Oxygen isotope in archaeological bioapatites from India: Implications to climate change and decline of Bronze Age Harappan civilization

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The antiquity and decline of the Bronze Age Harappan civilization in the Indus-Ghaggar-Hakra river valleys is an enigma in archaeology. Weakening of the monsoon after ~5 ka BP (and droughts throughout the Asia) is a strong contender for the Harappan collapse, although controversy exists about the synchrony of climate change and collapse of civilization. One reason for this controversy is lack of a continuous record of cultural levels and palaeomonsoon change in close proximity. We report a high resolution oxygen isotope (δ18O) record of animal teeth-bone phosphates from an archaeological trench itself at Bhirrana, NW India, preserving all cultural levels of this civilization. Bhirrana was part of a high concentration of settlements along the dried up mythical Vedic river valley ‘Saraswati’, an extension of Ghaggar river in the Thar desert. Isotope and archaeological data suggest that the pre-Harappans started inhabiting this area along the mighty Ghaggar-Hakra rivers fed by intensified monsoon from 9 to 7 ka BP. The monsoon monotonically declined after 7 ka yet the settlements continued to survive from early to mature Harappan time. Our study suggests that other cause like change in subsistence strategy by shifting crop patterns rather than climate change was responsible for Harappan collapse.

The rise of the post-Neolithic Bronze Age Harappan civilization 5.7–3.3 ka BP (ca. 2500 to 1900 year BC; all ages henceforth mentioned are in cal year BP) spread along the Indus Valley of Pakistan through the plains of NW India, including into the state of Gujarat and up to the Arabian Sea and its decline has remained an enigma in archaeological investigation. In the Indian subcontinent the major centers of this civilization include Harappa and Mohenjo-Daro in Pakistan and Lothal, Dholavira and Kalibangan in India (Fig. 1A). In recent years excavation at Rakhigarhi and few other places indicate that the civilization probably was more expansive than thought before. Whatever may be the extent most Harappan settlements grew in the floodplains of river systems including those of the Indus or now defunct Ghaggar-Hakra (mythical river Saraswati?) Climatically although these regions fall under the influence of the Indian summer monsoon, they are currently semi-arid receiving much lesser rainfall than the mainland India. Because the monsoon showed significant variation over, both on short and long term time scale, throughout the Holocene period, attempts have been made to relate the evolution of the Harappan civilization to the changes in monsoon. Accordingly, the flourishing Harappan civilization and its decline have been linked to the intensification of monsoon during the Mid-Holocene climate optimum and its subsequent weakening, respectively. The evidence comes from a variety of sources like distant lake sediments in the Thar desert, foraminiferal oxygen isotopes in Arabian sea cores, fluvial morphodynamics, and climate models. Although the collapse of the Harappan as well as several contemporary civilisations like Akkadian (Mesopotamia), Minoan (Crete), Yangtze (China) has been attributed to either weakening of monsoon or pan-Asian aridification (drought events) at ~4.1 ka, the evidence is both contradictory and incomplete. Either the climatic events and cultural levels are asynchronous or the climate change events themselves are regionally diachronous and references therein).

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Potential reasons for these conflicting interpretations is that the climate reconstructions were made from locations (e.g., Thar Desert or Arabian Sea) distant from the main Harappan settlement areas or that the climate proxies (e.g., sedimentology and geochemistry in lakes) could have been influenced by multiple local parameters apart from mere rainfall or temperature. To date no continuous climate record has existed close to or from the Harappan settlements. Here we report a high resolution bulk oxygen isotope (δ\(^{18}\)O) record of animal teeth and bone phosphates (bioapatites) from an excavated archaeological trench at Bhirrana, state of Haryana, NW India, to reconstruct a paleomonsoonal history of the settlement site itself. Based on radiocarbon ages from different trenches and levels the settlement at Bhirrana has been inferred to be the oldest (>9 ka BP) in the Indian sub-continent\(^8,17,18\). To check its validity we dated archaeological pottery from two cultural levels using optically stimulated luminescence (OSL) method and thus investigated the interrelationship between the cultural levels and climate change that occurred right at the settlement, a critical gap in information that exists in our present understanding of the Harappan civilization.

**Harappan civilization and archaeology of Bhirrana**

Archaeological chronologies of Harappan (Indus) civilization in South Asia\(^2,16,19\) are given in SI. Conventionally the Harappan cultural levels have been classified into 1) an Early Ravi Phase (~5.7–4.8 ka BP), 2) Transitional Kot Diji phase (~4.8–4.6 ka BP), 3) Mature phase (~4.6–3.9 ka BP) and 4) Late declining (painted Grey Ware) phase (3.9–3.3 ka BP\(^13,18,20\)). This chronology is based on more than 100 \(^{14}\)C dates from the site of Harappa and nearby localities. These periodization is temporally correlatable with the Indus valley civilisations from Baluchistan and Helmand province proposed by Shaffer\(^21\). While the first two phases were represented by pastoral and early village farming communities, the mature Harappan settlements were highly urbanized with several organized cities, developed material and craft culture having trans-Asiatic trading to regions as distant as Arabia and Mesopotamia. The late Harappan phase witnessed large scale deurbanization, population decrease, abandonment of many established settlements, lack of basic amenities, interpersonal violence and disappearance of Harappan script\(^22–24\). Although referred to as a ‘collapse’ of Harappan civilization, evidences rather suggest that smaller settlements continued albeit dispersed from original river valleys of Indus and Ghaggar-Hakra (Fig. 1A) to more distant areas of the Himalayan foothills and Ganga-Yamuna interfluves or Gujarat and Rajasthan\(^25–27\).

Based on the spatio-temporal distribution of the archaeological remains spread throughout the subcontinent a much older chronology has, however, been advocated by Possehl\(^2,16\). Accordingly the time spans of the above four phases have been suggested as ~9–6.3 ka BP, 6.3–5.2 ka BP, 5.2–3 ka BP and 3–2.5 ka BP respectively. Clearly the latter time scale pushes back the Harappan chronology to at least 1–2 ka older. Evidences of a post-Neolithic-Pre Harappan (often referred to as the Hakra ware) phase were first reported by Mughal\(^20,29\) in the Cholistan region east of the Indus valley along the Indo-Pakistan border, but have now been found from several localities in India. The Hakra settlements, spread along the Ghaggar-Hakra river valleys have been found at Kalibangan, Farmana, Girawad, Rakhigarhi and Bhirrana, the present site of investigation (Fig. 1A \(^30–33\)). A large number (~70) of conventional and AMS radiocarbon dates indeed support the antiquity of this phase in different parts of the Indus-Ghaggar Hakra river belts viz. Girawad (Pit-23, 6.2 ka BP), Mithathal (Trench A-1, 8.2 ka BP), Kalibangan...
The recent excavations at Rakhigarhi suggest hitherto unknown largest Harappan settlement in India preserving all the cultural levels including the Hakra phase (sample S-4173, 6.4 ka BP). A compilation of calibrated radiocarbon dates of the charcoal samples and OSL dates of pottery (see later discussion) from various cultural levels of Bhirrana (Lat. 29°33′N; Long. 75°33′E), retrieved during the excavation of 2005, is given in SI8,18. At Bhirrana the earliest level has provided mean 14C age of 8.35 ± 0.14 ka BP (8597 to 8171 years BP8). The successive cultural levels at Bhirrana, as deciphered from archeological artefacts along with these 14C ages, are Pre-Harappan Hakra phase (~9.5–8 ka BP), Early Harappan (~8–6.5 ka BP), Early mature Harappan (~6.5–5 ka BP) and mature Harappan (~5–2.8 ka BP8,17,18,20,34). Cultural stratigraphy of Bhirrana settlement depicting the periods, cultural levels, ages based on calibrated radiocarbon ages in different trenches and characteristic archeological artefacts and attributes are given in SI 8,17,20. A panoramic view of the excavation of the mature Harappan level at Bhirrana view from north-east is shown in Fig. 1B. Figure 2A shows the settlement pattern of pre-Harappan Hakra phase (period 1A9) along with locations of three major trenches at Bhirrana mound YF-2, A-1, and ZE-10. A schematic E-W cross section of the trench YF-2 depicting the cultural levels at Bhirrana is shown in SI. Fig. 2B (inset) shows the tentative lateral time correlation based on radiocarbon and OSL dates generated during present investigation (see later discussion). The Bhirrana settlement, close to the presently dried up Ghaggar-Hakra (Saraswati) river bed preserves all the major laterally traceable and time correlatable cultural levels. As expected in trench A-1, the central part of the archaeological mound, the Hakra or other phases are much thicker (>3 m) compared to the flanking trenches of YF-2 and ZE-10. At Bhirrana the Hakra ware culture period is the earliest and occurs as an independent stratigraphic horizon17,34. The Hakra phase was primarily identified by ceramics such as mud appliquè ware, incised ware, and bi-chrome ware, much similar to the Pre-Harappan phase in Cholistan (Figs 1A and 3C36) and was characterized by its subterranean dwelling, sacrificial and industrial pits8,17,34. The Early Harappan phase shows settlement expansion, mud brick houses with advanced material culture including arrow heads, rings and bangles of copper; beads of carnelian, jasper, and shell; bull figurines; chert blades; terracotta bangles, etc. (Fig. 3C17,32,34). The early mature to mature Harappan phases yielded ceramics with geometric, floral and faunal motifs; steatite bull seals; beads of semi-precious stone, shell and terracotta; animal figurines; bangles of faience and shell; copper bangles, chisels, rings, rods, etc.17,34. The excavations also yielded large quantities of faunal remains comprising bones, teeth, horn cores, etc. from all the four periods at Bhirrana and were identified at species levels37. Detail methods of faunal analysis for materials from the Bhirrana trench YF2 are given in the SI. Preliminary faunal investigations suggest presence of domestic cattle e.g., cow/ox (Bos indicus), buffalo (Bubalus bubalis), goat (Capra hircus) and sheep (Ovis aries) from the earliest levels. Besides the dietary use of cattle and goats, wild fauna such as nilgai (Boselaphus tragocamelus), Indian spotted deer (Axis axis) and antelope (Antilope cervicapra) were also a part of the diet17–40. Representative photographs of the artefacts and animal remains from various cultural levels of Bhirrana are shown in SI.
For retrieving information on past climatic changes we isotopically analysed bulk (see SI text) teeth and bone phosphates, wherever available, from the trench YF-2 which has both stratigraphic and sampling continuity (SI Table 2). To check the validity of the radiocarbon dates and the antiquity of the Bhirrana settlement we dated two pottery fragments (SI Fig. 1) in the same trench by OSL technique from both early mature and mature Harappan intervals. Detail methodology is given in SI text. The pottery at 42 cm, identified as mature Harappan level yielded mean 4.8 ± 0.3 (1σ) ka BP age (range 5120 to 4520 year BP) while the pottery from deeper level corresponding to early mature Harappan at 143 cm yielded 5.9 ± 0.25 (1σ) ka BP age (range 6185 to 5695 year BP). Within the experimental errors both the stratigraphically controlled new ages agree with the time scale based on archaeological evidences (as well as 14C ages) proposed by earlier workers8,17,18,34; Fig. 3C,D) and suggest that the Bhirrana settlements are the oldest of known sites in the Ghaggar-Hakra tract. Figure 3D,E show the comparison between the conventional chronology of the Harappan civilization with the proposed chronology at Bhirrana. Clearly the Bhirrana levels are few thousand years older. The 5.9 ka age at 143 cm along with the 8.38 ka age of the Hakra level below suggest that the base of the Bhirrana section, representing initiation of Harappan settlements (Hakra phase), is older than 8 ka BP. Below we show that isotope based paleoclimatic information also lends supports to the antiquity of Harappan settlements at Bhirrana.

**Oxygen isotope ($\delta^{18}O$) in bioapatites and past monsoon record at Bhirrana excavation site**

$\delta^{18}O$ [defined as $\delta (\%) = \{R_{sample} - R_{reference}\}/R_{reference} \times 1000$; $R = ^{18}O/^{16}O$ ratio] composition of fossil bone or tooth enamel bioapatite [carbonated hydroxyapatite41] is a robust tool for estimating the past meteoric water composition (drinking water for land animals41–46) compared to carbonates which are prone to diagenetic alteration. Near-continuous teeth and bone samples were available only in trench YF-2 and have been analysed. SI Fig. 4 shows the representative teeth and bone samples analysed from all the four cultural levels of Bhirrana. The samples comprise a large variety of bioapatites from mandibular and maxillary molar teeth of cattle, goat, deer and antelope to rib and vertebra bones. Since diagenetic alteration can alter isotopic signals we investigated the animal bones under electron microprobe that suggests preservation of original bioapatites suitable for isotopic analysis (see diagenetic investigation of bioapatites in SI). Detail methods of $\delta^{18}O$ analysis of bioapatites are given in SI text. Under a constant body temperature of ~37 °C, the $\delta^{18}O$ in mammalian phosphate ($\delta^{18}O_p$) essentially depends on the $\delta^{18}O$ value of water ($\delta^{18}O_w$) ingested by the organism. Between the water and phosphate, oxygen isotope is fractionated in two steps, i.e., between environmental and body water and between body water and phosphate in teeth and bones47,48. Large numbers of studies have been made on modern mammalian phosphates to constrain the interrelationship between $\delta^{18}O_p$ and $\delta^{18}O_w$. Although in general most large mammals have been found to preserve equilibrium isotopic signature, species specific fractionation equations have also been proposed by
several workers (ibid). For the Bhirrana mammals we used the taxon specific herbivorous mammal equations of Bryant and Froelich47. Because these equations are dependent on body mass it is desirable to infer paleoclimate from large body sized mammals. All Bhirrana mammals satisfy this criterion representing only cattle, deer or goats. δ18O, data of bioapatites and calculated δ18OW are given in Table 1 of SI.

Figure 3C shows δ18OW variation as a function of depth and against Harappan chronology at Bhirrana proposed by Rao et al.17 and Mani18. In general the bulk bioapatite δ18O in large mammals reflects the integrated mean annual δ18O of local meteoric water ingested by the animal during its life time. At several cultural levels we analysed multiple samples of either teeth or both teeth and bones. The spread in estimated δ18OW ranges from <1‰ to maximum ~4‰ and are probably due to the seasonal variation in δ18OW. Because our purpose was to retrieve the mean meteoric water δ18OW value from successive layers, we sampled bulk enamel or phosphate along the entire length of a single tooth or a bone (see SI text), yet the inter-sample seasonal signature might have been preserved in some cases. In spite of the inter-sample spread, the mean δ18OW values (dotted line in Fig. 3C) through the levels show a clear trend. At the base of the trench section (355 cm), equivalent to ~9 ka Pre-Harappan Hakra level, the δ18OW values are enriched (+3.75‰). The δ18OW values rapidly decreases towards the early Harappan phase reaching δ18O minimum of −9.01‰ at ~8 ka (trench depth ~308 cm). Thereafter the δ18OW monotonically gets enriched from early Harappan through early mature Harappan to mature Harappan, a time span from ~8 ka BP to 2.8 ka BP. We interpret this δ18OW variation through all the cultural levels at Bhirrana as major change in monsoon precipitation during the last 9.5 ka. We compare the Bhirrana record with available monsoon records from Arabian Sea (G. bulloides upwelling index; Fig. 3A)57 and composite gastropod–carbonate δ18O records from two inland lakes Riwas and Kotla Dahar, proximal to Bhirrana (Fig. 3B, re-plotted from supplementary information in refs 5 and 6). A weak monsoon phase is identified before 9 ka BP (lower part of Hakra phase). The well constrained monsoon intensification phase from 9 ka BP to 7 ka BP (late Hakra to middle part of early Harappan) is clearly discernible in all three records (blue shaded bars in Fig. 3A–C). Monsoon monotonically declined from 7 ka BP to 2 ka BP i.e., during later part of the early Harappan to mature Harappan phase (brown shaded bar) with concomitant lowering of lake levels (Fig. 3B). The early Holocene monsoon intensification and its subsequent decline, as recorded in Bhirrana archaeological bioapatites, have been widely documented in Asia and were principally driven by boreal summer insolation54,56. Presence of aeolian sands in lake Riwas, higher salinity in Bay of Bengal, lower G. bulloides upwelling intensity and enriched δ18O in Arabian speleothems suggest a weak monsoon phase before 10 ka BP throughout the Asia55–60. Correspondingly the 9–7 ka monsoon intensification phase is recorded in high lake levels (negative δ18O), lower oceanic salinity, increased upwelling, reduction in δ18O in speleothems from Arabia to Tibet, higher erosion rate in the Himalayas, and increased sedimentation in the Ganges deltaic plains (ibid). The late Holocene (7 ka onwards) gradual reduction in monsoon is also amply evident throughout the Asia.

Although compared to marine or lake archives the time resolution of the archaeological bioapatite based monsoon record is poor, preservation of the major phases of Holocene monsoon change combined with the OSL dates of potteries lend strong support to the antiquity of the Bhirrana settlement. To further constrain the change in paleo-meteoric water composition we generated time series δ18O of modern precipitation for successive three years at Hisar, a place 50 km SE of Bhirrana (Fig. 4) under the national program of ‘Isotopic Fingerprinting of Water in India (IFWIN)’. As in other places of north-western India, rainfall is highest during the summer months from June to September (Fig. 4A). The monsoon moisture originates in Bay of Bengal and successively rains inland towards north-western India (Fig. 1A). The continental effect thus causes depletion in precipitation δ18O from −5.4‰ near the coast to −6.5‰ in north western India67. The modern mean annual rainfall isobaths for this part of semi-arid NW India (Fig. 1A) show that all the Harappan settlement areas (including Bhirrana) receive 400 to 600 mm precipitation compared to >1000 mm in eastern and southern India67. At Hisar the modern precipitation δ18O ranges from ~+5‰ in non-monsoon (extreme evaporation) to ~15‰ in peak monsoon periods (depletion) with weighted mean annual δ18O value of ~7‰. The large monsoon depletion in δ18O results from well-known amount effect where excess rainfall is known to produce extreme depletion (an increase in 100 mm of rainfall associated with a decrease in δ18O by 1.5‰67). The most depleted paleo-meteoric value at Bhirrana is ~9.01‰ (SI Table 2; Fig. 3C). Considering the δ18OW value at each level represents mean annual precipitation and using a simple moisture flux model67, we estimate that the early Holocene (9–7 ka) monsoon precipitation at Bhirrana was ~100–150 mm higher than today. The subsequent enrichment from 7 ka onwards (by more than 6‰) reaching maximum towards the mature Harappan time indicates very low rainfall generating mean annual δ18OW similar to present day non-monsoon months. Such a climate scenario is indeed catastrophic and if persisted for several thousand years could easily convert large monsoon-fed perennial rivers to ephemeral or even dry ones.

Climate-culture relationship at Harappan Bhirrana

The climate reconstruction at Bhirrana demonstrates that some of the Harappan settlements in the Ghaggar-Hakra valley are the oldest in India and probably developed at least by the ninth millennium BP over a vast tract of arid-semi-arid regions of NW India and Pakistan. The Ghaggar (in India)-Hakra (in Pakistan) river, referred to as mythical Vedic river ‘Saraswati’ (Fig. 1A) originates in the Siwalik hills, ephemeral in the upper part with dry river bed running downstream through the Thar desert to Rann of Kachchh in Gujarat. More than 500 sites of Harappan settlements have been discovered in this belt during the last hundred years. Of these several sites both in India viz. Kalibangan, Kunal, Bhirrana, Farmana, Girawad7,9,31,33,69 and Pakistan viz. Jalipur, Mehrgarh in Baluchistan, Rehman Dheri in Gomal plains29,69,70 have revealed early Hakra levels of occupation preceding the main Harappan period. We infer that monsoon intensification from 9 ka onwards transformed the now dried up Ghaggar-Hakra into mighty rivers along which the early Harappan settlements flourished. That the river Ghaggar had sufficient water during the Harappan period is also attested by the faunal analysis. Frequency of occurrence of...
aquatic fauna like freshwater fish bones, turtle shells and domestic buffalo in these early levels of trench YF-2 is higher (compared to early or mature Harappan periods; SI) indicating a relatively wetter environment.

Study of fluvial morphodynamics coupled with detrital zircon analysis of river channel sands indicated presence of a more energetic fluvial regime before 5 ka across the entire Harappan landscape, stabilized alluvial systems during early Harappan (5.2–4.6 ka BP) and drying up of many river channels during post-Harappan period. Consequently floodplain agriculture helped in the expansion of the Harappan civilization which diminished as the monsoon waned during the late Holocene. Interestingly, the large scale droughts at ~8.2 and ~4.1 ka BP, recorded in the two lake records of Riwas and Kotla Dahar of Haryana correspond to the base of early Harappan and middle part of mature Harappan period at Bhirrana. These events were not local, extended from the Mediterranean through Mesopotamia to China and also are recorded as dust spike in Tibetan ice cores. Yet the settlements survived and evolved at several sites of Ghaggar-Hakra belt including at Bhirrana. The climate data and chronology of Bhirrana suggest that not only the Harappan civilization originated during the 8–9th millennium BP, it continued and flourished in the face of overall declining rainfall throughout the middle to late Holocene period. It is difficult to point to one single cause that drove the Harappan decline although diverse suggestions from Aryan invasion, to catastrophic flood or droughts, change in monsoon and river dynamics, sea-levels, trade decline to increased societal violence and spread of infectious diseases have been proposed. The continued survival of Harappans at Bhirrana suggests adaptation to at least one detrimental factor that is monsoon change. Although direct paleobotanical data from Bhirrana does not exist, archeobotanical study from nearby Farmana excavation, located ~100 km SW of Bhirrana clearly indicated change in crop pattern through cultural levels. At Farmana, compared to early levels a dramatic decrease in both ubiquity (from 61% to 20%) and seed density (1.5% to 0.7%) in wheat and barley in the later Harappan period has been documented. The study also indicates increasing dependence on summer crops like millet and has been inferred as a direct consequence of lesser rainfall. Such pattern have also been found elsewhere in Indus valley where the Harappans shifted their crop patterns from the large-grained cereals like wheat and barley during the early part of intensified monsoon to drought-resistant species of small millets and rice in the later part of declining monsoon and thereby changed their subsistence strategy. Because these later crops generally have much lower yield, the organized large storage system of mature Harappan period was abandoned giving rise to smaller more individual household based crop processing and storage system and could act as catalyst for the de-urbanisation of the Harappan civilization rather than an abrupt collapse as suggested by many workers. Our study suggests possibility of a direct connect between climate, agriculture and subsistence pattern during the Harappan civilization.

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Author Contributions
A.S. conceived the problem, prepared the figures, helped in analysis and wrote the paper. A.D.M. did the field work, collected samples and did the faunal analysis of teeth and bone samples, M.K.B. and B.D. carried out the chemical extraction of phosphates from bioapatites and did the stable isotope analysis, N.J. and P.M. did the OSL dating of potteries, R.D.D. coordinated the Hissar IWIN precipitation station and carried out the stable isotope analysis of rain water, V.S. provided input about Harappan archeology. Late L.S.R. excavated the Bhirrana archeological site.

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