Study of Preservation Status and Dietary Reconstruction in Human Remains Recovered from Roopkund Lake through Chemical Analysis of Faunal Remains

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The present study carried out on the bone samples collected from Roopkund Lake in Chamoli Garhwal, Uttarakhand, which is located 5029 meters above sea level in between Nanda Ghunghti and Trishuli peak. This historical site belongs to the 9th century A.D. All the samples selected for the study were dried in room temperature, as well as in hot air ovens at 32 degree celsius. The cleaning, pretreatment and digestion process of all faunal remains was done through established scientific methods. Chemical analysis i.e. concentration of different elements such as calcium, strontium, barium, magnesium and zinc as well as isotopic ratios of carbon and nitrogen was estimated with the help of Inductively Coupled Plasma Spectroscopy (ICP) and Atomic Absorption Spectrophotometer (AAS).

The results obtained from the chemical analysis are significant. Based on concentration of different elements and ratios of nitrogen and carbon isotopes, the dietary habits of the peoples buried in the Roopkund Lake have been identified, The results are also significantly helpful for knowing the preservation status of faunal remains in Roopkund Lake. This study also indicated the potentiality of chemical analysis for reconstructing the palaeodiet behaviour and preservation status of bone remains.

Keywords: *Roopkund Lake; Dietary Reconstruction; Chemical Analysis; Faunal Remains; Archaeological Site*

Introduction

This paper reports a general investigation into the status of preservation, degree and nature of post depositional changes as well as dietary reconstruction in the human bones recovered from Roopkund Lake of Garhwal (Fig. 1). It has been well established that bones are a rich source of information at an archaeological site (Stephen *et al.* 1993: 613-627), as they are used to get information about the fauna of the site and also to assess various aspects of behavioral activities of humans and carnivores (De Niro and Epstein 1978: 495-506; Binford 1981; Brain 1981; Klein and Cruz-Uribe 1984: 1-266).

It has also been worked out that dietary reconstruction through chemical analysis of bones is an important aspect of archaeological research. A number of studies on palaeodietary reconstruction have been carried out by various workers on the faunal remains recovered from various archaeological sites around the world (Antoine *et al.* 1988: 101-106; Borgognini 1989: 283-320; Burton and Price 1991: 787-795; Burton 1996: 327-333; Chisholm *et al.* 1982: 1131-1132; Farswan and Nautiyal 1997: 227-239; Nautiyal *et al.* 1995: 139-140; Price *et al.* 1985:419-448; Price *et al.* 1986: 365-375 and Lambert and Homeyer 1993: 279-29). The analytical methods used in dietary reconstruction were as follows; estimation of trace element contents, isotopic ratios of carbon and nitrogen in the faunal remains excavated from different archaeological sites, as these methods are found to be potentially significant for reconstructing the diet of ancient human populations.

The present study is mainly considered keeping in view of the potentiality of estimation of Carbon & Nitrogen isotope ratios and trace element analysis in archaeological faunal remains as well as importance of the state of preservation and palaeodietary reconstruction in the faunal remains of buried people (Fig. 2) from Roopkund Lake.

Before going through the materials and methods it is also equally important to discuss the history of buried human remains of Lake of Roopkund, which

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is situated at an altitude of 5029 meters (around 16750 feet) above sea level in the interior of district Chamoli, Uttarakhand. The Lake is rather shallow having a depth of 2 meters with the edges covered with snow for most of the year (Fig. 3). In the summer when the snow melts (Fig. 4), the human remains can be seen, some of them still having flesh. These remains have been preserved in the alpine conditions.

It is believed that these are the remains of about 300-500 people and as per the radiocarbon dating, estimated by Oxford University it is clear that these people died around the 9th Century AD. The origin

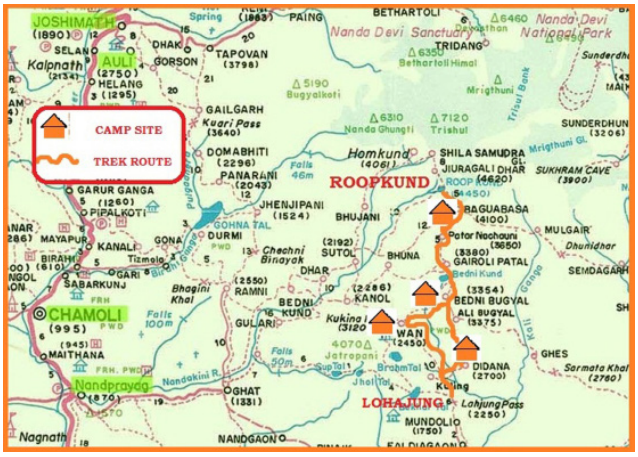

Fig. 1: Location of Roopkund Lake in Uttarakhand, India (Photo: Author).

of these people is still unknown. The legendary history of Garhwal Himalayan region indicates that these people were related to the legend of Nanda Devi Raj Jat. Because it is an important and existing event from the spirituous and cultural point of view, as the shrines of Nanda Devi are scattered all over the Central Himalayas. This spiritual and religious event is organized every 12 years.

Therefore, keeping in view of the significance of these remains present investigation is aimed at the study of preservation status and dietary reconstruction in faunal remains of Roopkund through elemental analysis and estimation of isotopic ratios of carbon and nitrogen.

For the purpose of chemical analysis all the basic treatment of faunal remains was done in our laboratory, while the elemental analysis and estimation of isotopic ratios has been done with the help of the Laboratory for archaeological science, Wisconsin University, Madison, Wisconsin, USA.

Meterials and Methods

Materials and methods includes collection and selection of bone samples, cleaning, pretreatment, elemental analysis, estimation of isotopic ratios and hydrogen ion concentration.

For the present study a total of 20 faunal remains of long bones were collected from different locations and depths of Roopkund Lake during the summer of 2005 (Fig. 5). The collected samples were cleaned and pretreated in the laboratory by using different mechanical and chemical fieldwork. All the flesh, tendon, cartilage, and any other non-bone tissue were removed from each bone sample by scraping with the glass edge.

The entire bone samples were then broken to expose the medullar cavity and the exposed surfaces were then abraded with a moto tool to remove contamination, such as, dust particles, calcification and other type of external deposition. Besides this the outermost layer of all bone samples

Fig. 2: *Human Bones in Roopkund Lake (Photo: Author).*

Fig. 3: *View of Roopkund Lake with snow(Photo: Author).*

Fig. 4: *Roopkund Lake with melted snow Diring Raj Jat of 2000 (Photo: Author).*

were also removed, since this part contains most of the digenetic contamination. For the betterment of chemical analysis it is necessary to select the shaft of long bones i.e. femur or humorous.

For elemental analysis, abraded pieces of bones were broken into small pieces and cleaned with aid of ultrasonic bath, allowed to acid wash in 1–Molar or 1-Normal acetic acid solution overnight, then ringed in de-ionized water and dried at 80-90 degree celsius for few hours. These dried samples were used for making the ash which was done with the aid of a muffle furnace. The ash samples were digested in 1ml concentrated Nitric acid for

Fig. 5: *Limb and skull bone samples from Roopkund (Photo: Author).*

one hour, after cooling 16 ml of 5% nitric acid was added to make a final volume of 17 ml. This solution was then directly aspirated to Atomic Absorption and ICP spectrophotometer. The results obtained in ppm were calculated statistically.

For the estimation of isotopic ratios of carbon and nitrogen the cleaned and acid washed bones were selected and collagen of every sample was extracted. The required carbon-containing components were separated for every sample which was combusted on line carbonate system of ratio recording mass spectrometer to obtain the required CO2. The liberated carbon dioxide was automatically fed to the dual beam isotope-ratio mass spectrometer. For carbon the internationally recognized standard pee dee belemnite carbonates (PDB), a marine carbonate. For nitrogen, the sample ratio is reported relative to AIR (ambient inhalable reservoir), which became the internationally recognized standard following the demonstration that the isotope ratio of N2 in the atmosphere is constant across the globe (Antoine *et al.* 1988:101-106 and Mariotti 1983: 685-687). Isotopic ratios of carbon and nitrogen which presented as delta (δ) values in parts per thousand ("per mil" represented by the symbol ‰) as shown in the following formulas (Schoeninger and Moore 1992: 247-296 and Van Der Merwe 1992: 247-264).

$$\delta^{13}C = \frac{13C/12C\,Sample}{13C/12C\,PDB\ Satndard} - 1$$

$$\delta^{15}N = \frac{15N/14N\,Sample}{15N/14N\,PDB\ Satndard} - 1$$

The values obtained from the previously mentioned equations are multiplied by 1000 to get the exact value of each sample.

To find out about the nature of the soil around the faunal remains, values of hydrogen ion concentration (pH) were also estimated at different locations of Roopkund Lake by using field pH meters. These values of soil pH are helpful for establishing the preservation status of faunal remains and archaeological artifacts as well. But in the case of the present study faunal remains selected for the analysis were also examined through ocular microscope and hand lances to see the post depositional changes in the morphology of bones.

Results and Discussion

As the main aim of the present study was to know about the preservation status of archaeological faunal remains recovered from Roopkund Lake as well as reconstruction of dietary behaviour of the people buried in the Roopkund lake around the 9th century AD. The results obtained from elemental analysis are presented in Tables.1-2, while isotope ratios of carbon and nitrogen estimated from 20 bone samples are shown in Table. 3. At the same time, it is also clarified that for checking the preservation status of faunal remains, the estimated hydrogen ion concentration of soil at different locations are mentioned in Table. 4.

However, before discussing the analytical results it is also important to note that trace element analysis and estimation of isotopic ratios of carbon and nitrogen have a great potential and significant features to reconstruct the palaeodiet and past history of ancient animal population (Farswan and Price 2002: 197-208; Thompson *et al.* 2005: 451-463; Bocherens *et al.* 2007: 10-27; Iacumi *et al.* 2006: 16-25; Hollund *et al.* 2010: 2971-2983; Al-Bashaireh and Al-Muheisen 2011:2606-2612). It is also well known that the archaeological remains in any archaeological sites are well preserved when environmental condition becomes favourable and for the chemical analysis only well preserved archaeological remains are selected, because digenetic remains lost their original chemical composition (Farswan and Price 2002: 197-208; Von Endt and Ortnet 1984: 47-153). Based on the same assumptions, in the first phase of the present study we have estimated the hydrogen ion concentration of soil at different locations of Roopkund lakes, as it has already been reported by so many workers

Table. 1: *Mean Values of Concentration Of Different Elements In The Humorous Bones of Human Remains From Roopkund Lake.*

Bones of	Name of Site	Concentration of Elements (in ppm ± S.D.)				
		Ca	Sr	Ba	Mg	Zn
Sample No.01	Roopkund	**439752.30 ±134.60**	**388.22 ±20.55**	**302.52 ±21.31**	**6265.22 ± 15.25**	**396.02 ± 10.3**
Sample No.02	Roopkund	41527.50 ± 140.4	372.25 ± 18.52	284.75 ± 22.25	2950.25 ± 13.50	234.05 ± 11.5
Sample No.03	Roopkund	42572.50 ± 125.65	367.50 ± 15.35	286.55 ± 20.15	2980.25 ± 15.75	228.09 ± 09.5
Sample No.04	Roopkund	**42570.45 ± 133.55**	**385.56 ± 18.57**	**314.52 ± 25.30**	**6904.50 ± 16.25**	**388.07 ± 12.8**
Sample No.05	Roopkund	39566.22 ± 119.15	372.25 ± 20.55	290.72 ± 23.33	2955.75 ± 113.25	295.06 ± 10.7
Sample No.06	Roopkund	**41525.25 ± 144.6**	**382.58 ± 10.77**	**316.45 ± 22.32**	**6775.25 ± 18.55**	**335.08 ± 11.4**
Sample No.07	Roopkund	40577.50 ± 110.43	369.52 ± 19.55	285.44 ± 19.24	2925.25 ± 113.25	219.09 ± 12.8
Sample No.08	Roopkund	39555.56 ±112.34	375.58 ±17.50	287.60 ±20.10	2805.25 ±23.25	216.03 ±11.2
Sample No.09	Roopkund	40755.45 ±117.62	368.75 ±16.33	278.58 ±21.32	2895.25 ±22.25	222.06 ±10.5
Sample No.10	Roopkund	**42650.50 ±111.44**	**373.53 ±19.25**	**315.75 ±25.30**	**6795.25 ±32.25**	**357.05 ±11.7**
Coastal Herbivore	Ref-1	37954.67 ±13.50	565.20 ±9.50	68.65 ±5.50	2553.64 ±22.25	89.22 ±5.11
Terrestrial Omnivore	Ref-2	36758.00 ±12.55	367.10 ±5.50	237.75 ±19.25	5937.00 ±32.54	218.55 ±10.5
Carnivore	Ref-3	**46557.28 ±24.55**	**278.54 ±10.42**	**366.50 ±27.65**	**7535.50 ±1.45**	**415.65 ±10.2**

that highly acidic and alkaline soil are responsible for the degradation of organic archaeological artifacts (Antoine *et al.* 1988: 101-106; Farswan and Nautiyal 1997: 227-239; Nautiyal *et al.* 1995: 139-140; Farswan and Price 2002: 197-208) and relatively less acidic and alkaline conditions of soil are the best preservative for the archaeological remains.

Results obtained from the examination of faunal remains of Roopkund lake indicated that majority of the faunal remains are well preserved, as no digenetic effects have been noticed in any samples considered for analysis. This is simultaneously verified by the positive values of hydrogen ion concentration (Table. 4) estimated from the soils of Roopkund, as it is ranging between 6.02-6.95, which is slightly acidic in nature and this condition of soil is suitable for the preservation of archaeological artifacts (Antoine *et al.* 1988: 101-106; Farswan and Nautiyal 1997: 227-239; Nautiyal *et al.* 1995: 139-140; Farswan and Price 2002).

It has also been confirmed from earlier analytical

Table. 2: *Mean Values of Concentration of Different Elements in the Femur Bones of Human Remains from Roopkund Lake.*

Bones of	Name of Site	Concentration of Elements (in ppm ± S.D.)				
		Ca	Sr	Ba	Mg	Zn
Sample No.11	Roopkund	41533.53 ± 99.62	375.55 ±21.55	323.53 ±29.32	2955.55 ± 11.09	231.02 ± 10.4
Sample No.12	Roopkund	**42752.55** ± 101.62	**368.22** ± 20.55	**322.52** ± 18.36	**6265.22** ± 12.27	**396.02** ± 10.3
Sample No.13	Roopkund	40768.82 ± 111.53	375.24 ± 18.11	324.75 ± 15.23	2980.25 ± 15.75	228.09 ± 09.5
Sample No.14	Roopkund	**40572.50** ± 127.54	**378.23** ± 19.53	**320.79** ± 19.24	**2934.15** ± 102.25	**295.33** ± 10.2
Sample No.15	Roopkund	**43656.75** ± 122.16	**376.51** ± 12.45	**320.72** ± 15.05	**6474.30** ± 18.55	**328.09 ± 19.4**
Sample No.16	Roopkund	40865.55 ± 144.44	366.54 ± 19.55	318.75 ± 12.22	2925.58 ± 111.23	199.03 15.8 ±
Sample No.17	Roopkund	**41577.52** ± 115.62	**381.26** ± 20.55	318.57 ± 25.30	**6965.58** ± 16.25	**345.06** 11.8 ±
Sample No.18	Roopkund	41432.53 ±144.6	362.55 ±20.55	326.43 ±25.30	2868.56 ±23.25	221.05 ±11.3
Sample No.19	Roopkund	**43578.58** ±144.6	**328.28** ±18.55	**324.75** ±15.22	**6795.25** ±25.33	**357.05** ±11.7
Sample No.20	Roopkund	42566.55 ±144.77	372.58 ±19.55	326.85 ±15.35	2895.25 ±22.25	222.06 ±10.5
Coastal Herbivore	Ref-1	37954.67 ±13.50	568.25 ±10.55	70.85 ±5.50	2553.64 ±22.25	89.22 ±5.11
Terrestrial Omnivore	Ref-2	36758.00 ±12.55	369.15 ±5.75	238.95 ±19.25	5937.00 ±32.54	218.55 ±10.5
Carnivore	Ref-3	**46557.28** ±24.55	**280.55** ±11.45	**364.25** ±27.65	**7535.50** ±1.45	**415.65** ±10.3

studies that continuous intake of terrestrial food reduce the concentration level of Mg, Sr and Zn in the animal bones, while it increases the values of Ba, but continuous intake of terrestrial food along with meat reversed the concentration values of these elements which is clearly shown in Table. 1 -2 (Pate and Hutton 1987; Pate 1994: 161-09; Antoine *et al.* 1988:101-106). Earlier studies also described that plant tissue contain a greater amount of Mg as compared to animal flesh but concentration of Mg

Table. 3: *Isotopic Ratios of Carbon and Nitrogen in the Faunal Remains Recovered from Roopkund Lake.*

Skeletal Remains of	Isotopic Ratio of Carbon Delta ^{13}C) in) (‰o (Per mill	Isotopic Ratio of Nitrogen Delta ^{15}N) in ‰o (Per) (mill
Sample No.01	- 22.75	09.25
Sample No.02	- 17.27	08.25
Sample No.03	- 18.78	07.25
Sample No.04	- 23.58	10.25
Sample No.05	- 16.75	07.25
Sample No.06	- 25.65	09.25
Sample No.07	- 19.25	07.25
Sample No.08	- 24.05	10.25
Sample No.09	- 18.82	08.25
Sample No.10	- 23.95	10.25
Sample No.11	- 18.34	08.25
Sample No.12	- 26.70	09.25
Sample No.13	- 17.65	08.25
Sample No.14	- 25.20	11.25
Sample No.15	- 24.29	09.25
Sample No.16	- 19.32	08.25
Sample No.17	- 26.65	10.25
Sample No.18	- 18.62	07.25
Sample No.19	25.33 -	10.25
Sample No.20	17.85 -	08.25

Table. 4: *Hydrogen Ion concentration (pH) in Soils from different locations of Roopkund Lake.*

Locations	Hydrogen ion (concentration (pH
Mts. Depth 0.5	**6.02**
East of Lake	**6.05**
West of Lake	**6.22**
North of Lake	**6.33**
South of Lake	**6.45**
Mts. Depth 1.0	
East of Lake	**6.72**
West of Lake	**6.85**
North of Lake	**6.75**
South of Lake	**6.95**

also records the relative amounts of vegetation and meat in the animal diet (Pate and Hutton 1987; Pate 1994: 161-09). It is evident from the analysis of present results shown in Tables. 1-2 that the values of concentration of Magnesium (Mg), Zinc (Zn) and Barium (Ba) are higher in the bones of Roopkund as compared to coastal reference samples, which indicate a high terrestrial component in their diet. Meanwhile, higher levels of Mg estimated from more than 50% of samples suggest a terrestrial and meat consumption diet for these people. A detailed analyses of bone sample from marine and terrestrial animals recovered from different archaeological sites, show a lower level of Ba in marine animals, while higher values of same elements in terrestrial animals (Burton and Price 1991: 787-795; Burton 1996: 327-333; Pate and Hutton 1987).

Simultaneously Zinc (Zn) is also a useful element for distinguishing carnivores from herbivores (Farswan and Price 2002: 197-208), as Zn is more concentrated in flesh than in most plant foods. Therefore, carnivores generally have high concentration of Zn than herbivores. concentration level of Zn obtained from the present study also indicates that the peoples of Roopkund were omnivore, although most of them showed signs of having greater percentage of meat in their diets (Tables. 1-2).

For further confirmation we have also carried out the estimation of isotopic ratios of carbon and nitrogen in the same faunal remains as These values are positively correlated to the diet of animals. The carbon isotopic ratios of for coastal skeletons range between -14 and 16 per mil (‰), while inland skeletons range between -17 and -19‰ Von Endt and Ortnet, 1984: 47-153. Marine foods represent the isotopic ratios of -12 and -18‰, while terrestrial foods (meat and plants) average about -25‰ (Von Endt and Ortnet 1984: 47-153). Similarly isotopic ratios of nitrogen are equally useful in dietary reconstruction. Previous studies on isotopic ratios of nitrogen indicated that in herbivores ratios of delta N is 4-7‰ and for carnivore these values are 7-19‰ and for Arctic hunter delta N value would be above 20 ‰ (Table. 5, Von Endt and Ortnet 1984: 47-153).

The estimated ratios of carbon and nitrogen from faunal remains of Roopkund (Table. 3) clearly indicated that these people were well nourished with higher percentage of terrestrial diet along with meat in their diet, and more than fifty percent of people in this group were having a higher percentage of meat and terrestrial material in their diet as it is verified from elemental and isotopic data (Tables. 1-3).

Table. 5: *$\delta^{13}C$ and $\delta^{15}N$ Values in Different Types of Diets.*

Types of Diet	$\delta^{13}C$ (‰)	$\delta^{13}N$ (‰)
C3 Plants only	- 26.6	5.5
Meat from Herbivore on C3 diets	- 25.7	9.5
C4 plants only	- 12.6	6.5
Meat from herbivore on C4 diets	- 11.4	9.6
Marine Plankton only	- 19.2	12.6
Meat from Marine Herbivore	- 18.7	15.5
Meat from Marine Carnivore	- 17.6	18.8

Conclusion

Results obtained from the estimation of isotopic ratios of carbon, nitrogen and trace element analysis of faunal remains recovered from Roopkund Lake, Central Garhwal Himalaya revealed that the faunal remains of peoples buries around the 9th century are well preserved and these peoples belongs to omnivore group with rich C4 and meat in their diet (Tables. 1-3). This clearly indicated that there were two groups of people in that journey.

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