Ancient Asia

Mahajan, S and Sathe, V. 2020. Scope and Limitations of Stable Isotope Analysis in Indian Archaeology. *Ancient Asia*, 11: 6, pp. 1–8. DOI: https://doi.org/10.5334/aa.190

SHORT REPORT

Scope and Limitations of Stable Isotope Analysis in Indian Archaeology

Sangeeta Mahajan and Vijay Sathe

Stable isotopes of lighter elements are frequently used to understand diet and ecology of ancient population in the western world. In India however, in spite of the infrastructure being available for stable isotope analysis, its usage to date is limited to geological and oceanographical studies. Its applications in investigating archaeological questions are still uncommon. Stable isotopes can be used to satisfy various hypotheses pertaining to an archaeological site in multiple ways. In this article, taking two recent stable isotope studies into consideration (one based on human teeth and another on faunal teeth), the scope and limitations of use of stable isotope analysis in archaeology are extended in detail. The differences in selection of samples, sampling procedures, methodologies and interpretations while working on human as against faunal teeth are discussed discretely.

1 Introduction

Recent decades have been very rewarding for the field of archaeology, thanks to the application of biochemistry in analysing and interpreting the material and biological data obtained from the archaeological excavations. Interpretations regarding the health and disease of past humans and animals depend largely upon keen observations of the morphological details and biometry of the skeletal remains, histological findings and radiological observations. These findings mostly provide information about health, growth pattern and disease prevalence in a community. Recent techniques like DNA analysis of the biological remains (Shinde *et al* 2019; Rai *et al* 2020) and occlusal microwear texture analysis (Fiorenza *et al* 2011; Daegling et al 2016) contribute to a better understanding of the genetic evolution and diet preferences, respectively. The assessment of diet and environment of a particular population is based principally on the analysis of botanical remains, faunal remains, palynological analysis and detailed study of material culture at a particular site. Trace element analyses are widely used to reveal knowledge about group specific nutrition and pollution with toxic substances (Horwood 1989; Ezzo 1994). The interpretations based on these findings though offer a picture of gross utilisation of food and ecology by the ancient people, certain caveats need to be addressed. Different organic remains like grains and bones are preserved to a variable extent at an archaeological site. This may be due to favourable/unfavourable burial conditions or scavenging activities by animals etc. The archaeological evidences provide a view into the quality of the diet consumed by the people but do not necessarily provide quantitative share of each food element in their diet. Moreover, the clue to the environment prevalent during that period needs to be based on careful observation of the floral and faunal elements prevalent in the particular time and space and cultural adaptation of humans to the changed environment.

Migrations to more resourceful locations have played an important role in the survival of early humans in the past. Climate changes made places inhabitable to the hominins initiating movements in search of a more habitable environment. Habitation or abandonment of a particular location by ancient humans exhibits their behavioural adaptation to the changing ecology. Understanding the reasons behind these movements is essential to reconstruct the past.

Reproduction and fertility are of utmost importance for survival and multiplication of a population. It has been observed that pregnancy and birth spacing are often manipulated by humans to optimally utilise available resources. In case of a heavy population burden or under unfavourable conditions prolonged breast feeding is used as a contraceptive method. Breast feeding is observed to postpone ovulation (Lewis *et al* 1991; Rivera *et al* 1988). In nomadic ethnic groups, where suitable weaning food is scarce and large family size puts the mother and group in jeopardy, infants are breast fed for a long time to increase birth spacing. On the contrary, in a sedentary food producing community, period of breast feeding is shortened. An infant is shifted to solid food much earlier. Easy availability of weaning food and more secure food economy culminates in a larger family size. Apart from the biological and ecological aspects, cultural traditions customary in an ethnic group also influence the age of weaning. Hence, determination of age of weaning has tremendous importance in archaeological and ethnographic studies (Fuller *et al* 2006)

Deccan College Post-Graduate and Research Institute, Pune, IN

Corresponding author: Sangeeta Mahajan (mahajansangeeta@yahoo.com)

In recent decades, stable isotope analyses have been reliably used to investigate diet, ecology, migration and age of weaning by researchers. Plethora of recent studies signifies the importance of stable isotope analysis in archaeology. However, it needs to be underlined that stable isotope analyses complement various other investigations rather than replacing them. For example, unless the crops cultivated and animals domesticated for food at a site are known, the quantitative isotopic characterisation is unworkable. Where botanical/faunal evidences are unavailable (like in the case of hominins) tooth wear analysis needs to be coupled with isotope analysis to decipher food strategies.

Here, we are considering two recent stable isotope studies performed in India as examples for discussion and comparison between human and faunal tooth enamel as regards to their scope and limitations–

> **[A]** The first study seeks to obtain inputs into the diet and ecology of the people of chalcolithic site of Inamgaon by analysing human tooth enamel (Mahajan 2019) and
> **[B]** The second study analyses buffalo and cattle tooth enamel in an attempt to distinguish between two different taxa (cattle and buffalo) through stable isotope analysis. (Kalwankar 2017).

This paper assesses the scope and limitations of applying stable isotope analysis to humans and animals under different contexts and how each case differs from the other in terms of the research planning and implementation. Here, taking reference from noteworthy past stable isotope studies worldwide, we will discuss the scope and limitations of stable isotope analysis, keeping in mind the case studies [A] and [B] as representatives of human and animal tooth enamel analyses in India, respectively. We will elaborate the topic step by step as evident in material selection, sampling methods, methodologies and interpretations.

2 Discussion

Since the advent of stable isotope analysis, a vast corpus of studies has been undertaken by researchers following different methodologies. Studies analysing carbon, nitrogen, oxygen, sulphur, lead, strontium stable isotopes are largely published. Here, we will restrict our discussion to carbon, nitrogen, oxygen and strontium isotopes. Many organic tissues (bone, nail, hair, lipids, amino acids, blood cells, breath etc) (Herrscher *et al* 2017; Petze *et al* 2005; Smith *et al* 2009; Bearhop *et al* 2002; Voigt *et al* 2008) and inorganic tissues (apatite) (MacFadden *et al* 1994; Keyoner *et al* 2013) of humans and animals have been analysed for stable isotopes. Here, we restrict ourselves to studies utilising bone, enamel and dentine.

2.1 Considerations in Material selection

Amongst tissues analysed for isotopes, hair, nails and teeth are incremental tissues and preserve records of seasonal variations as their mineralisation progresses. All other tissues including bones remodel periodically. Preservation of such tissues at archaeological sites is always uncertain. Extremely cold geological zones preserve tissues for a longer period. In hot and humid climate biological remains degenerate rapidly. In extreme hot and dry conditions bones and teeth are preserved well for a longer time. Hence, when it comes to material selection for stable isotope analysis, the choice may be limited. Consequently, teeth and bones make for most of the analysed samples.

Mahajan (2019) analyses carbon and oxygen isotopes of tooth enamel of the archaeological individuals from the three habitational phases at the ancient site of Inamgaon, District Pune, Maharashtra Total 20 archaeological teeth from 10 burials – two teeth from each individual – were selected. Based on $\delta^{13}C$ enrichment the observations strongly suggested that there was a shift to a more C4 diet towards the last habitational phase. Also, $\delta^{18}O$ enrichment towards the last habitational phase indicated increasingly arid climate.

In Kalwankar (2017), the teeth of five modern buffaloes and five modern cattle and two unknown fossil teeth samples from Narmada and Manjra valley were selected. The fossil samples were otherwise indistinguishable taxonomically (cattle v/s buffalo) from each other based on their morphological characteristics. Modern teeth were procured from the mandibles of slaughtered animals and method by Balasse and Ambrose (2005) was used to process the samples. The detailed results can be obtained from Kalwankar (2017). The results suggested that there was a definite pattern of isotopic distribution during the life span of the animal. This pattern was species specific.

We begin with the selection of material; tooth enamel in case of both these studies. At any given archaeological site faunal remains are generally abundant. These belong to a variety of domesticated and wild species. The domestic animals are either contributing to food economy in the form of meat or can be used as beasts of burden and in some cases they are reared as a source of milk production. The teeth of large animals are better preserved because of their sheer large size. The large size renders serial sampling along the long axis of the tooth easy. Just a dental drill bit is what is necessary to acquire enamel powder. Hence, even season wise stable isotope values can be obtained from a single tooth of an animal. The portion near the occlusal table represents older values in the life of an animal. Almost invariably, taphonomic location of domesticated animals also remains the same as origin. Wild animals are hunted for meat, horns, fur, bones etc. Consequently, faunal teeth are generally available in plenty.

Acquiring permissions to use archaeological faunal samples for a study is an easier process as compared to acquiring archaeological human samples. As in case of Mahajan (2019) and Kalwankar (2017) the sample size of archaeological teeth is inadequate. Deriving conclusions based on such scarce data can be misleading at times. Sample size, as large as possible, needs to be worked upon to arrive at a meaningful inference. Another way to tackle this problem is to add samples from living population in a study as is done in both these studies. This helps in two ways. The values received from living population give a general idea about the results expected. This is because most of the contributing factors like diet, environment and location are known. Also, it is a good idea to compare the archaeological samples with the modern ones to understand the deviation in the values brought about by causative factors through time and space.

As in case of Mahajan (2019) discovery of human teeth in an excavation is unpredictable and one has to make most of the available specimens. The reason is differential preferences for inhumation practices across populations. Many a times, only people from privileged class are inhumed in a proper burial. As in case of study [A], at Inamgaon only 243 burials were confronted over the total habitational period of around 1400 years. Rest of the deceased were probably either cremated or left as sky burial in the open etc. Many of the skeletons were deteriorated or fragmented, leading to loss of considerable number of teeth. There was no adult burial from Malwa phase. In whatever child burials were recovered, no skeletons possessed permanent second molar tooth, which was the requirement of the study. Even for the Early and Late Jorwe phases, only isolated permanent second molars had to be selected, where third molar would have been an ideal choice. Third molars are often impacted deep in the jaws. Removing attached teeth from a jaw can fracture the brittle jaw bone thereby destroying the specimens.

2.2 Considerations in Sampling

The biological remains under burial conditions are subject to chemical changes. Calcium in the tissues is replaced by leaching of minerals from the surroundings leading to diagenesis (Lee-Thorp 2002; Berna *et al* 2004). Diagenesis tends to influence the outcome of the analyses adversely. This compelled researchers to develop techniques to treat the samples prior to isotope analysis so that the diagenetic effects could be nullified (Gehre and Strauch 2003; Grimes and Pellegrini 2012). Several methods specific to tissue analysed were developed and such pre treatment of samples has now become a mandatory part of analysis of the archaeological remains.

The crown height of longest of the human tooth is less than 1cm. Most often than not, they are cracked, fractured or with some fragments missing. In such cases, it becomes difficult to do serial sampling along the long axis of the tooth which is so important to know the incremental variations in enamel composition. Serial samples offer a greater resolution with regards to the incremental layers of enamel representing birth to adulthood through various stages of life (Reade *et al* 2015). In Mahajan (2019) bulk average sampling was done as most of the teeth were fragmented. Also, drilling an ancient tooth to get the enamel powder tends to break the brittle tooth into pieces. Laser ablation is an ideal procedure to be followed in this case (Cerling and Sharp 1996; Passey and Cerling 2006). However, it was beyond the scope of Mahajan (2019). Powder of human tooth enamel was taken from the fresh surface of cuspal enamel of the polished section by a dental drill. Carbon and oxygen isotopes were measured as described in Agrawal *et al* (2013). In Kalwankar (2017) powder of buffalo and cattle teeth enamel was obtained as described by Balasse and Ambrose (2005).

To achieve the desired results, selection of appropriate tissue and sampling procedure is critical. In tooth studies, the location of obtaining sample varies depending upon whether the desired enamel or dentine is prenatal, natal or postnatal. The occlusal mineralisation takes place ahead of the cervical part. Bulk average method considers average of the occluso-cervical enamel and hence, represents the total crown formation period. Sequential sampling involves multiple samples along the long axis of the tooth so that incremental stable isotope values can be obtained. Each increment corresponds to a successively later part of life and exhibits changes occurred during tooth mineralisation stages.

Bone remains physiologically active throughout life. Depending upon the location of body, remodelling period varies. Hence, selection of bone sample is very important to fulfil the research requirements.

2.3 Considerations in Methodology and Interpretations

In Mahajan (2019), 4 samples from Malwa phase, 8 samples each from Early Jorwe and Late Jorwe phase were selected in such a way that two teeth from each individual would represent different stage of life of that individual. The carbon and oxygen stable isotope ratios in different tooth types of the concerned individual will provide information about the diet and change in climate during the life of that individual, depending upon the time of formation. Simultaneously, the variation in carbon and oxygen stable isotope values in the same tooth types across different habitational phases will throw light on the temporal change in subsistence strategies and climate during different phases. The age of weaning of infants can also be estimated by comparing isotope variation between first and second molars of a given individual.

Kalwankar (2017) on the other hand, analyses carbon and oxygen isotope ratios in the selected paleontological animals and compares it with the modern cattle and buffalos to understand the correlation between specific patterns of stable isotope values in a particular taxon.

The first ever stable isotope study was undertaken by Vogel and Merwe (1977). They concluded that maize being a food processor using C4 photo synthetic pathway possesses enriched $\delta^{13}C$ signature. The carbon stable isotope values are a reliable indicator for introduction of C4 plants in an otherwise C3 biome. $\delta^{13}C$ values are since been used to determine diet preferences in animals and humans. The analysis helps distinguish between browsers and grazers. Animals fed on agricultural waste and those foraged in the wild can be distinguished (Chase *et al* 2014). Nitrogen isotope analysis helps distinguish between terrestrial and marine food (Schoeninger and DeNiro 1983). Nitrogen isotope analysis is also used to identify trophic level (Robson *et al* 2016). In case Mahajan (2019), there was shift in staple diet from wheat in Early Jorwe phase to millets in Late Jorwe phase (Kajale 1988). Though recovery of few grains proves the cultivation of these grains, their exact contribution to the diet at each phase could be estimated by carbon stable isotope analysis (Mahajan 2019). The archaeological evidence pointed to the presence of wheat, millets, rice, pulses etc. but did not explain their individual contributions to the diet. The contribution of C3/C4 plants and the meat of animals eating these plants in diet are effectively indicated by carbon isotopes. But these interpretations are to be strictly arrived at by taking into consideration the archaeological evidences. In Mahajan (2019), the $\delta^{13}C$ enrichment is associated with greater consumption of millets and meat of wild grazers which was as a consequence of increased aridity indicated by $\delta^{18}O$ enrichment. The addition of nitrogen stable isotope analysis gives further resolution about the share of animal protein in the diet. Though, these prove to be very useful insights in archaeology, the relation between diet and $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ values is not always straightforward as discussed in Milner *et al* (2003). Kalwankar (2017) is also a noteworthy example. Though the diet of cattle and buffalos does not differ significantly, the $\delta^{13}C$ and $\delta^{18}O$ composition of tooth enamel is significantly different at different stages of maturity.

Carbon, oxygen and nitrogen stable isotope analyses in combination provide valuable details about duration of breast feeding and shifting to adult diet of an infant. Carbon isotopes tell us about the introduction of solid food to a breast feeding infant (Fuller *et al* 2003). Nitrogen isotopes denote shift to a complete adult food indicating change in trophic level (Schurr 1998). Oxygen isotopes point to complete cessation of breast feeding (Wright and Schwarcz 1998).

In animals raised on controlled diet the $\delta^{13}C$ values of whole body were noted to reflect the diet and average whole body enrichment of $\delta^{13}C$ was found to be 1 $\%_0$ relative to the diet (DeNiro and Epstein 1978). Similar studies gave a firm foundation to use stable isotope analyses for different diet related issues. Stable isotope studies on the native American populations before and after introduction of maize unfolded that though maize consumption increased to about 50% of the diet (based on $\delta^{13}C$ enrichment), there had been a steady consumption of meat and fish all along (based on $\delta^{15}N$). In such studies, $\delta^{15}N$ is essential to know the share of animal proteins in the diet (Schwarcz *et al* 1985). A study on Cenozoic mammals of South America investigated the appearance of C4 plants in a C3 environment in America (25 Ma to 7500 yr ago) based on $\delta^{13}C$ of tooth enamel. It was suggested that C3 plant eaters retained low crown heights whereas C4 consumers developed high crown heights (MacFadden *et al* 1994). In such studies, consideration should be given to the fact that the $\delta^{13}C$ values do not necessarily indicate foods available to organisms but rather food preferences opted by them. Evaluation of ethnographic analogues plays important role in archaeology to arrive at a logical conclusion. Based on ethnographic evidences Balasse *et al* (2003) established antiquity of ingestion of seaweed by sheep during winter season to c. 3000 BC using tooth enamel carbon and oxygen stable isotopes. Through an innovative approach estimating duration of reproductive period in cattle and sheep from tooth enamel oxygen isotope analysis Balasse and Tresset (2007) proposed that cultural modifications in the animal breeding practices like migrations and transhumance helped humans modify and improve fertility in animals. Oxygen stable isotopes are reliable in interpreting migrations and transhumance of humans and animals. Determination of habitat preferences by ancient taxa based on $\delta^{13}C$ and $\delta^{18}O$ values reveals the biome present during those periods and the changes in ecology (Zin-Maung-Maung-Thein *et al* 2011). Case of Kalwankar (2017) goes further on this line to identify two very distinct taxa – cattle and buffalo- based on carbon and oxygen stable isotope analysis. These two taxa consume similar diets and enjoy different habitats. Though, these two taxa are very dissimilar phenotypically, their archaeological remains are hard to distinguish from each other unless the diagnostic parts are available. Kalwankar (2017) shows very peculiar differences in isotopic values in both the taxa and promises to be a good diagnostic tool in the context of Indian archaeology. This is probably first of its kind of a study. Klein (2013) tracked the human evolution through carbon isotope analysis for diet and correlated bipedalism in humans to increased abundance of C4 plants in the C3 biome. To undertake such studies, more and more skeletal remains need to be analysed for isotopes. A very prototype study by Lee-Thorp *et al* (1989) to determine the $\delta^{13}C$ for collagen and apatite of an individual to understand the apatite-collagen spacing proposed that the relationship of collagen to apatite in an individual depended on the trophic level. This study holds importance in the process of interpreting values obtained in archaeology, as apatite and collagen of bone and tooth of same individual carry differential values. A classic example of importance of comprehensive investigation of archaeological evidences before interpreting stable isotope values is discussed by Milner *et al* (2003). It also reiterates that sample bias in the selection of archaeological samples plays an important role and thorough investigation of available data is therefore very essential. The usefulness in determination of relative proportions of variety of foods in ancient diets is the most important contribution of stable isotope analysis to archaeology. These observations when weighed against the case Mahajan (2019) on samples from Inamgaon, there are certain points to ponder. As the faunal and botanical analysis at Inamgaon suggests that the ratio of consumption of plants to animals in diet varied in different habitational phases, it is mandatory that nitrogen stable isotope analysis be done to get the exact ratio of consumption of plants against animals. As also, since millets was staple food of people of Inamgaon in all phases, it becomes necessary to know the exact source of enriched $\delta^{13}C$ values during Late Jorwe phase; was it the C4 grains or the meat of the grazers. This necessitates that for interpreting diet of an ancient populations both $\delta^{13}C$ and $\delta^{15}N$ need to be analysed simultaneously along with thorough inspection of the associated archaeological evidences.

The breeding season of cattle and sheep/goat was studied by Balasse and Tresset (2007) analysing sequential samples of tooth enamel for $\delta^{18}O$ to determine their birth cycle. This is a very path breaking approach to the cultural modifications used by ancient people in herd management through controlled fertility. This aspect has important implications on survival of cultures in harsh environments. This approach works well in case of static populations as well as those undergoing transhumance seasonally (Makarewicz *et al* 2017). Based on $\delta^{18}O$ and $\delta^{13}C$ of enamel and other tissues Cerling *et al* (2007) proposed that contrary to previous notion that *Hippopotamus amphibius* is a grazer, the isotopic results suggest that they even consume considerable amount of C3 biomass. Also, they are the most $\delta^{18}O$ depleted animals in the ecosystem due to their semi-aquatic habitat. Oxygen and carbon stable isotopes can be effectively used to determine pattern of survival and extinction of a species. Jukar *et al* (2019) have claimed to chronologically arrange the *Hexaprotodon* species in Indian subcontinent based on tooth enamel $\delta^{18}O$ and $\delta^{13}C$. Feranec (2004) analysed enamel of marmot cheek teeth for $\delta^{18}O$ and $\delta^{13}C$ in order to determine their diet and ecology. It was concluded that 8 month long hibernation without drinking water failed to distinguish oxygen isotope stages based on $\delta^{18}O$. $\delta^{13}C$ also was unable to distinguish the stages. It was suggested that a sequential tooth sampling identifying period of hibernation would help. Though, these analyses would work well on non hibernating obligate drinkers.

A study by Sarkar *et al* (2016) is a welcome effort in Indian archaeology. This study analysed animal teeth-bone phosphates for oxygen isotopes from Bhirrana, NW India, which exhibits continuous habitation by people of Harappan civilization. It was concluded that even after decline of monsoon after 7 ka, the civilisation still survived. Hence, aridity was not the cause of decline of Harappan civilisation but change in subsistence strategies by shifting crop patterns maybe the culprit.

Seasonal vertical migration of domestic animals in search of pasture has been a common practice since ancient times. Makarewicz *et al* (2017) analysed intra tooth $\delta^{18}O$ and $\delta^{13}C$ by sequential sampling sheep and goat tooth enamel from a chalcolithic site Kosk Hoyuk for vertical transhumance. It was found that the $\delta^{18}O$ and $\delta^{13}C$ values were cyclically inversely proportional. High summer $\delta^{18}O$ associated with low $\delta^{13}C$ indicated consumption of moist $\delta^{13}C$ depleted pasture at higher elevation and $\delta^{13}C$ enriched fodder during winter. This method is helpful in understanding human manipulation of herds.

$\delta^{18}O$ is widely used to reconstruct past climate. With the exception of few studies, it has hardly been used in Indian archaeology. The decline of Harappan civilisation, the growth and decline of Central Indian Chalcolithic cultures, frequent flooding associated with Gangetic cultures, glaciations and inter glacial period and its influence on ancient Indian cultures can be studied by analysing faunal and human tooth samples for oxygen stable isotopes. In case of Mahajan (2019), the $\delta^{18}O$ values of permanent second molars point to progressively increased aridity near the end of the habitational period at Inamgaon. Though lot of work has been done on $\delta^{18}O$ of speleothems and ice cores by geological and climate researchers in India, the results indicate a general trend of resolution on a centennial scale. Enamel $\delta^{18}O$ in collagen and apatite unfolds precise turn of events during the lives of the individuals at the habitational site which can be reliably used to arrive at conclusions. Large animals with slow metabolism who are obligate drinkers are the best candidates for oxygen isotope analysis for the study of palaeoecology (Luz *et al* 1984).

Another important implication of $\delta^{18}O$ is determining the mobility of an individual. The $\delta^{18}O$ of tooth enamel mainly depends on the water consumed by an organism and in case of browsers, on the leaf water as well (Kohn *et al* 1996; Fricke *et al* 1995). $\delta^{18}O$ of meteoric water depends upon the precipitation and the distance from the source of precipitated water. Hence, by comparing tooth enamel $\delta^{18}O$ with local meteoric water, mobility of an individual can be traced (Dury *et al* 2018). This method can widely be used to prove the migration hypotheses in Indian archaeology.

A study by Keyoner *et al* (2013) analysed tooth apatite and bone collagen from individuals buried at Harappa cemetery in Pakistan and cemetery at Ur in Mesopotamia. The strontium isotopic ratio of tooth and bone of an individual was used to determine the place of childhood and the place of birth. The $\delta^{87}Sr/^{86}Sr$ of teeth and bone is related to the geological strontium signatures of a particular location. It was observed that there was a substantial variation in the strontium values of Harappan individuals suggesting foreign places of origin. The individuals from cemetery of Ur did not show much variation indicating local origin. In case of Mahajan (2019), the people of Malwa phase are first to migrate to Inamgaon. Strontium isotope analysis should help identify their place of origin. But unfortunately, adult skeletons are absent from this phase. However, semi-nomadic life of Late Jorwe people can be traced by this method.

Valentine *et al* (2015) conducted strontium and lead stable isotope analyses on the tooth enamel of individuals from Harappa and Farmana cemetery. Three tooth types from each individual representing three cohorts were selected. The faunal teeth and soil samples at the sites were also analysed to understand the local isotopic values. The results indicated that the first molar cohort of almost all individuals at Farmana exhibited values of non-local origin. The other two cohorts were well within the range of local samples. This meant that these individuals were born and brought up in early childhood non-locally and migrated to Harappa and Farmana later. Hence, it was proposed that only first generation immigrants were offered inhumation in these cemeteries and it was a specialised cultural custom which was not presented to local people. This probably is the only isotope study on human tooth enamel prior to that on Inamgaon population (Mahajan 2019). In Indian archaeology, this method holds promise to evaluate the pattern and route of individual and mass migration or seasonal migration of humans and animals and their origins.

Hughes *et al* (2014) performed human tooth enamel oxygen and strontium stable isotope analyses at Berinsfield cemetery to understand the origins of the individuals. The Anglo-Saxon conquest of Roman period Briton has always been a point of controversy as to whether the conquest brought about almost complete replacement of the indigenous population. This study claims that only 5.3% population of all individuals analysed were from Europe, rest all were local born. These results endorse the theory of acculturation rather than complete replacement. In Indian archaeological context, Aryan invasion vs. acculturation has always been a point of hot debate. A strontium isotope analysis of the deceased individuals from Harappan civilisation will certainly help in providing a scientific answer to this question.

3 Concluding remarks

India is a vast peninsula with different geographical and environmental features. A large number of sites have been excavated belonging to different locations and different periods. There are hundreds of human skeletons recovered during excavations in the last few decades (Mushrif *et al* 2016) which are lying idle after the basic investigations. These biological remains are bound to undergo degenerative changes faster in the repositories than in the burial environment. Temperature and humidity regulated environments for these precious samples are rare to find in India. Without further archaeochemical investigations they will be lost along with the treasure of information they are packed with, forever. Till date, faunal tooth stable isotopes analyses are in an incipient stage (Sarkar *et al* 2016; Chakraborty 2019). Moreover, human tooth stable isotope analytical studies are absent. The implications of stable isotope analyses in animals and humans are completely different. Study of stable isotopes in animals helps understand the baseline values of a particular location, though domesticated animals are often manipulated by humans to their advantage. Humans however, adapt themselves to the changed ecology by changing their food strategies, migrating to more favourable places and producing their own food. Their behavioural abilities facilitate them to survive and thrive in the given circumstances. Such may not be the case with the animals. Hence, interpretations of isotope values in both need to be carried out differently. Unless both are used in combination, a comprehensive understanding of an archaeological site may not be achieved.

Exploiting stable isotope analyses to answer these questions has not gained momentum in India. There is a huge scope for use of stable isotopes in Indian archaeology, provided the traditional approach of excavation methods is expanded further into serious endeavours towards timely treatment of materials for various analytical methods of archaeological sciences.

Competing Interests

The authors have no competing interests to declare.

References

Balasse, M and **Ambrose, SH.** 2005. Distinguishing sheep and goats using dental morphology and stable carbon isotopes in C4 grassland environments. *Journal of Archaeological Science*, 32: 691e702. DOI: https://doi.org/10.1016/j.jas.2004.11.013

Balasse, M and **Tresset, A.** 2007. Environmental constraints on the reproductive activity of domestic sheep and cattle: what latitude for the herder? *Anthropozoologica*, 42(2): 71–88.

Balasse, M, Tresset, A and **Ambrose, SH.** 2005. Stable isotope evidence ($\delta^{13}C$, $\delta^{18}O$) for winter feeding on seaweed by Neolithic sheep of Scotland. *Journal of Zoology*, 270: 170–176. DOI: https://doi.org/10.1111/j.1469-7998.2006.00104.x

Bearhop, S, Waldron, S, Votier, SC and **Furness, RW.** 2002. Factors That Influence Assimilation Rates and Fractionation of Nitrogen and Carbon Stable Isotopes in Avian Blood and Feathers. *Physiological and Biochemical Zoology*, 75(5): 451–458. DOI: https://doi.org/10.1086/342800

Berna, F, Matthews, A and **Weiner, S.** 2004. Solubilities of bone mineral from archaeological sites: the recrystallization window. *Journal of Archaeological Science*, 31: 867–82. DOI: https://doi.org/10.1016/j.jas.2003.12.003

Cerling, CE and **Sharp, ZD.** 1996. Stable carbon and oxygen isotope analysis of fossil tooth enamel using laser ablation. *Palaeogeology, Palaeoclimatology, Palaeoecology*, 126(1–2): 173–186. DOI: https://doi.org/10.1016/S0031-0182(96)00078-8

Cerling, TE, Harris, JM, Hart, JA, Kaleme, P, Klingel, H, Leakey, MG, Levin, NE, Lewison, RL and **Passey, BH.** 2007. Stable isotope ecology of the common hippopotamus. *Journal of Zoology*, 276: 201–212. DOI: https://doi.org/10.1111/j.1469-7998.2008.00444.x

Chakraborty, P. 2019. Impact of animal exploitation as seen in the bones from chalcolithic sites in Western India, with special reference to skeletal microstructure. PhD thesis submitted to Deccan College Post Graduate and research Institute.

Chase, B, Meiggs, D, Ajithprasad, P and **Slater, PA.** 2014. Pastoral land-use of the Indus civilisation in Gujarat: faunal analysis and biogenic isotopes in Bagasra. *Journal of Archaeological Science*, 50: 1–15. DOI: https://doi.org/10.1016/j.jas.2014.06.013

Daegling, DJ, Hua, L-C and **Ungar, PS.** 2016. Role of food stiffness in dental micro wear feature formation. *Archives of Oral Biology*, 71: 16–23. DOI: https://doi.org/10.1016/j.archoralbio.2016.06.018

DeNiro, MJ and **Epstein, S.** 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta*, 42: 495–506. DOI: https://doi.org/10.1016/0016-7037(78)90199-0

Dury, G, Lythe, A, Marquez-Grant, N, Garcia-Rubio, A, Graziani, G, Mari, J, Ziriax, M and **Schulting, R.** 2018. The Islamic cemetery at 33 Bartomeu Vicent Ramon, Ibiza: investigating diet and mobility through light stable isotopes in bone collagen and tooth enamel. *Archaeological and Anthropological Sciences*. DOI: https://doi.org/10.1007/s12520-018-0644-4

Ezzo, JA. 1994. Zinc as a Paleodietary Indicator: An Issue of Theoretical Validity in Bone-Chemistry Analysis. *American Antiquity*, 59(4): 606–621. DOI: https://doi.org/10.2307/282336

Feranec, RS. 2004. Stable Carbon and Oxygen Isotope Analysis of Marmot Cheek Teeth from the Pit Locality. *Biodiversity Response to Climate Change in the Middle Pleistocene: The Porcupine Cave Fauna from Colorado*, Barnosky, AD (ed.). Berkeley: University of California Press. DOI: https://doi.org/10.1525/california/9780520240827.003.0024

Fiorenza, L, Benazzi, S, Tausch, J, Kullmer, O, Bromage, TG and **Schrenk, F.** 2011. Molar macro wear reveals Neanderthal eco-geographic dietary variation. *PLoS ONE*, 6(3): 1–11. DOI: https://doi.org/10.1371/journal.pone.0014769

Fricke, HC, O'Neil, JR and **Lynnerup, N.** 1995. Oxygen Isotope Composition of Human Tooth Enamel from Medieval Greenland: Linking Climate and Society. *Geology*, 23: 869–872. DOI: https://doi.org/10.1130/0091-7613(1995)023<0869:SROICO>2.3.CO;2

Fuller, BT, Fuller, JL, Harris, DA and **Hedges, REM.** 2006. Detection of Breastfeeding and weaning in Modern Human Infants with Carbon and Nitrogen Stable Isotope Ratios. *American Journal of Physical Anthropology*, 129: 279–293. DOI: https://doi.org/10.1002/ajpa.20249

Gehre, M and **Strauch, G.** 2003. High-temperature elemental analysis and pyrolysis techniques for stable isotope analysis. *Rapid communications in mass spectrometry*, 17(13): 1497–1503. DOI: https://doi.org/10.1002/rcm.1076

Grimes, V and **Pellegrini, M.** 2012. A comparison of pretreatment methods for the analysis of phosphate oxygen isotope ratios in bioapatite. *Rapid communications in mass spectrometry*, 27(3): 375–390. DOI: https://doi.org/10.1002/rcm.6463

Herrscher, E, Goude, G and **Metz, L.** 2017. Longitudinal study of stable isotope compositions of maternal milk and implications for the palaeo-diet of infants. *Bulletins et Mémoires de la Société d'anthropologie de Paris*. Springer Verlag, 29(3–4): 131–139. DOI: https://doi.org/10.1007/s13219-017-0190-4

Horwood, M. 1989. Trace element analysis of human bone from the prehistoric Moriori of the Chatham Islands, with special reference to diet. *Journal of the Royal Society of New Zealand*, 19(1): 59–71. DOI: https://doi.org/10.1080/03036758.1989.10426456

Hughes, SS, Millard, AR, Lucy, SJ, Chenery, CA, Evans, JA, Nowell, G and **Pearson, DG.** 2014. Anglo-Saxon origins investigated by isotopic analysis of burials from Berinsfield, Oxfordshire, UK. *Journal of Archaeological Science*, 42: 81–92. DOI: https://doi.org/10.1016/j.jas.2013.10.025

Jukar, AM, Patnaik, R, Chauhan, PR, Li, H-C and **Lin, J-P.** 2019. The youngest occurrence of *Hexaprotodon* Falconer and Cautley, 1836 (Hippopotamidae, Mammalia) from South Asia with a discussion on its extinction, to be published in *Quaternary Interntional*. DOI: https://doi.org/10.1016/j.quaint.2019.01.005

Kajale, MD. 1988. Plant economy, Excavations at Inamgaon, 1(2). Deccan College Post Graduate and Research Institute.

Kalwankar, C. 2017. *Stable isotopes and bovine taxonomy*. Dissertation submitted to Deccan College Post-Graduate and Research Institute, Pune for the Degree of Master of Arts in Archaeology.

Kenoyer, JM, Price, TD and **Burton, JH.** 2013. A new approach to tracking connections between the Indus Valley and Mesopotamia: initial results of strontium isotope analyses from Harappa and Ur. *Journal of Archaeological Science*, 40: 2286–2297. DOI: https://doi.org/10.1016/j.jas.2012.12.040

Klein, RG. 2013. Stable carbon isotopes and human evolution. *PNAS*, 110(26): 10470–10472. DOI: https://doi.org/10.1073/pnas.1307308110

Kohn, MJ, Schoeninger, MJ and **Valley, JW.** 1996. Herbivore tooth oxygen isotope compositions: Effects of diet and physiology. *Geochimica et Cosmochimica Acta*, 60(20): 3889–3896. DOI: https://doi.org/10.1016/0016-7037(96)00248-7

Lee-Thorp, JA. 2002. Two decades of progress towards understanding fossilisation processes and isotopic signals in calcified tissue minerals. *Archaeometry*, 44: 435–46. DOI: https://doi.org/10.1111/1475-4754.t01-1-00076

Lee-Thorp, JA, Scaly, JC and **van der Merwe, NJ.** 1989. Stable Carbon Isotope Ratio Differences Between Bone Collagen and Bone Apatite, and their Relationship to Diet. *Journal of Archaeological Science*, 16: 585–599. DOI: https://doi.org/10.1016/0305-4403(89)90024-1

Lewis, PR, Brown, JB, Renfree, MB and **Short, RV.** 1991. The resumption of ovulation and menstruation in a well-nourished population of women breastfeeding for an extended period of time. *Fertility and Sterility*, 55(3): 55–529. DOI: https://doi.org/10.1016/S0015-0282(16)54180-6

Luz, B, Kolodny, Y and **Horowitz, M.** 1984. Fractionation of oxygen isotopes between mammalian bone-phosphate and environmental drinking water. *Geochimica et Cosmochimica Acta*, 48: 1689–1693. DOI: https://doi.org/10.1016/0016-7037(84)90338-7

MacFadden, BJ, Wang, Y, Cerling, TE and **Anaya, F.** 1994. South American fossil mammals and carbon isotopes: a 25 million-year sequence from the Bolivian Andes. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 107: 257–268. DOI: https://doi.org/10.1016/0031-0182(94)90098-1

Mahajan, S. 2019. *Stable isotopic analysis of human tooth enamel for palaeo diet and palaeo climate at Inamgaon, District Pune, Maharashtra*. Dissertation submitted to Deccan College Post-Graduate and Research Institute, Pune for the Degree of Master of Arts in Archaeology.

Makarewicz, CA, Arbuckle, BS and **Oztan, A.** 2017. Vertical transhumance of sheep and goats identified by intra-tooth sequential carbon and oxygen isotope analyses; Evidence from chalcolithic Kosk Hoyuk, Central Turkey. *Journal of Archaeological Science*, 86: 68–80. DOI: https://doi.org/10.1016/j.jas.2017.01.003

Milner, N, Craig, OE, Bailey, GN, Pedersen, K and **Andersen, SH.** 2013. Something fishy in the Neolithic? A re-evaluation of stable isotope analysis of Mesolithic and Neolithic coastal populations. *Antiquity*, 78(299):

9–22. DOI: https://doi.org/10.1017/S0003598X00092887

Mushrif-Tripathy, V, Chakraborty, KS and **Lahiri, S.** 2016. Where Are They Now? The Human Skeletal Remains from India. In: *A companion to South Asia in the past*, 496–553. Wiley online library. DOI: https://doi.org/10.1002/9781119055280.ch32

Passey, BH and **Cerling, TE.** 2006. In situ stable isotope analysis ($\delta^{13}C$, $\delta^{18}O$) of very small teeth using laser ablation GC/IRMS. *Chemical Geology*, 235(3–4): 238–249. DOI: https://doi.org/10.1016/j.chemgeo.2006.07.002

Petzke, KJ, Boeing, H, Klaus, S and **Metges, CC.** 2005. Carbon and Nitrogen Stable Isotopic Composition of Hair Protein and Amino Acids Can Be Used as Biomarkers for Animal-Derived Dietary Protein Intake in Humans. *The Journal of Nutrition*, 135(6): 1515–1520. June. DOI: https://doi.org/10.1093/jn/135.6.1515

Rai, N, Verma, SK, Gaur, A, *et al.* 2020. Ancient mtDNA from the extinct Indian cheetah supports unexpectedly deep divergence from African cheetahs. *Sci Rep*, 10: 4618. DOI: https://doi.org/10.1038/s41598-020-60751-7

Reade, H, Stevens, RE, Barker, G and **O'Conell, TC.** 2015. Tooth enamel sampling strategies for stable isotope analysis: Potential problems in cross-method data comparisons. *Chemical Geology*, 404: 126–135. DOI: https://doi.org/10.1016/j.chemgeo.2015.03.026

Rivera, R, Kennedy, K, Ortiz, E, Barrera, M and **Bhiwandiwala, PP.** 1988. Breast-feeding and the return to ovulation in Durango, Mexico. *Fertility and Sterility*, 49: 780. The American Fertility Society. DOI: https://doi.org/10.1016/S0015-0282(16)59883-5

Robson, H, Andersen, SH, Clarke, L, Craig, OE, Gron, KJ, Jones, AKG, Karsten, P, Milner, N, Price, TD, Ritchie, K, Zabilska-Kunek, M and **Heron, C.** 2016. Carbon and nitrogen stable isotope values in freshwater, brackish and marine fish bone collagen from Mesolithic and Neolithic sites in central and northern Europe. *Environmental Archaeology*, 21(2): 105–118. DOI: https://doi.org/10.1179/1749631415Y.0000000014

Sarkar, A, Deshpande-Mukherjee, A, Bera, MK, Das, B, Juyal, N, Morthekai, P, Deshpande, RD, Shinde, VS and **Rao, LS.** 2016. Oxygen isotope in archaeological bioapatites from India: Implications to climate change and decline of Bronze Age Harappan civilization. *Scientific Reports*, 6: 26555. DOI: https://doi.org/10.1038/srep26555

Schoeninger, MJ and **DeNirO, MJ.** 1983. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. *Geochimica n Cosmochimica ACIP*, 48: 625–539. DOI: https://doi.org/10.1016/0016-7037(84)90091-7

Schurr, MR. 1998. Using stable nitrogen-isotopes to study weaning behavior in past populations. *World Archaeology*, 30(2): 327–342. DOI: https://doi.org/10.1080/00438243.1998.9980413

Shinde. *et al* 2019. An Ancient Harappan Genome Lacks Ancestry from Steppe Pastoralists or Iranian Farmers. *Cell*, 179: 729–735. DOI: https://doi.org/10.1016/j.cell.2019.08.048

Smith, CI, Fuller, BT, Choy, K and **Richards, MP.** 2009. A three-phase liquid chromatographic method for d13C analysis of amino acids from biological protein hydrolysates using liquid chromatography–isotope ratio mass spectrometry. *Analytical Biochemistry*, 390: 165–172. DOI: https://doi.org/10.1016/j.ab.2009.04.014

Valentine, B, Kamenov, GD, Kenoyer, JM, Shinde, V, Mushrif-Tripathy, V, Otarola-Castillo, E and **Krigbaum, J.** 2015. Evidence for Patterns of Selective Urban Migration in the Greater Indus Valley (2600–1900 BC): A Lead and Strontium Isotope Mortuary Analysis. *PLOSONE*, 10(4): e0123103. DOI: https://doi.org/10.1371/journal.pone.0123103

Vogel, JC and **van der Merwe, NJ.** 1977. Isotopic Evidence for Early Maize Cultivation in New York State. *American Antiquities*, 42(2): 238–242. DOI: https://doi.org/10.2307/278984

Voigt, CC, Baier, L, Speakman, JR and **Siemers, BM.** 2008. Stable carbon isotopes in exhaled breath as tracers for dietary information in birds and mammals. *The Journal of Experimental Biology*, 211: 2233–2238. DOI: https://doi.org/10.1242/jeb.018523

Wright, L and **Schwarcz, LP.** 1998. Stable Carbon and Oxygen Isotopes in Human Tooth Enamel: Identifying Breastfeeding and Weaning in Prehistory. *American Journal of Physical Anthropology*, 106: 1–18. DOI: https://doi.org/10.1002/(SICI)1096-8644(199805)106:1<1::AID-AJPA1>3.0.CO;2-W

Zin-Maung-Maung-Thein, Takai, M, Uno, H, Wynn, JG, Egi, N, Tsubamoto, T, Thaung-Htike, Aung-Naing-Soe, Maung-Maung, Nishimura, T and **Yoneda, M.** 2011. Stable isotope analysis of the tooth enamel of Chaingzauk mammalian fauna (late Neogene, Myanmar) and its implication to paleoenvironment and paleogeography. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 300: 11–22. DOI: https://doi.org/10.1016/j.palaeo.2010.11.016

How to cite this article: Mahajan, S and Sathe, V. 2020. Scope and Limitations of Stable Isotope Analysis in Indian Archaeology. *Ancient Asia*, 11: 6, pp. 1–8. DOI: https://doi.org/10.5334/aa.190

Published: 20 August 2020