Gilan Iron Age Diet: Results based on chemical analysis on samples of human and animal bones

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Trace element analyses was carried out using Atomic Absorption Spectrophotometer and XRF on a small collection of bones and teeth from horse and human bone samples to check the ratio of strontium and calcium and other elements like zinc and copper. Interestingly the copper representation in humans is much higher compared to that of Sr/Ca. This shows that their diet consisted mainly of animal meat and fish rather than other vegetarian foods. However, the high representation of copper found in horse bone samples makes this scientific evidence an anomaly and needs to be seen with caution and to be checked with a larger set of samples. likewise the zinc

Samples have a very weak representation. Of course the bones examined in this study came from museum repositories and did not accompany the soil samples hence the values presented here represent only the bone chemistry.

Keywords: *XRF analysis, AAS analysis, Gilan Iron Age, bone, Diet*

Introduction

Experiments on human bones and animal remains of ancient times are the best way to understand diet and other ecological processes that till this study has not yet been mentioned in the archaeological research of Gilan. Here for the first time due to a few sample examinations we are able to know the quantity of copper, zinc, calcium, strontium, etc in animal and human excavated bones.

A small section of samples of human and horse bones from three Iron Age sites in Gilan, viz. Toul in Talesh, Khusro Khani in Dailaman, Boye in Amlash area were examined to see the representation of any specific trace elements that may give evidence about the type of diet and/or ecology of the areas studied.

The samples subjected to Trace element analyses were examined at the Tata Institute of Fundamental Research (TIFR), Mumbai and Sheetal Analytical Laboratory, Pune. At TIFR the bones were examined using the X-Ray Fluorensce (XRF) while Sheetal Laboratory by Atomic Absorption Spectrophotometer (AAS) of Thermo Fischer Scientific.

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Importance and the method of studies on the skeletal remains

The animal bones found in archaeological excavations are undoubtable food debris. Those killed for food, hide, and bones as well as kept for secondary usage like traction, ploughing, carriage etc. are a primary and perennial source of subsistence in ancient civilizations. Another category that has been in the focus of archaeological research is the bones, teeth and ivory of animals, which are one of the extensively exploited raw materials by ancient societies for making objects like tools, decorative beads, dice, animal figurine, spindle, whistles, musical instruments like flute and rattle, etc. The use of ostrich eggshells used for making beads is also a well-known instance of the earliest examples of prehistoric art. Eventually all the bone artifacts fall in the category of 'use assemblage' according to the definition of taphonomic history of an archaeofaunal assemblage advocated by Gilbert (1979). Identification of the source animal whose bones were used as raw material is yet another

analytical method in the field of archaeolozoology where chemical methods are emerging today.

Human burials are one of the indispensable pieces of evidence which provide significant clues to the identification of age, gender, social hierarchy, pathological lesions (if any) and health status of the contemporary society by examining the mode of disposal of the dead, as well as bone morphology, morphometrics and chemical examinations of bones. Eventually the applications of archaeological chemistry for two types of analysis like isotopic and trace elements are currently in practice for assessment of past dietary behaviour. Identifying animal species in the face of taphonomic contrition of skeletal preservation (Sathe and Joshi 2013), dietary status of animals and humans (Pharswan and Farswan 2011) and palaeoecology (Cerling *et al*, 2008) are some of the recent milestones in the chemical analysis of bones from Archaeological sites.

Bones are composed of structurally complex material, both organic and inorganic chemicals. Living bone consists of three major components: organic matter, principally proteins; minerals in the form of calcium phosphates; and water. The water contents of buried bones and the sediments that surround them play as an important a role in their future integrity over archaeological time-scales as the chemistry and availability of biological fluids do during life. A definite ratio of trace elements in the bones (Parker and Toots 1980) has emerged as a meaningful tool in reconstructing the palaeodiet of an individual. Field research in the modern ecosystems indicate that inter and intra regional geological variability in elemental concentration produces variations in dietary and bone elemental values within trophic levels (Sealy 2004).

The precise presence of trace elements such as Strontium (Sr), Barium (Ba) and Magnesium (Mg) are 99% and 93% and 65% respectively reside in the skeleton of the animal. Strontium-calcium ratios are normally reduced at higher trophic levels in food webs, due to discrimination against strontium in favour of calcium by animals. Sr is an element most widely used in dietary reconstruction and its 99% presence exclusively within the bone implies that analysis of bone mineral could disclose the feeding habits of various animals. Its representation can be determined by studying trophic chain processes for example grass consumption by a herbivore. Carnivores will have a far lesser representation because of the difference of diet and thus carnivores and herbivores could be separated with a fair degree of accuracy on the basis of mean strontium contents. Sr and Ca are similar elements in terms of their chemical properties. Ca is however, preferentially extracted in the mammalian digestive tract, and strontium is preferentially removed in the kidneys. Therefore Sr is depleted relative to Ca in the tissues of herbivores as a result of the plants they eat. Similar process exists in carnivores too where their tissues are even more depleted in Sr compared to the tissues of the herbivores. Thus the ratio in tissues should reflect the Sr/Ca ratios in the bedrock and the trophic levels of animals that produced the tissue (Sealy 2004) were the first to show the application of analysing tertiary fossils in identifying the role of trace elements in palaeodietary reconstruction. They (Parker & Toots 1980) went on further to infer that even the browsers could be discriminated against the grazers presumably reflecting higher strontium concentrations in succulent herbs than in grasses. The research on trace elements over more than the last five decades in accordance with advances in methods and techniques of archaeological sciences has amply shown its usefulness in characterising the palaeoecological behaviour of animals, and in turn their dietary response over time across the continents (Sathe and Paddayya 2013 & Pharswan and Farswan 2011 & Gogte and Kshirsagar 1988).

Table. 1: *Correlation of diet with mineral content of bones*

Diet	**Sr.**	**Ba**	Mg	**Zn**
Herbivorous	High	High	High	Low
Omnivorous	Intermediate	Intermediate	Intermediate	Intermediate
Carnivorous	Low	Low	Low	High
Marine	High	Low	High	-
Terrestrial	Low	High	Low	-

Table.2: *Diagenetic profile of trace elements in bone*

Element	Food source	Trophic chain	Comments
Ba	Plant fibre, berries, tubers, vegetables, nuts, meat	Herbivore-carnivore	Diagenetic
Cu	Crustaceans, molluscs, viscera, meat, nuts, honey	Carnivore-herbivore	Similar to Barium
Mg	Green plants, cereals , vegetables, nuts, meat	Herbivore-Carnivore	High levels are related to cereals-rich diets
Sr	General marine and plant foods	Herbivore-Carnivore	Useful in palaeodiet analysis
V	Tubers, vegetables, nuts, milk	Herbivore-Carnivore	Of little use yet in palaeodiet analysis
Zn	Crustaceans, molluscs, meat, cereals	Carnivore-Herbivore	Diagenetically stable. Very used in palaeodiet analysis

Glimpse into position and results of the studied sites

The samples which were selected from three sites in Gilan include: Toul in North West, Boye in south east and khosro khani in south of Gilan that are seen in the map (Fig. 1), that being said the results are generalizable to the entire Gilan. The following are the visible explanations of the three selected sites.

Burial Customs in Toul

The site of Toul is located in the West of Karganrood Valley in a high mountainous area at the central county of Talesh in Gilan (Fig. 2). The GPS of cemetery is (37˚44′07″ N) & (48˚36′25″ E) and an altitude is 1640 MSL.

The excavations in Toul cemetery were carried out between 2003 to 2004 (Khalatbari 2004, 2007).

47 graves had been identified in Toul. The discovered graves can be divided into three distinct types in terms of shape as follows: 1. Enclosure Graves (four walls) 2. Simple Vacuolar Graves 3.Megalithic Graves.

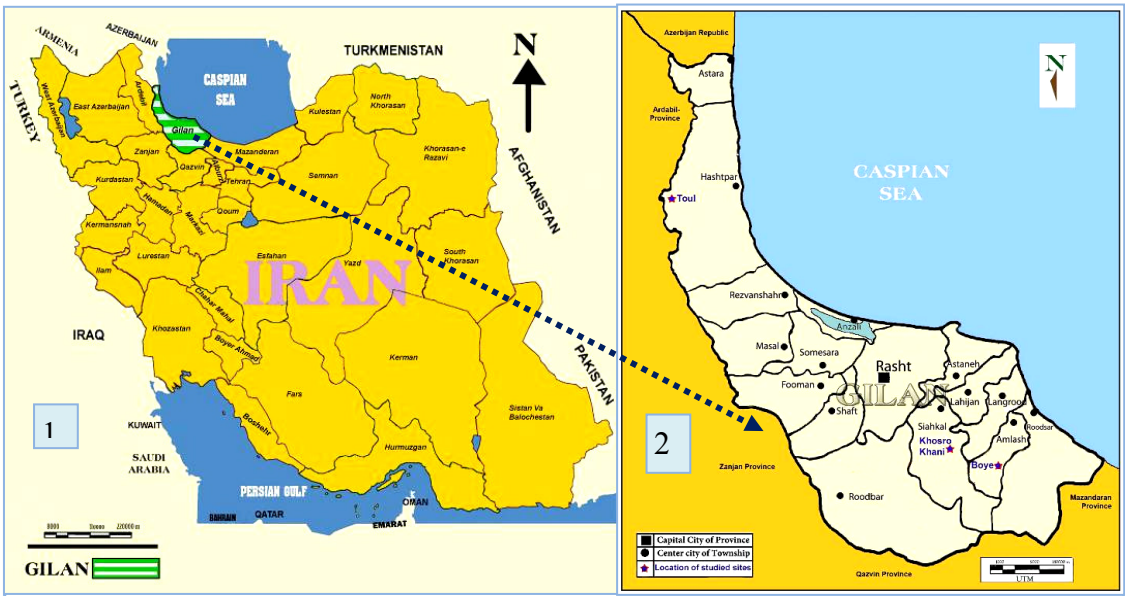


Fig. 1: *(right one) The Map of Iran showing all provinces, with Gilan highlighted and (left one) the map of Gilan showing all townships and location of studied sites.*

Forty seven skeletons had been excavated in Toul that can be divided into 3 groups in terms of direction of burials including: First the skeletons that are buried in the east and west direction (22 items). The second group refers to those that had been buried in the north and south direction (18 items). The last group consists of 7 individuals buried entirely in the North- South direction.

Burial conditions at Toul have their own diversity and difference. Generally, the dead bodies had been buried resting on the left shoulder or supine.

From the 47 explored graves in Toul, a large number of objects and cultural material had been discovered mostly made of clay, bronze, iron, silver, glass paste, stone, and bone.

The discovered pottery in Toul is generally dark gray, glossy black and red. They are a combination of sand, gravel, mica, with some plant material to strengthen it. They are wheel-made pottery but some of the artifacts are handmade and have usually no decoration. There are also some cases having carved, distressed, or added designs on the neck, handle and body.

A number of tools and equipment made of bronze had been discovered in Toul. Some silver decorative items were found in Toul. In addition, a large number of decorative beads made of agate, glass paste, glass and bone and stone spindle were found in Toul cemetery. The most important artifact that had been discovered in the excavations of Toul is a bronze bracelet. The inner rim of this bracelet is decorated with Orartoee cuneiform.



Fig. 2: *The site of Toul in the time of excavation (2003)*

During the two excavations which had been carried out in Toul, many pieces of animal bones which are related to cow, wolf, sheep etc. Were discovered however the samples related to horse bones are the most important discovery, because of high frequency of horse burials found in the site of Toul during the Iron Age period of Gilan, therefore the sample of horse teeth and bone from this site were selected for analysis.

Burial Customs in Boye

Boye is located in the political divisions of Amlash city, but archeologically, has been considered in the cultural domain of Dailaman and Amlash. Eight of the historical sites there are in the village of Boye but one of the most important sites is Gardanesar (Fig. 3).
The latitude of this site is: 36˚51′34/4″ and the longitude: 50˚ 06′ 20″ and at an altitude equal: 1478 MSL.
Gardanesar of Boye was excavated three times as follows: the first had been in 1961 (Moghaddam 1961), the second in 1996 (Sadr kabir 1996) and the third had been carried out as an emergency case in 2006 (Aslani 2008).
Four types of the graves including: enclosure graves (36 items), Simple vacuolar graves (7 items), stone stack graves (3 items) and crock graves were found.

The fourth type had just been discovered in the second season of excavation, at a depth of 120 cm surrounded by 6 large stone blocks. This crock grave contained human infant bones inside. Thus, it can be said that crock burials were used for children and infants in Boye.

Most of the pottery had been discovered in the second stage of excavations. These were divided into three groups of red, gray and red-brown clay pottery. In the third season eight different types of potteries had been found. The discovered pottery can be divided into four groups regarding decorative, added, distraught, highlights of different types of geometric shapes.

In the Boye excavations, metal objects made of

respectively bronze and iron had been discovered. Bronze artifacts include various blades of dagger, dirk, and knife and several arrows and spears in two different types. Also in the third season of excavations, small golden objects and bronze object such as: rings, bracelets, beads, swords and arrows and other material containing stone and glass had been discovered.
There were not any animal's bones in Boye cemetery which were related to wild or domestic animal, so just human skeletons were selected for chemical analysis. The culture of Boye cemetery belonged to the early period of Iron Age.



Fig.3: *The view of Boye site (Gardanesar) in the time of excavation (2008).*

Burial Customs in Khosro Khani

The site of "Kharsa khani" or "Khosro khani" is located at Deylaman city in Siakal Township, the site is in the shape of an oval with an opening to the northeast and southwest corners. The dimensions are about 120 to 200 meters (Fig. 4). The site registered under number 9943 is in the national index of 2003. The GPS of the site is: (36˚ 53′ 47.06″ N) & (49˚ 54′ 47.90″ E). The excavation of Khosro khani is carried out by present author in 2008 (Fallahian 2004).
The cemeteries section has been cluttered due to unauthorized digging in the past, however during the excavation evidence showed that the structure of graves are simple vacuolar in most parts with an enclosed stone structure in some parts such as fifth trenches.
The study confirms the holes of graves were about 170 to 135 cm and the burial in this size of holes were likely in a supine position. Moreover, the depth of graves was between 1 and 2 m.

According to the excavation, two era cemeteries appear in the Khosro Khani most part of cemetery contain the graves of Iron Age and a small part contain Parthian period graves, but an important point is that the Parthian graves in this precinct is not totally the depiction of the Parthian culture found in the central area of Iran but also maybe the impact of local culture that is mostly similar to the culture of Iron Age in Gilan.
During the excavation the fragments of animal and human bones have been discovered from the Khosro khani and a few pieces of human bones were selected for chemical analysis the result of these testing seen here.



Fig.4: *The site of Khosro Khani in Deylaman (2008).*

XRF analysis (TIFR, Mumbai)

XRF is an analytical method to determine the chemical composition of all kinds of materials which can include solids, liquid, powder, filtered or other form. It is a fast, nondestructive and accurate method requiring a minimum amount of specimen; the specimen preparation is done with bare minimum requirements. The elements that can be analyzed and their detection levels mainly depend on the spectrometer system used. The concentration range goes from (sub) ppm levels to 100%. Generally, the elements with high atomic numbers have better detection limits than lighter elements.

As soon as the sample is irradiated, the elements in the sample will emit fluorescent X-ray radiation

with discrete energies (determining the colours) of the radiation emitted by the sample, thereby determining the elements present. By measuring the intensities of the emitted energies (colours) one can determine how much of each element is present in the sample.

The samples (Table. 3) were soaked in de-ionised water for a couple of hours later thoroughly washed using plastic brushes and air dried, placed before the x-ray gun and time was set for 13mts on each so that an adequate spectra could be obtained. The values for each of the specimens with reference to the quantitative assessment of elements present are given in Table 5.3.

The objectives of these analyses aimed at identifying the representation of Ca/Sr ratio and other elements, and to see as to what could be the representative difference between the values found in animals as well as humans. It is a known fact that in the trophic chain, the humans may have lower levels of Sr. compared to the herbivores and in that case it is interesting to see how the horse bones responded to the taphonomic process and trophic levels of elemental absorption.

AAS analysis (Pune)

Fragments (and/or teeth) of the four samples examined under the XRF were also subject to Atomic Absorption Spectrophotometer, at Sheetal Laboratory in Pune city. The samples weighing 5gms each were exhumed in a muffle furnace at 650^{o} C and concentrated Hydrochoric and Nitric Acids were added to ash and reflure for an hour. The solution was aspirated on the AAS for different elements following the protocol and wavelengths were measured on the basis of the spectra for Cu (copper), Z (Zinc), Ca (Calcium) and Sr (Strontium). The values are enumerated below (Table.4):

The interpretations for the values are on one hand the first ever data set for a snapshot of the dietary preferences while on the other are seriously limited by the number of specimens with reference to the skeletal parts and number of individuals of the same taxon. The limited number of specimens is naturally due to the legal and logistical constraints of transporting archeological materials from Iran to India for analysis.

For example, a single horse (1*n*) bone and a tooth from Toul Talash cannot be a representative of the entire assemblage. Such is also the case with the limited number of human bones from Boye Amlash and Khusro Khani Dailaman. The results are indeed a window to the palaeodietary reference to Iron Age of Gilan and stand open to revision. Statistically satisfying numbers of specimens have to be drawn from each of skeletal elements with desired number of individuals of each taxon, since the bone histology and histo-chemistry has significant bearing on the elemental distribution within and across the skeleton. It is worthwhile to give an example of a recent study by Santos *et al.* (2008) who have demonstrated a clear correlation between strontium and calcium concentrations and proximity to warmer/colder parts of the body of

Table. 3: Concentration of trace elements in bone samples according to XRF Analysis

Toul (Horse Bones)		**Boye (Human Bones)**		**Khosro Khani (Human Bones)**	
Component	**Conc.**	**Component**	**Conc.**	**Component**	**Conc.**
Ca	84.93274	Ca	94.56838	Ca	76.55723
Sr	0.2252084	Sr	0.3481013	Sr	0.9564478
Fe	8.861819	Fe	4.220777	Fe	20.55997
Cu	0.2070791	Cu	0.2016272	Cu	0.387594
Mn	0.5060682	Mn	0.3444579	-	-
Zn	0.3287168	Zn	0.3166487	-	-
Ti	0.2356365	-	-	Ti	1.538758
P	4.702727	-	-	-	-

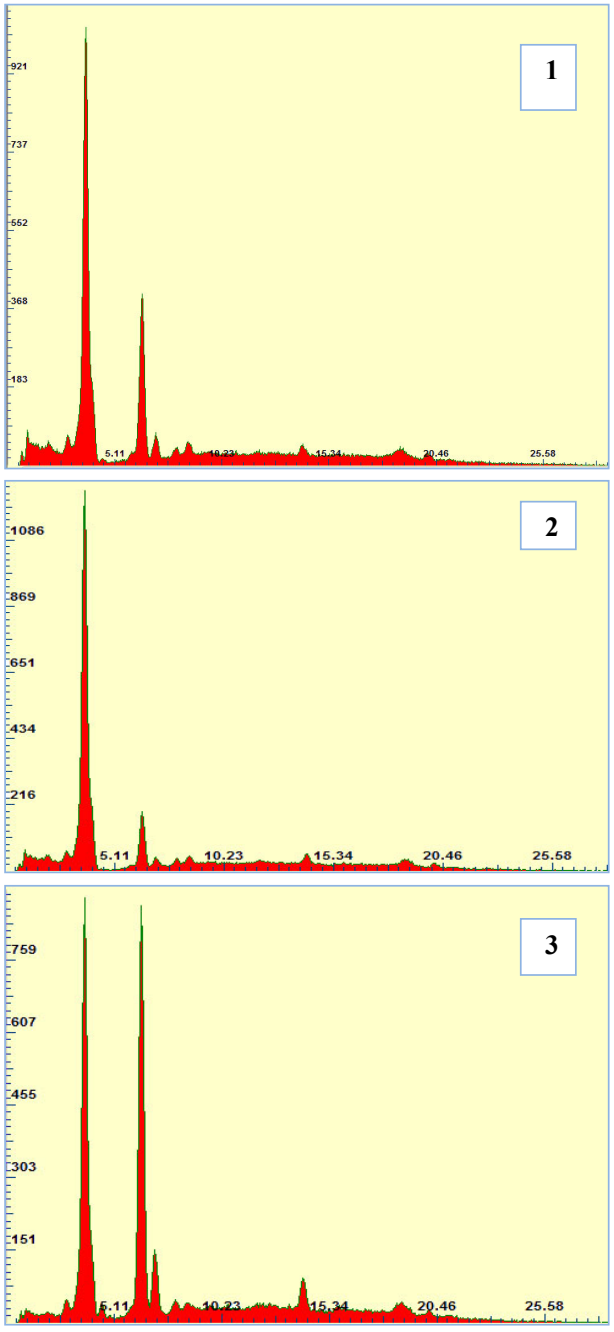


Fig. 5: *Spectrum of human bone from 1. Toul, 2. Boye, 3. Khosro Khani*

a crocodile, implying higher Sr/Ca ratios for the bones which are closer to the heart.

AAS experiments bring out the values for Cu, Zn, Ca and Sr., which show an interesting pattern (Table: 4). the values for copper (Cu) are predominantly much higher compared to that of Calcium and Strontium, while the Zn exhibits negligible variations. However, the value in horse tooth and bone are competitive with those of humans and shows the need of a larger bone samples from all the groups for a fair and reasonable elemental representation with respect to the taxon and their dietary regime. The values of Zn, Ca and Sr. in horse are significantly low except Cu which appears much higher and is not expected in herbivores.

As far as the Cu content in Human bones from the sites concerned, the specimen from Khosro Khani is significantly high and shows strong possibility of consumption of animal meat and fish. On the other hand, the specimen from Boye has a much lower Copper content, perhaps indicating that the individuals in question consumed comparatively lower amounts of meat and fish. This is interesting given the literary evidence of palaeodiet in Gilan, which tends to emphasise on the role of meat and fish in the diet of the Iron Age people. Similar studies carried out on more than 165 human bones from the Chalcolithic site of Inamgaon in Pune Dist. in India have clearly shown higher copper/ Zinc values for the human bones which come from the archaeological levels well known to have experienced drought like conditions and people's excessive dependence on animal meat and fish for subsistence (Gogte and Kshirsagar 1988).

Besides dietary interpretations, this study also intended to see as to how bones have interacted with the sediments and can this be documented at trace elemental levels. Titanium (T) is an example whose representation on the bone surfaces was exceptionally higher but when the x-ray probe had passed through the cortical bone the presence had reduced to zero, and when the bone was further cleaned, even the surfaces showed dismal presence of titanium. It has been the part of the protocol of elemental analysis to include soil samples, to assess the elemental profile of the soil that has been found in association with the bone. The concentration in soil is always higher than in bones but the degree of *in situ* fossilisation is also ascertained with precision. The bones examined in this study came from museum repositories and did not accompany the soil samples hence the values presented here represent only the bone chemistry.

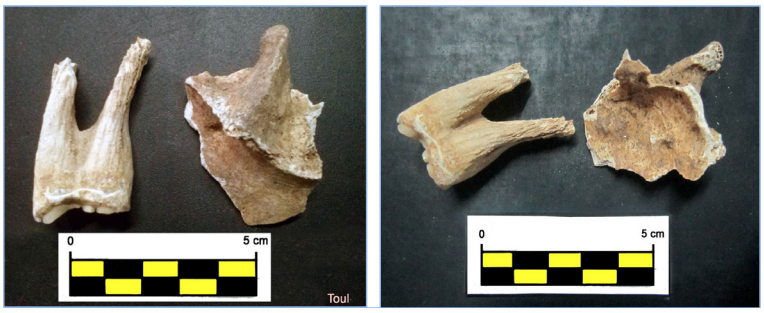


Fig.6: *Horse tooth and bone for analysis from Toul- Gilan*

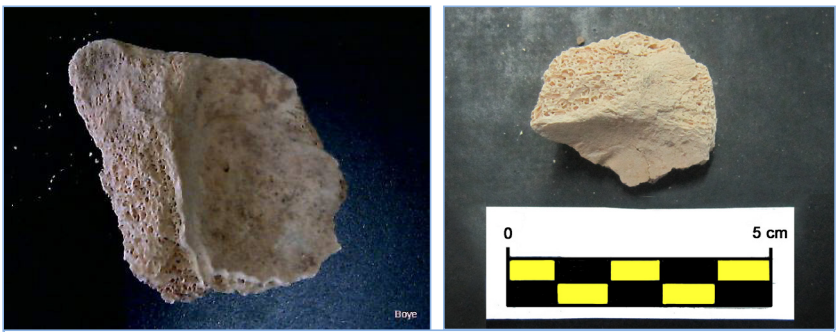


Fig.7: *Human Hip bone fragment from Boye*

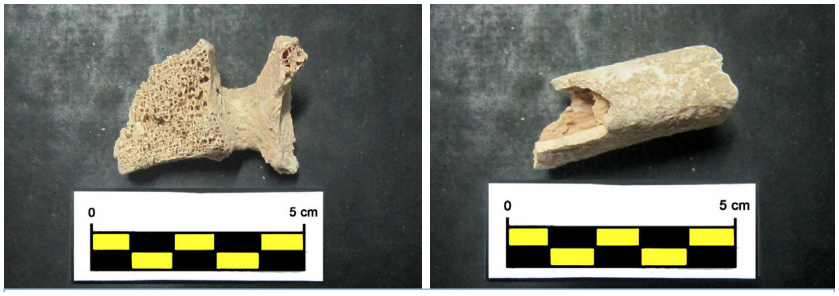


Fig. 8: *Human vertebra and long bone fragment from Khosro Khani*

Table.4: *Trace element analysis of Iron Age bones from Gilan using AAS Analysis*

Sr. No.	Name of Site and area	Research Method & Year	Supervisor of excavation	Number of samples	Taxon	Copper (Cu)	Zinc (Zn)	Calcium (Ca)	Strontium (Sr)
1	Toul in Talesh	Excavation 2003	M. Khalatbari	2	*Equus Caballus*	49.3 ppm	0.33%	7.37%	0.014%
2	Boye in Amlash	Excavation 2008	G. Aslani	2	*Homo Sapiens*	9.34 ppm	0.35%	8.15 %	0.154%
3	Khosro Khani in Dailaman	Excavation 2004	Y. Fallahian	2	*Equus caballus*	37.7 ppm	0.29%	8.95 %	0.0165%
					Wavelength used	324.8	307.6	422.7	460.7

Strontium/Calcium ratios too contibute to our understanding of the proportion of meat and vegetables in the diet of human populations Statements like comparison of dietary composition of meat and vegetables between archaeological sites or the region may not be possible in this study but obviously the beginning has been made in the field of palaeobiochemistry.

Result

The trace element analyses of bones and a tooth of horse and human bones clearly highlight the importance of chemical analyses of bones from archaeological sites in understanding the dietary profile of ancient humans and animals and also in understanding the cultural and ecological dynamics of the ancient landscape. Since small sample sizes from three sites of Iron Age of Gilan were examined, a synoptic view of palaeodiet is made available by this study. The predominance of copper hints at the consumption of animal meat and fish while the ratios of Sr and Ca correspond to range of values known for the herbivores and man. The results may pertain to just a couple of individuals from the entire complex of Iron Age sites but being the first ever approach in Gilan Archaeology, it has opened up fresh avenues for archaeological sciences with especial reference to palaeobiochemstry.

References

Aslani, G.
2008 *Emergency exploration of Gardanesar in Boye,* Archive report, Rasht: Document center in the Cultural heritage and Tourism Administration in Gilan province.

Cerling, T.E.; G. M. Harris; J. A. Hart; P. Kaleme; H. Klingel; M. G. Leakey; N. E. Levin; R.L. Lewison & B.H. Passey
2008 Stable isotope ecology of the common hippopotamus, *Journal of zoology*, ISSN No: 0952- 8369

Fallahian, Y.
2004 *The excavation of Khosro Khani site in Daylaman,* Archive report, Tehran: Document Center in the Cultural Heritage and Tourism Organization Iran.

Gilbert & S. Alan
1979 *Urban Taphonomy of Mammalian Remains from the Bronze Age of Godin Tepe, Western Iran*, Ph.D. Dissertation, Columbia University, University Microfilms Int'l, Ann Arbor, Michigan.

Gogte, V.D. & A. Kshirsagar
1988 Chalcolithic diet and trace element analysis of human bones, In Dhavalikar, M.K. and Sankalia, H.D., Ansari (eds.) *Excavations at Inamgaon,* vol. I part ii, 991-1000, Pune: Deccan College.

Khalatbari, M.R
2004 *Archaeological Investigations in Talesh, Gilan-1: Excavations at Toul-e Galan,* Translator: Fereshte Kasraei Moghim, Publisher: Gilan Cultural Heritage Org. Rasht.

2007 Analysis of archaeological research in the Toul of Gilan *(Tahlili bar Natayej-e Tahghighat-e Bastanshenasi dar Toul-e Gilan), Second Conference on the culture and Civilization of Talesh,* publication by Cultural heritage Administration in Hashtpar, 6-64.

Moghaddam, M.
1965 *Archaeological Reports, East of Gilan province,* Report No. 13, Tehran: General Administration of Archaeology and Folklore.

Parker R.B & H. Toots
1980 Trace Elements in bones as Palaeobiological Indicators, In Behrensmeyer A.K. and Hill A. P. 1980, *Fossils in the Making Vertebrate Taphonomy and Palaeoecology*. Midway Reprint, University of Chicago Press

Pharswan, J.S. & Y.S. Farswan
2011 Role and Utility of Trace Elements in Palaeodietary Reconstruction, *New York Science Journal*, 4 (11), 43-48.

Sadr Kabir, R.
1996 *Preliminary Report of Emergency excavation of Gardanesar in Boye*, Archive report, Rasht: Document center in the General Administration of Cultural Heritage and Tourism in Gilan Province

Santos, P. R.; N. Added; J. H. Aburaya & M. A. Rizzutto
2008 Measurements of Sr/Ca in bones to evaluate differences in tempeterature, *Nuclear Instruments and Methods in Physics Research* B 266, 1616-1618

Sathe, V. & S. Joshi
2013 X-Ray diffraction analyses of Bioapatite for taxonomic discrimination in archaeozoology, *Bulletin of Deccan College Postgraduate and Research Institute,* Pune: Deccan College.

Sealy, J.
2004 Body tissue chemistry and palaeodiet, In *Handbook of Archaeological Sciences* (ed. Brothwell, D.R. and A.M. Pollard), pp. 269-279, John Wiley, Chichester, U.K.