Cannabis sativa (Cannabaceae) in ancient clay plaster of Ellora Caves, India

M. Singh[1] and M. M. Sardesai[2,]*

[1]Archaeological Survey of India, Science Branch, Western Zone, Aurangabad 431 004, India
[2]Department of Botany, Dr Babasaheb Ambedkar Marathwada University, Aurangabad 431 004, India

The present research trend is to explore sustainable construction materials having least environmental impact that also encapsulate in terms of our natural resources. The present communication discusses the use of raw hemp as an organic additive in the clay plaster of the 6th century AD Buddhist Caves of Ellora, a World Heritage Site. *Cannabis sativa* L. admixed in the clay plaster has been identified using scanning electron microscope, Fourier transform infrared spectroscopy and stereomicroscopic studies and the results are compared with fresh specimens. The study indicates that many valuable properties of hemp were known to the ancient Indians in the 6th century AD.

Keywords: Ancient caves, *Cannabis sativa*, clay plaster, World Heritage Site.

CANNABIS is one of the oldest domestic plants in the history of mankind, probably being utilized for more than 10,000 years[1,2]. Hemp grows in most climates and enriches poor soils after every crop[3]. Owing to its fast growth in both temperate and tropical climates, hemp has been used for centuries to make a variety of items such as rope, paper, clothing, sails, etc. The fibrous strands of hemp are ideal for fabric owing to its better durability over cotton.

Hemp originated from Central Asia and has been grown there for thousands of years. It is mentioned in Roman literature from around 100 BC, claiming that the plant was grown in southern Europe[4]. The remains of hemp (*C. sativa* L.) unearthed mainly in Eurasia have either carbonized or decayed due to climatic change and storage conditions[2,5]. The oldest example of hemp remains with macroscopic/microscopic structure has been reported from Yanghai Tombs located in Turpan Basin of China[6]. This material has provided significant information on ancient civilization of the Turpan area and about utilization of hemp.

In India, hemp domestication dates back to 5000–4000 BCE (ref. 7), cultivated for multiple purposes, including use of its fibre from stem, edible food/oil from its achene, medicine and psychoactive substances from its resin glands[1]. *Cannabis* is popularly known as ganja or bhang in India. The Ayurvedic and Siddha systems of Indian medicine contain a number of references about *Cannabis*. The medicinal use of *Cannabis* was first recorded in India in the medical work '*Sushrita*' compiled around 1000 BC (refs 8, 9) and finds mention in the ancient Persian literature *Zend Avesta*[8]. *Cannabis* has also been listed in Indian texts such as *Tajnighuntu* and *Rajbulubha*[8,9]. According to these texts, *Cannabis* is used in the treatment for clearing phlegm, expelling flatulence, inducing costiveness, sharpening memory, increasing eloquence, as an appetite stimulant, for gonorrhea, and also as a general tonic. Moreover, the Hindus consider *Cannabis* as a holy plant, and it is used in Hindu festivals like Shivratri even today.

The stalk of hemp plant consists of fibres (soft and flexible) and hurds (rigid and hard). Processing of *C. sativa* results in three basic constituents, namely shives or hurds (~62%) by weight plant fibres (~35%), and seed and dust with particle size less than 0.5 μm (~4%). Hemp is an environment-friendly plant that does not require pesticides or herbicides to grow. Hemp hurds are acid-free and unlikely to deteriorate over time. Hemp is also resistant to rodents, fungus and many weeds. It is also a heterogeneous plant, its microstructure is extremely complicated and therefore *Cannabis* itself can be regarded as composite system[10,11].

The basic unit of *Cannabis* consists of cellulose polymeric chain aligned in micro fibrils. They are linked to each other by lignin, pectin and hemi cellulose. The strength and stiffness of the fibres are provided mostly by hydrogen bonds between the different chemical components. The hemp composition and properties of each component are responsible for its thermal stability, resistance to UV rays and biodegradation of *Cannabis*. Hemps are low-density light-weight materials which can exhibit mechanical strength parameters[12,13]. Hemp hurds can be combined with lime to form concrete-like substance called hempcrete. A combination of hemp hurd, lime, clay, cement and water in various proportions has been used for centuries in the construction of building. Hempcrete has been discovered in a bridge abutment in France built in the 6th century AD. *C. sativa* was chosen for industrial use due to its long, soft, durable fibrous properties that provide immense amount of strength.

The significant advantage of hempcrete is that it is greenhouse negative, as it absorbs more carbon dioxide from the atmosphere than it produces in its life cycle, thereby reversing the effect of global warming. Hempcrete produces no toxic by-products. It has a high thermal mass and insulating properties, and therefore lowers the heating and cooling emission to maintain a healthy and comfortable living environment. Hempcrete buildings have long life due to material properties of the durable fibre. Hempcrete also exhibits higher vapour permeability that regulates moisture content and internal humidity, and also improves indoor air quality and health of the building occupants. A hemp home also maintains the acoustic

*For correspondence. (e-mail: sardesaimm@gmail.com)

Figure 1. General view of Ellora caves, a World Heritage Site in Aurangabad, India.

requirement of buildings. Incorporation of hemp will also result in aggregate reduction to save our natural resources[14–16].

The production of building materials for construction is responsible for consumption of energy from fossil fuel resources and depletion of non-renewable materials[17]. Nowadays the trend is shifting towards finding production technologies that encapsulate saving of our natural resources. Because of their positive environmental impact, the sustainable construction materials are now gaining more ground worldwide. New bio-composites containing natural fibres and/or particles, lignocelluloses wastes are the subject of current research worldwide.

The Ellora Caves, dating back from 6th to 11th century AD are located near a village locally known as Verul at a distance of 29.8 km to the northwest of Aurangabad city, Maharashtra, India (Figure 1). This is a group of 34 rock-cut caves dedicated to the three main religions of India – Buddhism, Hinduism and Jainism. In 1983, the caves were declared a World Heritage Site (WHS) by UNESCO. The caves of Ellora are breathtaking examples of rock-cut architecture that stands testimony to the imagination and artistry of its creators. Unlike the Ajanta Caves, a nearby WHS site, the caves of Ellora lie on an ancient trade route and have almost remained in public eye. The caves run approximately in north–south direction for about 2 km. At the southern end are 12 Buddhist caves, while in the north there are 6 Jain caves and in between lie 17 Brahmanical caves. The Buddhist caves of Ellora are the earliest (AD 550–700) among the group.

The Buddhist cave no. 12, from where clay plaster samples were examined, is a remarkable three-storeyed building with indistinguishable traces of paintings on the ceilings and walls of the inner shrine (Figure 2). The designs painted on the wall and ceilings represent floral and creeper patterns and other geometric shapes, jeweller designs and wood work. It is obvious that the colour of these paintings is dull and insipid owing to deposition of soot and weather conditions as the caves are exposed to sunshine and rainfall. The clay plaster samples admixed with vegetal plant remains were extracted from cave no. 12 for laboratory study (Figure 3).

Specimens of *C. sativa* were isolated from the clay plaster. The isolated specimens include pounded pieces of shoots, fragmented leaves and single (male?) flower. The sample of *Cannabis* was examined using scanning electron microscope (SEM), light microscope, stereomicroscope and Fourier transform infrared spectroscopy (FTIR) techniques.

For SEM examination, the dried specimens were directly placed on the stubs and sputter-coated with gold using a SPI-MODUEL Sputter coater. The *C. sativa* samples were examined and photographed under EDAX model JEOI–SEM360. The fresh specimen of *Cannabis* was also similarly examined.

For FTIR examination, the pounded pieces of shoots were examined using Agilent Technologies Cary 600 Series FTIR spectroscopy along with fresh specimens of *C. sativa* for comparative study.

For light microscopic examination, the pounded pieces of shoots were examined and measured with a calibrated ocular micrometer and photographed under a LABOMED vision 2000 and lab vision Madstar light microscope.

For stereomicroscopic examination, the flowers and leaves of *C. sativa* were examined and photographed under a Olympus Magnus MSZ and LABOMED CSM 2 stereo microscope.

For comparative studies, fresh specimen (Pardeshi 3723) was collected from Jalna, Aurangabad district, Maharashtra and also from the roadside on the outskirts of Delhi city (Sardesai 1500). The specimens are stored in the Herbarium of Dr Babasaheb Ambedkar Marathwada University, Aurangabad.

The temperature and relative humidity were monitored both inside and outside the caves during the period November to December 2014. For measurement of temperature and humidity inside cave no. 12, an Oakton RH/T data logger was temporarily fixed near the Buddha chamber in the inner part of the cave. The temperature and Humidity data outside the cave were derived from

Figure 2. Paintings on the walls of cave no. 12 at Ellora.

Figure 3. Small piece of clay plaster showing *Cannabis* fibres.

weather monitoring station installed in front of cave no. 21, at Ellora in collaboration with Indian Space Research Organization (ISRO), Bengaluru.

Cannabis is highly polymorphic in nature, which has resulted in consideration of more species in the genus by many authors[1,18–22]. However, based on its morphology, anatomy, phytochemistry and genetic studies, *Cannabis* is now considered as monotypic by the botanists[8,23–26].

The leaves have been preserved as small fragments in the clay plaster. The cuticle of the leaves is round with raised epidermal cells. The epidermal cells are irregular in outline with undulated walls (Figure 4). The adaxial epidermis of the leaves has conical non-glandular trichomes with a warty surface. Their tips are erect or inclined towards the leaf apex. Nearly all trichomes have a rounded enlarged base (Figure 4). The length of the trichomes varies from 45 to 115 μm and the diameter of the base varies from 68 to 134 μm.

The morphology of the trichomes is well discussed in the literature[25,26]. Two types of trichomes occur on the abaxial epidermis, i.e. glandular and non-glandular. The non-glandular ones are conical, 85–175 μm long with an enlarged bulbous base. The bases vary from 17.8 to 29.5 μm in diameter (Figure 4). The trichomes around the principal veins display a warty surface, while those between the veins are only slightly warty or smooth. The tips of the trichomes are inclined towards the leaf apex at various angles. The glandular trichomes are sessile, brown-red in colour, and rounded in shape with an average diameter of 37.4 μm. They are sparsely and uniformly distributed with an average density at $28/mm^2$.

The non-glandular and sessile glandular trichomes occur on the epidermis. The former are conical, curved and simple with a warty surfaces inclined towards the apex of the leaf. The latter are globular, light brown in colour with smooth surfaces.

The hurd of *C. sativa* collected from the clay plaster was observed under Agilent Resolution Pro FTIR spectrophotometer. The fresh specimen of *Cannabis* was also observed under FTIR spectroscopy. Figure 5 shows the FTIR image of both the samples. It can be observed that the main peak for cellulose is around 2900 cm^{-1} for both samples. The peak around 1000 cm^{-1} observed in the ancient sample is due to the presence of silicate in the clay plaster. The FTIR image confirms the mixing of *C. sativa* in the clay plaster of Ellora Caves, as the peaks of the both samples are similar and support the investigations carried out using other techniques.

Light microscope examination reveals that the small fragments of leaves are preserved in the clay plaster. The raised epidermal cells with irregular outline and undulate

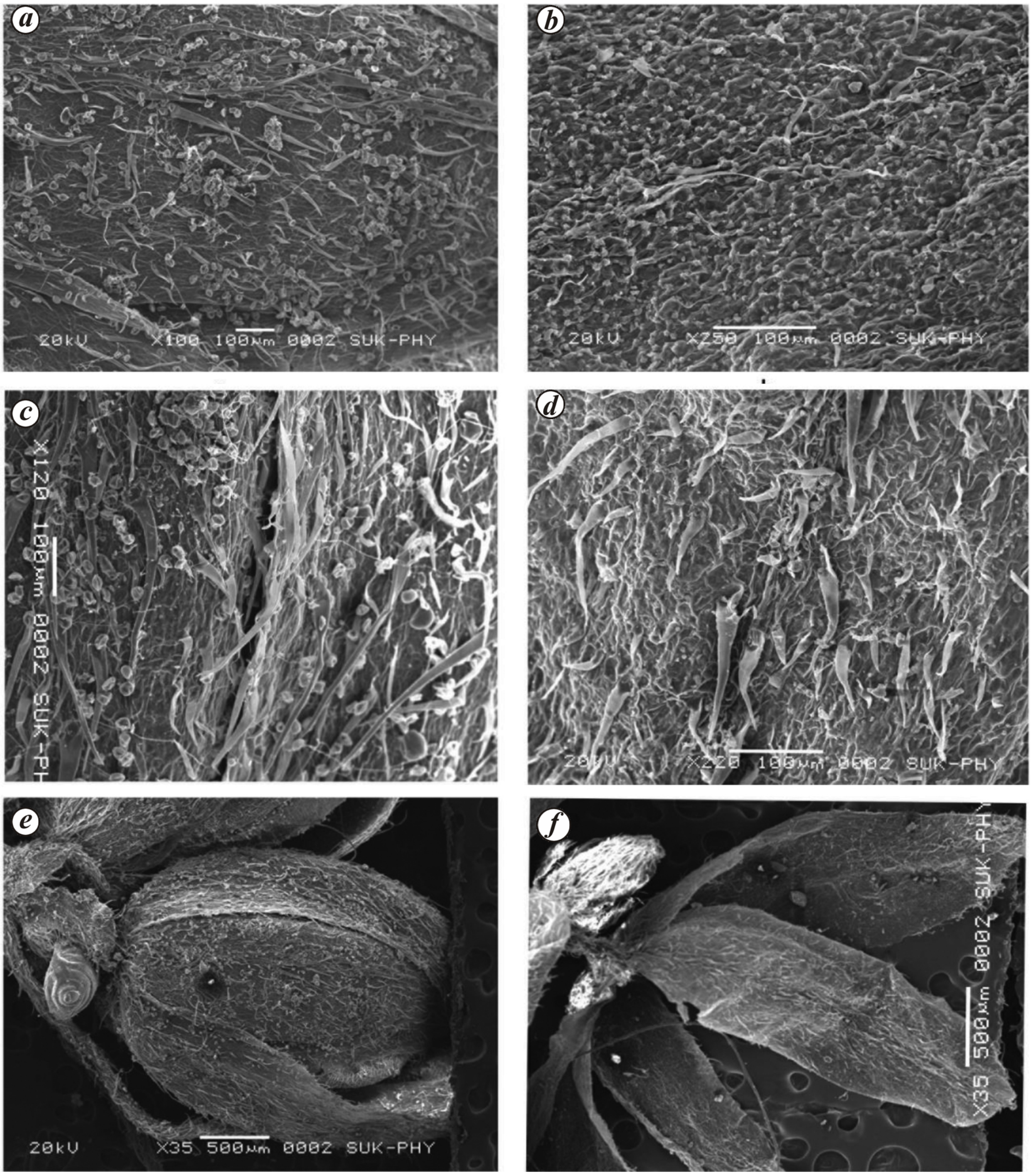

Figure 4. SEM photographs of fresh samples and archeological samples of leaf and flower. (***a***), (***c***) and (***e***) are from living plants while (***b***), (***d***) and (***f***) are from the remains. ***a***, ***b***, Adaxial epidermis of the leaf. ***c***, ***d***, Abaxial epidermis of the leaf. ***e***, ***f***, Flower.

walls can be seen. The upper epidermis shows conical, non-glandular trichomes with tips erect or inclined towards the leaf apex. Trichomes have a rounded enlarged base.

The sample preserved in the clay was seen under stereromicroscope. Morphological characters of fragmented leaves and flower were studied in comparison with fresh hemp (Figure 4).

The temperature inside the caves does not show much fluctuation and varies from 25.5°C to 27°C (Figure 6*a*). However, the temperature outside the caves shows large fluctuation and varies from 18°C to 32°C (Figure 6*b*); and solely depends on the weather conditions in the climatic zone. This shows that the caves are thermally stable during all weather conditions.

Relative humidity shows large fluctuation both inside and outside the caves. During the period of monitoring, relative humidity recorded inside the caves was found to vary from 27% to 45% (Figure 7*a*) while outside the caves it varied from 25% to 55% (Figure 7*b*). The data

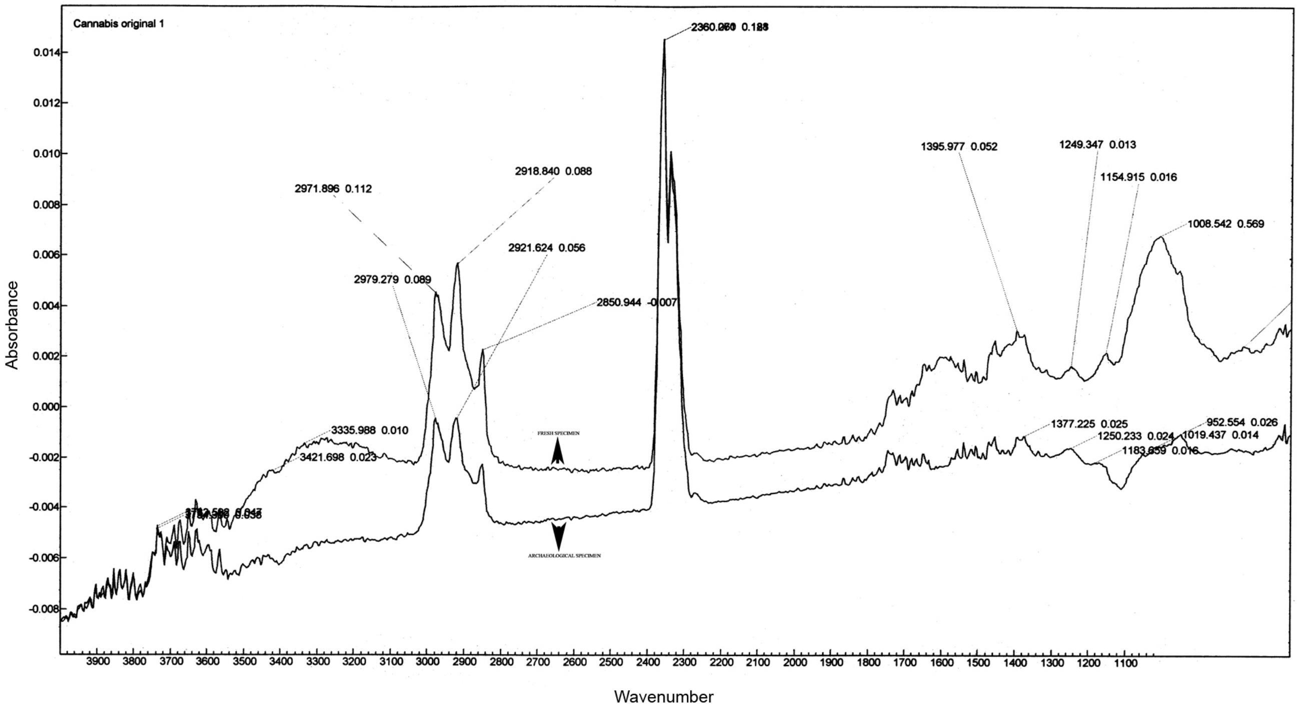

Figure 5. Comparative FTIR graph of archaeological specimens and fresh specimens of *Cannabis sativa*.

show that although the caves are thermally stable, much variation in the humidity within the caves persist that causes the mud plaster to flake.

The plant remains of *Cannabis* of Ellora Caves display chiselled and pounded shoots, small fragments of leaves, non-glandular and glandular trichomes on the epidermis, a male flower, perianth with parallel and undulated epidermal cell walls, etc. These features suggest that the remains belong to *C. sativa* L. (Cannabaeacae), as no difference was observed between the archaeological sample and fresh specimen of *C. sativa* L.

According to Rhydwen *et al.*[27], poor moisture control in heritage and stone-walled buildings in the United Kingdom has led to mould growth and structural damage. There are concerns regarding an increased risk of internal condensation of moisture with regard to maintaining integrity of heritage buildings. For the heritage structure, air tightness improves interstitial wall moisture ingress either due to membrane failure or liquid water surface diffusion.

Hemp as binder is an insulating matrix used as an internal insulating render that can provide improved efficiency in both heating and cooling seasons although its overall thermal effectiveness remains unreported. It also potentially controls both internal and interstitial moisture fluctuations due to its hygroscopic properties, thus reducing the need for increased air flow to prevent surface condensation[27–29].

The cave no. 12 at Ellora is wide open to atmosphere and microclimatic condition in the cave is in consonance with external conditions. This has led to uncontrolled amount of humidity inside the cave during rainy season. Moreover, there is an additional potential for interstitial condensation on the outer surface. Further penetration of rainwater into the external parts of the wall and permeating inwards results in fluctuation in the temperature gradient. This moisture leads to condensation and has the tendency to build up in the walls as external evaporation is reduced and internal evaporation is prevented[27]. These effects also enhance the likelihood of damage to plaster and stone members of heritage structures.

The remains of *Cannabis* from the sample of clay plaster of Ellora suggest that it was used with clay/lime binder[30] as insulating agent as well as to provide a degree of strength to the plaster. Studies in Europe have estimated 600–800 years of life span to the hempcrete wall system[31], but hemp in the clay plaster of Ellora has survived more than 1500 years. The long life of clay plaster at Ellora, despite damaging environmental parameters, may be attributed to the material properties of hemp, which is fibrous and durable. The hempcrete plaster of Ellora must have provided a healthy, comfortable and aesthetically pleasing living environment to the Buddhist monks to stay. Further, as the hemp plaster has the ability to store heat, is fire-resistant[3] and absorbs about 90% of airborne sound, a peaceful living environment for the monks has been created at Ellora Caves. The study also suggests that raw and unprocessed hemp was mixed in the clay plaster at Ellora. Recent research has shown that the use of both shives/hurd and fibre of hemp did not give

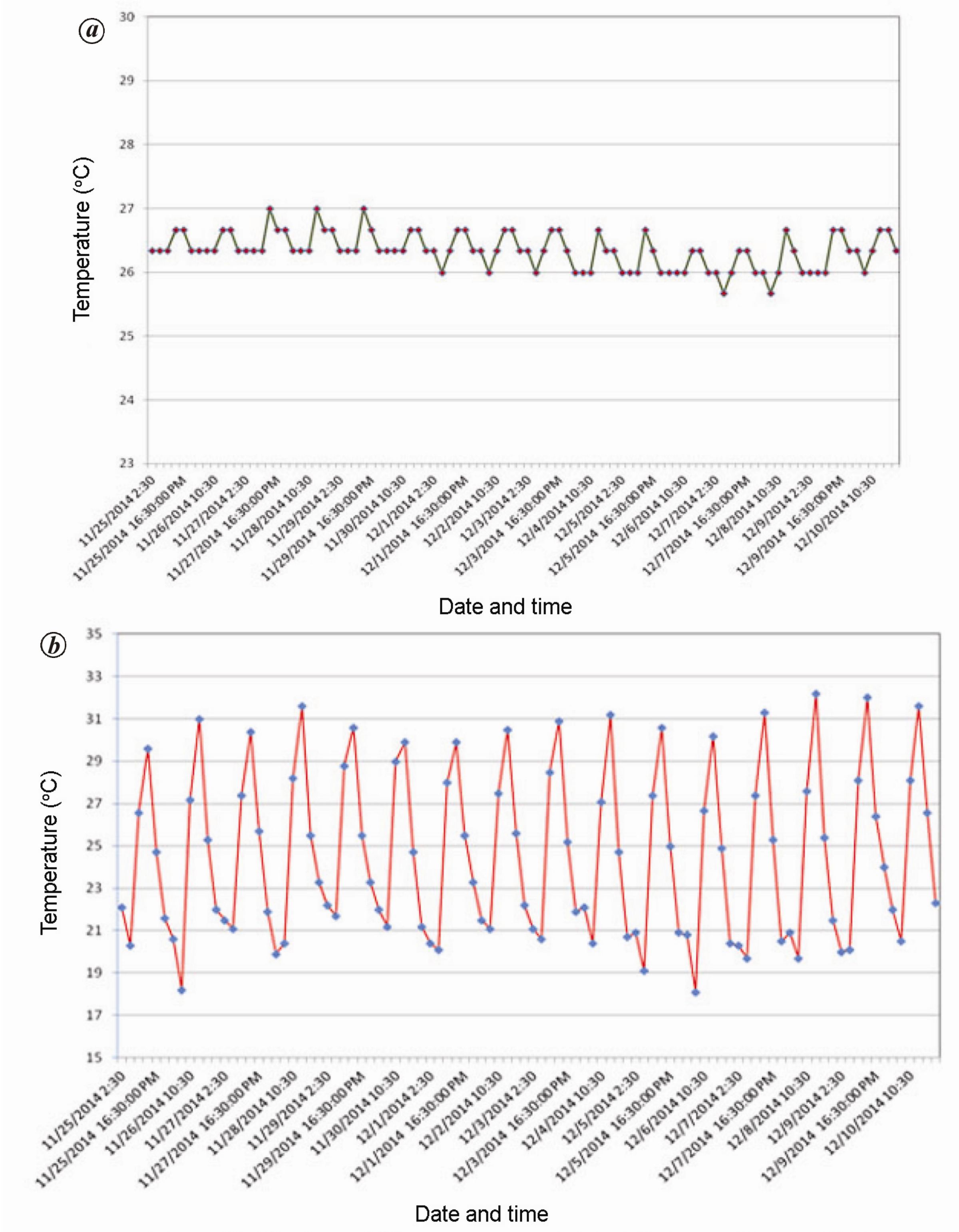

Figure 6. Temperature recorded (***a***) inside and (***b***) outside cave no. 12 at Ellora.

different mechanical properties compared to when only hemp shives were used. Probably, the makers of Ellora clay plaster were familiar with the characteristic features of hemp and used raw hemp for their work.

Studies of ancient fibre of fresh specimen using various techniques confirms the presence of *Cannabis* in the earthen mix of Ellora Caves. These studies also establish inclusion of hemp as vegetal additives in the earthen

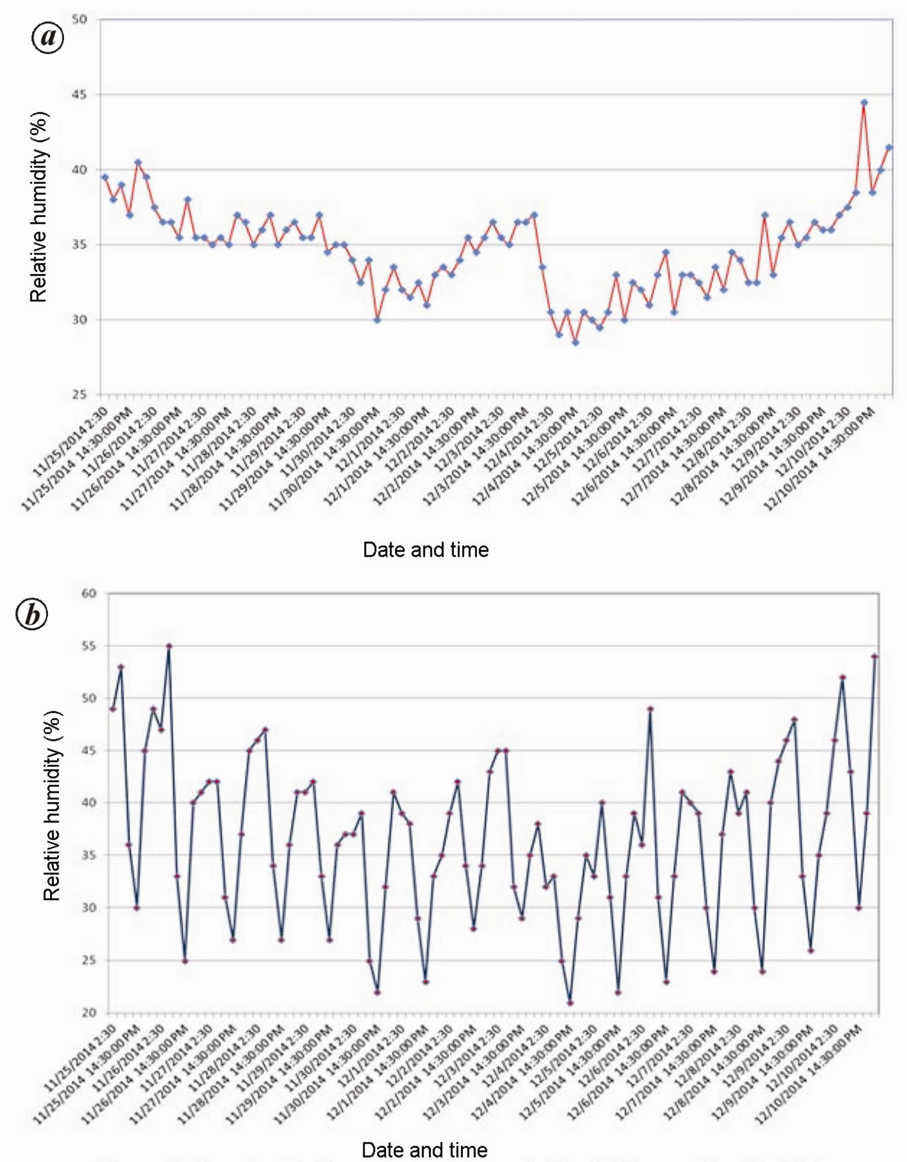

Figure 7. Relative humidity recorded (***a***) inside and (***b***) outside cave no. 12 at Ellora.

plaster mix due to its long, soft, durable fibres that provided strength to the plaster. It appears that properties of hemp fibres such as the ability to regulate humidity inside the cave, pest resistance, fire-retardant, non-toxicity, high vapour permeability, hygroscopic properties, etc. were known to the inhabitants of Ellora in the 6th century AD.

1. Schultes, R. E., Klein, W. M., Plowman, T. and Lockwood, T. E., *Cannabis*: an example of taxonomic neglect. *Bot. Mus. Leafl., Harv. Univ.*, 1974, **23**, 337–367.
2. Merlin, M. D., Archaeological evidence for the tradition of psychoactive plant use in the old world. *Econ. Bot.*, 2003, **57**, 295–323.
3. American Lime Technology, Hemp lime technology, 2008; www.americanlimetechnology.com
4. Abel, E. L., *Marijuana: The First Twelve Thousand Years*, Plenum Press, New York, 1980.
5. Fleming, M. P. and Clarke, R. C., Physical evidence for the antiquity of *Cannabis sativa* L. *J. Int. Hemp Assoc.*, 1998, **5**, 80–92.
6. Jiang, H. E. *et al.*, A new insight into *Cannabis sativa* (Cannabaceae) utilization from 2500-year-old Yanghai Tombs, Xinjiang, China. *J. Ethnopharmacol.*, 2006, **108**, 414–422.
7. Kajale, M. D., Archaeology and domestication of crops in the Indian subcontinent. *Diversity*, 1996, **12**(3), 23–34.
8. Bouquet, R. J., Cannabis. *Bull. Narc.*, 1950, **2**, 14–30.
9. Schultes, R. E., Random thoughts and queries on the botany of *Cannabis*. In *The Botany and Chemistry of Cannabis* (eds Joyce, C. R. B. and Curry, S. H.), J. and A. Churchill Publishers, London, 1970, pp. 11–38.
10. Dai, D. and Jan, M., Characteristic and performance of elementary hemp fiber. *Mater. Sci. Appl.*, 2010, **1**(6), 336–342.
11. Brett, C. and Waldron, K., *Physiology and Biochemistry of Plant Cell Walls*, Chapman and Hall, London, 1996, 2nd edn.
12. Sankari, H. S., Comparison of bast fibre yield and mechanical fibre properties of hemp (*Cannabis sativa* L.) cultivars. *Indu. Crops Prod.*, 2000, **11**, 73–84.

13. de Bruijn, P. B., Hemp concretes – mechanical properties using both shives and fibres. Licentiate thesis. Swedish University of Agricultural Sciences, Alnarp, 2008.
14. Awwad, E., Mabsout, M., Hamad, B. and Khatib, H., Preliminary studies on the use of natural fibers in sustainable concrete. *Leban. Sci. J.*, 2011, **12**(1), 109–117.
15. Awwad, E., Mabsout, M., Hamed, B., Farran, M. and Khatib, H., Studies on fiber-reinforced concrete using industrial hemp fibers. *Constr. Build. Mater.*, 2012, **35**, 710–777.
16. Sedlbauer, K. and Kunzel, H. M., Frost damage of masonry walls – a hygrothermal analysis by computer simulation. *J. Therm. Envelope Build. Sci.*, 2000, **23**, 227–281.
17. Pacheco-Torgal, F. and Jallali, S., Cementitious building materials reinforced with vegetal fibers, a review. *Constr. Build. Mater.*, 2011, **25**(2), 575–581.
18. Anderson, L. C., A study on systematic wood anatomy in *Cannabis*. *Bot. Mus. Leafl., Harv. Univ.*, 1974, **24**, 29–36.
19. Anderson, L. C., Leaf variation among *Cannabis* species from a controlled garden. *Bot. Mus. Leafl., Harv. Univ.*, 1980, **28**, 61–69.
20. Emboden, W. A., *Cannabis* – a polytypic genus. *Econ. Bot.*, 1974, **28**, 304–310.
21. Hillig, K. W., A chemotaxonomic analysis of terpenoid variation in *Cannabis*. *Biochem. Syst. Ecol.*, 2004, **32**, 875–891.
22. Hillig, K. W. and Mahlberg, P. G., A chemotaxonomic analysis of cannabinoid variation in Cannabis (Cannabaceae). *Am. J. Bot.*, 2004, **91**, 966–975.
23. Miller, N. G., The genera of Cannabaceae in the southeastern United States. *J. Arnold Arbor., Harv. Univ.*, 1970, **51**, 185–203.
24. Davidyan, G. G., Botanicheskaya kharakteristika konopli. *Tr. Prikl. Bot., Genet. Sel.*, 1972, **48**, 17–52.
25. Small, E. and Cronquist, A. A., Practical and natural taxonomy for *Cannabis*. *Taxon*, 1976, **25**, 405–435.
26. Klimko, M., Morphological variability of *Cannabis sativa* L. *Bull. Amis Sci. Lett. Poznan. Ser. D.*, 1980, **20**, 127–134.
27. Rhydwen, R., Wright, M., Miskin, N., Flower, A. and Butler, A., Dry-lining versus a hemp and lime insulating render for internal thermal renovation of a stone cottage in West Wales, including embodied energy assessment, interstitial wall monitoring, *in-situ* U-value and WUFI modelling. In Retrofit 2012 Academic Conference, Salford University, UK, 2012.
28. Morgan, J., Rhydwen, R. and Wijeyesekera, D. C., An investigation into structural capabilities and suitability for mainstream construction of hemp and clay lightweight blocks. In Advances in Computing and Technology: 7th Annual Conference, University of East London, 2012.
29. Sadler, C. H., Rhydwen, R. and Wijeyesekera, D. C., An investigation of the acute hygric properties of hemp and binder. In Advances in Computing and Technology: 7th Annual Conference, University of East London, 2012.
30. Singh, M. and Arbad, B. R., Characterization of traditional mud mortar of the decorated wall surfaces of Ellora Caves. *Constr. Build. Mater.*, 2014, **65**, 384–395.
31. Department of Agricultural Economics, Industrial hemp, Global operations, local implications, 1998; www.Uky.edu/classes/GEN/101/Hemp/HEMP 98.PDF

ACKNOWLEDGEMENTS. We thank Professor Cheng-Sen Li and Dr. Yi-Feng Yao, Beijing, China for their help; the Director General and Director (Science) Archaeological Survey of India for support; and the authorities of Dr Babasaheb Ambedkar Marathwada University, Aurangabad for providing laboratory facilities.

Received 9 April 2015; revised accepted 24 October 2015

doi: 10.18520/cs/v110/i5/884-891

Induction of sperm-head abnormality in Swiss albino mice *Mus musculus* by administration of fresh and processed betel nut extracts

Pritikana Saha* and Sekhares Bhattacharya

Department of Zoology, M.B.B. College, Agartala 799 004, India

Chewing betel nuts is a worldwide masticatory habit. However, these nuts have many harmful effects on human body. Sperm-head abnormality test is an easy and rapid *in vivo* technique to determine the ability of an agent to introduce abnormal change in the process of spermatogenesis. In the present study this test has been adopted to compare the dose-dependent toxic potentialities of fresh betel nuts of Tripura and processed nuts (tambul) of Assam. Both are capable of inducing significant dose-dependent toxic changes as well. Aqua leaching of small pieces of fresh betel nuts or tambul for 24 h only, makes them much less harmful in terms of this potentiality.

Keywords: Betel nut, *Mus musculus*, spermo-head abnormality, toxic potentiality.

PLANTS and various plant products are no doubt the main dietary ingredients of animals, including human being. However, in recent years, different reports are accumulating on the hazardous roles of different consumed plant products. One of these is the betel nut[1], which many people almost throughout the world consume as a masticatory habit[2]. Many oriental people use this nut[3,4] with or without betel leaves (leaves of *Piper betle*) along with other ingredients like slaked lime and tobacco[3,4]. The nuts are also used in different ways: in raw as well as processed form. In Tripura, it is generally used in raw form, while in Assam it is mainly processed and known as 'tambul'[5].

The main objective of the present study is to expand the existing pool of information regarding betel nut extract and to give a fresh insight into the toxic potential in terms of inducing significant and dose-dependent sperm-head abnormalities of two different types (unprocessed and processed) of betel nut collected from two different states of Northeast India.

In this study we adopt the assay of sperm-head abnormalities in mice. This *in vivo* technique assists in the rapid identification of the ability of an agent to cause an increase in the incidence of abnormality of the sperm-head in animals, regardless of the mechanism involved. When a compound induces a positive response in the sperm-head abnormality assay, it indicates that the compound may likely induce heritable genetic changes in

*For correspondence. (e-mail: pritikanasaha@rediffmail.com)