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RELATIVE DATING OF CHEHRABAD (IRAN) SALT MINE MOMMIES ON THE BASIS OF FLUORINE, URANIUM, AND NITROGEN CONTENT (FUN)

Masoud B. Kasiri*, Bistoon Alizadeh

Department of Archaeometry, Faculty of Applied Arts, Tabriz Islamic Art University, Tabriz, P.O. Box: 51385-4567, IRAN

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**Corresponding author: (m.kasiri@tabriziau.ac.ir)*

ABSTRACT

Relative dating of archaeological bones by the measurement of fluorine, uranium, and nitrogen content (FUN method) is a well-established method. In this study, bone samples of Salt Men mummies of Chehrabad salt mine of Zanjan, IRAN, were relatively dated on the basis of FUN method. The amount of fluorine in the selected samples was measured by UV-Vis spectrophotometry, while the amount of uranium and nitrogen was determined by ICP-MS, and CHNS and Kjeldahl techniques, respectively. The results obtained suggest that firstly, corpse no. 3, then corpse no. 5, and finally corpse no. 2 have been buried. Comparing results obtained from FUN tests with radiocarbon ages of the samples showed that this methode could adequetly determined the sequence of the archaeological bones and their burial times. Moreover, this study revealed the high accuracy and precision of FUN relative dating test in the archaeological sites such as salt mines, where the samples are well-preserved, and also showed suitable consistent of the results obtained with the results of radiocarbon dating method.

KEYWORDS: Bones dating; Chehr Abad salt mine; FUN dating; Mummies bones; Relative dating

1. INTRODUCTION

One of the most important concerns of archaeologists, after the discovery of objects during the archaeological excavations, is determining the age of them or in other words, their dating. Developments that have been made at the beginning of 20th century so far in collecting and processing data and archaeological studies have led scientists to finding new approaches to data analyzing, especially to absolute and relative dating methods. For this purpose, different techniques have been developed in the archaeological science to determine the age of an object or to date an event or period. (Renfrew and Bahn, 2008)

Biologists, paleontologists, and pathologists are doing comprehensive studies on the archaeological bone findings, where the first step at such studies is determining the age of findings. Datelining the findings such as bone, provides the possibility to investigate the precedence, posterior or concurrency of different discovered human species in a certain region. There are several ways to determine bone age, that can be classified into two main groups including direct dating and indirect dating methods. In indirect dating of findings, the age of samples is estimated by dating the upper and lower layers that circled the bones. For example, it could be referred to dating the other findings such as pottery and charcoal that could be possibly dated by precise direct dating methods. For direct dating and in possession of a sufficient amount of sample, the absolute dating methods such as radiocarbon (^{14}C) dating or Electron Spin Resonance (ESR) are used. Choosing the method of dating depends on sample rate, the time limit for dating with the considered method, the required precision, the available navigation equipment, and the costs associated with the dating tests (Hashemi Zarj Abad, 2004). If there is no possibility of the absolute dating of samples, direct comparative methods of relative dating can be used. In this case, only the chronological sequence of findings in a certain place is obtained (Bahrololumi, 2014). This idea that something is older (or younger) to something else is the basis of relative dating.

In the relative dating, if the available sample is sufficient (in milligram amounts) the sequence of samples to each other can be determined using the methods of chemical detection such as fluorine, uranium, and nitrogen measurement (FUN method) or amino acid racemization technique.

FUN dating method is based on the measurement of content of three elements of fluorine, uranium, and nitrogen in the samples of the studied bones. Fluorine (F) usually exists in nature as calcium fluoride (CaF_2) and is abundant in the groundwater (Woittiez and Das, 1980). Hydroxyl groups of hydroxyapatite in the structure of the bone or teeth mineral matrix could be substituted by fluoride ions existing in mineral water. (Eq. 1) (Kottler et al., 2002)

$$Ca_{10}(PO_4)_6(OH)_2 + 2F^- \rightarrow Ca_{10}(PO_4)_6F_2 + 2OH^- \quad (1)$$

Due to the intense reaction of fluorine with any other molecules and very poor reaction between fluorapatite and hydroxyl ions, the fluorapatite does not exchange the additional ions anymore. Thus, that is why its concentration at the time of aggregation or accumulation will be up to and stay at about 3.8% of the bone weight. The accumulation of fluorine in bones depends on weather conditions, the chemical composition, moisture content, and status of groundwater and surface flows (Kottler et al., 2002). It has been experimentally found that the concentration of fluorine in different time periods are significantly different, so that the producing of a universal calibration curve has not yet been successfully done. For example, a bone with an age of several thousand years may contain fluorine of those who lived several million years ago (Table 1) (Goksu et al., 1991).

Table 1. Fluorine absorption content (%) in different geological periods (Goksu et al., 1991)

The contemporary era	≤0.3
Pleistocene (the quaternary period of Geology)	1.5
Tertiary (the third period of geology)	2.3
Mesozoic	3.4
Paleozoic	3.7

Due to climate changes during the past years, which could alter the concentration of fluorine in water and given that the reconstruction of past climates is not always possible, instead of comparing the fluoride concentration in the samples with control samples, determining the ratio of fluorine for example to phosphorus pentoxide (P_2O_5), is considered. It should be noted that the new bone may contain about 42% of P_2O_5 (Bahrololumi, 2014).

Uranium (U) that is available in the groundwater, is also absorbed slowly and over a relatively long time into the bone structure, replacing the calcium of hydroxyapatite mineral. Uranium uptake in bone, teeth, and horns, undergoes a process similar to fluorine absorption in these samples, while the main difference is in the rate of uranium absorption. In general, the concentration of uranium is different significantly with the geological environment, so that the granite and volcanic rocks tend to produce large amounts of uranium in the underground water, while the deposits usually contain significantly lower amounts of uranium (Hedges and Millard, 1995). In a process simulated artificially in the laboratory, it was determined that the archaeological samples had a proper fitness with the absorption of uranyl ion (U_2^{2+}) available in the underground water via ion exchange mechanism (Goksu et al., 1991). The uranium dating method, due to the impact of environmental conditions on its rate, is used only for the relative dating. (Bahrololumi, 2014)

Nitrogen (N) exists in amino acids of the collagen structure of the bones and teeth. New bone typically contains 4 to 5% of nitrogen. A study conducted by Jarvis revealed that older people gradually will store less nitrogen in their bones while the nitrogen amount of young people is high (Figure 1). Therefore, a special attention should be paid to this issue and before measuring the nitrogen content of the bone, the approximate age of the body should be determined and then, according to these points, the results must be interpreted. (Jarvis, 1997)

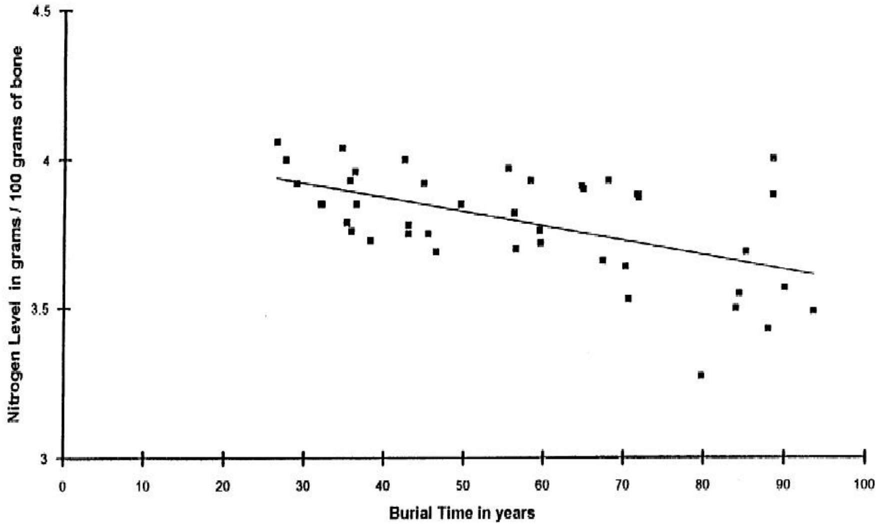

Figure 1. Linear regression of nitrogen content in 100 grams of bone versus burial time (Jarvis, 1997)

The rate of bone proteins degradation depends strongly on the environmental conditions, such as temperature and humidity. Microorganisms also play an important role in removing bone protein (Reiche et al., 2003). But in general, after 250 000 to 300 000 years, the amount of nitrogen in the bones at different temperatures will be very low and almost non-detectable.

In spite of all the above-mentioned points, FUN dating method could be used to determine the relative age of bone samples. (Bahrololumi, 2014)

The present study is a try to evaluate the performance of FUN relative dating method in saline environments, where the priority and posteriority sequence of the salt men bone samples will be determined. To materialize this purpose, fluorine, uranium, and nitrogen content of the samples taken from Salt men mummies of Chehrabad mine, IRAN, were analyzed and the sequence of their burial time was determined.

It must be mentioned that the absolute dating of these mummies has been realized by radiocarbon dating method. (Table 2) (Pollard et al., 2008)

Table 2. Results of radiocarbon dating of Salt men of Chehrabad (Pollard et al., 2008)

OXA number	Mummy	Sample description	C14 age	error	Delta 13C	Delta 15N	Calibrated date BC/AD (95.4%)
OXA-16831	SM2	Human skin	1504	27	-18.2	14.4	80 AD (95.4%) 110 AD
OXA-15222	SM3	Human soft	2336	29	-20.3	13.7	410 AD (95.4%) 540 AD
OXA-16832	SM5	Human skin	2286	28	-20.2	15.1	510 BC (95.4%) 360 BC

2. MATERIALS AND METHODS

Review of published literature shows that many pieces of the skeleton have been selected for elemental analysis, probably depending on the type of bones, the extent of damages, and the storage temperature. Graph has explained the manner and the importance of sampling of ancient bones as well as the scientific basis of the analysis of each part of the skeleton (Grupe, 1988). Considering this scientific rules, the ribs and legs bones of the salt men were selected for realizing the FUN relative dating tests.

2.1. SAMPLE BONES OF THE SALT MEN

Salt mines are places where due to the limited activity of destructive bacteria, the organic materials, like bones, are preserved and remained well. The mummies studied in this research have been found in Chehr Abad salt mine in Zanjan, north-west of IRAN, which is located in a semi-mountainous area containing clay and salt deposits (Figure 2). The skeletons no. 2, no. 3, and no. 5 found at this salt mine were selected for this study and, Figure 3 shows the selected bones of these mummies for running the FUN experiments.

Figure 2. Map of Chehrabd Salt mine located in a semi-mountainous area containing clay and salt deposits

2.2. PREPARATION OF SAMPLES AND MEASURMENT PROCEDURES

The measurement of fluorine, uranium, and nitrogen content of the samples was realized on the basis of different standard methods, as follows:

2.2.1. FLUORINE

First of all, 2 g of bone samples was washed thoroughly to remove the surface sediments, while in the case of high contamination, an ultrasonic bath was also used. Then, the sample was heated up to 70 °C for 48 hours, cleaned using mechanical cleaners, and was powdered by a mortar. One gram of this powder was weighted and poured in a clean flask. Then, 12 ml of perchloric acid ($HClO_4$) along with 12 ml of distilled water is injected into the flask, and finally, 24 ml of Tizab water buffer was added to it (Worbel, 2007). The concentration of fluorine in this solution was measured by an UV-Vis spectrophotometer, model Spectronic Helios-Alpha (England) and a calibration curve. This analysis was repeated two times to minimize the errors of the measurements.

It should be noted that the presence of phosphonate ions causes the test results to be false. These phosphate ions cause the fluoride ions be removed from the bone environment and consequently, its concentration be appeared lower than its actual concentration. Subtraction of phosphate ions content from the measurement results, can provide more verifiable data. (Chlubek et al., 1996)

The following equation is used to calculate the amount of net fluorine in the samples. (Woittiez and Das, 1980) (Eq. 2)

$$\text{(Net fluoride value) } A = \frac{F\%}{P_2O_5\%} \times 100 \qquad (2)$$

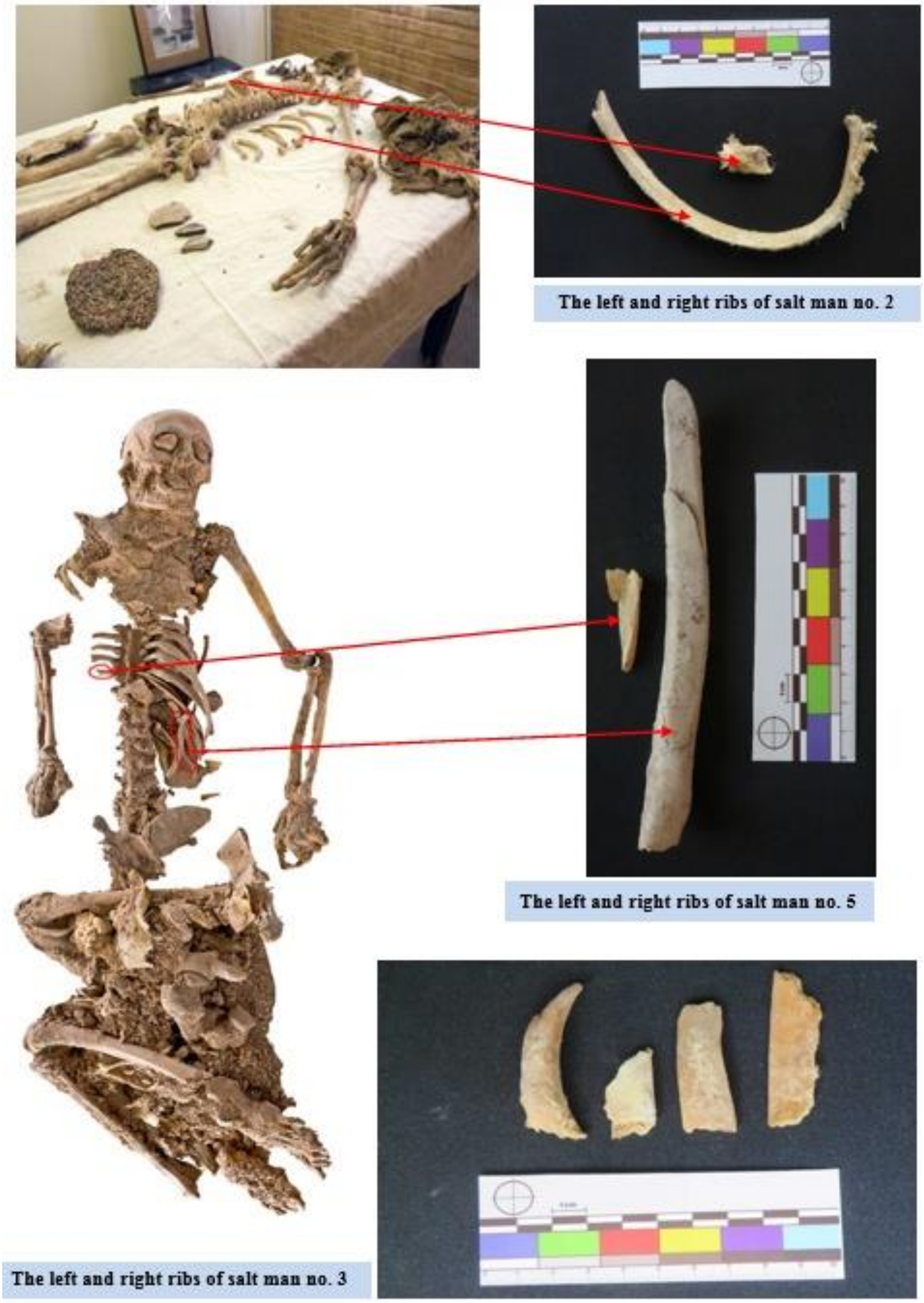

Figure 3. Salt men of Chehrabad mine and the selected bones from mummies no. 2, no. 3, and no. 5

2.2.2. URANIUM

To measure the amount of uranium, the inductively coupled plasma-mass spectrometry (ICP-MS), model Varian Radial 735, manufactured by Agilent America Corporation, was used. The measurement of uranium concentration is as the percentage of U_3O_8 (uranium oxide) that its value in the fresh samples is about 0.02% while in samples dating back 1 million years ago, its value reaches to 600 ppm. (Turner-Walker, 2007)

2.2.3. NITROGEN

To prepare the sample and to measure the concentration of nitrogen content, the samples were first cleaned with distilled water using an ultrasonic bath, and then were powdered with the mill. Globally, different methods can be used to determine the nitrogen concentration, for instance in the chemical method of collagen extraction, 10 mg of the sample is heated to 70 °C in sulfuric acid (H_2SO_4), and then the solution is neutralized by the addition of sodium hydroxide (NaOH) during the cooling. In this study, the amount of nitrogen existing in the bone structure was measured by two methods, CHNS and Kjeldahl techniques (Thompson, 2008). The CHNS analyzer instrument used in this study was a Euro EA 3000 model (Italy). The Kjeldahl method is also the standard method for determining nitrogen content of archaeological samples (Fearon, 1920; Persson, 2008).

To statistically analyze the results obtained and to find out how the relationships between independent and dependent variables are, SPSS software (v 20) was employed.

3. RESULTS AND DISCUSSION

The theory of using the measurement of concentration of the elements in FUN relative dating test is based on the fact that, when the bone uptakes more fluorine and uranium, it is older, and when the amount of nitrogen is high, the age of bone is less.

Measurement of the concentration of fluorine, uranium and nitrogen elements in salt men bone samples, no 2, no.3 and no. 5, and comparing the results obtained, can lead to determine the priority and posterity of their burial time or in other words, their relative dating.

3.1. FLUORINE ANALYSIS

Preparation of samples and measurement of their fluorine content with a UV-Vis spectrophotometer for each sample repeated 2 times and the obtained average results are shown in Table 3. Moreover and using Eq. 2, the net value of fluoride was also calculated for each bone samples. It could be seen that sample no. 2 has absorbed less fluorine and is younger one. Mummy bone no. 5 has absorbed more fluorine than sample no. 2 and less amount than sample no. 3. Therefore, it is older than no. 2 and younger than no. 3.

Table 3. Obtained results of measuring the amount of fluorine, P_2O_2 and calculated net fluorine

Sample type	2			3			5		
	Fluorine (%)	P_2O_5(%)	Net fluoride	Fluorine (%)	P_2O_5(%)	Net fluoride	Fluorine (%)	P_2O_5(%)	Net fluoride
Right ribs	0.98	23.00	4.26	1.44	16.0	9.00	1.01	18.0	5.61
Left ribs	0.97	25.00	3.88	1.40	15.0	9.30	1.06	23.0	4.60

Figure 4 shows, the interval time between 1400 and 2500 years ago, the range of salt men of Chehrabad Salt mine ages, based on the results of the net fluoride content versus the radiocarbon ages.

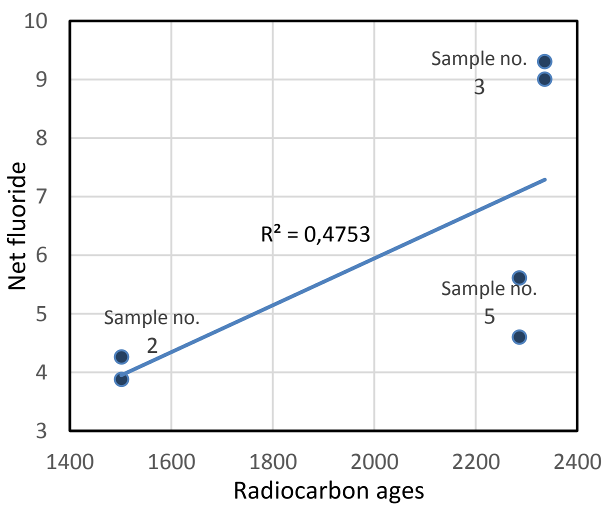

Figure 4: Control chart of the net fluorine content of the samples versus their radiocarbon ages

It is clear that the net fluoride content of the samples is proportional to their absolute radiocarbon ages, but as discussed earlier, there is not an acceptable linear relationship between net fluoride measured and absolute ages. This is due to the different variable affecting the rate of fluorine uptake process by the bone structure. Consequently, a calibration curve could not be obtained for absolute dating of bone samples by net fluoride measurement. Based on the obtained results of the above analysis on the bone samples no. 2, no. 3, and no. 5 (Table 3 and Figure 4), it was determined that the salt man no. 3 has absorbed more fluorine than salt man no. 2 and no. 5. As a result, it is older than two other salt men. The salt man no. 5 has also absorbed more fluorine than salt man no. 2, and indicates that it is older than the salt man no. 2. At the end, the salt man no. 2 has absorbed less fluorine than salt man no. 3 and no. 5, and shows a new burial.

3.2. URANIUM ANALYSIS

The uranium in groundwater is slowly and in relatively long periods of time has been inserted into hydroxyapatite mineral structure (Tomassettil et al., 2014). In this research, the right and left gear of salt men no. 2, no. 3, and no. 5 were sampled and their uranium content was measured two times to achieve high precision. Table 4 shows the results of this measurement.

Table 4. Results of measurements of uranium (ppm) in the bone samples of the salt men samples

Sample no.	2	3	5
Right ribs	0.47	0.67	0.52
Left ribs	0.50	0.93	0.52

The results of the linear regression analysis between the dating of radiocarbon and uranium oxide are presented in Table 5. Based on these results, since the value of Sig. (0.019) in coefficients is less than 0.05, the variables are independent. The amount of b is also greater than zero (1.310), and consequently, there is a direct relationship between the variables selected. Also, the value of R equals to 0.807, and hence, the correlation coefficient of data is also relatively high.

Table 5. Linear regression and coefficient of reliability of uranium ratio with the results of radiocarbon dating

Model	Unstandardized coefficients		Model Summary R	Standardized coefficients	Sig.
	b	Std. error		beta	
(Constant) f.p. ratios	1.310 0.000	0.125 0.000	0.807	0.703	0.068 0.019

The simplest expression of the relationship between two measurements is a straight line. When the data all placed along with each other at the right line, it is said that there is a complete linear relationship between the two variables. The whole power to analyze regression depends on the interpretation of this line and how these data are put on it. (Niknami, 2008)

Figure 5 shows the time interval between 1400 and 2500 years ago in the Chehrabad Salt men's mine area based on the results of measuring the uranium content versus the radiocarbon ages.

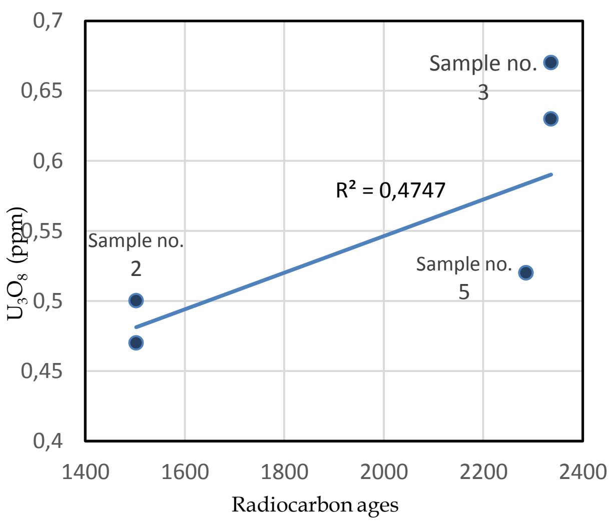

Figure 5. Control graph of the uranium content versus the radiocarbon ages of the salt men

As it could be seen on Figure 4, there is a direct relationship between uranium content of the samples and their absolute radiocarbon ages. But, here again, this relationship is not linear and hence, a calibration curve could not be achieved. Based on the results presented in Figure 5 and Table 4, the salt man no. 3 has absorbed more uranium than no. 2 and no. 5. As a result, it is older than two other salt men. Also, the salt man no. 5 has absorbed more uranium than salt man no. 2, which means that it is older than salt man no. 2. And finally, salt man no. 2 has also absorbed less uranium than salt men no. 3 and no. 5, which shows a new burial.

3.3. NITROGEN ANALYSIS

To judge about the dating of specimens based on the measurement of the amount of nitrogen in the bone, the control charts should be plotted using the ratio of nitrogen to phosphorus (N/P) and determination of this ratio with age-specific samples, as well as the preparation of control charts using the ratio of carbon to nitrogen (C/N). The results obtained from the analysis of salt men ribs bone samples by ICP-MS, are shown in Table 6. Due to the effect of environmental conditions in the process of reducing the concentration of nitrogen in the bone, the control charts should be plotted for different regions individually using well-known examples. Nitrogen dating method is particularly useful in cases where the sample size is insufficient for annual aging, however, the results of this type of dating should not be interpreted as the absolute age of the sample.

Table 6. Amount of carbon and phosphorus (%) in bone samples of the salt men

Sample no.	2		3		5	
	C	P	C	P	C	P
Right ribs	13.20	2.75	7.25	2.50	8.00	2.25
Left ribs	13.00	3.00	6.00	2.00	12.50	2.70

The results of nitrogen measurement by CHNS and Kjeldahl analysis that are shown in Table 7, indicate that the salt man no. 2 has more nitrogen in the bone tissue, where this value is lower in salt men no. 5 and no. 3, respectively.

Table 7. The results of nitrogen measurement (%) analyzed by CHNS and Kjeldahl analysis

Sample no.	2		3		5	
	CHNS	Kjeldahl	CHNS	Kjeldahl	CHNS	Kjeldahl
Right ribs	3.48	3.20	2.15	2.45	2.40	2.30
Left ribs	3.55	3.25	1.80	2.45	3.30	2.95

According to the measurements on the bones of salt men (Tables 6 and 7), it could be concluded that salt man no. 3 has less N/P than salt men no. 2 and no. 5 in the bone tissue (The ratio obtained by the CHNS and Kjeldahl methods is 0.865 and 0.901, respectively), which means that it has lost more nitrogen and, consequently, is older than two other salt men. The value of N/P of salt man no. 5 using these two methods is 0.906 and 1.022, respectively, which consequently has more N/P in the bone tissue than salt man no. 3 and is, therefore, newer than it. The N/P value of salt man no. 2 is 1.265 and 1.183, respectively. As a result, its N/P is more than salt men no. 3 and no. 5, which means that it is the youngest.

Figure 6 shows that there is a direct relationship between N/P ratio of the samples and their absolute radiocarbon ages. But, due to the non-linearity of this relationship, a calibration curve could not be achieved for absolute dating of the unknown samples.

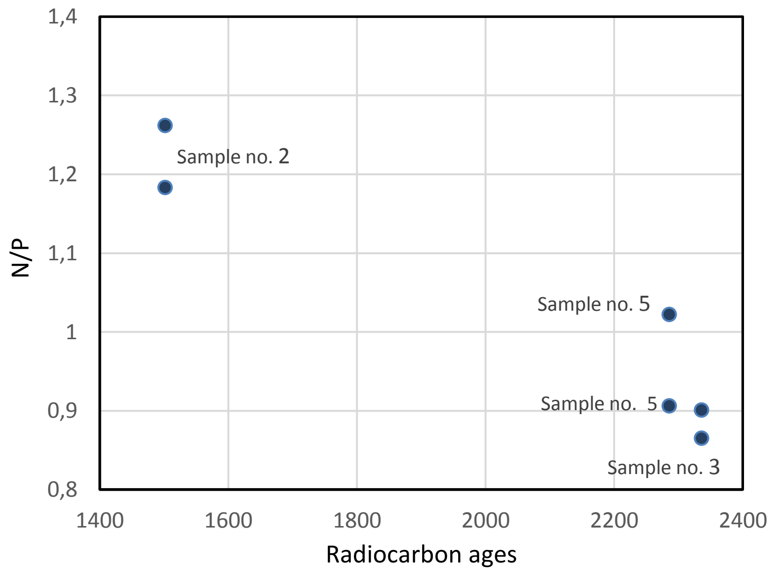

Figure 6. Control graph of N/P ratio versus specific radiocarbon ages of salt men

4. CONCLUSION

In this study, bone samples of Salt Men mummies of Chehrabad mine of Zanjan, IRAN, were successfully dated on the basis of FUN method. The results obtained suggest that firstly, corpse no. 3, then corpse no. 5, and finally corpse no. 2 have been buried. Comparison of the results with the ages obtained from radiocarbon dating showed that the relative FUN dating could successfully indicate the precedence and posterity of these three samples of salt man mummies. Meanwhile, there are not a linear relationship between fluorine, uranium, and nitrogen content of the samples and their absolute ages, and consequently, a universal calibration curve could not be obtained for absolute dating of the samples by FUN method. The results of this research indicated high accuracy and precision of FUN relative dating test in the archaeological sites such as salt mines, where the samples are well-preserved, and also showed suitable consistent with the method of radiocarbon dating.

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