

# ELEMENTAL ANALYSIS OF BEYBAĞ-MUĞLA (TURKEY) BYZANTINE SKELETONS

## BEYBAĞ-MUĞLA (TÜRKİYE) BİZANS DÖNEMİ İSKELETLERİNİN ELEMENT ANALİZİ

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**Anahtar Kelimeler:** İz Element, Kemik Analizi, Paleopatoloji, Beslenme, Bizans Dönemi

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### ABSTRACT

*Skeletal material forms the basis of paleoanthropological studies and they are also valuable sources of information for the assessment of elemental analysis which may provide clues about nutritional status of ancient populations. Study material comprises skeletons from the necropolis of Beybağ-Muğla (Turkey) region belonging to Byzantine period and excavated during 2008 period. 52 femoral samples (19 men, 18 women, 15 children) and 3 soil samples were studied with the aid of XRF spectroscopy. Macro elements such as calcium, iron and phosphorus as well as trace elements such as arsenic, barium, lead, strontium and zinc were evaluated in order to figure out nutritional status of this Byzantine population with the help of skeletal elemental analysis. In addition it was also aimed to assess different parts of femoral bone within the study in order to determine the effect of sampling area in this population. Results were evaluated with Kruskal-Wallis and Tukey tests. Strontium values for groups were found significantly higher than soil samples. Similarly zinc and phosphorus values for groups were also found significantly higher than soil. However barium, iron and iodine values were found similar to soil samples and affected by diagenetic rather than a biogenetic source. Phosphorus content of bone material was high and Ca/P rate was found lower than observed values in literature which is possibly due to diagenesis. Log (Ba/Sr) rate was*

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*altered because of possible diagenesis for Ba. Since those two ratios are used as indicators of nutrition of the individual, presence of potent diagenesis prevents conclusion on nutrition and diet choices such as marine/ terrestrial for Beybağ-Muğla population. Since aim of this study was to assess bone material of this high anemia and other paleopathological disease bearing population in elemental analysis a malnutritional situation was expected. However observation of high zinc and phosphorus compared to literature suggests no lack of protein in diet. In addition low lead and low arsenic values suggest no adverse impact of those toxic elements on physiological state of Beybağ people. According to these findings diet of Beybağ population can be considered within omnivorous limits and without apparent insufficiency of nutrition. However presence of anemia in all of the sample material suggests infections diseases and low hygiene conditions in this population and needs further assessment on burial locations and burial types with less influence of diagenetic processes in order to figure out nutritional status of this population.*

## ÖZET

*İskelet materyali paleoantropolojik çalışmaların temel materyalini oluşturur ve element analizinden elde edilen bilgilerle antik toplumların beslenme durumu hakkında ipuçları sağlar. Çalışma materyali Beybağ-Muğla (Türkiye) bölgesi nekropolünden 2008 yılındaki kazıda elde edilmiş Bizans dönemine ait iskeletleri içermektedir. 52 femur örneği (19 erkek, 18 kadın, 15 çocuk) ve 3 toprak örneği XRF spektroskopisi aracılığıyla araştırıldı. Bizans toplumunun beslenme durumunun iskelet element analizi yardımıyla ortaya konulabilmesi için kalsiyum, demir ve fosfor gibi makro elementlerin yanı sıra arsenik, baryum, kurşun, stronsiyum ve çinko da söz konusu çalışmada incelendi. Buna ek olarak femurun farklı kısımlarının örneklenmesinin bu topluma ait eser element analizindeki etkisini araştırmak da hedeflendi. Sonuçlar Kruskal-Wallis ve Tukey testleri ile değerlendirildi. Gruplar için stronsiyum değerleri toprak örneklerine göre anlamlı derecede yüksek bulundu. Benzer şekilde grupların çinko ve fosfor değerleri de toprağa göre anlamlı derecede yüksek tespit edildi. Ancak baryum, demir ve iyot değerleri toprak örnekleri ile benzer bulundu ve biyogenetik bir kaynaktan çok diyagenetik olaylardan etkilenmiş olarak tespit edildi. Kemik materyalinin fosfor içeriği yüksekti ve Ca/P oranı muhtemelen diyagenezle bağlı olarak literatürden yüksek bulundu. Log (Ba/Sr) oranı muhtemelen baryumun diyagenezden etkilenmesine bağlı olarak farklı bulundu. Bu iki oran bireyin beslenmesinin belirteçleri olarak kullanıldığı için muhtemel diyagenezin güçlü etkisi Beybağ-Muğla toplumunun beslenmesi ve denizel/karasal gibi diyet tercihleri hakkında yargıya varmayı engellemektedir. Bu çalışmanın amacı yüksek oranda anemi ve diğer paleopatolojik hastalıkları taşıyan bu toplumun kemik materyalinin element analiziyle değerlendirilmesi olduğu için bir malnütrisyon durumu beklenmekteydi. Ancak çinko ve fosforun literatüre göre yüksek düzeyde gözlenmesi diyetle protein açısından bir eksikliğin olmadığını düşündürmektedir. Buna ek olarak düşük kurşun ve düşük arsenik değerleri bu toksik elementlerin Beybağ toplumunun fizyolojik durumuna olumsuz bir etkisi olmadığını düşündürmektedir. Bu bulgulara göre Beybağ toplumunun diyetinin omnivor limitler içinde olduğu ve belirgin bir beslenme bozukluğunun gözlenmediği yargısına varılabilir. Ancak incelenen materyallerin tümünde aneminin varlığı bu toplumda enfeksiyon hastalıklarını ve düşük hijyen koşullarını düşündürmektedir. Bu toplumun beslenme durumunun ortaya konulabilmesi için diyagenetik süreçlerden daha az etkilenen gömü alanlarının ve gömü tiplerinin değerlendirilmesine ihtiyaç duyulmaktadır.*

## 1. INTRODUCTION

Human body is composed of elements dominated by carbon, hydrogen and oxygen but also encompasses more than 80 elements classified in the periodic table. Many of them can be incorporated into the bone tissue<sup>1</sup>. Amount consumed in foods defines the term macro and microelements in diet. Macro elements which are consumed in large quantities are carbon, hydrogen, nitrogen, oxygen, calcium, potassium, phosphorus (P) and sodium (Na). Whereas strontium (Sr), barium (Ba), selenium (Se), lead (Pb) are classified as micro or trace elements. These are found in very low quantities in bone tissue. This quantity may be as low as parts per million (ppm) or billion (ppb)<sup>2</sup>. However this minute contribution into bone tissue does not lessen the physiological importance of trace elements. In various parts of the world alterations in the trace elements in soil is correlated with certain diseases such as Kashin-Beck disease (osteoarthritis deformans endemica) found in Northern China, Northern Korea and Siberia. This disease is found to be related by low selenium content of soil<sup>3</sup>.

Use of physicochemical analysis techniques in anthropological studies brought a new area to obtain more data from the skeletal materials in this field. Presence of many of the diseases which existed during historical periods was found with the help of science of paleopathology which deals with the remnant skeleton material of humans. Metabolic diseases which form an important portion of those diseases have direct relation with nutrition. Stable isotopes of some elements and trace elements in the periodic table are among the mostly investigated substances. Both techniques base on the analysis of bone and teeth which are the most durable tissues in the body that can withstand the disintegration occurred during postmortem period. Amount of those elements in those tissues and their ratios to one another gives valuable information in the field of nutrition and related paleopathological conditions<sup>4</sup>. Since elemental compositions of soils from different parts of the world are vastly dissimilar, this situation avoids comparison between anthropological materials from different studies. Nevertheless comparisons of elemental content of skeleton materials from the same burial site give valuable information<sup>5</sup>. Another important detail in this subject is the dissimilarity of data obtained from teeth and skeletal material. Teeth are almost completely formed at the early stages of life. On the contrary bone tissue is a

dynamic structure in which osteoclastic and osteoblastic cells continue to break down and build up bone tissue throughout lifetime. This disparity in the exposure of teeth and bone tissues to different environmental and nutritional impacts give way to assess different conditions faced in distinct phases of lifetime<sup>6</sup>. Elements widely used for clarifying nutritional status and nutrition of past humans are Ba, Sr, Iron (Fe), Copper (Cu), Cadmium (Cd), Silicon (Si), Pb, Se and Iodine (I)<sup>7</sup>. Accumulation of those elements in the bone tissue may be affected from various factors. Food choice of humans may include more animal or plant based food or a balanced diet. This variation is reflected in the bone elemental composition. Calcium (Ca), Ba and Sr which are all members of 2A group of the periodical table are found in soil, plant and animals within the food web. Close physicochemical properties of those elements cause similar accumulation in bone tissue.

Animal body can incorporate all three elements however they favour Ca when it is available. Since absorption of Ca and excretion of Sr is high in the kidneys, Ca accumulation in the organism is much higher than Sr in animals compared to plants and in carnivores compared to herbivores<sup>8</sup>. Sr and Ba are high in plant food resources and so in bones of individuals which have higher plant sources in the daily nutrition. Similarly silicon can be found in higher amounts when plant based food dominates the diet<sup>9</sup>.

Pb is accepted as a toxic element for organisms and its physiological significance is not known yet. Although its toxicity and related symptoms are most visible at central nervous system its accumulation occurs mostly at bone tissue. Copper is another element which can cause toxicity however its adequate amounts are necessary for enzymes of antioxidant system in the body. It also accumulates in the bones. Those elements can occur in food as well as they can be found as a result of contamination from the storage cups to the food<sup>10</sup>. Similarly water pipes can contaminate the water inside them. Apart from the accumulation of such elements in the skeleton due to the food or water consumed by the people, diagenesis can be a source of augmented copper or zinc (Zn) content<sup>11</sup>.

Although Pb and mercury (Hg) can cause very distinct intoxications when they are found in very low quantities, all elements may cause toxicity above certain levels. Fe is an important element for oxygen transport via hemoglobin as well as for oxidant/antioxidant enzyme systems. Its decrease in the body leads to anemia whereas its over accumulation

<sup>1</sup> Smrcka 2005, Bowen 1979, Emsley 1998

<sup>2</sup> Smrcka 2005.

<sup>3</sup> Sokoloff 1988.

<sup>4</sup> Trimble/Macko 1997; Lee-Thorp/Sponheimer/Vander Merwe 2003.

<sup>5</sup> Katzenberg/Saunders 2008.

<sup>6</sup> Grupe 1995.

<sup>7</sup> Smrcka 2005.

<sup>8</sup> Smrcka 2005.

<sup>9</sup> Aksoy 2007.

<sup>10</sup> Smrcka 2005.

<sup>11</sup> Zapata/Perez-Sirvent/Martinez-Sanchez/Tovar 2006.

in the body may lead to pathological conditions<sup>12</sup>. Under normal circumstances over accumulation of a trace element in the body is rare but it may occur due to geological conditions of the inhabited region, food preparation techniques and used materials for food and water.

Various techniques are applied for the analysis of elements from bone or earth material. Gas chromatography, atomic absorption spectroscopy, atomic emission spectroscopy XRF (X-Ray fluorescence), ICP-MS are examples for such techniques<sup>13</sup>. Such techniques depend on the fact that properties of atoms in absorbing and emitting energy are different. By their different properties elements can be discriminated from each other. The principles of X-Ray Fluorescence (XRF) analysis is related to optical dispersion spectrograph principles. However in the X-ray fluorescence method excitation of atom happens through X-rays (instead of heat). As the atom numbers increase in the elements, the amount of electrons they have and thus their number of orbitals on which the electrons move increase. If any atom is excited with a high energy radiation like X-Ray, this high energy input brings the electrons in the near orbitals to a higher energy level; when the excited electrons go back to the initial energy level, they discharge the extra energy they had gained in the form of X-Rays. This secondary X-Ray emission is called "fluorescence radiation". The wavelength of these radiations that an element emits is characteristic for that element. In other words, these radiations are like fingerprints of the related elements. By identifying the wave-length of the radiation, the type of the element and by measuring the density of this identified ray, the concentration of the element in that substance can be determined<sup>14</sup>.

Porous structure which gives bone tissue its strength and flexibility also causes its close interference with blood elemental values such as Ca and P<sup>15</sup>. This porous structure is the key for exchange of elements between bone and blood during lifetime and between bone and soil after death. Following the death of individual this close interrelation of bone with blood ends and another interaction starts with soil and burial environment. Some elements in the bone are lost whereas some others may start to accumulate to the bone from the soil such as manganese, Ca and Fe<sup>16</sup>. Ba is more affected from diagenesis although it is a better indicator than Sr for plant based food<sup>17</sup>. Therefore Ba

content of soil should be assessed prior to the use of Ba as a plant based food in order to discriminate whether this Ba is originated from soil diagenesis or the food consumed by the individual. More abundance of an element in soil rather than bone material suggests a diagenetic process. Since Sr and Zn are less affected from diagenesis unlike the Ba it is much safer to use them in nutrition assessment studies<sup>18</sup>. But still soil elemental analysis is essential for confirmation.

In addition to soil analysis, Ca/P ratio in bone can be used as an indicator of diagenesis. Ca/P rate is found at a constant rate in humans. This ratio is 2.16<sup>19</sup>. An attenuation of this value or its increase can be considered as a diagenetic process to the skeletal material. Diagenesis is known to effect bone and dental tissue differently. Teeth are more resistant to diagenesis<sup>20</sup>. Also skeletal tissue is not uniformly affected by diagenesis. Cortical bone is less affected than trabecular bone and surface is more affected than deeper parts of bone<sup>21</sup>. In order to lessen the effect of diagenesis cortical bone surface obtained from areas such as femoral neck is scraped with a suitable material and less affected parts are used for analysis<sup>22</sup>. There are reports stating the impact of sampling area on the bone on the results. Femoral head (spongiosa part of the femoral head) is used but also compact bone extracted from the proximal part (femoral neck) is also assessed<sup>23</sup> since it is known that compact bone is less influenced from diagenesis<sup>24</sup>. Even spatial accumulation of elements is also observed in same site of bone<sup>25</sup>. Therefore additional samplings may aid evaluation of accumulation of elements. Generally bone which have more surface/volume ratios such as ribs are more affected from diagenesis. Due to same physical surface/volume ratio adult bones are less affected from diagenesis compared to children bones<sup>26</sup>.

Another ratio frequently used is the Sr and Ca ratio. High Sr/Ca ratio is an indicator of plant based diet and vice versa<sup>27</sup>. Higher levels in food pyramid causes less Sr/more Ca<sup>28</sup> since animal tissues prefer Ca in favor and Sr levels attenuate compared to lower levels of this pyramid<sup>29</sup>. Humans place somewhere in between carnivorous and herbivorous animals because of their omnivorous

<sup>12</sup> Allen/Gurrin/Constantine/Usborne/Delatycki/Nicoll/McLaren/Bahlo/Nisselle/Vulpe/Anderson/Southey/Giles/English/Hopper/Olynyk/Powell/Gertig 2008.

<sup>13</sup> Katzenberg/Saunders 2008; Smrcka 2005; Johansson/Campbell 1988.

<sup>14</sup> Shackley 2011.

<sup>15</sup> Katzenberg/Saunders 2008.

<sup>16</sup> Sillen/Sealy/vander Merwe 1989

<sup>17</sup> Farnum/Sandford 1997.

<sup>18</sup> Ezzo 1994.

<sup>19</sup> Posner 1969.

<sup>20</sup> Kyle 1986.

<sup>21</sup> Lambert/Weydert-Homeyer 1993.

<sup>22</sup> Lambert/Weydert-Homeyer 1993.

<sup>23</sup> Smrcka 2005.

<sup>24</sup> Katzenberg/Saunders 2008.

<sup>25</sup> Pemmer 2013.

<sup>26</sup> Mays 1998.

<sup>27</sup> Sillen 1992.

<sup>28</sup> Smrcka 2005.

<sup>29</sup> Katzenberg/Saunders 2008.



Figure 1: Muğla - Beybağ Region / *Muğla Beybağ Bölgesi*

property<sup>30</sup>. Also augmented Log Ba/Sr rate in the skeletal material may indicate an augmented marine food in the diet of this human<sup>31</sup>.

Turkey with its long human settlement history starting from prehistoric times provides a vast study area for elemental analysis from burial sites. There are increasing number of studies on the subject presenting different nutritional status of individuals and different burial conditions in the scope of diagenetic processes which alters elemental content of skeletons. Among them Beybağ-Muğla Byzantine population has been studied by different researchers in archaeological and anthropological view. In this population various paleopathological conditions has been found (PhD thesis by S.K. Arıhan). This reminds a possible malnutrition or intoxication case. This study aims to assess nutritional status of this population with the aid of certain chemical elements found in bone material in order to figure out any possible interrelation between those pathologies and nutritional state.

## 2. MATERIALS AND METHODS

### 2.1. Skeletal Material

Research material consists of samples from an excavation study conducted at Beybağ Region (Muğla-Turkey-Byzantine Period) by Prof Dr. Ahmet Tırpan from Konya Selçuk University Department of Archaeology in 2008

<sup>30</sup> Lambert/Vlasak-Simpson/Szpunar/Buikstra 1984.

<sup>31</sup> Smrcka 2005; Burton/Price 2002.

(Fig. 1). This site had been inhabited between 10th century AD and 13th century AD<sup>32</sup> (Fig. 2). Skeletons belonging to 52 individuals were studied in this research. Chronologic determination of skeletons was performed according to archaeological findings. Burials were in different types but all were found with close contact with soil material.

Different settlement periods are shown in different colors as: Bronze age, Byzantine period and Ottoman period

### 2.2. Age And Sex Determination

Determination of age and sex are important subjects in anthropological studies. Following cleaning and repair on skeletal material age and sex determination were performed. Methods accepted for this purpose were applied according to literature records. Morphological features on pelvis and skull of adult individuals were chosen for sex determination. For sex determination Brothwell<sup>33</sup>, Buikstra and Ubelaker<sup>34</sup>, Krogman and İşcan<sup>35</sup>, Olivier<sup>36</sup>, Steele and Bramblett<sup>37</sup> and criteria determined at Workshop of European Anthropologists (1980) were used.

<sup>32</sup> Tırpan/Sögüt/Büyüközer 2009.

<sup>33</sup> Brothwell 1981.

<sup>34</sup> Buikstra/Ubelaker 1994.

<sup>35</sup> Krogman/İşcan 1986.

<sup>36</sup> Olivier 1969.

<sup>37</sup> Steele/Bramblett 1988.

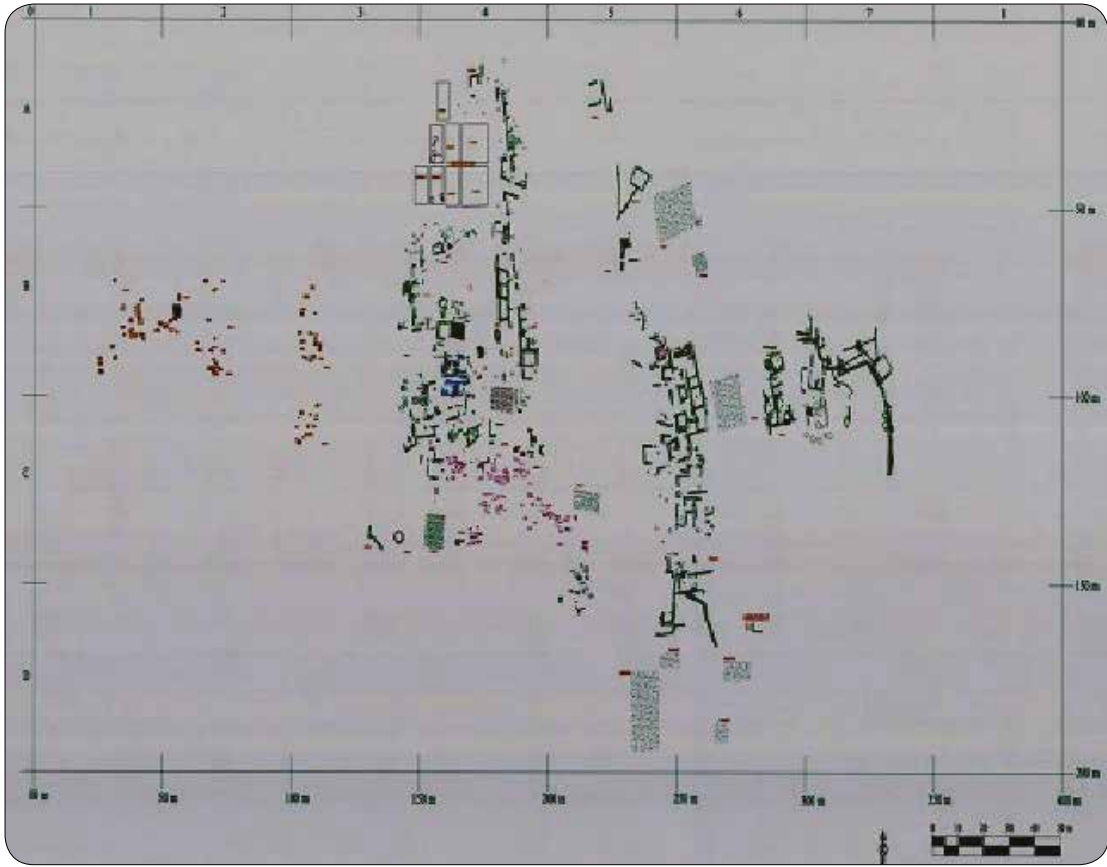


Figure 2: Beybağ Region Excavation Site (Beybağ Excavation Archive) Different Settlement Periods are Shown in Different Colors as: Bronze Age, Byzantine Period and Ottoman period / *Beybağ Bölgesi Kazı Alanı (Beybağ Kazı Arşivi). Farklı Yerleşim Dönemleri Farklı Renklerle Gösterilmiştir: Bronz Çağı, Bizans Dönemi ve Osmanlı dönemi*

For age determination of adults symphysis pubis found on pelvis<sup>38</sup> and auricular surface morphology<sup>39</sup>, degree of sutural closure on skull<sup>40</sup>, cross section of clavícula body<sup>41</sup>, teeth erosion<sup>42</sup>, changes of costae on sternal end<sup>43</sup> proximal cross section of humerus and femur<sup>44</sup> were preferred.

For age determination of young adults methods such as epiphyseal aging and symphyseal aging<sup>45</sup>, dental erosion<sup>46</sup>, complex aging<sup>47</sup> were used.

Epiphyseal aging in young adults<sup>48</sup> and tooth eruption as well as root length were chosen for children<sup>49</sup>. For aging

of childrens and babies measurement of maximal long bones<sup>50</sup>, and tooth eruption times were<sup>51</sup> chosen.

Mean age of males, females and childrens were 39±12, 41±9 and 5±4 respectively. At least one of the pathological signs of anemia (cribra orbitalia, porotic hyperostosis and thickening of skull bones) were observed in all of the individuals, studied in this research (PhD thesis by S.K.Arihan)

### 2.3. Elemental Analysis

A total of 52 skeletal (19 male, 18 female and 15 childrens) and 3 soil samples were investigated for this population. 3 samples from man and woman are from the same individuals sampled from two different areas of the femoral bone; femoral head (spongiosa part of the femoral head) and femoral neck (at the opposite position of lesser trochanter). Therefore we aimed to assess different parts of the femoral bone to determine the effect of sampling area.

<sup>38</sup> McKern/Stewart, 1957.

<sup>39</sup> Lovejoy/Richard/Meindl/Thomas/Pryzbeck/Robert 1985.

<sup>40</sup> Olivier 1969.

<sup>41</sup> Kaur/Jit 1990.

<sup>42</sup> Brothwell 1981.

<sup>43</sup> Krogman/İşcan 1986; Loth/İşcan, 1989.

<sup>44</sup> Szilavssy/Kritscher 1990.

<sup>45</sup> White/Black/Folkons 2012.

<sup>46</sup> Brothwell 1981.

<sup>47</sup> Workshop of European Anthropologist, 1980.

<sup>48</sup> Brothwell 1981.

<sup>49</sup> Ubelaker 1978.

<sup>50</sup> Workshop of European Anthropologist 1980.

<sup>51</sup> Ubelaker 1978.

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Figure 3: Burning Samples in Stove / *Örneklerin Fırında Yakılması*



Figure 5: Prepared Samples of Males, Females and Children (Left to Right Respectively) / *Hazırlanmış Erkek, Kadın ve Çocuk Örnekleri (Soldan sağa doğru)*



Figure 4: Sample Crushing in Agate Mortar / *Örneklerin Agat Havanda Ezilmesi*

without any hand contact. Ultrasonic device was cleaned with distilled water following cleansing of each sample in order to avoid any cross contamination between samples.

Following washing procedure drying had been performed in stove (Raypa) for 12-15 hours at 100 - 110°C. Dried samples were placed in numbered crucible and burned at stove (Nuve MF 120) for 3 hours at 550°C (Fig. 3). Samples were crushed until they are fine as dust in agate mortar, sieved (Retsch 250 µm) and placed in locked plastic bags. In order to avoid contamination between samples, agate mortar and sieve was cleaned with air pump and alcohol after each sample preparation (Fig. 4, 5).

Samples were prepared for analysis at Historical Material Research and Conservation Laboratory-MAKLAB at Gazi University (Faculty of Fine Arts, Department of Conservation and Restoration of Cultural Properties). By the suggestions made by Katzenberg and Saunders<sup>52</sup> in sample preparation, our bone samples were processed to make them ready for analysis. Samples were mostly chosen from femoral and humerus head. Contact of samples with metal substances was avoided. All samples taken were washed at ultrasonic bath (Transsonic 470/H) for 10 minutes for each sample with distilled water

<sup>52</sup> Katzenberg/Saunders 2008.

The element contents of the skeleton/bone samples from Beybağ-Muğla were determined by using X-Ray Fluorescence (XRF) Analysis Method (PED-XRF). After pulverizing the analysis samples selected for the analysis in an agate mortar, 32 mm discs were formed, each disc was mixed with a chemical (wacks) used in the XRF analysis and placed on the sample stage of the device and was analysed. In this study, X-Lab 2000 model PED-XRF (Polarized Energy Dispersive-XRF) brand spectrometer was used. X-Lab 2000 PED-XRF spectrometer has a property of being capable of analysing the elements from sodium (Na) with atom number 11 to uranium (U) with atom number

Element	Man femoral head	Man femoral neck	Woman femoral head	Woman femoral neck	Children	Soil	P
Arsenic	7.4±0.7 <sup>b</sup>	5.8±1.9 <sup>b</sup>	9.0±1.2 <sup>b</sup>	3.2±0.9 <sup>b</sup>	7.5±0.6 <sup>b</sup>	26.5±9.3 <sup>a</sup>	*
Barium	250.7±14.4 <sup>a</sup>	117.5±23.0 <sup>b</sup>	260.1±20.2 <sup>a</sup>	179.3±22.0 <sup>ab</sup>	292.4±22.2 <sup>a</sup>	237.9±8.9 <sup>ab</sup>	*
Calcium	41.8±1.6% <sup>cd</sup>	54.4±0.8% <sup>ab</sup>	43.3±1.5% <sup>bc</sup>	55.3±0.9% <sup>a</sup>	42.9±1.6% <sup>c</sup>	31.5±4.1% <sup>d</sup>	*
Copper	17.4±1.3 <sup>ab</sup>	1.5±0.2 <sup>b</sup>	18.5±1.9 <sup>ab</sup>	1.53±0.1 <sup>b</sup>	27.7±5.9 <sup>a</sup>	24.17±4.37 <sup>ab</sup>	*
Iodine	10.1±1.2 <sup>a</sup>	3.2±0.7 <sup>b</sup>	9.1±1.1 <sup>a</sup>	2.4±0.0 <sup>b</sup>	7.3±0.9 <sup>a</sup>	6.1±2.0 <sup>a</sup>	*
Iron	2.4±0.3% <sup>a</sup>	0.1±0.1% <sup>b</sup>	2.5±0.3% <sup>a</sup>	0.1±0.0% <sup>b</sup>	2.7±0.3% <sup>a</sup>	3.7±0.3% <sup>a</sup>	*
Lead	13.8±1.1 <sup>a</sup>	4.9±0.8 <sup>b</sup>	14.5±1.0 <sup>a</sup>	4.6±1.1 <sup>b</sup>	16.9±1.2 <sup>a</sup>	17.6±1.5 <sup>a</sup>	*
Phosphorus	27.5±0.9% <sup>bc</sup>	36.3±1.4% <sup>a</sup>	25.6±1.2% <sup>c</sup>	34.1±0.9% <sup>ab</sup>	25.6±1.2% <sup>c</sup>	2.3±1.3% <sup>d</sup>	*
Strontium	131.2±6.7 <sup>a</sup>	133.0±19.0 <sup>a</sup>	144.6±7.1 <sup>a</sup>	138.4±16.1 <sup>a</sup>	131.9±6.7 <sup>a</sup>	92.9±8.0 <sup>b</sup>	*
Zinc	177.5±31.9 <sup>a</sup>	189.4±65.6 <sup>a</sup>	195.7±45.6 <sup>a</sup>	184.7±74.2 <sup>a</sup>	182.1±47.2 <sup>a</sup>	69.4±23.7 <sup>b</sup>	*

Table 1: Results of Analysis from Skeletal Samples from Femoral Head from Man, Woman and Children and Soil Samples. Data were Statistically Evaluated with Kruskal-Wallis and Tukey Tests. Statistically Homogenous Subsets for Groups are Shown in a, b, c and d Letters. Statistical Significance was Accepted as \*P<0.05. Data are Presented as Mean ± Standard Error of Mean. (n=19 for Man, n=18 for Woman, n=3 for Soil). Iron was Given in the Form of % Fe203, Calcium in the Form of % Ca0 and Phosphorus in the Form of P205. / Erkek, Kadın ve Çocuk Femur İskelet Örnekleri ve Toprak Numuneleri için Analiz Sonuçları. Veriler Kruskal-Wallis ve Tukey İstatistik Testleriyle Değerlendirildi. İstatistik Açısından Homojen Gruplar a, b, c ve d Harfleri ile Gösterildi. İstatistik Anlamlılık \*P<0.05 Olarak Kabul Edildi. Veriler Ortalama ± Standart Hata Olarak Gösterildi. (Erkekler için n=19, Kadınlar için n=18, Toprak için n=3). Demir % Fe203 Cinsinden Verildi. Kalsiyum % Ca0 Biçiminde ve Fosfor P205 Cinsinden Verildi.

92. The sensitivity threshold of the device is 0.5 ppm for heavy elements and about 10 ppm for light elements. In this study approximately 50 elements could be identified. Because of the weight loss (LOI) at high temperature (950°C) lithium, boron and fluorine cannot be detected. In the analysis major and minor elements are given in the form of oxide percentages (%) and trace elements are given in one in a million (ppm) concentration. In the analysis the USGS (U.S. Geological Survey) standards and as reference GEOL, GBW-7109, and GBW-7309 were used.

## 2.4. Statistical Analysis

Data were statistically evaluated with Kruskal-Wallis and Tukey test. P<0.05 was accepted as statistically significant. Data is presented as mean ± standard error of mean.

## 3. RESULTS

Sr values were found as 131.2 ppm±6.7 for man femoral head, 144.6 ppm ±7.1 for woman femoral head, 131.9

ppm±6.7 for children and 92.9 ppm±8.0 for soil samples. Values of all three groups were found significantly higher than soil samples (p<0.05). However, no significance were found between man, woman and children groups. Femoral neck values were found as 133.0 ppm ±19.0 in man and 138.4 ppm ±16.1 in woman (Table 1).

Ca values were found as 41.8 % ±1.6 for man femoral head, 43.3 %±1.5 for woman femoral head, 42.9 % ±1.6 for children and 31.5 % ±4.1 for soil samples. Values of all three groups were found significantly higher than soil samples (p<0.05). Femoral neck values were 54.4 % ±0.8 for man and 55.3 % ±0.9 for woman (Table 1).

Ba values were found as 250.7 ppm ±14.4 for man femoral head, 260.1 ppm ±20.2 for woman femoral head, 292.4 ppm ±22.2 for children and 237.9 ppm ±8.9 for soil samples. No difference was observed with groups and soil (p>0.05). Femoral neck values were 117.5 ppm ±23.0 for man, 179.3 ppm ±22.0 for woman (Table 1).

Zn values were found as 177.5 ppm ±7.2 for man femoral head, 195.7±11.86 for woman femoral head, 182.1 ppm ±12.2 for children and 69.4 ppm ±13.7 for soil samples. All values of groups were found significantly higher than



soil samples ( $p < 0.05$ ) (Femoral neck value was found as 189.4 ppm  $\pm 37.9$  for man and 184.7 ppm  $\pm 42.8$  for woman. Table 1).

P values were found as, 27.5 %  $\pm 0.9$  for man femoral head, 25.6 %  $\pm 1.2$  for woman femoral head, 25.6 %  $\pm 1.2$  for children and 2.3 %  $\pm 1.3$  for soil samples. Values found for P for man, woman and children groups were significantly higher than soil samples ( $p < 0.05$ ). No significant difference was found between man, woman and children. Femoral neck values were found as 36.3 %  $\pm 1.4$  for man and 34.1 %  $\pm 0.9$  for woman (Table 1).

I values were found as 10.1 ppm  $\pm 1.2$  for man femoral head, 9.1 ppm  $\pm 1.1$  for woman femoral head, 7.3  $\pm 0.9$  for children and 6.1 ppm  $\pm 2.0$  for soil samples. Femoral neck values were 3.2 ppm  $\pm 0.7$  for man and 2.4  $\pm 0.0$  for woman. Femoral neck values were found significantly lower than those for femoral head and soil sample values ( $p < 0.05$ ).

Fe were found as 2.4 %  $\pm 0.3$  for man femoral head, 2.5 %  $\pm 0.3$  for woman femoral head, 2.7 %  $\pm 0.3$  for children and 3.7 %  $\pm 0.3$  for soil samples. Femoral neck values were found as 0.1 %  $\pm 0.1$  for man and 0.1 %  $\pm 0.0$  for woman. Femoral neck values were found significantly lower than femoral head samples ( $p < 0.05$ ) (Table 1).

Cu were found as 17.4 ppm  $\pm 1.3$  for man femoral head, 18.5 ppm  $\pm 1.9$  for woman femoral head, 27.7 ppm  $\pm 5.9$  for children and 24.2 ppm  $\pm 4.4$  for soil samples ( $p > 0.05$ ). Femoral neck values were found as 1.5 ppm  $\pm 0.2$  for man and 1.5 ppm  $\pm 0.1$  for woman. Femoral neck values were found significantly lower than femoral head samples ( $p < 0.05$ ) (Table 1).

Pb were found as 13.8 ppm  $\pm 1.1$  for man femoral head, 14.5 ppm  $\pm 1.0$  for woman femoral head, 16.9 ppm  $\pm 1.2$  for children and 17.6 ppm  $\pm 1.5$  for soil samples ( $p > 0.05$ ). Femoral neck values were found as 4.9 ppm  $\pm 0.8$  for man and 4.6 ppm  $\pm 1.1$  for woman. These values found for man and woman were significantly lower other groups ( $p < 0.05$ ) (Table 1).

As were found as 7.4 ppm  $\pm 0.7$  for man femoral head, 9.0 ppm  $\pm 1.2$  for woman femoral head, 7.5 ppm  $\pm 0.6$  for children and 26.5 ppm  $\pm 9.3$  for soil samples. Femoral neck values were found as 5.8 ppm  $\pm 1.9$  for man and 3.2 ppm  $\pm 0.9$  for woman. Values of all groups were found significantly lower compared to soil samples ( $p < 0.05$ ) (Table 1).

Sr/Ca rate was found as 3.14 for man femoral head, 3.34 for woman femoral head and 3.07 for children samples. Same ratio was found as 2.44 for man femoral neck and 2.5 for woman femoral neck samples.

Log (Ba/Sr) rate was found as 0.28 for man femoral head, 0.25 for woman femoral head and 0.35 for children. Same rate was found as -0.05 for man femoral neck and 0.11 for woman femoral neck samples.

Ca/P rates were found as 1.52 for man femoral head, 1.69 for woman femoral head and 1.68 for children. Same rate

was found as 1.50 for man femoral neck and 1.62 for woman femoral neck samples.

#### 4. DISCUSSION

In this study elemental analysis of 52 samples from Beybağ-Muğla (Turkey) Byzantine population was conducted. On the bone samples 3 macro (Ca, P and Fe) and 6 trace elements (As, Ba, Pb, Sr, Zn and I) analyses were performed. For the analyses, femoral head samples were used. In order to determine any possible differences in different parts of bone material, femoral neck from 3 samples of man and woman were compared with samples obtained from femoral head.

Sr values of man, woman and children are significantly higher than soil samples from the graves, suggests that this Sr content reflects food origin rather than diagenesis. Comparison of trace element content of bone material and soil samples are a common method for determining the impact of diagenesis<sup>53</sup>. Similarly close values observed in femoral neck and femoral head samples for Sr supports the possibility that diagenetic processes did not have a significant impact for this element. No significant difference between man and woman were observed (Table 1). Beybağ values (man 131.2 ppm, woman 144.6 ppm) were similar with an ancient Kelenderis population (man 198.2, woman 179 ppm)<sup>54</sup> whereas much lower than found in ancient İkiştepe population where values for adults vary between 674 to 757 ppm. Soil Sr for İkiştepe population (139 ppm)<sup>55</sup> was also higher than Beybağ population (92.9 ppm).

Ba is a good indicator of plant based diet however it was shown that it is affected from diagenesis<sup>56</sup>. In Beybağ samples diagenesis is also apparent therefore together with Sr values it avoids conclusion on plant or animal based nutrition for man or woman.

Log (Ba/Sr) rate which is an indicator of marine/terrestrial feeding<sup>57</sup> is found at similar rates in man (0.28) and in woman (0.25) compared to childrens (0.35). It was expected to observe negative values for this ratio whereas we observed altered values compared to literature. Findings for man and woman are at the limit of terrestrial feeding however strong diagenetic impact on Ba avoids such conclusions. Although higher Ba content in children can be related with breast feeding however high diagenesis obscures such conclusions.

<sup>53</sup> Smrcka 2005.

<sup>54</sup> Çirak 2010a.

<sup>55</sup> Özdemir/Erdal 2009.

<sup>56</sup> Katzenberg/Saunders 2008.

<sup>57</sup> Smrcka 2005.

Ca in Beybağ for adults was found higher (adults 41 to 43% and soil 31%) than İkiztepe population population (37 to 41% and soil 10%) (Özdemir and Erdal 2009). Accumulation of Ca and Sr may show different patterns according to their presence in the diet and fiber content of the food. Increased plant fiber may decrease Ca but enhance Sr. In addition Ca content in bone tissue may be subject to diagenesis. According to our study results, Ca was found significantly higher than soil samples except males.

Sr/Ca rate is observed higher in woman (3.34) than man (3.14) and children (3.07). These values are higher compared to İkiztepe bronze age population (Özdemir and Erdal 2009).

Phosphorus was observed in similar quantities in man (27.5±0.9%), in woman (25.6±1.2%) and in children (25.6±1.2%). Although P content was significantly high compared to soil samples (2.3±1.3%) ( $p<0.05$ ), values observed in Beybağ bone samples were much higher compared to literature. This situation reminds strong diagenesis for this element<sup>58</sup>. were found P as 18 % for adults and 4.3 % for soil. Excessive phosphorus due to agricultural practices interferes with biological content of bone post-mortem. In addition to that in a study by Nielsen and Kristiansen<sup>59</sup> a prehistoric Celtic field system in Denmark possessed augmented levels in Na, K, P, Ca, Sr and Mn, whereas an attenuation in Co compared to a reference soil. Beybağ site has been inhabited at least since Bronze age therefore such enhancements in soil is also expected which in turn alter chemical composition of buried bones.

One of the most important indicators of diagenesis is the Ca/P rate<sup>60</sup>. Ca/P rates are 1.52 for man, 1.69 for woman, 1.68 for children at femoral bone samples. This rate is lower than 2.16 which is found as a standart value in other studies<sup>61</sup>. Ca/P values in our study are much lower than other studies performed in Turkey such as, Özdemir 2008, Çırak 2010a and Özdemir and Erdal 2009. This finding seems related with high P content of sampled bone material due to diagenesis since Ca ratios are in parallel with other elemental analysis studies from Turkey. However there are studies which determine Ca/P rate as 5.3 which suggest that this ratio can change and can not be used as a constant for every site<sup>62</sup>.

Zn was found as 177.5±7.2 ppm for man, 195.7±11.86 ppm for woman and 182.1±12.2 ppm for children and

found statistically higher than soil which is 69.4 ppm ±13.7 ppm ( $p<0.05$ ). Zapata<sup>63</sup> found a mean of 133 ppm for a late ancient period, Martinez-Garcia et al (2005) found a mean of 131 ppm in Catagena, Spain. From Anatolian settlements, Ezci<sup>64</sup> found 102.2 ppm for man, 76.5 ppm for woman and 32.3 ppm for soil and Çırak<sup>65</sup> found 154.3 for man and 158.5 ppm for woman. Higher values were found by Güner<sup>66</sup> as 194.3 ppm for man, 156.9 for woman and 72.2 ppm for soil in Adramytteion population. Zn values found for man, woman and childrens in Beybağ population are within the range (150-400 ppm) of omnivors in which the humans are categorized<sup>67</sup>. Conditions where Sr was low whereas Zn was high were related with a diet high in protein with meat, kazein (milk protein), peanut and other protein sources<sup>68</sup>. Çırak<sup>69</sup> concluded on a vegetation dominant diet in ancient Kelenderis population due to findings on Sr (198.2 ppm for males and 179 ppm for females) and Zn (154.4 ppm for males and 158.7 ppm for females). In Beybağ population Sr values were lower and Zn values were higher than values found by Çırak<sup>70</sup>. Therefore, higher protein intake can be considered for Beybağ population compared to this population. Observation of high Zn in our study suggests a diet high in protein. This protein richness is not just related with meat consumption<sup>71</sup>. Therefore according to our results it is not possible to state high meat consumption in this population. Zn is known to incorporate to bone tissue differently due to seasonality<sup>72</sup>. However it is not possible to make such correlations since it is not known the exact burial season of Beybağ samples.

Fe is an important element for nutrition and its low intake may result in diseases such as anemia. Fe was also assessed in our study. Although femoral head has a higher blood circulation compared to femoral neck cortical bone and it's the site of medullary hematopoiesis (blood production) higher Fe content is expected but when high Fe content in the soil samples are considered this discrepancy in both sampling sites reminds an effective diagenesis. In relevant scientific literature Fe in soil was also found in high quantity<sup>73</sup>. In Beybağ population iron was found as 2.4 % for man, 2.5 % for woman, 2.7 % for childrens and 3.7 % for soil ( $p>0.05$ ). This situation prevents evaluation of this important element in this aspect. However femoral

<sup>58</sup> Özdemir/Erdal 2009.

<sup>59</sup> Nielsen/Kristiansen 2014.

<sup>60</sup> Zapata/Perez-Sirvent/Martinez-Sanchez/ Tover 2006.

<sup>61</sup> Posner 1969.

<sup>62</sup> Klepinger/Kurn/Williams 1986.

<sup>63</sup> Zapata/Perez-Sirvent/Martinez-Sanchez/ Tover 2006.

<sup>64</sup> Ezci/Kaya/Erdem/Akay/Kural/Soykut/Boçoğlu/Fenyurt/Kılıç/Temiz 2013.

<sup>65</sup> Çınar 2010a.

<sup>66</sup> Güner/Türksoy/Atamtürk/Duyar 2012.

<sup>67</sup> Lambert/Vlasak-Simpson/Szpunar/Buikstra 1984.

<sup>68</sup> Lambert/Weydert-Homeyer 1993.

<sup>69</sup> Çırak 2010a.

<sup>70</sup> Çırak 2010a.

<sup>71</sup> Katzenberg/Saunders 2008.

<sup>72</sup> Lambert/Weydert-Homeyer 1993.

<sup>73</sup> Larsen 2008.

neck samples present much less iron content showing impact of diagenesis on femoral heads. Ezci<sup>74</sup> found 230 ppm for males, 295 ppm for females and 1278 pp for soil in Camihoyuk population.

Woman was expected to have more I than man because of pregnancy and breastfeeding however results for man (10.1 ppm) and woman (9.1 ppm) are similar and also statistical insignificance with soil shows effect of diagenesis. Nevertheless although different from soil, similar results were observed for man (3.2 ppm) and woman (2.4 ppm) for femoral neck samples.

Insignificant results in copper content of man, woman and soil samples in Beybağ population state high diagenesis for this element. In literature records mean Cu value for ancient populations varies as 12 ppm<sup>75</sup>, 13.4 ppm<sup>76</sup>, 28.5 in İkištepe population<sup>77</sup>, 38.4 in Resuloğlu population<sup>78</sup> and 52.5 ppm for a Jordanian population<sup>79</sup>. In ancient Tios population Cu was found as 28.2 ppm for males and 35.3 for females. In Adramytteion population Cu values were found as 40.6 ppm for man, 48.3 ppm for woman and 30.6 ppm for soil<sup>80</sup>. Tios population Cu levels are also high (28 ppm for man and 35 ppm for woman)<sup>81</sup> as Adramytteion population. Cu values were 27.3 ppm for males and 29.8 ppm for females in İkištepe population<sup>82</sup> which show similar values with Tios population. Since augmented grain consumption attenuates Zn absorption and leading high Cu level in bones<sup>83</sup> it can be interpreted as an indicator of animal based diet. In this aspect low Cu content in femoral neck samples (1.5 ppm for males and females) of Beybağ population suggests a low plant based and high animal food based diet. Compared to other literature records, Beybağ population Cu values are similar to Camihoyuk population (1.2 ppm for males and 0.47 ppm for females)<sup>84</sup>.

As values in Beybağ population presents low arsenic content in bones (man 7.4 ppm, woman 9 ppm and children 7.5 ppm) however soil As content was high (26.5 ppm). In another Anatolian settlement Adramytteion population As values were found higher as 26.6 ppm for man, 35.5 ppm for woman and 7.5 ppm for soil

in<sup>85</sup>. Values of As for another population in Anatolia (İkištepe Early Bronze Age population) were found in between Adramytteion and Beybağ populations. In this population As values were 16.9 ppm for males, 15.0 ppm for females, 14.1 ppm for childrens and 6.0 ppm for soil samples<sup>86</sup>. No metal object findings within graves in Beybağ population eliminates such an interaction with burial objects however significantly lower As content observed in femoral neck samples (5.8 ppm for man and 3.2 ppm for woman) suggests partial impact of diagenesis on this burial site for this element.

Lead is a toxic element with well known physiological outcomes. It accumulates in bones. It has a mean of 5 ppm in ancient populations<sup>87</sup>. Among most potent reasons for lead accumulation in bones, use of lead containing cookware is being accused. In İkištepe population in Northern Turkey high lead values were related with the use of lead made cookware<sup>88</sup>. In Beybağ population information about lead containing cookware is not present however high lead content of soil compared to finding in Minnetpınarı population (male 5.78 ppm, female 5.67 ppm, children 4.63 ppm)<sup>89</sup> reminds a strong diagenetic process. Pb values in ancient populations may vary vastly. Zapata et al<sup>90</sup> found mean Pb for population as 225.0 ppm whereas Gonzalez-Reimers et al<sup>91</sup> found mean Pb for population as 4.06 for a Bronze age population in Spain. Another low Pb finding was from Camihoyuk, Turkey as 3.43 ppm for males, 2.71 ppm for females and 0 ppm for soil samples<sup>92</sup>.

## 5. CONCLUSION

Results of elemental analysis of Beybağ samples present a high diagenetic impact of burial environment on Ba, I, Fe and P. This situation hampers evaluation of Log (Ba/Sr) and assessment of diet choices of Beybağ population such as animal/plant or terrestrial/marine based diet. Although sample sizes are small for femoral neck samples they show great difference with femoral head samples for the elements Ba, Cu, I, Fe and Pb.

High Ba content in all of the groups, observation of Ca/P rate altered from the standart limits in humans and dissimilarity of femoral neck with femoral head for certain

<sup>74</sup> Ezci/Kaya/Erdem/Akay/Kural/Soykut/Boçoğlu/Fenyurt/Kılıç/Temiz 2013.

<sup>75</sup> Zapata/Perez-Sirvent/Martinez-Sanchez/ Tover 2006.

<sup>76</sup> Martinez-Garcia 2005.

<sup>77</sup> Özdemir 2010.

<sup>78</sup> Güner 2011.

<sup>79</sup> Grattan/Abu Karaki/Hine/Poland/Gilbartian/Al-Saad/Pyatt 2005.

<sup>80</sup> Güner/Türksoy/Atamtürk/Duyar 2012.

<sup>81</sup> Çırak/Akyol 2015.

<sup>82</sup> Özdemir/Erdal/Demirci 2010.

<sup>83</sup> Ezzo 1994.

<sup>84</sup> Ezci/Kaya/Erdem/Akay/Kural/Soykut/Boçoğlu/Fenyurt/Kılıç/Temiz 2013.

<sup>85</sup> Güner/Türksoy/Atamtürk/Duyar 2012.

<sup>86</sup> Özdemir/Erdal/Demirci 2010.

<sup>87</sup> Becker/Spadaro/Berk 1968.

<sup>88</sup> Özdemir 2008.

<sup>89</sup> Çırak/Akyol 2014.

<sup>90</sup> Zapata/Perez-Sirvent/Martinez-Sönehez/Tovar 2006.

<sup>91</sup> Gonzales-Reimers/Velasco-Vázquez/Arnay/de/la/Rosa/Santolaria/Fernández/Galindo/Martin 2001.

<sup>92</sup> Ezci/Kaya/Erdem/Akay/Kural/Soykut/Başoğlu/Fenyurt/Kılıç/Temiz 2013.

elements demonstrate a strong impact of diagenesis for this burial site. When Zn is considered as a non affected element from diagenetic processes it is possible to state that man and woman in this population had similar protein intake. Similar content of Sr in both genders suggests a similar plant source was incorporated into diet. However Sr/Ca rate observed in woman (3.34) is higher than man (3.14) and childrens (3.07). More animal food in the diet was mentioned in past populations such as İkiztepe population<sup>93</sup>.

Low As, Cu and Pb levels in Beybağ samples show no sign of toxication by those elements. This can be concluded as no potential impact of those elements on the observed paleopathological signs on this population samples.

Although sample sizes are small for femoral necks, comparison of femoral head to femoral neck bone values for certain elements reveals importance of sampling area from bone tissue. In areas such as Beybağ region where burial types are simple earth grave, agricultural practices are frequent and contamination with diagenetic processes are augmented, sampling from femoral neck may prevent loss of information from elemental analysis.

As a result it can be concluded from the data obtained from this study, correlation of reported paleopathological findings in Beybağ Byzantine population with any toxication from assessed toxic elements or any apparent lack of macro or micro nutrients is not evident. However presence of anemia signs in all of the sample material suggests infectious diseases and low hygiene conditions in this population and needs further assessment on burial locations and burial types with less influence of diagenetic processes in order to figure out nutritional status of this population.

This study presents an example for assessment of nutritional status of individuals belonging to a high paleopathological disease bearing ancient population with the help of elemental analysis.

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<sup>93</sup> Özdemir 2008.

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