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Carbon and Oxygen Isotope Analysis of Modern Cattle *(Bos indicus)* Molars from the Central Narmada Valley, India

RESEARCH PAPER

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ABSTRACT

The carbon and oxygen isotopic composition of tooth enamel is connected to the diet and environment in which it develops. Enamel is invariably preserved for a long time and hence provides the best material for chemical analysis. Teeth are known to reflect a record of dietary and environmental changes taking place during their growth. This paper presents the results of intra-tooth oxygen and carbon isotope values ($\delta^{18}O$, $\delta\delta^{13}C$) of first, second and third molars obtained from five modern cattle collected from two locations: Dhansi and Hathnora from the Central Narmada Valley, India. The specimens chosen for this study are individuals presumed to have died naturally and/or disposed of by local farmers. The isotopic analysis of tooth enamel is broadly indicative of a C_3 diet with values of $\delta^{13}C$ (enamel bioapatite) ranging from –6.4‰ VPDB to –27.31‰ VPDB with an average of –16.68‰ VPDB. The $\delta^{18}O$ values measured in the enamel samples range between of 1.76‰ to 25.15‰ with a mean value of 22.17‰ VSMOW. These present day dental enamel values of modern cattle were compared against the published enamel isotope values of Bos namadicus, that occupied this region during the Pleistocene era, in order to understand the possible shift in diet and environment and their inter-relationship between the modern and the Pleistocene Era. The fossil sample produced enriched values of carbon isotopes compared to the modern taxa, indicating a C_4 rich diet, while the diet of the modern cattle is extensively dominated by C_3 type vegetation. We also observed an enriched oxygen isotope values for the fossil sample compared to the modern samples, indicating a possible effect of diagenesis and/or a shift in the temperature and rainfall.

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1. INTRODUCTION

Isotopic studies have emerged as an important data tool and have been employed in multiple fields and disciplines, from forensics to climate studies, to augment the authenticity of the food source. Isotopic methods can provide information on the behaviour and physiology of individuals as well (Koch et al. 2009). Using stable isotope carbon isotope signatures, multiple problems have been addressed which include estimating the amount of C_4 plant derived food in the diet, including agricultural maize, identifying closed-canopy habitats, evaluation of herd-management strategies of ancient pastoralists, estimating age at weaning (Passey et al. 2005; Mahajan 2019). Besides carbon, isotopes of other elements are also routinely used to interpret changes in the animal dietary habits, their migration patterns, past climate conditions from hypsodont tooth enamel, and ecological background to ancient settlements and sites (Zazzo et al. 2005; Stacy 2008; Valentine et al. 2015; Sarkar et al. 2016; Patnaik et al. 2015; Chakraborty et al. 2018). In the last few years, the isotope study is being extensively used to address the problem of taxonomic discrimination between closely related ungulate taxa as well (Balasse et al. 2005).

Collagen was the first tissue used to reconstruct and trace the animal's diet patterns (DeNiro & Epstein 1978), and since then it has been extensively used for dietary and climatic reconstructions (e.g., van der Merwe & Vogel 1978; Ambrose & DeNiro 1986a, 1986b; Schwarcz 1991; Bocherens et al. 1994; Cormie et al. 1994; Iacumin et al. 1996). Along with collagen, important isotopic inferences were drawn using bioapatite from tooth enamel as well, that not only helped in the reconstruction of past diet and its impact on the environment, but provided valuable information on ecology, climate, species tropic level and seasonal pattern of birth (e.g.; Lee-Thorp & van der Merwe 1987; Lee-Thorp et al. 1989a, 1989b; van der Merwe et al. 1990; Vogel et al. 1990; Bocherens et al. 1996; MacFadden & Cerling 1996; Cerling et al. 1997).

Enamel bioapatite is an important tissue for isotopic analysis primarily because of its resistance to diagenetic alterations and its ability to preserve almost an intact isotope record (Lee-Thorp 1989; Lee-Thorp et al. 1989; Lee-Thorp & Van der Merwe 1991; Shahack-Gross et al. 1999). Enamel bioapatite is preferred because of its larger, more tightly packed structure and highly organized apatite crystals. It is also preferentially used because it forms the exterior coating of teeth, and therefore, it is more easily accessible that allows minimal damage to the specimen (Zazzo et al. 2005). Along with enamel bioapatite, various other tissues from animals that are routinely used are dentine, bones, hair, etc. (Schoeninger & DeNiro 1984; Ambrose & Norr 1993: Hobson et al. 1993; Tieszen & Fagre 1993; Fischer & Fox 1998; Balasse et al. 1999; Balasse et al. 2001; Sponheimer et al. 2003; Ayliffe et al. 2004). Carbon and oxygen isotope time-series records can be reconstructed by analysing samples procured from incrementally growing tissues of an animal.

The growth and development of mammalian teeth take about 1 to 2 years, during which it record seasonal variations in diets, local weather etc. (e.g., Koch et al. 1995; Fricke & O'Neil., 1996; Fricke et al. 1998; Sharp & Cerling 1998; Dettman et al. 2001; Balasse et al. 2003; Nelson 2005; Spoonheimer et al. 2006). Many herbivores display high crowned teeth also known as 'hypsodonts', which translates to their growth taking place sequentially from the cusp at the occlusal surface towards the cervix at the junction of root and the crown (Brown et al.1960; Hillson 2005). Therefore, by studying and analysing a series of intra tooth samples, a dataset on seasonal variations in isotopes of carbon and oxygen can be developed (Fricke & O'Neil 1996). However, it has been observed by studying various models of forage composition for different areas that different animals in these regions show considerable differences in their isotope composition, these differences arise as the animals with different physiologies, and metabolism will document and record the climate and environment in a distinct way (Kohn 1996).

We endeavour to carry out a first ever comparative study between molar teeth from present-day cattle and their fossil ancestors from the localities of Central Narmada Valley in central India. The study involves measuring carbon and oxygen isotope ratios from the dental remains of cattle *Bos indicus*, from two localities: Dhansi and Hathnora, located in the Central Narmada Valley near Hoshangabad district. This area is particularly well known for its rich Quaternary fossiliferous deposits of mammalian fauna. The information will be used first to develop a general idea about the isotopic composition of these animals in the area and subsequently compared to the isotope readings from the fossilized remains of now-extinct *Bos namadicus*. The data generated will be used to examine the dietary changes that may have incurred on

account of changing environmental conditions along with human interference that may have occurred over the last forty thousand years. These values are a point of reference to assess ecology and environment for the Pleistocene fauna with a special reference to large bovines. This paper also aims to provide the modern-day isotopic standards for modern livestock in the Narmada valley region that can potentially be used for both future paleontological and archaeological endeavours in this region.

1.1. STABLE ISOTOPE RATIOS

Many plants generally photosynthesize by one of the two primary photosynthetic pathways: either by C_3 or by C_4 pathway. Some plants also employ an in-between pathway known as CAM, however we have restricted our discussion to only C_3 and C_4 plants here.

Plants using these two distinctive types of pathways display considerably different stable carbon isotopic ratios. C_3 plants consist of most trees, shrubs, and cool-season grasses have $\delta^{13}C$ values ranging from − 20 to − 35‰, with an average of − 27‰ (O'Leary 1988; Farquhar et al. 1989; Cerling et al. 1997). The C_3 plants growing in shaded environments may produce even low $\delta\delta^{13}C$ values (Nygaard et al. 2004). C_4 plants constitute the warm-season grasses and have $\delta\delta^{13}C$ values ranging from − 9 to − 17‰, and averaging to − 13‰.

This distinctive isotopic difference between plants undergoing different photosynthetic pathways is passed along with the food chain to the animal tissues. However, physiological processes may cause further isotopic fractionation, and the end-member isotopic values may be quite different from that of the food consumed (Williams & Elliott 1979). Numerous studies have reported that the tooth enamel $\delta^{13}C$ values of large ruminant animals are 12–14‰ enriched compared to their diet (Tieszen et al. 1983; Passey et al. 2005; Zazzo et al. 2005).

If a large ruminant animal consumes predominantly C_3 type vegetation, it will produce enamel bioapatite carbon isotope values close to – 13‰ or lower; however, this value can be much higher close to – 6.5‰ if the growth occurs in a water – stressed environment. On the contrary, large ruminant animals consuming predominantly C_4 type vegetation would produce enamel bioapatite carbon isotope values close to or higher than +1‰. Any values falling in between +1‰ and – 6.5‰ would indicate a mixed diet consisting of both C_3 and C_4 type vegetation (Sternberg et al. 1984a, 1984b; Cerling et al. 1997; Zhang et al. 2012; Chakraborty et al. 2018).

Stable oxygen isotope ratios ($\delta^{18}O$) of the tooth enamel are directly linked to the available water taken by the animals. It also depends on physiology and the drinking behaviour of the animal, as body water is a function of the meteoric water consumed directly by drinking and indirectly through the water in food and inspired air (Longinelli 1984; Luz et al.1984; Bryant & Froelich, 1995; Kohn et al. 1996; Podlesak et al. 2008). It is widely known that the mammalian bone and tooth hydroxyapatite are formed at a constant body temperature, which is not disturbed by the variations in the temperature in the environment around. Therefore the $\delta^{18}O$ of biogenic apatite in bone and tooth is directly related to the $\delta\delta^{18}O$ of body water (Longinelli 1984; Luz et al. 1984; Nagy 1989; D'Angela & Longinelli 1990; Iacumin et al. 1996). The oxygen isotopic composition of body water is controlled by multiple variables such as the isotopic values of drinking water and the water in food, physiological processes, dietary/drinking behaviour, and so forth (Longinelli 1984; Luz et al. 1984; Bryant & Froelich 1995; Kohn, 1996). The oxygen isotopic composition of the tooth enamel of large animals, therefore, contains information about this meteoric water (Longinelli 1984; Luz et al. 1984; Bryant et al. 1996). Oxygen has multiple pathways by which it enters and exits a system; the input fluxes can be via inhaled air, which has water vapour, through diet and most presumptively through drinking water. The out fluxes can be via expiration i.e. exhaling carbon dioxide, through fecal waste, other bodily secretions such as milk, sweat, urine and transcutaneous water vapour, these are largely controlled and regulated by the physiology of the organism and certain environmental influences besides atmospheric processes are also active which determine the isotopic composition of the meteoric water (Chakraborty et al. 2016).

1.2 DEVELOPMENT OF CATTLE MOLARS

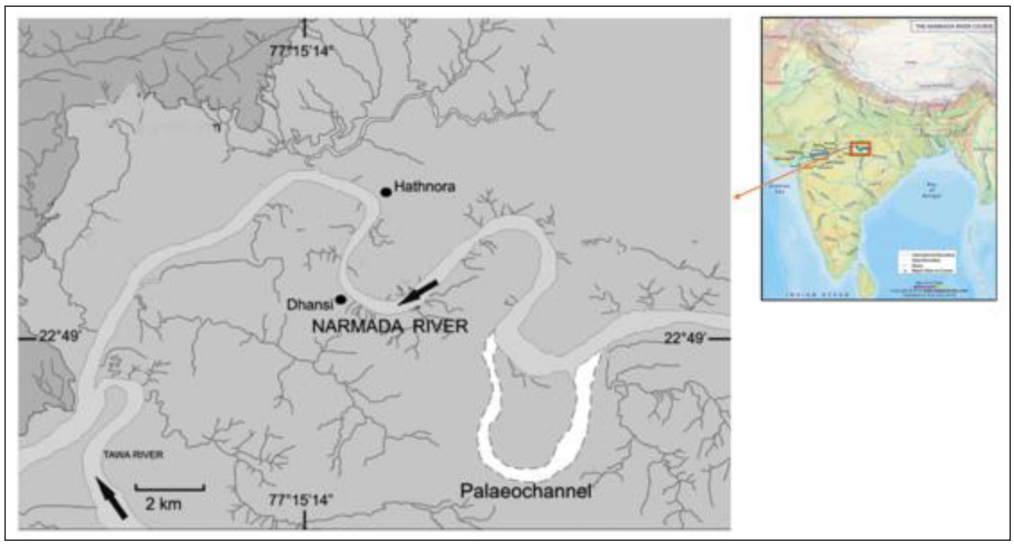
The development of cattle teeth, particularly the molars can be described simply as a sequential progression from the cusp to the cervix over a period of several months. According

to various studies, cattle molar enamel at any position on the crown takes at least 6-7 months to complete the mineralization process (Balasse 2002; Zazzo et al. 2005; Montgomery et al. 2010). Brown et al. (1960) through radiographic technique, established a chronology of molar development for modern cattle.

According to Brown et al. (1960), the first molar M_1 develops prior to birth, by the time the calf is born around one third of its crown is already calcified. For all the five individuals studied, the M_1 was the smallest as expected and was found to be lying in its follicle posterior to the last deciduous molar. Since M_1 calcification begins prior to birth,the neonatal line was visible for M_1 teeth of all the five studied individuals. The M_2 i.e., the second permanent molar, generally erupts at around twelve months; the second lower molar (M_2) crown grows from the second month till the end of the first year of life of the animal (Brown et al. 1960). At nine to thirteen months, formation and calcification of the third molar M_3 begin (Brown et al. 1960). The eruption of the M_3 generally happens between two to three years of life. The order of the eruption is the second premolar, followed by the third molar, the first premolar, and finally the third premolar. These erupt together as a group. Therefore, a detailed analysis of carbon and oxygen isotopes from all three molars will provide dietary and environmental information of an animal starting from before birth to the end of the second/third year of it's life (Brown et al. 1960).

1.3 THE STUDY AREA

Narmada River is a major river in peninsular India, originating at the Amarkantak hills of Madhya Pradesh, it flows westwards across the country to meet the Arabian Sea; it is bordered by Vindhyans in the north and Satpura basin in its south (Verma & Rao 2011). It covers a distance of nearly 1312 km before draining into the Gulf of Khambhat (Utpal 2017). Both the sampling localities: Hathnora and Dhansi are diverse, wherein Hathnora is located right next to the river, and Dhansi is within the paleochannel and at present in a semi forested region (see ***Map 1***).



Map 1 The Narmada River and the two sampling sites (black dots). Courtesy Maps of India. (modified from Verma & Rao, 2011).

Dhansi village (22°.50'13.89"N, 77°.51'41.35"E) is found in the Babai Tehsil of Hoshangabad district in the state of Madhya Pradesh, India. It is situated 28 km away from the sub-district headquarter Babai and 48 km away from the district headquarter, Hoshangabad. The mandibles were collected from a semi forested locality near the village (***Figure 1A***).

Hathnora: (77° 53'N: 22°52'E) is situated on the northern banks of the Narmada River c. 40 km northeast of Hoshangabad. Hathnora is primarily famous for its fossiliferous conglomerate beds of fluvial origin. The site is also known for its unique fossil find of the archaic hominid from the Indian subcontinent. The mandibles were collected from the fossil beds itself next to the river (***Figure 1B***).

1.4 CLIMATE AND VEGETATION OF THE STUDY AREA

The sites are located in rural settings with agricultural fields around them. This area, in general, follows the semi-arid template. The environment of Narmada Basin is humid and tropical,

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Figure 1 A: Dhansi village showing the field site, **B:** Village Hathnora with Narmada river in the background, **C:** Samples as observed in the field site before collection.

although, at places, extremes of heat and cold are often registered. The overall climate of Madhya Pradesh is categorized into the following seasons: (Shah et al. 2007).

(i) Winter (January– February),

(ii) Hot summer (March–May),

(iii) South west monsoon (June–September),

(iv) Post monsoon or transition period (October–November).

Climate wise the mean monthly temperature and precipitation data from A.D. 1901–1997, is available at Hoshangabad climate station (22°46' N Lat. and 77°6' E Long.) a record of highest and lowest temperatures can be obtained wherein May (34.4°C) and December–January (19.1°C) record the maximum and the minimum temperatures respectively. July receives the highest rainfall (408.8 mm), and April experiences the driest weather recording only 3.9 mm of precipitation (Shah et al. 2007).

The Forest cover of the Narmada basin has been divided into three levels: an upper canopy at 15–25 m, an understory 10–15 m, and 3–4 m undergrowth can be noticed in the forest. Teak (*Tectona grandis*) succeeds in the canopy. It is closely linked with *Diospyros melanoxylon, Anogeissus latifolia, Lagerstroemia parviflora, Terminalia tomentosa, Lannea coromandelica, Hardwickia binata,* and *Boswellia serata*, *Holoptelea, Sterculiaurens, Acacia* spp. and *Ziziphus mauritiana* (Champion & Seth 1968). Riparian habitats with trees such as *Terminalia arjuna, Syzygium cumini, Salix tetrasperma, Homonoia riparia,* and *Vitex negundo* constitute the moist forests.

The mixed tropical deciduous forest is an assortment of 80–100 tree species. *Butea monosperma, Diospyros melanoxylon, Anogeissus latifolia, Terminalia tomentosa*, *Boswellia serata, Agle Marmelos* in associated teak (*Tectona grandis*) are major components. The mixture of these forests and their sub-types is due to the heterogeneity of environmental factors in the region and insufficient ecological amplitude of species (Bhatia 1958). In addition, the other common trees in all types of forest are *Madhuca indica, Manilkara hexandra, Azardiracta india* and *Mumuso pelengi* (Verma & Rao 2011).

Cultivation is being actively practiced in this region, the problem of irrigation is solved by the construction of the Tawa Dam, which ensures the water supply for irrigation throughout the year. Crop rotation is being practiced by the farmers; major crops grown today are: *Triticum aestivum* (Wheat), *Vigna radiata* (Mung beans), *Saccharum officinarum* (Sugarcane), *Glycine max* (Soya Bean).

2. MATERIALS AND METHODS

Four mandibles and one maxilla from five individuals were collected from the two locations (***Figure 1C***) Dhansi and Hathnora, the collections were made from the surface, assuming the animals were locally available. The samples were selected on the basis of their wear-n-tear and efforts were made to include samples of different ages so as to develop a reliable data set. Care was taken to collect specimens with molars, which showed minimum damage. Mandible no 1 was identified to be of a young adult (***Figure 2A***), Mandible no 2 of an adult (***Figure 2B***). A Maxilla no 3 from Hathnora was also included (***Figure 2C***). Mandible no 4 was again of a sub-adult collected from Dhansi (***Figure 2D***), and Mandible no 5 was identified as an adult again from Dhansi. Refer to ***Figure 2***. Mandible no 5 showed maximum damage. Due to restriction in photography by the local administration, no photograph was provided. A total of 14 teeth were analysed, four mandibular first molars (M_1), four mandibular second molars (M_2), and four mandibular third molars (M_3). From the Maxilla first molar (M_1) and second molar (M_2) were analysed.

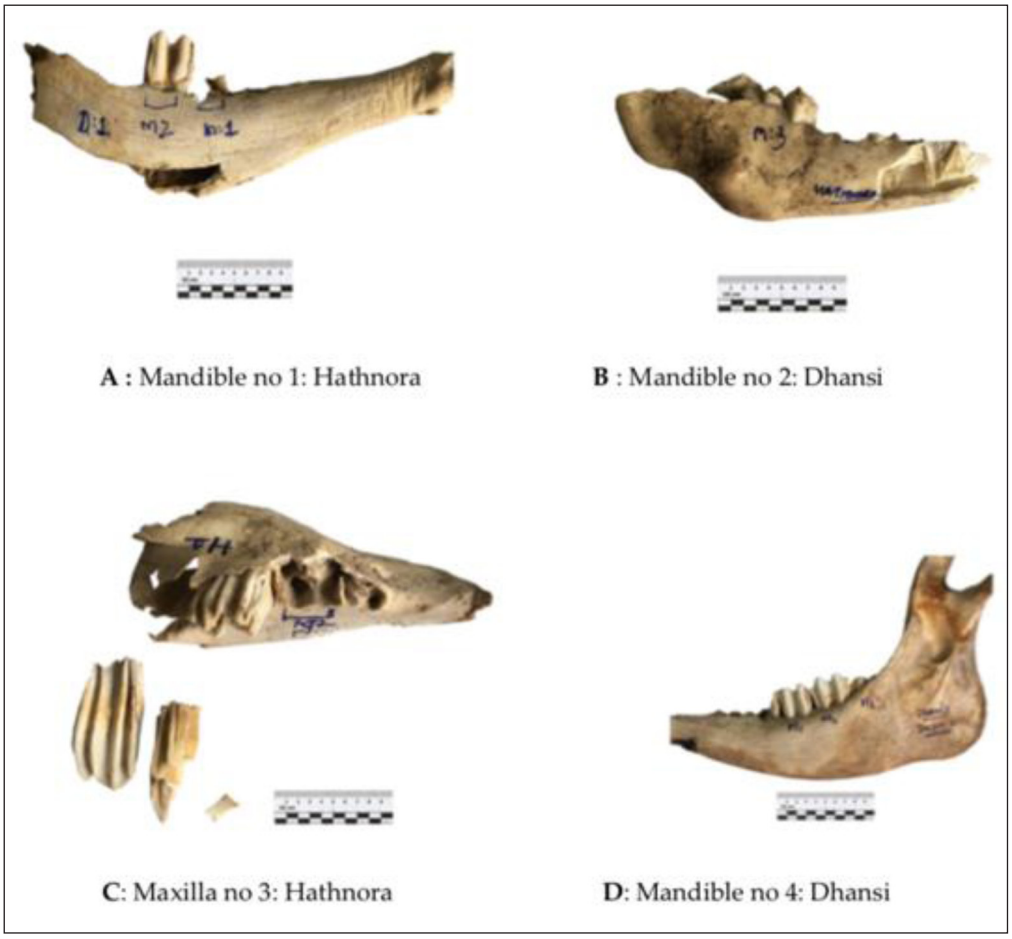

Figure 2 Analysed Specimens, **A:** mandible no 1 from Hathnora, **B:** mandible no 2 from Dhansi, **C:** maxilla from Hathnora, **D:** mandible no 4 from Dhansi.

2.1 SAMPLE PROCESSING

The tooth samples were first cleaned and prepared by removing dirt, grit, and other such materials. Every Individual tooth was then sonicated for 10 min (×2) to remove any adherent sediments. A total of fourteen molar teeth were sampled from the five individuals, from the Mandibles 1,2,4,5: three molar teeth (M_1, M_2, and M_3) were sampled. From the Maxillae 3 only two molars M_1 and M_2 were sampled. Sequential samples weighing approximately 3 mg were obtained in bands perpendicular to the growth line from the cervix to the cusp, using a diamond

drill bit. Each enamel sample was then numbered and stored in centrifuge tubes (5 ml). For each tooth, a total of ten sequential samples were analysed, thus generating a data set of one hundred and forty readings (Chakraborty et al. 2018).

2.2 SAMPLE PREPARATION FOR CARBON AND OXYGEN ISOTOPE ANALYSIS

The enamel powders were first treated with 3% NaOCl for 24 h (0.1 ml/mg), followed by 0.1 M acetic acid for 4 h (0.1 ml/mg) as proposed by Balasse et al. (2002). After each step, the samples were rinsed 5 times with double distilled water. After drying in an oven at 25°C the samples were air dried at room temperature (Chakraborty et al. 2018).

Isotope measurements were carried out using a Delta V Plus Isotope Ratio Mass Spectrometer of Thermo Fisher Scientific at the Stable Isotope Laboratory of the Indian Institute of Tropical Meteorology, Pune. The treated enamel samples were reacted with 100% phosphoric acid at 25°C, and the carbon and oxygen isotopic ratios of the CO_2 produced were analysed. NBS-19 and PRL Lab Standard were used to standardize our isotopic measurement (Chakraborty & Ramesh 1992).

The instrument was standardized and checked for reproducibility/accuracy with a large number of primary and secondary laboratory standards of water and carbonate samples. The analytical precision (based on replicate analyses of NBS-19 and VSMOW and several other lab standards processed with each batch of samples) is ±0.1‰ for $\delta\delta^{13}C$ and ±0.2 for $\delta^{18}O$. As a standard practice and to be able to compare with published fossil data, the VPDB scale of oxygen was converted to VSMOW using the equation proposed by Coplen (Coplen 1988; Chakraborty et al. 2018).

$$\left(\delta^{18}O_{SMOW} = 1.03091 \times \delta^{18}O_{PDB} + 30.91\right)$$

Isotope ratios are expressed as $\delta^{18}O$ and $\delta\delta^{13}C$, and the units are per mil (‰) (Sharp 2007, Libes & Susan M. 1992). Oxygen and carbon isotopes are reported as $\delta = [(R_{sample}/R_{standard})-1]*1000$ where $R = {}^{13}C/{}^{12}C$ or ${}^{18}O/{}^{16}O$, and reported against the VSMOW and VPDB scale for oxygen and carbon respectively.

3. RESULTS

Using tooth eruption patterns (Wilson et al. 1982), the modern specimens from Hathnora are of individuals identified to be of different ages. For each mandible, three molars M_1, M_2,M_3, were sampled. At birth, the first molar (M_1) is already partially formed, it further develops and grows rapidly after birth and is completely formed within the first few months itself (Hillson, 1986; Brown et al. 1960).

The data for isotopic ratios are represented in ***Table 1***. For Mandible no 1 which has been identified as a young adult from the locality of Hathnora, three molars were studied. For M_1 teeth an increase in the values for carbon and oxygen is recorded from the base to the apex, indicative of an isotopic enrichment. A similar pattern is seen for M_2 and M_3 molars for this mandible, the values are suggestive of a C_3 diet.

Table 1 Isotopic Data obtained from the tooth samples.

LOCALITY	SPECIMEN NO	AGE	TOOTH TYPE	SAMPLE NO	DISTANCE FROM CERVIX(MM)	$\delta^{13}C$ (VPDP)	$\delta^{18}O$ (VSMOW)
Hathnora	Mandible 1	Young Adult	M_1	M1P1	0.0–2.2	–8.60	22.08
	Mandible 1			M1P2	2.2–4.6	–9.31	22.04
	Mandible 1			M1P3	4.6–7.0	–10.01	20.01
	Mandible 1			M1P4	7.0–10.5	–10.06	18.17
	Mandible 1			M1P5	10.5–13.4	–11.03	16.05
	Mandible 1			M1P6	13.4–16.0	–12.91	15.09
	Mandible 1			M1P7	16.0–19.5	–14.06	13.20
	Mandible 1			M1P8	19.5–23.5	–16.04	11.18

(Contd.)

LOCALITY	SPECIMEN NO	AGE	TOOTH TYPE	SAMPLE NO	DISTANCE FROM CERVIX(MM)	$\delta^{13}C$ (VPDP)	$\delta^{18}O$ (VSMOW)
	Mandible 1			M1P9	23.5–25.0	–18.02	10.90
	Mandible 1			M1P10	25.0–27.5	–21.03	10.60
	Mandible 1	Young Adult	M_2	M2P1	0.0–2.5	–7.60	24.03
	Mandible 1			M2P2	2.5–5.5	–8.01	24.90
	Mandible 1			M2P3	5.5–7.0	–10.03	27.31
	Mandible 1			M2P4	7.0–9.4	–11.06	28.04
	Mandible 1			M2P5	9.4–12.3	–12.13	29.32
	Mandible 1			M2P6	12.3–15.0	–13.04	31.32
	Mandible 1			M2P7	15.0–17.5	–16.92	33.60
	Mandible 1			M2P8	17.5–19.0	–19.83	35.87
	Mandible 1			M2P9	19.0–22.6	–22.05	39.13
	Mandible 1			M2P10	22.6–25.0	–23.06	40.01
	Mandible 1	Young Adult	M_3	M3P1	0.0–3.0	–6.41	24.03
	Mandible 1			M3P2	3.0–5.0	–6.92	24.08
	Mandible 1			M3P3	5.0–7.5	–8.21	26.40
	Mandible 1			M3P4	7.5–9.0	–10.80	28.31
	Mandible 1			M3P5	9.0–11.5	–13.05	31.72
	Mandible 1			M3P6	11.5–13.0	–14.91	31.94
	Mandible 1			M3P7	13.0–15.5	–17.01	33.52
	Mandible 1			M3P8	15.5–17.5	–19.83	33.60
	Mandible 1			M3P9	17.5–19.0	–19.72	34.10
	Mandible 1			M3P10	19.0–21.5	–20.01	36.20
Hathnora	Maxilla 3	Young Adult	Maxilla	M1P1	0.0–2.3	–16.02	25.45
	Maxilla 3			M1P2	2.3–4.4	–17.30	24.00
	Maxilla 3			M1P3	4.4–6.0	–17.91	23.49
	Maxilla 3			M1P4	6.0–8.5	–18.72	22.77
	Maxilla 3			M1P5	8.5–10.5	–21.03	22.53
	Maxilla 3			M1P6	10.5–12.4	–22.40	21.12
	Maxilla 3			M1P7	12.4–14.8	–23.60	20.40
	Maxilla 3			M1P8	14.8–16.0	–25.32	19.44
	Maxilla 3			M1P9	16.0–18.5	–27.21	16.79
	Maxilla 3			M1P10	18.5–20.5	–27.94	14.84
	Maxilla 3			M2P1	0.0–2.5	–13.72	20.40
	Maxilla 3		M_2	M2P2	2.5–4.5	–14.24	16.79
	Maxilla 3			M2P3	4.5–6.0	–15.95	14.84
	Maxilla 3			M2P4	6.0–8.2	–16.70	12.47
	Maxilla 3			M2P5	8.2–10.5	–17.81	10.73
	Maxilla 3			M2P6	10.5–13.3	–19.32	11.97
	Maxilla 3			M2P7	13.3–15.6	–19.84	14.23
	Maxilla 3			M2P8	15.6–17.5	–21.26	16.50
	Maxilla 3			M2P9	17.5–19.5	–22.34	17.12
	Maxilla 3			M2P10	19.5–21.0	–23.01	14.75

(Contd.)

LOCALITY	SPECIMEN NO	AGE	TOOTH TYPE	SAMPLE NO	DISTANCE FROM CERVIX(MM)	$\delta^{13}C$ (VPDP)	$\delta^{18}O$ (VSMOW)
Dhansi	Mandible 2	Adult	M_1	M1P1	0.0–2.5	–14.60	13.60
	Mandible 2			M1P2	2.5–4.4	–15.03	12.47
	Mandible 2			M1P3	4.4–6.2	–15.91	12.35
	Mandible 2			M1P4	6.2–8.0	–16.13	11.13
	Mandible 2			M1P5	8.0–10.5	–16.79	10.61
	Mandible 2			M1P6	10.5–12.3	–17.41	10.00
	Mandible 2			M1P7	12.3–15.0	–18.22	9.48
	Mandible 2			M1P8	15.0–17.5	–22.31	6.60
	Mandible 2			M1P9	17.5–19.0	–22.85	3.20
	Mandible 2			M1P10	19.0–22.5	–23.70	1.96
	Mandible 2		M_2	M2P1	0.0–2.0	–11.06	22.65
	Mandible 2			M2P2	2.0–4.5	–13.05	23.66
	Mandible 2			M2P3	4.5–6.0	–16.30	23.28
	Mandible 2			M2P4	6.0–8.2	–16.91	22.87
	Mandible 2			M2P5	8.2–10.5	–17.82	21.43
	Mandible 2			M2P6	10.5–12.4	–18.03	20.30
	Mandible 2			M2P7	12.4–15.6	–21.20	18.96
	Mandible 2			M2P8	15.6–17.8	–23.70	18.24
	Mandible 2			M2P9	17.8–19.5	–25.01	17.10
	Mandible 2			M2P10	19.5–21.0	–25.03	15.15
	Mandible 2		M_3	M3P1	0.0–2.5	–15.20	24.31
	Mandible 2			M3P2	2.5–4.5	–15.81	23.80
	Mandible 2			M3P3	4.5–7.5	–17.43	23.49
	Mandible 2			M3P4	7.5–9.5	–18.01	27.40
	Mandible 2			M3P5	9.5–12.2	–19.20	22.25
	Mandible 2			M3P6	12.2–15.9	–22.70	18.85
	Mandible 2			M3P7	15.9–17.0	–24.31	16.69
	Mandible 2			M3P8	17.0–19.5	–25.50	15.97
	Mandible 2			M3P9	19.5–21.5	–26.01	14.12
	Mandible 2			M3P10	21.5–23.0	–27.42	10.82
Dhansi	Mandible 4	Subadult	M_1	M1P1	0.0–2.5	–11.30	20.09
	Mandible 4			M1P2	2.5–4.0	–11.91	20.85
	Mandible 4			M1P3	4.0–6.5	–12.60	19.27
	Mandible 4			M1P4	6.5–8.2	–13.21	18.75
	Mandible 4			M1P5	8.2–10.5	–14.01	17.31
	Mandible 4			M1P6	10.5–12.3	–14.82	15.87
	Mandible 4			M1P7	12.3–14.0	–16.24	14.53
	Mandible 4			M1P8	14.0–16.5	–16.76	11.64
	Mandible 4			M1P9	16.5–19.0	–17.40	11.95
	Mandible 4			M1P10	19.0–22.3	–19.51	13.09
	Mandible 4			M2P1	0.0–2.0	–9.02	27.40
	Mandible 4		M_2	M2P2	2.0–4.5	–10.13	23.90

(Contd.)

LOCALITY	SPECIMEN NO	AGE	TOOTH TYPE	SAMPLE NO	DISTANCE FROM CERVIX(MM)	$\delta^{13}C$ (VPDP)	$\delta^{18}O$ (VSMOW)
	Mandible 4			M2P3	4.5–7.2	–12.51	23.67
	Mandible 4			M2P4	7.2–10.5	–13.83	22.77
	Mandible 4			M2P5	10.5–13.3	–14.35	22.25
	Mandible 4			M2P6	13.3–17.2	–15.40	25.55
	Mandible 4			M2P7	17.2–19.5	–17.90	26.52
	Mandible 4			M2P8	19.5–22.0	–18.21	24.52
	Mandible 4			M2P9	22.0–24.5	–19.72	22.87
	Mandible 4			M2P10	24.5–27.0	–22.35	22.36
	Mandible 4		M_3	M3P1	0.0–2.5	–10.01	24.93
	Mandible 4			M3P2	2.5–4.0	–13.07	25.45
	Mandible 4			M3P3	4.0–7.5	–14.20	23.80
	Mandible 4			M3P4	7.5–10.0	–14.90	23.49
	Mandible 4			M3P5	10.0–12.5	–15.42	22.05
	Mandible 4			M3P6	12.5–14.0	–16.08	21.22
	Mandible 4			M3P7	14.0–16.5	–17.20	20.30
	Mandible 4			M3P8	16.5–18.5	–18.03	21.43
	Mandible 4			M3P9	18.5–20.0	–21.50	18.96
	Mandible 4			M3P10	20.0–22.5	–22.09	17.62
Dhansi	Mandible 5	Adult	M_1	M1P1	0.0–2.5	–7.70	27.20
	Mandible 5			M1P2	2.5–4.0	–8.91	25.15
	Mandible 5			M1P3	4.0–7.5	–9.11	20.19
	Mandible 5			M1P4	7.5–10.0	–11.30	18.96
	Mandible 5			M1P5	10.0–13.5	–12.92	18.24
	Mandible 5			M1P6	13.5–16.0	–13.20	15.46
	Mandible 5			M1P7	16.0–19.2	–14.61	17.31
	Mandible 5			M1P8	19.2–21.5	–15.82	14.63
	Mandible 5			M1P9	21.5–24.3	–16.30	11.03
	Mandible 5			M1P10	24.3–26.0	–16.70	11.85
	Mandible 5		M_2	M2P1	0.0–2.0	–8.20	23.80
	Mandible 5			M2P2	2.0–4.5	–10.30	23.39
	Mandible 5			M2P3	4.5–7.5	–11.91	22.87
	Mandible 5			M2P4	7.5–10.2	–12.01	22.15
	Mandible 5			M2P5	10.2–13.6	–13.09	21.43
	Mandible 5			M2P6	13.6–15.5	–15.33	20.30
	Mandible 5			M2P7	15.5–17.0	–16.92	22.97
	Mandible 5			M2P8	17.0–19.5	–17.84	22.36
	Mandible 5			M2P9	19.5–21.5	–19.21	18.03
	Mandible 5			M2P10		–21.03	17.72
	Mandible 5		M_3	M3P1	0.0–3.5	–9.30	23.90
	Mandible 5			M3P2	3.5–7.5	–11.60	24.11
	Mandible 5			M3P3	7.5–11.0	–12.52	26.89
	Mandible 5			M3P4	11.0–14.2	–14.81	25.55

(Contd.)

LOCALITY	SPECIMEN NO	AGE	TOOTH TYPE	SAMPLE NO	DISTANCE FROM CERVIX(MM)	$\delta^{13}C$ (VPDP)	$\delta^{18}O$ (VSMOW)
	Mandible 5			M3P5	14.2–17.3	–15.20	23.18
	Mandible 5			M3P6	17.3–21.5	–17.92	22.65
	Mandible 5			M3P7	21.5–23.0	–19.44	20.40
	Mandible 5			M3P8	23.0–25.5	–21.31	18.75
	Mandible 5			M3P9	25.5–27.8	–22.80	19.88
	Mandible 5			M3P10	27.8–29.0	–23.72	18.24

For Mandible no 2 identified as an adult, three molars were samples M_1, M_2, M_3. The values again show an increase from base to the apex for both carbon and oxygen values.

For Maxilla no 3 from Hathnora, two molars were studied: M_1, M_2. These are again indicating a C_3 diet with enrichment in the values for both the isotopes.

Mandible no 4 and 5 are both from Dhansi, Mandible no 4 is of a sub-adult and mandible no 5 of an adult. For the three studied molars for each sample, enriched values were observed from base to cervix. The overall values indicate a C_3 based diet for all the modern mandibles.

The average readings from both the specimens from Hathnora for carbon isotope are –13.72‰ for Mandible 1 and –20.08‰ for Maxilla no 3. Both these readings are indicative of a C_3 diet. For specimens from Dhansi, Mandible no 2, recorded –19.42‰ whereas the other two individuals, Mandible no 4 and 5 recorded –15.45‰ and –14.70‰, respectively. Again the readings are indicative of an enriched C_3 diet (***Figure 3***: Mandible no: 2,4,5).

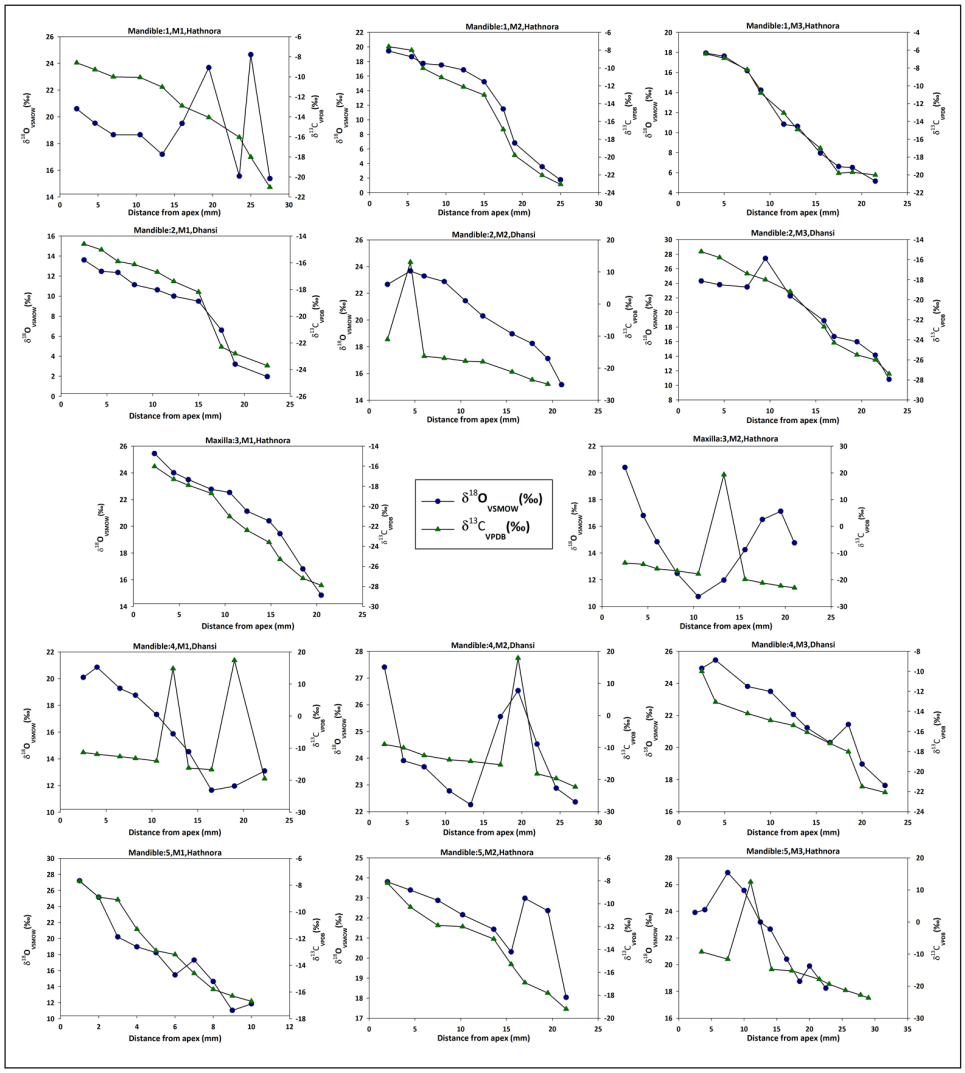

Figure 3 Intra-tooth variation of carbon ($\delta^{13}C$) and oxygen ($\delta\delta^{8}O$) isotope ratios of the tooth enamel bioapatite of Cattle.

The mean oxygen values for cattle from Hathnora for Mandible no 1 and Maxilla no 3 is 30.86‰ and 14.98 ‰. The mean oxygen values from Dhansi for mandible 2, 4, and 5 are 20.07‰, 23.05‰, and 21.93‰. The mean values have only been calculated for M_2 and M_3 molars as the M_1 molar forms prior to birth. The oxygen ratios in all the specimens are showing a depreciating trend in their values from the base of the teeth until their cervix.

The correlation co-efficient has been calculated between oxygen and carbon isotope values for individual tooth of each specimen. Except for one individual (mandible 1) that indicates a strong negative correlation, other individuals indicate a strong positive correlation (***Figure 3***). This is indicative of a seasonally varied and dependent diet.

4. DISCUSSION

The specimens were collected from the surface of the two above mentioned locality. In the present day, these localities are part of the rural set up where these animals were kept by the villagers. The site of Hathnora is next to the river, the specimens were collected when the river was flowing low. In rural India, it is not uncommon to see skeletal remains of domesticated animals next to riverbeds. On the other hand, it could not be ensured that the specimen collected had died in the site and was not carried from elsewhere. Both sites are located in semi-arid climate where summers are usually harsh.

In ruminating animals such as cattle the food is fermented in first of the four stomachs also known as the rumen, this process produces carbon dioxide and methane (McDonald et al. 1988). In domesticated animals the diet mirrors the resources and the feed given to the livestock by humans, it is also indicative of the ways the animals are integrated and made part of the human subsistence economies and their day to day life. According to our data, the feeding pattern is indicative of agricultural feed substantiated with seasonally available natural vegetation found at the vicinity of these localities. These animals not only consumed agricultural leftovers, but were allowed to graze in the pastures. However, there are no natural grasslands near these localities, and possibly the cattle were left to graze in fields left fallow or even on the edge of forested land and river banks that supplemented their daily diet, which was likely comprised of shrubs, bushes and leaves from the low lying branches. The recent changes in vegetation pattern in the modern day context is attributable to anthropogenic disturbances wherein the natural grasslands (C_4) are subsequently being replaced by shrubs and woodland plants (C_3). Though agricultural lands and fields dominate the horizon, the Narmada basin is still endowed with rich and diverse ecosystems. Two major types of forest formation can be distinguished, i.e., Teak forest and Mixed forest, and the effect of the closed canopy can be visible in our modern samples as they produced extremely depleted carbon isotope values.

The most common and age-old feeding strategy followed in rural India is mixing the green grasses with dry ones, and it is further fortified with cottonseed cakes, rice, bran, coconut cakes, etc. However, due to the demands on the dairy industry and also on the drought animals, this feed is slowly being replaced with a Compound Cattle Feed (CCF). The major factors responsible for this are (I) Loss and reduction in the pastures and grazing lands due to (ii) Need for specialized feeds arising from the use of high yield cattle, (iii) Marked shift in eating habits of people because of urbanization, with an increased intake of milk and other cattle-based products, etc. (Manoj 2015). Homemade feeds are also prevalent. In oil seed cakes category, soybean, groundnut, mustard, linseed, sesame, and sunflower are used in cattle and poultry feeds. Most compound feeds contain a limited amount of grains and oilseed cakes (Uppal et al. 2004). At present, the low yielding cattle is fed with grass mixed with fallen leaves, hay, dried stocks of paddy, wheat straw, and other agro and domestic wastes of vegetables and fruits (Francis, 2003). However, this situation was different during the Pleistocene, as indicated by the fossil remains. The carbon isotope data from the fossil remains indicate that unlike now, C_4 grasses dominated the Pleistocene era. Generally, C_4 plants are found in tropical and warm temperate regions. Throughout the Holocene (~3 kyr) the climate has been sub humid with a mixed vegetation of C_3–C_4 *type with little change in their relative proportions*. In cattle grazing fresh pasture, a large proportion of the ingested water is in the form of leaf water, Leaf water is enriched in $\delta^{18}O$ due to isotopic fractionation during transpiration (Flanagan et al.1991). Plants via transpiration contribute a substantial amount of water vapour into the atmosphere; besides adding water vapour they also consume a large amount of soil water to carry out their various

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physiological activities. Evaporation of soil water results in isotopic enrichment and this enriched state is maintained in the water transpired by the trees and plants. The evaporation of soil water primarily depended on humidity, which in turn was modulated by rainfall (Chakraborty et al. 2018a). The isotopic composition of the $\delta\delta^{18}O$ of water in grass fluctuates on an hourly scale. This effect is even seen in the silage water, which again varies and changes according to the ambient conditions. Another complexity is that of intercepted rain and dew in the grass, which, when ingested by the animal again, adds another source of oxygen in the body; this is also recorded in the tooth enamel. Hence the $\delta\delta^{18}O$ of daily total feed moisture is a complex and difficult variable that is affected not only by the ambient environmental conditions but also by the feed administered to the animal.

Many studies have shown that the diets of modern taxa within control environments have strong influences on the isotopic composition of ingested water, which leads to sympatric herbivores to show a variation in their $\delta\delta^{18}O$ values by as much as 8–9% (Bocherens et al. 1996; Kohn, Schoeninger & Valley 1996). It has been concluded that the browsing and herbivores feeding on mixed diets tend to have enriched $\delta^{18}O$ values as compared to herbivores, which employ grazing as a means of subsistence. Interestingly it has also been observed that the grazing taxa drink more water; the browsers get their water requirements met via feed only as the shrubs and trees that they feed on have deep roots capable of tapping the water underground (Goldstein & Sarmiento 1987; Estes, 1991). The feeding strategy changes with the season in temperate latitudes, fresh grass is usually fed in the warm season, which is the main growing period, whereas silage and hay are provided in winter; these sources differ in their $\delta^{18}O$ values (Chen et al. 2017). The $\delta^{18}O$ of water also exhibits substantial geographic variation related to the effects of latitude, altitude, amount of precipitation, and even distance from the coasts (e.g. Bowen & Wilkinson 2002).

Besides the above mentioned complexities, there are numerous other factors, both ecological and physiological, that affect oxygen isotope composition and makes its interpretation complex. One example is panting; this is basically an adaptation to heat stress; if we compare the oxygen isotope composition of water vapour lost during panting to that of sweat, we find it to be depleted (Wong et al. 1988). Also, some animals may drink more water in specific environments at certain times, whereas their conspecifics may show a completely opposite behaviour (Spinage 1986; Estes 1991). Due to the stated shortcomings and difficulties faced with oxygen isotope data, for this study, oxygen isotopes are not being interpreted for microclimate reconstruction.

The average mean carbon isotope values from molar teeth of fossil taxa from Narmada is recorded at –0.032‰ for carbon isotopes, based on a study done by Kalwanker (2013) on fossil samples (***Table 2***). For the purpose of comparison and to follow the standard practice, the oxygen values mentioned by Kalwanker (2013) have been converted to VSMOW from VPDB standard and presented in ***Table 2***. When compared against the modern values from cattle (***Figures 4*** and ***5***), the fossil carbon isotope values are considerably enriched than that of the modern ones (***Figure 4***), indicative of a mixed diet with a greater influence of C_4 type

S.NO	SPECIMEN NO	TAXON	LOCALITY	$\delta^{13}C$ VPDB	$\delta^{18}O$ VSMOW
1	UNMD1	*Bos namadicus*	Narmada	–0.13	31.92
2	UNMD2	*Bos namadicus*		–0.36	30.93
3	UNMD3	*Bos namadicus*		–0.95	30.08
4	UNMD4	*Bos namadicus*		–0.72	30.07
5	UNMD5	*Bos namadicus*		–1.55	29.73
6	UNMD6	*Bos namadicus*		0.75	32.72
7	UNMD7	*Bos namadicus*		0.24	32.12
8	UNMD8	*Bos namadicus*		0.51	32.35
9	UNMD9	*Bos namadicus*		0.57	31.82
10	UNMD10	*Bos namadicus*		1.32	33.02

Table 2 Carbon and Oxygen ratios from Fossil Teeth of *Bos namadicus, taken from* Kalwanker (2013).

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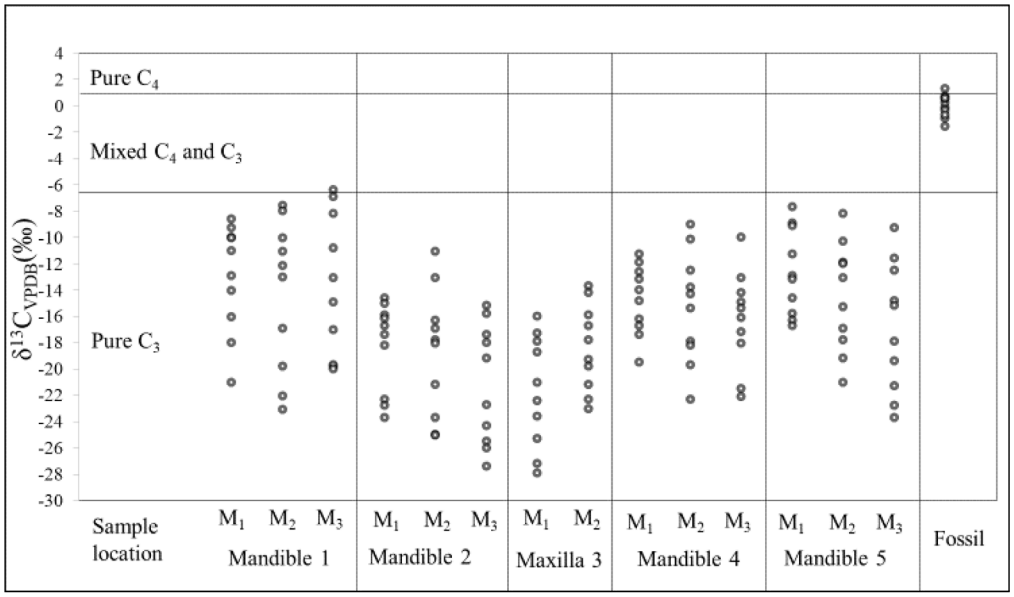

Figure 4 A Comparative graph of $\delta^{13}C_{PDB}$ between the values of Modern samples: Mandible 1,2,4,5 and Maxilla 3 and Fossil samples of cattle from Narmada (fossil values were taken from Kalwanker (2013).

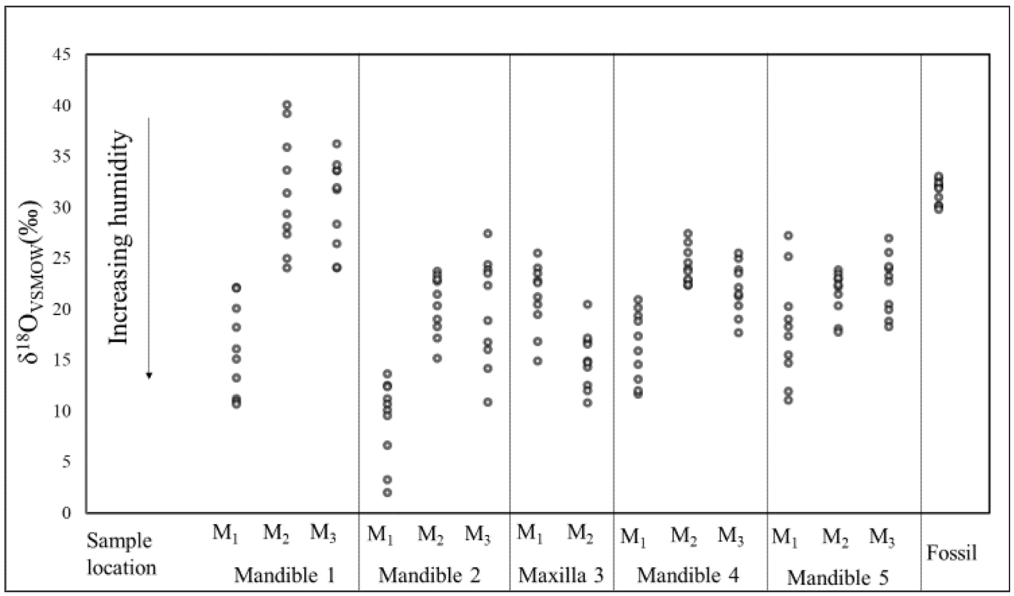

Figure 5 Variation of $\delta^{18}O$ of modern samples Mandible 1,2,4,5 and Maxilla 3 from Narmada. Fossil values were taken from (Kalwanker 2013), and the oxygen isotopic values were converted to VSMOW scale.

vegetation. Present-day populations display a C_3 signature, this is due to the feed administered to them along with anthropogenic activities and weather conditions which have affected the modern-day landscape in the study area, resulting in a more C_3 dominated environment where traditionally C_4 plants should have been present.

But even though we can conclude that there is a definite shift in the diets of this taxa from being C_4 grazers during the Pleistocene to now being primarily C_3 feeders with diets being guided by the anthropogenic practices. This change in diet, besides being attributed to anthropogenic factors, is also due to the variable rainfalls in these regions. Based on studies and findings, the annual rainfall from 1901–1960 had a positive trend in the Central parts of the Indian Subcontinent, but from 1960 onwards, annual rainfall over the major river basins in Central India, including the Narmada basin, experienced a decreasing trend (Parthasarathy & Dhar 1974; Sontakke et al. 2008; Sanikhani et al. 2018). Also post 1950, there has been a considerable increase in the frequency and intensity of heavy rain events and consecutive dry days; these have been more clearly recorded in regions where the monsoon system is active and is the primary factor in the climate (Kumar et al. 2013; Mallya et al. 2016; Malik et al. 2017). Besides a reduction in rainfall, the mean annual temperatures have increased for the period 1901–1982 (Hingane et al. 1985; Sanikhani et al. 2018). Along with the overall rise in temperatures, an increase is also recorded in the surface temperatures. This increase is by about 1°C and 1.1°C in winters and the months thereafter. This warming trend has continued till 2007 with a mean increase of 0.20°C per decade and has been primarily attributed to increasing temperatures in winter months

and the post-monsoon months (Dash et al. 2007; Sanikhani et al. 2018). In the present day context, the climate in the Narmada valley is a temperate sub-tropical type dominated by the monsoons (Kotlia & Joshi 2008).

Based on the oxygen isotope data from fossil remains which is slightly enriched than that of the modern values from Dhansi but comparable to the values obtained from Hathnora. it may appear that *Bos namadicus* during the Pleistocene Era might have experience a drier climate than today, a climate suitable for the growth of C_4 type grasses (Osborne & Sack 2012). Based on studies done on various lizards, rodents, bird fossils from Narmada, the environment during the Mid -Late Pleistocene is that of wooded grasslands with ponds, lakes, streams. Microfauna studies have revealed that during this period, the climate was that of a warm tropical type with seasonal pools and water bodies dotting the landscape. This observation has been supported by the presence of mega vertebrates such as Elephus (Pilgrim 1905), Bos namadicus and Bubalus (Lydekker 1886; Pilgrim 1939), Antelopes (Pilgrim 1939), Eqqus (Biswas 1988; Biswas et al. 2005). However, this type of climate was not constant, the absence of animals such as Crocodiles from such a well-watered environment indicates aridity. Also, presence of Lizards and Golundas are suggestive of small aridity events in the region. Hence according to the studied evidence, the climate of this region during the Mid-late Pleistocene seems to have been that of warm, humid type interspersed with arid events (Patnaik 1995; Patnaik & Sahni 1995; Mangerud et al. 1996; William et al. 1999; Kotlia & Joshi 2008).

The values, however not exceptionally enriched compare to the values, especially for Hathnora. The slight enrichment that we observed when compared against the values from Dhansi could be an effect of diagenetic effects due to prolonged burial, through which lighter isotopes might have leached out, enriching the original values. It is, therefore, essential to maintain caution while interpreting the oxygen isotope values of fossilized remains.

The isotopic data generated for modern Indian fauna may have important implications for broad ecological comparisons between extant and extinct cattle species and their adaptive contexts over time. Limited by the isotopically unexplored rich skeletal record of humans and animals from excavations in India (Mahajan & Sathe 2020), isotope analyses with special reference to modern descendants/ representative taxa will provide a continuum of dietary and ecological data, which is desideratum today.

6. CONCLUSIONS

The results of this preliminary study indicate that the modern cattle (*Bos indicus*) from the middle Narmada Valley region were fed primarily a C_3 dominated diet that is considerably different from the Pleistocene Era when C_4 type vegetation dominated the landscape. The modern cattle were domesticated by the local villagers, and the isotopic values observed in this study are concurrent with the type of feed provided to them. Also, even though this area should have more C_4 plants because of the climate and geology, but due to recent anthropogenic activities, C_3 plants might have gained dominance. In comparison with fossil taxa from the same region, we find that the animals in the past *Bos namadicus* that grazed on the naturally available grasses, consumed a more C_4 dominated diet. The oxygen values of the fossil sample from Narmada is comparatively enriched than the values from Dhansi, but similar to the values from Hathnora.

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COMPETING INTERESTS

The authors have no competing interests to declare.

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