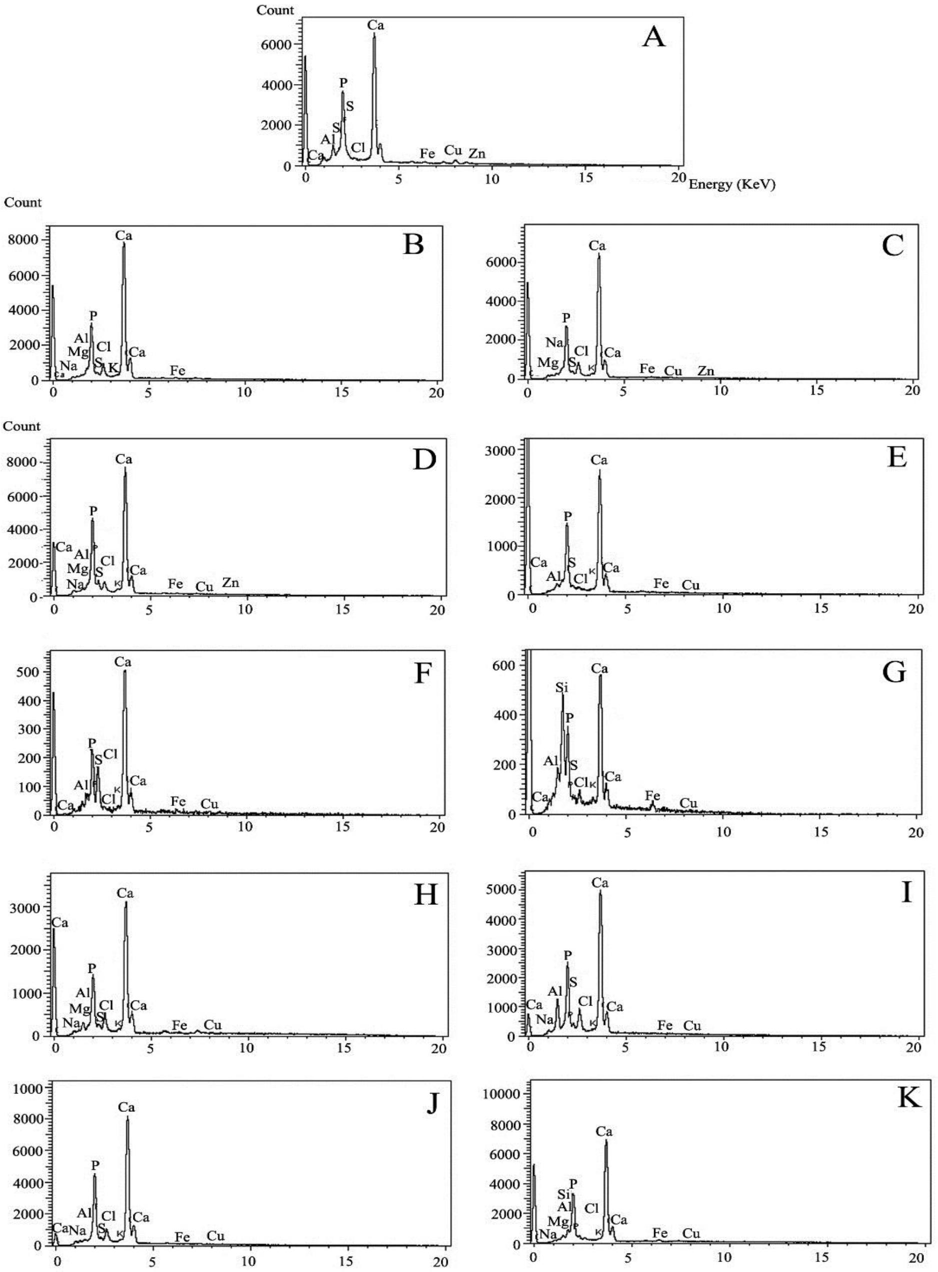

Figure 3. Scanning Electron Microscope equipped with EDX unite patterns of archaeological bones samples were studied (A) Modern sample (control), (B) Alexandria, (C) Tanta, (D) Quesna, (E) Ain Shams (El Matariya), (F) Giza (storehouse of Ahmed Fakhry), (G) Saqqara, (H) El Bahariya Oasis, (I) Asyut, (J) El Kharaga Oasis, (K) El Dakhla Oasis.

Table 2. EDX analysis results of modern and archaeological bones

Samples	Elements	Ca	P	Cu	Al	Zn	Fe	S	Cl	K	Si	Mg	Na	Total
A (Standard)	Wt%	57.52	21.23	7.38	5.34	4.16	1.36	1.06	0.69	0.65	0.60	-	-	100%
	At%	54.91	26.23	4.45	7.57	2.43	0.93	1.27	0.75	0.64	0.82	-	-	100%
B (Alex.)	Wt%	69.28	17.62	-	0.72	-	1.12	1.91	4.77	0.80	-	1.01	2.77	100%
	At%	63.53	20.91	-	0.99	-	0.74	2.18	4.42	0.75	-	1.53	4.42	100%
C (Tanta)	Wt%	68.84	18.45	0.21	0.82	1.34	1.07	2.01	5.67	0.97	-	0.62	-	100%
	At%	64.58	22.40	0.12	1.14	0.77	0.72	2.36	6.01	0.93	-	0.96	-	100%
D (Quesna)	Wt%	60.62	25.02	0.37	0.51	0.43	0.57	4.68	3.93	1.35	-	0.39	2.13	100%
	At%	54.77	29.25	0.21	0.69	0.24	0.37	5.28	4.02	1.25	-	0.58	3.35	100%
E (Ain shams)	Wt%	65.52	24.46	0.15	1.69	-	1.18	3.50	0.96	2.54	-	-	-	100%
	At%	60.29	29.12	0.09	2.31	-	0.78	4.03	0.99	2.40	-	-	-	100%
F (Giza)	Wt%	65.74	13.14	0.87	1.00	0.17	3.88	13.45	0.91	0.83	-	-	-	100%
	At%	61.81	15.98	0.52	1.40	0.10	2.62	15.80	0.97	0.80	-	-	-	100%
G (Saqqara)	Wt%	35.33	19.72	0.46	12.09	-	2.57	2.91	2.36	2.51	22.04	-	-	100%
	At%	29.13	21.04	0.24	14.81	-	1.52	3.00	2.20	2.12	25.93	-	-	100%
H (Bah. Oasis)	Wt%	62.18	19.80	0.41	1.87	-	0.57	2.55	6.72	1.06	-	1.28	3.56	100%
	At%	55.80	23.00	0.23	2.49	-	0.37	2.86	6.82	0.98	-	1.89	5.56	100%
I (Asyut)	Wt%	55.85	18.18	0.66	10.05	-	0.40	2.81	6.84	2.29	-	-	2.91	100%
	At%	49.13	20.69	0.37	13.13	-	0.25	3.09	6.80	2.07	-	-	4.47	100%
J (Kharaga)	Wt%	64.83	22.67	0.36	1.10	-	0.45	1.94	5.02	0.91	-	-	2.72	100%
	At%	58.87	26.64	0.20	1.48	-	0.30	2.20	5.15	0.85	-	-	4.31	100%
K (Dakhla)	Wt%	64.68	22.03	0.29	1.65	-	2.64	2.48	1.20	0.58	2.26	0.59	1.60	100%
	At%	58.93	25.97	0.17	2.24	-	1.72	2.82	1.24	0.54	2.94	0.89	2.55	100%

3.3. Fourier Transform Infrared Spectroscopy

Data pertaining to the FTIR spectra of the modern and archaeological samples are recorded in Fig. 4. All spectra of the archaeological samples differ from the modern samples and provide information regarding the effect of soil environment on archaeological bones.

3.3.1. Organic compound phase (Collagen)

It was clear from all archaeological samples studied (Fig. 4) that the band at 3401.82 cm^{-1} in the modern sample (control) assigned to a broad band represents (OH) hydroxyl stretching due to intermolecular hydrogen bonding of the hydroxyl group. The band at 2928.38 cm^{-1} was assigned to a symmetric C-H (residual alkane group) that are found in a very large number of compounds. The C-H stretching vibrations occur in the region 2928.38cm^{-1} stretching of aliphatic groups, and was found in some samples that were studied (Fig 4 - E, F, G, H, I, J) that the C-H vibrations occur in the region 2931.27 $cm^{-1,}$ 2927.41 cm^{-1}, 2931.27 cm^{-1}, 2929.34 cm^{-1}, 2923.56 cm^{-1} , 2932.23 cm^{-1}. The position of this band was very similar to the band of the modern sample.

Collagen exhibits a series of absorptions from 1700 cm^{-1} to 1200 cm^{-1} Band at 1663.3 cm^{-1} (C=O stretching) in the modern sample is assigned to amide I. The position of this band decreased when the samples were exposed to a heat source (Abdel-Maksoud, 2010), It was clear from data (Fig. 4) that the position of this band decreased in all archaeological samples. This was confirmed by Abdel-Maksoud (2010) that increasing or decreasing of C=O is dependent on the physical state of the sample. In the solid phase, the frequency of the vibration is slightly decreased. The presence of hydrogen bonding is an important contributing factor to this decrease in frequency. The similarity of the position of this band was noticed the bands at 1567.84 cm^{-1} (NH, CN stretching) in the modern sample are assigned to amide II, which disappeared in the most archaeological samples except the sample (Fig. 4 E) which appear in 1553.38 cm^{-1} and the sample No. I which appear in 1575.56 cm^{-1}.

The band at 1244.83 cm^{-1} is assigned to amide III which involves C-N stretching and N-H bending. The observed wave number of these peaks depends on the secondary structure of the protein (e.g., α-helix, β-sheet, β-turn, random coil) (Figueiredo et al., 2012). This band appeared only with the modern sample and disappeared with the other archaeological samples except the sample (Fig. 4 F) which appear in 1247.72 cm^{-1}. Collins et al. (2002) reported that at high temperatures, the rate of collagen loss will be accelerated. According to Götherstrom et al. (2002), the collagen was influenced by the presence or absence of water.

3.3.2. Inorganic phase

Bone mineral is a poorly crystalline calcium-deficient apatite, with a Ca:P ratio that differs from 1.67, which is the theoretical value for pure hydroxyapatite. The non-stoichiometric biological apatites contain several ion substitutions. For example, Na+ and Mg_{2+} may substitute Ca_{2+} ions, HPO_{42-} ions may substitute phosphate ions, and Cl- and F may replace OH-. Additionally, carbonate ions, the most abundant substitutions (3-8 wt. %), may occupy either the OH- (type A apatite) or PO43- (type B apatite) positions in the crystal lattice. The mineral component of bone is usually closer to B-type apatite (Landi, 2003; Figueiredo et al., 2012).

For the inorganic part of the studied bone samples, band at 1448.28 cm^{-1} in the modern sample is assigned to carbonate ($-CO_3$).

It was clear from data (Fig. 4) that the band at 873.596 cm^{-1} relates to carbonate ($-CO_3$). The strongest bands appeared at 1027.84 cm^{-1} (plus or minus) and are assigned to (PO_{3-4}) symmetric stretch, which are mainly from hydroxyapatite. The band at 602.64 cm^{-1} and the band at 566.005 cm^{-1} (plus or minus) are assigned to (PO_{3-4}) (antisymmetric phosphate).

The carbonate/phosphate ratio (C/P) was calculated using the peaks at around 1448.28 cm^{-1} ($-CO_3$) and 1027.84 cm^{-1} (PO_{3-4}) according to (Koon et al. 2003). The carbonate ratio was higher than the phosphate ratio in the modern sample (1.409). The archaeological samples ratio is sample No. B (1.373), No. C (1.398), No. D (1.374), No. E (1.370), No. F (1.392), No. G (1.375), No. H (1.419), No. I (1.376), No. J (1.415), No. K (1.376).

The data obtained from FTIR stated that degradation of collagen (loss of amide I, amide II and amide III) and the ratio of carbonate/phosphate.

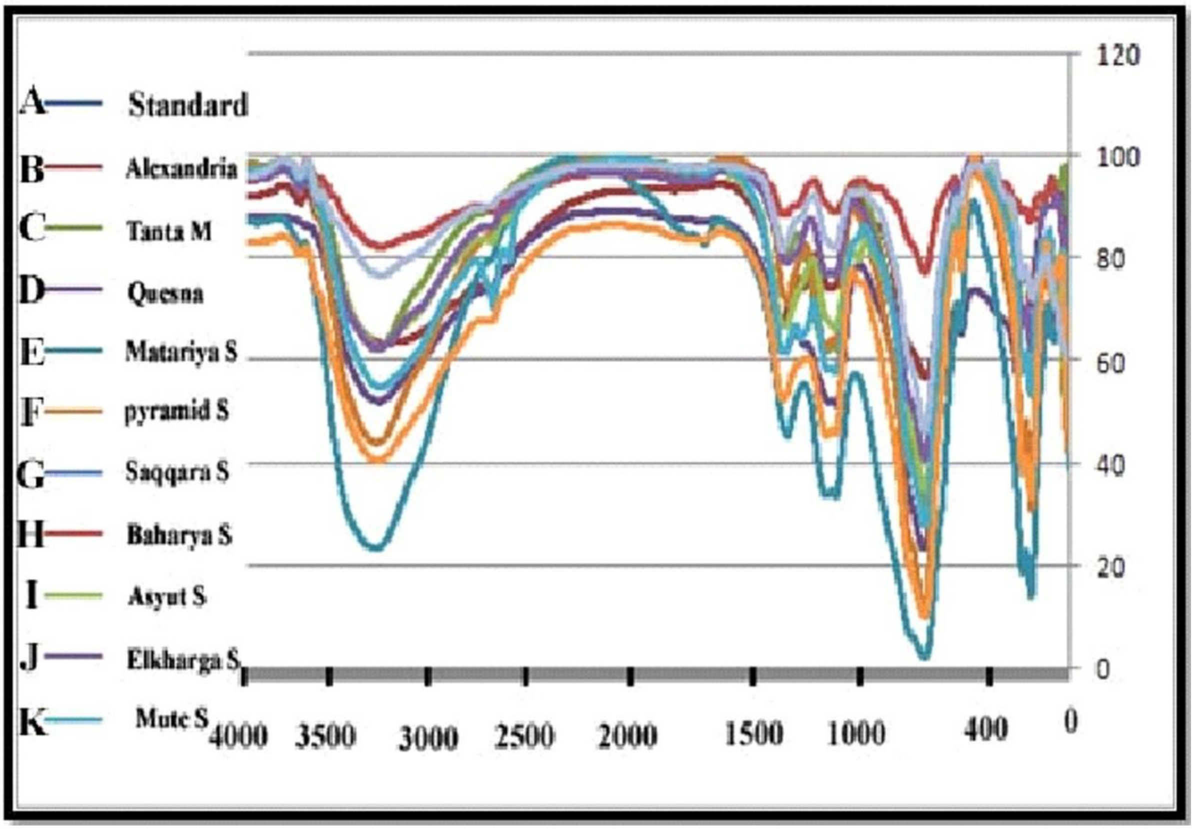

Figure 4. FTIR patterns of archaeological bones samples were studied (A) Modern sample (control), (B) Alexandria, (C) Tanta, (D) Quesna, (E) Ain Shams (El Matariya), (F) Giza (storehouse of Ahmed Fakhry), (G) Saqqara, (H) El Bahariya Oasis, (I) Asyut, (J) El Kharaga Oasis, (K) El Dakhla Oasis.

4. CONCLUSIONS

- X-ray diffraction analysis proved that all studied samples collected from different burial environments have high crystallinity compared to the control sample, but the crystallinity of the samples collected from dry burial environments were higher than those collected from humid burial environments.

- EDX analysis showed elements of Ca, P, Sr, Ba, Pb, Al, S, Fe, Cu, Si and Zn. It was also noticed that Ca and P elements increased in archaeological bone samples compared to the control. This may be due to some amounts of calcium element in the chemical composition of calcium hydroxyapatite of bone and the other amounts obtained from burial environments. The increasing of phosphor may be due to nutrient types in ancient population.

- FTIR analysis showed the deterioration of amides groups in archaeological bone compared to the control samples. This may be due to a combination of factors (physical, chemical and biological factors) in burial environments.

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