Mediterranean Archaeology and Archaeometry, Vol. 17, No 1, (2017), pp. 251-263

DOI: 10.5281/zenodo.400781

BIOARCHAEOLOGY, CONSERVATION AND DISPLAY OF A 16K-HUMAN SKELETON, JORDAN

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Received: 08/03/2017
Accepted: 01/04/2017
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ABSTRACT

The Jordanian Museum of Cultural Heritage houses the oldest human skeleton unearthed in Jordan; Radiocarbon analysis revealed a date of approximately 16000 years BP. The purpose of the study is to reconstruct the biology and way of life of an individual who lived in an era that is still ignored by archaeologists in the region. The methods of the reconstruction include bioarchaeological investigation, XRD, XRF, FTIR, pH meter, and microbiology. The reults indicate a 31-years-old male with an estimated height of about 172.4-175.2 cm. The bone pathology on the vertebrae and long bones suggests that the individual endured hard daily life activites. His teeth showed oblique dental wear that is attributed to using them as tools. The cause of death was probably due to a blunt force trauma to the left side of the skull. The bone analyses using XRD, XRF, FTIR, and pH meter ruled out bone diagenesis, which nominate the skeleton for further chemical analyses. The microbiological tests revealed the presence of a wide range of microorganisms: *Aspergillus viger*, *Pencillium chryogenum*, *Penicillum digitatum, yeast*s, *Corynebacterium equantium*, *Corynebacterium pyogemes*, *Escherichia coli*, *Pseudomonas pseudoaclingenes*, *Staphylcococus aureus*, *Salmonella enterica*, and *Corynebacterium pseudodiphthriticum.* For the purpose of exhibiting the skeleton at the Museum and ensuring longer survival, the previous conservation materials (P.V.A) were replaced by new ones (Paraloid B 72).

KEYWORDS: Bioarchaeology, Conservation, Skeleton, Jordan, XRD, XRF, FTIR.

1. INTRODUCTION

The archaeological skeletal remains are one of the most abundant findings at the Jordanian archaeological sites and probably the most useful. Studying them adds to the understanding of the health, growth, diet, environment, and migrations of past human societies (Larsen, 2015; Al-Shorman, 2007), especially when archaeolohgical records stand effete in reconstructuing the past history as triggered by the absence or scarcity of other archaeological materials. An example of such an archaeological period in Jordan is the period when there was a shift from hunting and gathering to agriculture (Rolston, 1982). Archaeologists agreed that there was a decline in the overall health for the generations of people who lived during this shift (Latham, 2013). This deterioration was triggered by greater physiological stress due to under nutrition and infectious diseases (Ulijaszek et al., 1991:271). However the period before 15000 BP has received little if any archaeological studies. In this period, the cold and harsh environment possessed extra demand on the human adaptation (Olszewski, 2008), especially in arid and semi arid regions of the Near East. Al-Kharaneh, eastof Jordan, is one of these areas were fortunately an Epipaleolithic human skeleton was recoved in 1982. Studying this skeleton adds to the general archaeological picture of this ingnored period. Consequently, a special attention should be directed toward the preservation and conservation of recovered skeletal materials from this period since discovery because they are sensitive and fragile (Plenderletih and Werner, 1971; Muller and Reiche, 2006).

Studying archaeological bone materials icludes a wide array of chemical and analytical methods. For example, Fourier Transform Infrared Spectroscopy (FTIR) has been used extensively to examine the bone organic material and its crystallinity (Wright and schwarcz, 1996; Lee-Throp and Sponheimer, 2003; Alvarez-Lioret et al., 2006; Brock et al., 2010), where the FTIR peaks at 565 cm^{-1} and 605^{-1} increased while the peak at 595^{-1} decreased (Stiner et al., 2001). On the other hand, XRD uses X-rays of known wavelengths to determine the lattice spacing in the bone crystal lattice and therefore identifies chemical compounds of the sampled bones (Al-Shorman, 2013). An example of one of the earliest use of XRD in archaeological bones analysis is the study by Hassan et al. (1977) who evaluated the chemical composition of bones for radiocarbon dating. Another study was conducted on four archaeological sites from Jordan to examine the crystallinity index using XRD (Al-Shorman, 2010) and found that the burial environment is a major factor in bone digenesis. Another technique is X-ray Fluorescence (XRF), which measures the elements of bone samples. For example, Todd and Landriagan (1993) and Little et al. (2014) measured the heavy metal content in archaeological bone using XRF. This technique was used also to examine the ionic exchange between soil solutions and bone (Pate et al., 1989). The burial environment has also received the archaeologists attention (Nord et al., 2005), where the soil acidity is a major factor in skeletal preservation (Gordon and Buikstra, 1981). The microbial attack of archaeological bones has also received a significant consideration especially during the early stages of digenesis (Muller et al. 2011). Such studies were able to visualize the microstructural damage cause by the bacteria (Nielsen-Marsh and Hedges, 2000; Jans et al., 2004), identify the various microbial species colonizing bones (White and Booth, 2014) and then inferring the biological burial context (Child, 1995).

However, the inclusion of modern chemical analyses in the field of archaeological skeletal analysis has contributed to the advancement of knowledge regarding the biological and social contexts of buried human remains. There are several factors that need to be considered in studying bone diagenesis: microbial attack, collagen loss, crystallinity increase, dissolution, and uptake of groundwater solutes (Hedges, 2002), porosity, bulk density, carbonate to phosphate ratio, and cracking (Smith et al., 2007). The current case study is an example, but one, that elucidates on the intertwined methods in extracting information from skeletal materials.

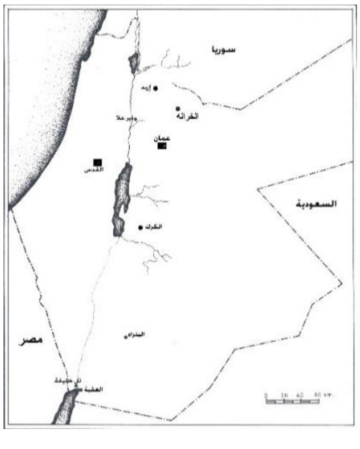

Figure 1. A Map of Jordan that shows Al-Kharaneh archaeological site.

The studied skeleton is displayed at the Jordanian Museum of Cultural Heritage/Yarmouk University, Jordan. It was recovered from a pit at the archaeological site of Al-Kharaneh, Al Azraq desert, 70 Km east of Amman (Fig. 1). The individual was buried in an extended position, where the head and legs were covered by large boulders and two antlers (Fig. 2). However, the common burial practices during this period (Epipaleolithic) was a flexed position in a small pit (Al-Shorman, 2007), which questions the manner of death as the study clarifies later.

Figure 2. The human skeleton during its discovery in 1982 (Muheisen, 1988).

Radiocarbon analysis revealed a date of approximately 16000 years BP (Rolston, 1982), which puts the skeleton on top of the oldest skeletal collections in Jordan. This skeleton tells the story of a vague period in the history of the transition from hunting and gathering to agriculture. The previous conservation has technical problems; the conservation materials were inappropriate and contributed to further deterioration. and the method of application did not follow 'thin film' techniques. In addition, the skeleton was displayed on dirt with the presence of insects (Silverfish). The temperature and humidity of the showcase are not appropriate for preserving organic materials. The average humidity is about 74% during summer days and 34% during nights; this fluctuation contributes to the deterioration of the organic matter of the skeletal materials at the museum (Khasawneh, 2006). In summer, the temperature may reach 30˚C during the day, and the difference in temperature between day and night may reach 10˚C, which enhances further deterioration especially by insects and microbes (Caple, 2000). The skeleton was not exhibited in an anatomical position.

2. MATERIAL AND METHODS

This study comprised one human skeleton that was recovered during a field excavation in the year of 1982. Radiocarbon dating revealed a date of about 16000 years BP- the Epipaleolithic Period (22500-10500 BP). Accordingly, the individual lived in a period just before humans were semi sedentary living in larger and organized groups (Al-Shorman and Khwaileh, 2011). The methods of the study include anthroposcopic examination of the human skeleton after Buikstra and Ubelaker (1991) while the stature was estimated after (Bass, 1987). The other method is analytical chemistry techniques (XRD, XRF, and FTIR). The sample size for analytical chemistry is 1 mg each (after grinding to a fine powder) and obtained from bone fragments (total number is 3). For the X-ray diffraction (XRD, Schimatzo 6000), the results were calibrated to a synthetic hydroxyapatite curve, where the used voltage is 40 Kv at 2 theta with a continuous speed of 1 deg/min. The other technique is X-ray fluorescence (XRF, Philips Minipal PW4025), which was standardless as the results do not aim to quantify the concentrations but to characterize the presence of elements. Fourier Transform Infrared Spectroscopy (FTIR, Bruker-Tensor 27) was also performed after mixing the grinded samples with 30 mg of KBr to ensure noise elimination of the peaks. The pH meter (PH315i/set) was used to measure the acidity of soil sample after calibration with known standards. The methods of conservation include cleaning, sterilization, consolidation, and coating to protect the skeleton from the surrounding environment using Paraloid B72 (Ambrose and Paine, 2007). For microbial testing, sterilized swabs were taken from the skeleton and cultivated in nutrient agars after Barrow and Feltham (2003). Fungi Complete media was also used to obtain pure cultures of them. After 3 days of growth at 37 ºC, fungal colonies were observed. Morphological identification of fungal isolates was performed microscopically after Elserogy et al. (2016). (Fig. 3).

A

B

Figure: 3. The method of taking swabs for microbial culture from (A) the skull and (B) the spine.

3. RESULTS AND DISCUSSION

3.1. Bioarchaeological assessment

The skeleton displays a poor preservation condition, where most of the bones were fragmented, bleached, and fragile. The bioarchaeological assessment extracts the demographic elements of the individual as well as the health status. Based on the shape of the Greater Sciatic Notch of the pelvis, Sacrum, Supra Orbital Ridge, and Mastoid process, the sex of the individual was male. The age of the individual was determined based on teeth, bone fusion, and the morphology of the auricular surface to be 30-31 years old (Table 1).

Table 1: The age estimation based on teeth, bone fusion, and the morphology of the auricular surface.

Feature	Description	Estimated age
Teeth	All of the teeth were fully erupted	+18 years
Bone fusion of the clavicle	Medial and lateral epiphyses are fused	+30 years
Morphology of Auricular surface of the pelvis	No apical activity, slight retroauricular activity, coarse granulity on superior demiface and significant striae on inferior demiface	31 years

There is myositis ossificans on the medial side of the left tibial midshaft (Fig. 4). This type of pathology is usually occurred at the insertion of muscles due to traumatic origin (Vargova et al., 2016), where after prolonged inflammation calcification starts to accumulate. Rolston (1982) in his report on the skeleton to the Jordanian Department of Antiquities mentioned that the left tibia is 9mm shorter than the right one. However, the right tibia is currently missing. The left fibula shows a healed fracture at its distal end (Fig. 5). Both of the clavicles show bone lesions on their inferior medial surfaces (rhomboid fossa), the site at which costaclavicuar ligament is attached. When prominent, the rhomboid fossa may be mistaken for an osteolytic lesion (Kumar et al., 1989) (Fig. 6). Osteoarthritis is apparent at the synovial joints and severe osteophytes mostly on the lumbar vertebrae (Fig. 7), which could be attributed to carrying heavy loads on the back (Kim et al., 2012; Alrousan and Abu Dalou, 2013). Although osteoarthritis advances with aging, the current case as being young is a clear indicator of living in a very harsh environment and at the same time enduring a tough daily living activity. The skull exhibits multiple fractures that probably happened perimortem as each fracture did not extend beyond the previous one. Accordingly, the sequence of the fractures can be reconstructed as shown in figure 8 below. The fracture labeled 1 occurred first followed by 2 and then 3. This type of fracture is caused by a blunt force trauma to the left side of the skull. This trauma explains the manner of death, where the individual was found buried in extended not flexed position and at the same time not in a cemetery.

Unfortunately, the bones of the face are missing where nothing can be said about the maxillary teeth. The mandible shows that the left lower second incisor was lost long time before death as the tooth socked was completely healed. The rest of the teeth were still in occlusion at the time of death. Periodontal disease is substantial as there were extensive alveolar bone resorption of the lower jaw. The left and right premolars and molars show extensive oblique dental wear caused by using teeth as tools (Al-Shorman, 2003; Al-Shorman and Khalil, 2006; Alrousan, 2009; 2016) (Fig. 9). The left side shows more oblique wear compared to the right one, which concludes a person who wasprobably left-sided.

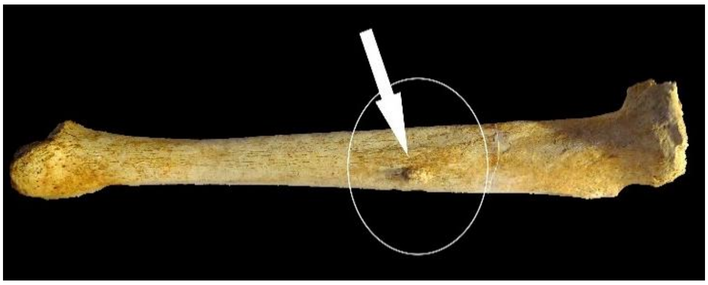

Figure 4: Myositis ossificans of the midshaft of the left Tibia.

Figure 5: Healed fracture of the left fibula.

Figure 6: Right and left clavicle lesions at the rhomboid fossa.

The stature of the individual was estimated using the maximum lengths of the humerus and tibia (31.1 cm and 36.9 cm respectively). The stature based on the humerus is 172.4-181.4 cm and 167.2-175.2 cm based on tibia. The final estimate would be 172.4-175.2 cm. This value falls within the range of the male living population in the country of Jordan (Abu Dalou, 2016).

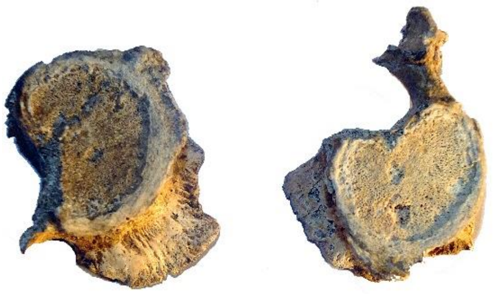

Figure 7: Osteophytosis of the lumbar vertebrae.

Figure 8: The pattern of skull fracture.

Figure 9: the oblique dental wear of the lower jaw.

3.2. The previous conservation of the skeleton

Unfortunately, the previous conservation method and procedures were not documented, which required extra examination and evaluation of the preservation condition of the skeleton. For example, the previous consolidation depended on the use of a regular glue without prior cleaning, and the application method was not thin films. Furthermore, the vertebrae were glued together using the same material without cleaning. Some of these vertebrae glued together using a paste that resembles gums (Figs. 10, 11, and 12). In addition, a piece of newspaper was found glued to some vertebrae.

(A) (B) (C)

Figure 10: (A) The previous conservation in which the adhesive material was used excessively (B) the use of a gum to glue vertebra broken parts together, (C) A piece of a newspaper was placed on one of the vertebrae.

(A) ***(B)***

Figure 11: (A and B) The adhesive material was placed over the dirt.

(A) ***(B)***

Figure 12: (A) The excessive adhesive material inside a bone. (B) The mandible was partially cleaned and it appears with multiple colors.

The length of the display area was 95 cm; not convenient to the stature of the skeleton. The presence of the skeleton in a pit of dirt helps gather insects and fungi (Fig. 13), where they have the ability to digest bone collagen (Metcalf et al., 2016). In addition, these insects can digest calcium and phosphorus of the bones (Brady et al., 2008); a recovered example is silverfish bugs (*Lepisma saccharina*). Humidity plays an important role in the biological deteriorations as a chemical reaction between bone elements and SO_2 or CO_2. Humidity also enhances further microbial attack (Collins et al., 2002). The microbes consume a huge amount of phosphorus for their growth: an element that constitute about 18% of bone weight. In addition, anaerobic microbes produce a number of organic acids (Metcalf et al., 2016), which convert phosphorus insoluble compounds to soluble ones and dissolve the inorganic lattice of bone (Hydroxyapatite: $Ca_{10}(PO_4)_6(OH)_2$). In addition, fungi are among the agents that are responsible for the deterioration of organic materials and produce a number of enzymes that contribute to collagen autolysis (Hiller et al., 2004).

Figure 13: The method of old display, the bones were placed incorrectly.

3.3. pH value

The pH was measured for soil samples that were attached to the surface of the bones using pH mater in order to control further diagenesis. Three readings were taken at different time intervals. The results show an average pH of 5.9, which means an acidic environment. Some bacterial species are able to live in such an environment (Gerardi, 2006). Furthermore, the acidic soil dissolves the hydroxyapatite of bones (Abdel-Maksoud and Abdel-Hady, 2011) and soften collagen (Al-Shorman, 2013).

3.4. The microbiological test

The method isolates, identifies, and treats the microbes that are responsible for the deterioration of the archaeological bone. The results revealed the presence of *Aspergillus niger*, *Penicillium chryso-genum*, *Penicillium digitatum*, and yeasts. The bacterial species are *Corynebacterium aquatium*, *Corynebacaterium pyogemes*, *Escherichia coli*, *Pseudomonas pseudoaclingenes*, *Staphylococcus aureus*, *Salmonella enterica*, and *Corynebacterium pseudodiphthriticum*. The study also shows that the concentration of the bacteria (Colony-Forming Units/ml: CFU/ml) on the sampled bones is very high compared to the other displayed artifacts (Fig. 14).

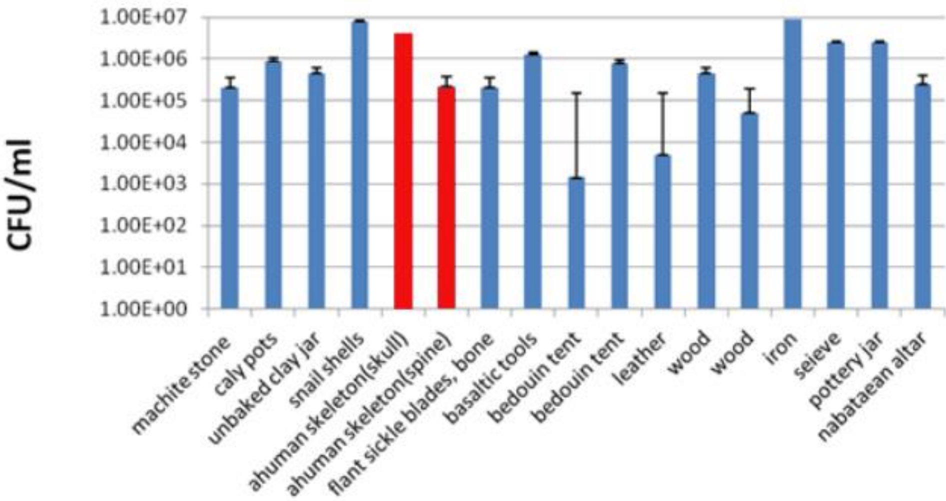

Figure 14: The percentages of bacteria in the skeleton and the other materials in the museum.

3.5. X-Ray diffraction

This technique characterizes the various crystal compounds attached to the bone surface or incorporated into its lattice structure due to soil bone exchange. Samples for XRD analysis were taken from the broken parts of the skeleton. The results showed the presence of fluoroapatite (Fig. 15). The fluorine enters the bones after they are buried as fluorides and/or dissolved fluorine, where it replaces the

(OH) group in the hydroxyapatite forming fluroapatite ($Ca_{10}(PO_4)_6F_2$). The percentage of fluorine increases in bones as along as the burying period increases. The formation of fluoroapatite took place while the skeleton had been buried not during exhibition.

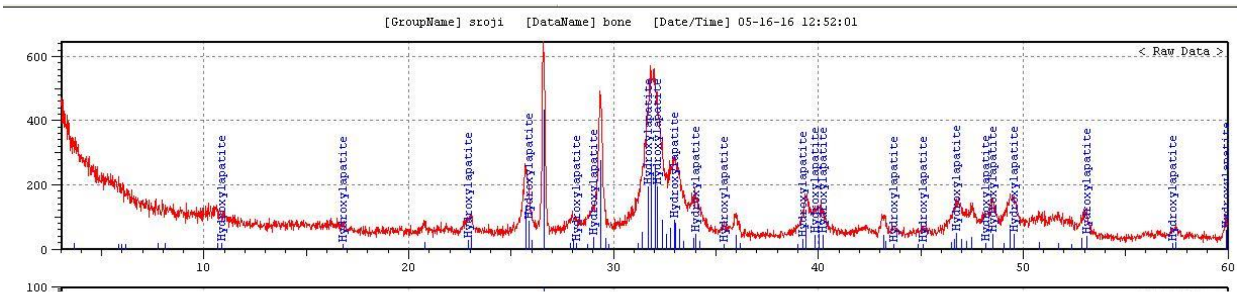

Figure 15. XRD results for a sample from the skeleton

3.6. X-Ray fluorecence

X-ray fluorescence is a technique that evaluates the presence of exogenous elements in the sampled bones to evaluate the preservation condition of the skeleton. The XRF analysis of the sampled bones (Fig. 15) showed no exogenous minerals attached either to the surface of the samples or the chemical structure except for fluoroapatite. This means that there is no chemical damage to the samples.

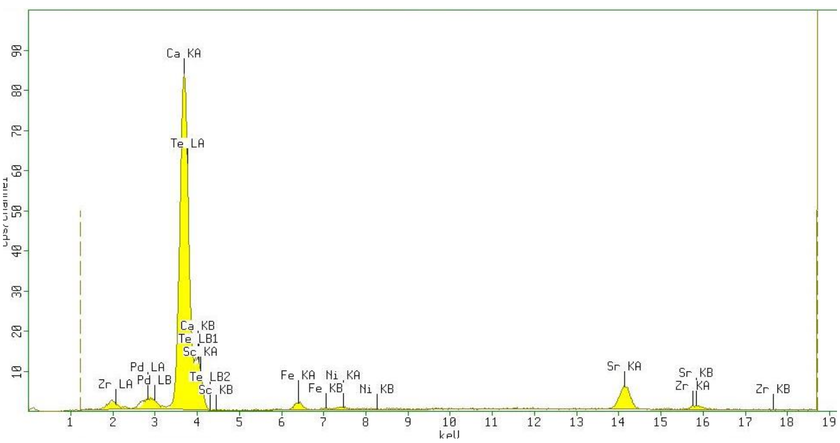

Figure 16: XRF results for a sample from the skeleton (a fragment from Tibia).

3.7. Fourier transform infrared spectroscopy

Although FTIR has the potential to characterize the organic component of the sampled bones, it is used here to evaluate digenesis through examining the crystallinity of bones. The results of the FTIR analysis showed a peak of absorption at 571 cm^{-1} and 608 cm^{-1} (Fig. 17). The heights of these peaks were used to calculate the Crystallinity Index (CI) of the sampled bone after Holland et al. (2013) and Al-Shorman (2013). A baseline correction then was drawn between 750-495 cm^{-1}. The CI is calculated to be 3.1 and thus indicating no digenesis (Cruz Baltazar, 2001), and thus supporting the previous XRF results. In addition, the FTIR analysis supports the XRD one regarding the presence of fluoroapatite in the samples, where a peak at 1096 cm^{-1} is noticed.

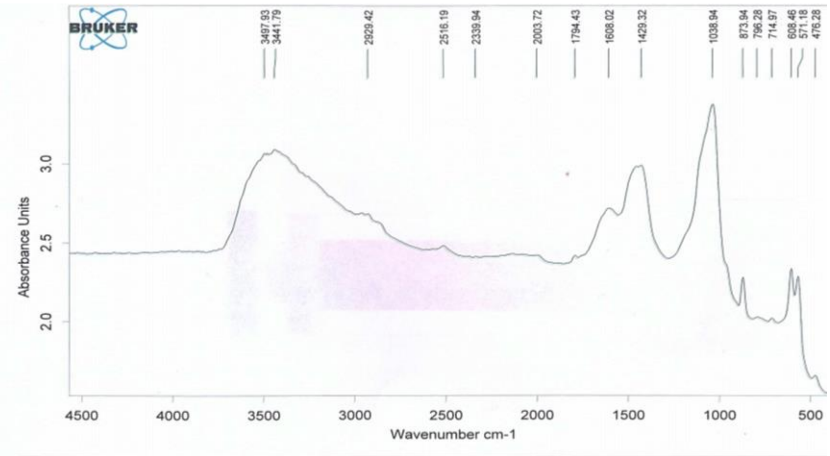

Figure 17. FTIR results for a sample from the skeleton (A fragment from a rib as it's the most vulnerable to digenesis due to its thin cortical bone).

4. Removal of the previous conservation materials

The skeleton was cleaned mechanically by the use different types of paint brushes in order to remove the accumulated dirt (Fig. 18). Then the cleaning process continued using a wood spatula, a medical scalpel, and dentist's tools in order to remove the calcified mud on the bones. Chemical cleaning was then used to remove old consolidants using organic diluted solutions (Ethyle Alcohol: C_2H_5OH) with a concentration of 80% after Johnson (1994).

A **B**

Figure 18. The mandible (A) before and (B) after the cleaning and conservation processes.

Gluing the broken parts

After the application of the mechanical and chemical cleaning, several broken bones were carefully identified and glued using Paraloid B 72 with a concentration of 10%(Podany et al., 2001). Previous studies revealed that Paraloid B 72 is considered as one of the best adhesive materials in bone conservation and can be used under room temperature. Paraloid B 72 is a reversible adhesive material, transparent, and its color is temperature resistant (Brielery, 2010).

Consolidation and coating of the skeleton

The consolidation and coating is one of the most important steps in conservation of archaeological bones. A thin film of Paraloid B 72 w/aceton (5% concentration) was applied for consolidation (Podany et al., 2001). The consolidation procedure was applied under room temperature (Turner, 2007).

The display of the skeleton

The skeleton was displayed in a showcase (Fig. 19). It was laid according to the anatomical position taking into consideration the stature of the skeleton. Light-Emitting Diode was used in the showcase, which is not harmful compared to previous ultraviolet or infrared lights. Ultraviolet and infrared lights generate heat that degrade the organic materials in bones. Furthermore, the glass showcase ensures a clean environment with no dirt, dust, or pollutants that may affect the archaeological bones negatively (Ambrose and Paine, 2007).

A

B

Figure 19: (A) before conservation and (B) after conservation.

5. CONCLUSIONS

The individual of this study had lived 1000 years before the climate began to ameliorate as glaciers in the northern latitudes began a relatively rapid retreat (Olszewski, 2008). Around 15000 BP vegetation communities like the Mediterranean forest became much broader in distribution, as temperatures became somewhat warmer and humidity increased (Baruch and Bottema 1991: 16). In other words, the individual lived in a very harsh environment with limited food resources that impacted his health. His death was tragic as he died of a blunt force trauma to the head. The previous preservation, conservation and display that were performed after its recovery in 1982 were scientifically incorrect and could have been attributed to further physical and chemical damages. The skeleton has little if any diagenesis as reported by the results of FTIR, XRD and XRF. However, there is considerable microbial attack that could lead to further degradation of the bones.

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