Organic residue analysis in archaeological ceramics from Lahuradewa, India: role of contaminants

Organic residue, often amorphous or invisible to the naked eye, cannot be characterized by using traditional archaeological techniques. Such residues are a result of plant and animal product processing either by heating or due to mechanical action[1–4]. Organic residues, in contrast to the food crusts, are often better preserved in the mineral matrices of the pottery fabric that protect the organic molecules from microbiological degradation[4,5]. The biomolecular components of organic residue are used as a tool to identify the source of the residue[6], and to glean information on economic and subsistence practices associated with prehistoric cultural and technological traditions[4,7–9]. This makes organic residue analysis a well-established tool in geoarchaeology.

Degraded animal fats are the most common organic residue observed in archaeological ceramics[10]. The fats are identified by characteristically high abundance of easily detectable saturated fatty acids, particularly palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids. For example, a fatty acid (FA) distribution, characterized by a high ratio (>0.5) of stearic to palmitic acids indicates significant contribution from terrestrial animal lipids. On the other hand, vegetable oils are rich in C_{18} polyunsaturated acids and the presence of $C_{18}\omega$-(o-alkylphenyl) alkanoic acids indicates their presence of vegetable oil in prehistoric ceramics[11]. Lipid residues of aquatic and marine animals are rich in C_{16}-C_{22} ω-(o-alkylphenyl) alkanoic acids, and have a fatty acid distribution characterized by a low $C_{18:0}/C_{16:0}$-ratio[12,13]. Phytanic acid, an isoprenoid alkanoic acid produced through decomposition of chlorophyll, indicates the presence of green vegetables. Presence of ruminant lipids (from meat or milk), in general, is reflected by the high ratio of $C_{17:0}$ to $C_{18:0}$ fatty acids[14], although to effectively separate non-ruminant, ruminant adipose and dairy lipid residues, single compound stable carbon isotope analyses are required[4,15]. Vessels used for alcoholic fermentation and baking, using yeast, may show traces of ergosterol (5,7,22-ergostatrien-3β-ol)[16]. However, pottery may also be used for storage and processing of tar and pitch[17]. In addition, traces of terpenoids, such as dehydroabietic acid, may be left by wood smoke.

Archaeological excavations adjacent to Lake Lahuradewa (Figure 1) in the Sant Kabir Nagar District in Uttar Pradesh, revealed a 4 m thick cultural layer, representing a five-fold cultural sequence[18] and significant human activities since the onset of the Holocene. At this site, evidence of domesticated rice and use of pottery dates back to the 7th millennium BC[19,20]. Further, evidence of domesticated wheat and barley and other artefacts indicate contacts with Harappan culture from the 3rd millennium BC onwards[18,21]. The outcome of excavations has marked Lahuradewa as one of the most significant and prominent archaeological sites in the Middle Ganga Plain (MGP), where extensive excavations revealed sequences of transition from a subsistence based on gathering and selective hunting (in the epipalaeolithic period) through incipient food-production (proto-Neolithic) to settled village farming during the Neolithic time[22–24]. The evidence shows a long-term continuity that gradually led to husbandry. Despite the archaeological significance of the excavation, to the best of our knowledge no work has been published on lipid biomarker based organic residue analysis in ceramics excavated from the site adjacent to Lake Lahuradewa. The objective of the study is to characterize the soluble organic matter extracted from the Lake Lahuradewa ceramic samples.

Lipid residues were extracted from 5 g of homogenized ceramic powder from 15 ceramic sherds using chloroform and methanol (2 : 1, v : v). The lipid extracts were treated with 100 µl of *bis*(trimethylsilyl)trifluoroacetamide with 10% (v) chlorotrimethylsilyl at 70°C for 20 min. The reagents were evaporated and the samples dried under a gentle stream of nitrogen gas. The derivatized lipid residues were diluted in 500 µl of *n*-hexane and analysed on a HP 6890 gas chromatograph (GC) equipped with a SGE BPX5 capillary column (30 m × 220 mm × 0.25 µm). The injection was done by pulsed splitless (pulse pressure 17.6 Psi) technique at 325°C through a Merlin Microseal High Pressure Septum using an Agilent 7683B Autoinjector. The injection volume used was 1.0 µl. The oven was temperature-programmed with an initial isothermal of 2 min at 50°C, with 10°C increment per minute to 360°C, followed by a final isothermal at this temperature for 15 min. Helium was used as carrier gas at a constant flow of 2 ml per minute throughout the analysis. The gas chromatograph was connected to a HP 5973 mass selective detector via an interface with a constant temperature of 360°C. The separated compounds were fragmented by electronic ionization (EI) at 70 eV. The temperature at the ion-source was 230°C. The mass filter was set to scan between *m/z* 50 and 700, providing 2.29 scans per second. The temperature of mass filter was 150°C. The chromatographic reproducibility of the system is in the order of ±0.05 min in retention time and the detection limit of lipid species in the powdered ceramic is estimated to be at least 0.06 µg/g, varying with the fragmentation pattern of specific lipids. Pre-cleaned and combusted glassware, and only GC-grade solvents and reagents were used. The results were evaluated using the MSD Chemstation software.

The result of the lipid biomarker analysis is presented in Table 1. One out of the 12 samples analysed (not shown in Table 1) contains only traces of lipid residues and phthalates, which is most likely derived from plastic packing materials. Samples 2–9 have a fairly similar fatty acid distribution primarily dominated by palmitic acid ($C_{16:0}$). The presence and distribution of long-chain alkanols, branched fatty acids, and monounsaturated fatty acids vary within the samples. The variation in long-chain alkanols could indicate variation in plant wax contributions[25]. Significant presence of stearic acid ($C_{18:0}$) in samples 10 and 11 could indicate contribution from terrestrial animal lipids[26]. Presence of cholesterol in all samples, especially in samples 10 and 11, indicates contribution from animal adipose lipids[25]. However, there is no clear trace of ruminant or dairy lipids in the samples. Notably, the presence of squalene and cholesterol in all samples (Table 1) indicates significant contribution of lipids from recent human

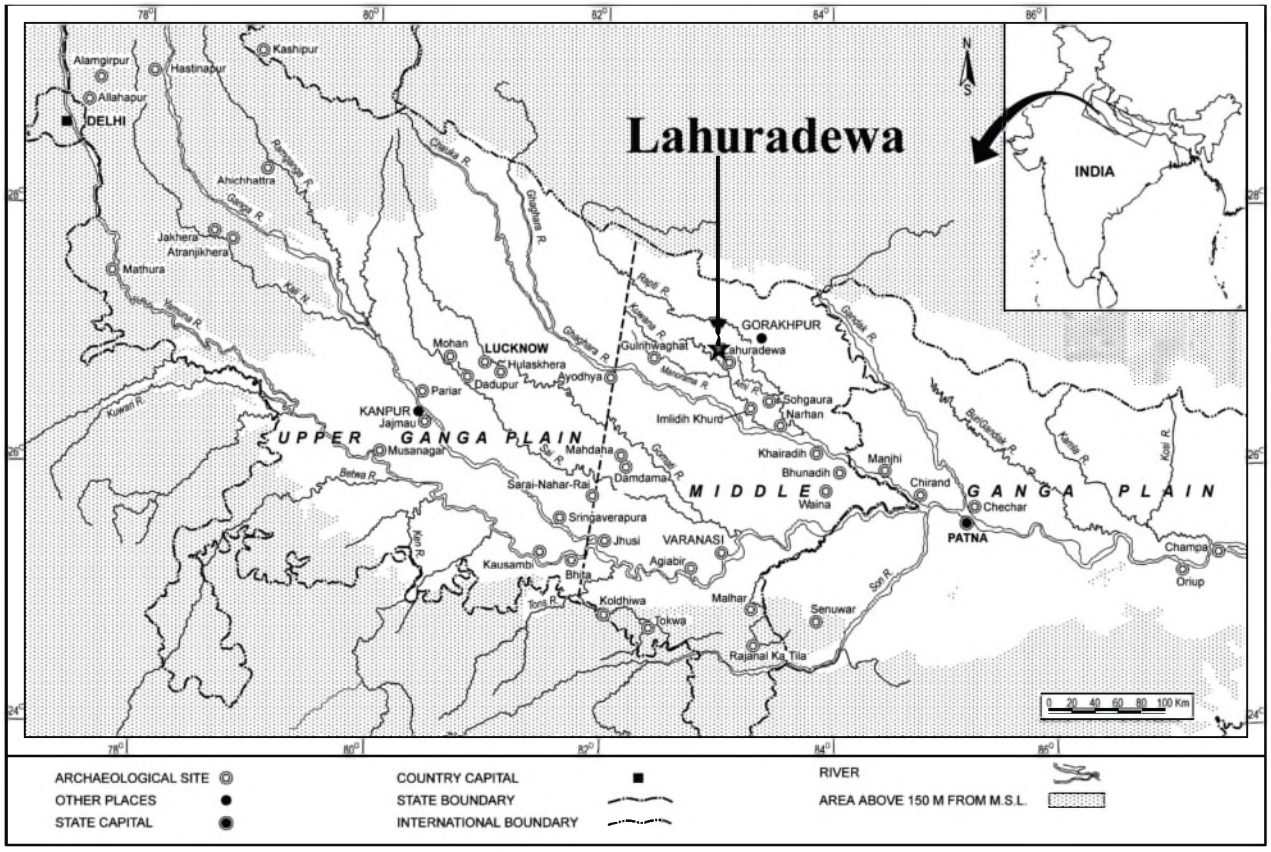

Figure 1. Position of Lake Lahuradewa in Uttar Pradesh, India.

Table 1. Lipid biomarkers in the organic residue extracted from the ceramics that were excavated from the site at Lake Lahuradewa. Fatty acids (FA) and long chain alkanol (LCAL) distributions are presented using the format: $C_n(C_k)C_m$, where C_n is the shortest carbon chain-length, C_m is the longest carbon chain-length and C_k is the chain-length of the dominating biomarker molecules. A high $C_{18:0}/C_{16:0}$-ratio characterizes a fatty acid distribution dominated by the stearic fatty acid ($C_{18:0}$) and a low ratio characterizes a distribution dominated by palmitic acid ($C_{16:0}$). A high ratio (>0.8) is indicative of terrestrial animal lipids and a low ratio indicates plant or aquatic animal lipids[28]. The carbon chain-lengths are given for the branched fatty acids (BR), and carbon chain-length and the number of double-bonds are given for monounsaturated (FA (uns))

Cultural periods	Sample no.	Sample name	FA	$C_{18:0}/C_{16:0}$	LCAL	BR	FA (uns)	Cholesterol	Squalene	Phthalates
Early farming phase										
Period IB	11	YA2 QDT 4	$C_{10}(C_{18})C_{22}$	4.07	C_{22}-C_{30}	C_{13}-C_{18}	16 : 1, 18 : 1	•	•	•
(*c.* 5000–4000 BP)[17]	10	YA1 QDT-1#	$C_{12}(C_{18})C_{22}$	1.44	$C_{24}(C_{28})C_{32}$	C_{15}-C_{17}	16 : 1, 18 : 1, 22 : 1	•	•	•
	9	YA1 QDT 1 TC	$C_9(C_{16})C_{18}$	0.16	$C_{24}(C_{28})C_{30}$	C_{15}, C_{17}	16 : 1, 18 : 1	•	•	•
	8	YA3 QDT-2	$C_{12}(C_{16})C_{18}$	0.16	$C_{24}(C_{28})C_{30}$	C_{15}	16 : 1, 18 : 1	•	•	•
	7	YA2 QDT-4	$C_{10}(C_{16})C_{22}$	0.26	n.d.	C_{15}-C_{17}	16 : 1, 18 : 1, 22 : 1	•	•	•
	6	YA2 QDT 1	$C_{12}(C_{16})C_{18}$	0.28	$C_{24}(C_{26})C_{28}$	C15	16 : 1, 18 : 1	•	•	•
Period IA	5	YA X 2	$C_9(C_{16})C_{18}$	0.25	n.d.	n.d.	16 : 1, 18 : 1	•	•	•
(*c.* 9000–5000 BP)[17]	4	YA1 QCH1	$C_{12}(C_{16})C_{18}$	0.38	n.d.	C_{15}-C_{17}	16 : 1, 18 : 1	•	•	•
	3	YA2 QDT-1#	$C_9(C_{16})C_{18}$	0.17	$C_{24}(C_{26})C_{28}$	C_{15}, C_{17}	16 : 1, 18 : 1	•	•	•
	2	YA2 adt-4	$C_{10}(C_{16})C_{18}$	0.2	n.d.	C_{15}, C_{17}	16 : 1, 18 : 1	•	•	•
	1	YA1 QH1	$C_{14}(C_{16})C_{18}$	0.24	n.d.	n.d.	16 : 1, 18 : 1	•	•	•

n.d. = not detected; • = detected; #Black and Redware pottery.

fingerprints[27–29]. Human skin lipids contain a range of lipid classes including glycerides, fatty acids, wax esters, squalene, sterols and sterols esters. The presence of human skin lipids implies that organic residue extracted from the ceramics are not suitable for geoarchaeological interpretation of the content and/or use of the ancient potteries. We suggest that caution be exercised during collection and storage of ceramics of archaeological importance for organic

residue analysis. Ceramics suitable for residue analysis should be selected during excavation, and should not be handled with bare hands. Washing of shreds as well as use of glues, varnishes or marker pens on such ceramics should be strictly avoided. To limit contamination from plasticizers from packing materials, such as plastic bags, ceramic samples chosen for organic residue analyses should be wrapped in pre-combusted (at 450°C) aluminium foil before storing in plastic bags. In addition, ceramics should be stored in a cool dry place and away from direct sunlight.

1. Chang, T. T., *Euphytica*, 1976, **25**, 425–441.
2. Stern, B., Heron, C., Serpico, M. and Bouiau, J., *Archaeometry*, 2000, **42**, 399–414.
3. Evershed, R. P., Dudd, S. N., Lockhart, M. J. and Jim, S., *Handbook of Archaeological Science*, Chichester, 2001.
4. Evershed, R. P., *Archaeometry*, 2008, **50**, 895–924.
5. Regert, M., Bland, H. A., Dudd, S. N., van Bergen, P. F. and Evershed, R. P., *Proc. R. Soc. London, Ser. B*, 1998, **265**, 2027–2032.
6. Gregg, M. W. and Slater, G. F., *Archaeometry*, 2010, **52**, 833–854.
7. Barnard, H. *et al.*, *J. Archaeol. Sci.*, 2007, **34**, 28–37.
8. Craig, O. E. *et al.*, *Nature*, 2013, **496**, 351–354.
9. Lucquin, A. *et al.*, *Proc. Natl. Acad. Sci. USA*, 2016, **113**, 3991–3996.
10. Dudd, S. N. and Evershed, R. P., *Science*, 1998, **282**, 1478–1481.
11. Isaksson, S., Olsson, M. and Hjulström, B., *Fornvännen*, 2005, **100**, 179–191.
12. Hansel, F. A., Copley, M. S., Madureira, L. A. S. and Evershed, R. P., *Tetrahedron Lett.*, 2004, **45**, 2999–3002.
13. Olsson, M. and Isaksson, S., *J. Archaeol. Sci.*, 2008, **35**, 773–780.
14. Hjulström, B., Isaksson, S. and Karlsson, C., *Acta Archaeol.*, 2008, **79**, 62–78.
15. Evershed, R. P., Stott, A. W., Raven, A., Dudd, A. N., Charters, S. and Leyden, A., *Tetrahedron Lett.*, 1995, **36**, 8875–8878.
16. Isaksson, S., Karlsson, C. and Eriksson, T., *J. Archaeol. Sci.*, 2010, **37**, 3263–3268.
17. Isaksson, S., *Curr. Swedish Archaeol.*, 2009, **17**, 131–149.
18. Tewari, R., Srivastava, R. K., Singh, K. K., Saraswat, K. S., Singh, I. B. and Chauhan, M. S., *Pragdhara*, 2006, **16**, 35–68.
19. Tewari, R., *Man Environ.*, 2004, **XXIX**, 102–116.
20. Joglekar, P. P., *Pragdhara*, 2006, **18**, 309–321.
21. Saxena, A., Prasad, V., Singh, I. B., Chauhan, M. S. and Hasan, R., *Curr. Sci.*, 2006, **90**, 1547–1552.
22. Sharma, G. R., Misra, V. D., Mandai, D., Misra, B. B. and Pal, J. N., Beginnings of agriculture: (Epi-Palaeolithic to neolithic; excavations at Chopani-Mando, Mahadaha and Mahagara), Abinash Prakashan, Allahabad, India, 1980.
23. Possehl, G. L. and Rissman, P. C., In *Chronologies in Old World Archaeology* (ed. Ehrich, R. W.), The University of Chicago Press, 1992, 3rd edn, vol. 1.
24. Saraswat, K. S., *Pragdhara*, 2004, **15**, 145–177.
25. Weyermann, C., Roux, C. and Champod, C., *J. Forensic Sci.*, 2011, **56**, 102–108.
26. Baeten, J., Jervis, B., De Vos, D. and Waelkens, M., *Archaeometry*, 2013, **55**, 1150–1174.
27. Romanus, K., Poblome, J., Verbeke, K., Luypaerts, A., Jacobs, P., De Vos, D., and Waelkens, M., *Archaeometry*, 2007, **49**, 729–747.
28. Emerson, B., Gidden, J., Lay Jr, J. O. and Durham, B., *J. Forensic Sci.*, 2011, **56**, 381–389.
29. Buchanan, M. V., Asano, K. and Bohanan, A., *SPIE*, 1997, **2941**, 89–95.

ACKNOWLEDGEMENTS. We acknowledge financial help of UGC for the study. We are also grateful to Directorate of Archaeology, Lucknow and Rakesh Tewari for providing samples and information on Lahuradewa. The UGC is further acknowledged for offering a position to Supriyo Kumar Das under FRP.

Received 20 July 2017; accepted 24 July 2018

SUPRIYO KUMAR DAS[1,*]
SANTANU GHOSH[1]
SVEN ISAKSSON[2]
KERSTIN LIDÉN[2]
ARUNABHA DEY[1]

[1]*Department of Geology, Presidency University, Kolkata 700 073, India*
[2]*Department of Archaeology and Classical Studies, Stockholm University, 106 91 Stockholm, Sweden*
**For correspondence.*
e-mail: sdas.geol@presiuniv.ac.in