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ORIGINAL PAPER

Life after death: a physicochemical study of materials used by the ancient Maya in human bone ointments

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Abstract

Ancient Maya believed in life after death. They used to prepare dead bodies during burial ceremonies whose purpose was to celebrate the dead and to help them passing through the way from earthly life to the beyond one. Bodies preparation included coloured scented body ointment application, with a deep symbolic connotation and probably also a conservative purpose. The aim of this research was to characterize pigments and binders used by ancient Maya in the preparation of body ointments used to paint human bones. Emblematic painted bone samples from Xcambó (Maya archaeological site located in the municipality of Dzemul, in the Mexican state of Yucatán) were investigated through a non-destructive and micro-destructive analytical techniques. Results pointed out the presence of two mainly red pigments, i.e. red ochre and cinnabar, as already observed in other Maya painted bones. The new insight of the research is the identification of the organic compounds used as binding media in the ointments: a mixture of vegetable drying oil (probably Chia seed oil) mixed with an aromatic compound (bitumen). This knowledge, together with that obtained in the last decades, is important to reconstruct the cultural habitat and social customs of this pre-Hispanic civilization and transfer them to today's context.

Keywords Maya culture · Xcambó · Painted human bones · Ritual · SEM–EDX · GC–MS · Py-GC–MS

Highlights

- New insights into the Maya body ointment preparation in burial ceremonies.
- The binders used to paint human skeletons in Maya burial ceremonies were identified by direct analysis on real human bone samples.
- Thanks to UV-VIS and EDX (elemental microanalysis) investigations we identified red pigments as red earth (or ochre) and cinnabar.
- Results obtained on organic fractions suggest the presence of a vegetable drying oil mixed with a bituminous material.

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Introduction

Among the ancient Maya, it was common practice to cover the bodies of the dead with red paint. There are two distinct ways to apply the red paint. On some occasions, this paint was laid on top of the cloth covering the deceased bodies, as in the case of the Calakmul royals (Rigon et al. 2020). In other cases, however, no cloth bandages were used, and instead the bodies were wrapped in layers of pigment(s) and resin(s) that overlapped each other. Much is known about the use of the colour red in funerary contexts, not only among the Maya. In fact, the use of red pigment as a part of the mortuary treatment of the body was not exclusive to the ancient Mayans. This practice was widespread throughout the ancient world (Sommer 2008; Batta et al. 2012; Cervini-Silva et al. 2012; Hovers et al. 2003; Quintana Owen et al. 2014; Wreschner Ernest et al. 1980; Vázquez de Ágredos-Pascual 2018a). This association was already known to Paleolithic hunter-gatherers and to their sedentary Neolithic successors (Attard Montalto 2010).

Other cultures from antiquity had similar practices in the shrouding and embalming (Vázquez de Ágredos-Pascual

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2018a). In the Andean region, for example, many tombs and burials that contain bodies covered in cinnabar (mercury sulphide, HgS), hematite (iron oxide, Fe_2O_3) and red ochre were discovered. These pigments were used on their own and/or mixed. Mummies from the Chinchorro culture (7000–1.500 BC) also attest this practice among the populations that lived in the Atacama Desert (Northern Chile) and reaffirm the ancestral belief associated to this practice: the existence of life beyond physical death (Arriaza Bernardo 1995; Dillehay 1995).

This kind of practice, in which colour is linked to funeral rituals, was also common in other ancient non-American cultures. In this sense, in the Chinese Hunan province, a mummy was found that was completely covered by cinnabar, including the hair (Wreschner Ernest et al. 1980). In accordance with ancient Chinese religion, medicine and philosophy, the use of mercury sulphide establishes a link between cinnabar and alchemy, conducive to the rebirth that must occur after physical death (Attard Montalto 2010). Another example is evidenced by the discovery of minium (red lead) pigment found on mummies from Roman Egypt (Vázquez de Ágredos-Pascual 2018a). All of these examples make it clear that this funerary practice was not circumscribed exclusively to Mesoamerica.

Shrouding a body in mercury, arsenic and lead-based pigments (or alternatively in iron pigments and red colorants of intense hue) responds to the principle of homeopathic magic, insofar as these colours imitate and reproduce the materiality of sacred blood. In this way, through matter and materiality, and following the logic that "same begets same", shrouds become metaphors for warm-blooded, life-sustaining "placentas" which revitalize the dead body that they envelop (Vázquez de Ágredos Pascual et al. 2000).

Recent research in Maya human bones from Calakmul has shown that these coloured shrouds also incorporated excipients, generally of vegetal origin, such as animal fat and the natural resin from *Hevea brasiliensis* that presumably had been used to spread more easily the coloured ointments (Rigon et al. 2020).

These substances were of great interest in the beliefs and worldview of this pre-Hispanic culture.

Likewise, these mixtures of colour and aromas have also been identified in the funerary beds where the Mayan royalty rested. In this way, and at least in the case of kings and queens, it seems that the usual practice was that the bodies of these high dignitaries rested or were enveloped by colours and fragrances that would favour their rebirth.

This funerary custom of the ancient Maya was registered at least from the Late Pre-Classic (300 AC–300 DC), as shown Burial 1 of Structure 7A at Tak'alik Ab'aj, Guatemala (ca 100 AD), where the red pigment that covered the funerary bed of the ruler was hematite mixed with a type of acacia gum, known in Guatemala as "cacho de toro" or *ixcanal* (*Acacia farnesiana (L.) Willd*) (Vázquez de Ágredos Pascual et al. 2000). From a later period, the Early Classic (300–600 AD), we have red pigment mixed with aromatic excipient in the grave goods of the Tomb 19 at Río Azul, Guatemala. In this case, a mixture of cinnabar and hematite was fixed on an excipient base that the organic analysis identified as animal fat (Rigon et al. 2020; Vázquez de Ágredos Pascual et al. 2018b). Finally, the best examples identified of red pigments mixed with aromatic excipients come from the archaeological sites of Calakmul and Palenque. In the Burial III-9 of Calakmul, placed at Lundell Palace, was discovered the body of an Early Classic king of 35–45-year-old who suffered from arthritis and whose cranium had been elongated and inclined artificially during his first infancy (Tiesler et al. 2001). His corpse was shrouded in an intricately woven blanket under which five ceramic plates were set (Pincemin 1994; Tiesler et al. 2013; Pereira and Michelet 2004) and covered with a fragrant unguent composed of hematite, cinnabar and pine aromatic excipient, in accordance with the Py-GC/MS results (Vázquez de Ágredos Pascual et al. 2018b). The cinnabar-hematite mixture that covered the body of the Red Queen of Palenque, belonging to the Lady *Tzak Bu Ahaw*, wife of the king *K´inich Janaab´Pakal,* offers us the last example. Her body was found in Temple XIII-sub, adjacent to *Janaab' Pakal's* own monumental mortuary mausoleum beneath the Temple of Inscriptions, and completely embalmed in what until now had been identified simply as red pigment. However, recent archaeometric data inform us that this pigment was mixed with the sacred copal resin (Vázquez De Ágredos et al. 2015).

The presence of these aromatic excipients in the funeral shrouds of Mayan royalty contributed to the rebirth of these dignitaries, thus reinforcing the function of the red pigment. It should be remembered that the ancient Mayans considered resins, gums and other organic substances that exist inside plants, trees and flowers as the vital liquid equivalent to the human or animal blood. They called this vital liquid *itz*, and this substance was considered the very matter of creation, fertility and life. In fact, Mayan art shows how the gods were born wrapped in the scent of flowers, especially lilies. In this way, and in the same way that the deities needed to imbue themselves with sacred fragrance to be born, also the royalty is wrapped with sacred fragrant substances for their rebirth in the afterlife. This means that these shrouds of colour and perfume were conceived as the "placenta" from which birth or rebirth would be possible, hence their sacred and symbolic meaning.

Research aim

The study of the coloured samples from Xcambó was performed by analysing ten samples of painted bones using complementary analytical techniques (optical microscopy,

SEM–EDX, UV–VIS spectroscopy, GC–MS, Py-GC–MS) with the aim of gaining more insights into the materials employed by ancient Mayans in the preparation of human bone ointments and, thus, into their funeral customs. This knowledge, moreover, allows us to better understand some of the funeral traditions of the Mayans of today, as evidenced by the use of certain fragrances and incenses in cemeteries and funeral ceremonies that are still performed in homes.

A further aim was to compare the information obtained in the current paper results with a previous study done by the same research group on samples of similar nature from Calakmul archaeological Maya site (Rigon et al. 2020). Analogous results have been observed in the samples of Xcambó regarding the nature of the pigments employed, but significant differences concerning the organic fraction have been highlighted.

Materials and methods

Bone fragments

Ten bone fragments with well-preserved painted areas coming from Maya archaeological site of Xcambó (Fig. 1) were studied.

The complete list is reported in Table 1, with all the excavation and labelling details.

Figure 2 illustrates some of the analysed bone fragments. The painted areas have similar brown reddish colour shades. Just in a few cases, the samples showed a thin transparent film on the surface, probably due to a superficial organic layer.

The first step was the collection of pigment dust by scraping the pigmented surface of the bones.

Fig. 1 Map of the Maya archaeological site of Xcambó (Mexico)

Table 1 Description of the samples considered in the study

Sample	Description	Burial of origin	Structure	Chronology	Colour
X1	Long bone fragment	6	21-A	Late Classic (around 600–850 AD)	Red brownish shades
X2	Skull fragment	39	Courtyard-IX	Late Classic	Red brownish shades
X3-1	Long bone fragment	241	Courtyard-XIII	Late Classic	Red brownish shades
X3-2	Long bone fragment	241	Courtyard-XIII	Late Classic	Red brownish shades
X4	Rib fragment	80	NE-7A	Late Classic	Red brownish shades
X5	Scapula fragment	21	NE-3	Late Classic	Red brownish shades
X6	Long bone fragment	8	NE-7	Late Classic	Red brownish shades
X7	Distal radius fragment	76	NE-7A	Late Classic	Red brownish shades
X8	Rib fragment	214-A	29	Late Classic	Red brownish shades
X9	Fragment of left femur	23	2	Late Classic	Red brownish shades

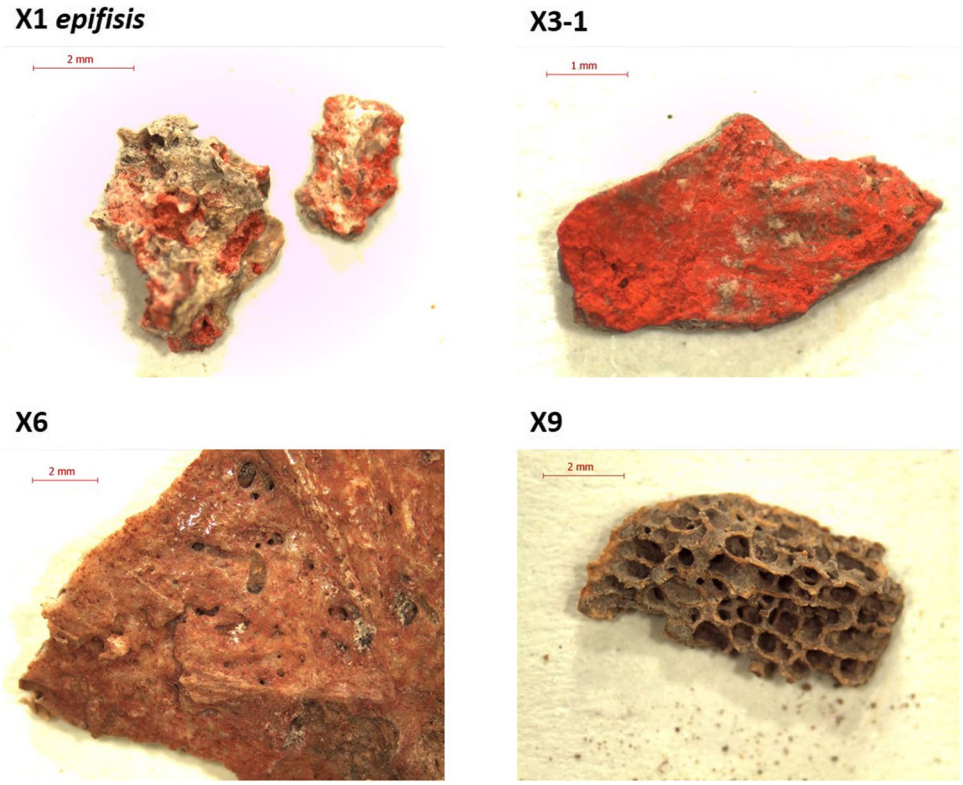


Fig. 2 Picture of samples X1 epifisis, X3-1, X6 and X9: fragments of human bones with well-preserved coloured areas. The sample X3-1 was the only one entirely covered with a homogeneous layer of pigment

Methods

An integrated multi-analytical approach was adopted to study the morphology and the composition of the red colouring materials and ointments use to paint the analysed bone fragments. The techniques and the experimental conditions used are reported in the following paragraphs.

Light optical microscopy

Preliminary observation of the painted bone fragments has been performed by a stereo-microscope Leica EZ4HD (zoom 4,4:1) with HD camera to investigate superficial morphology and macroscopic characteristics.

UV–VIS spectroscopy

UV–VIS diffuse reflectance analysis was performed by Cary 60Fiber Optic UV–VIS spectrophotometer fitted with the remote fibre optic diffuse reflectance accessory (Harrick Scientific Products). A 200 up to 1000 nm spectra collecting range was selected, and CaryWinUV Agilent Technologies software was used for data processing.

Spectra from bone surface were collected as well in order to "isolate" the reflectance information coming from matrix bone. Literature data and some standard Kremer pigments spectra—such as red ochre (arch. ref. 11,585), brown earth (arch. ref. 11,620), cinnabar (arch. ref. 10,625) and jarosite (arch. ref. 11,520)—were used for comparison to identify the unknown red brownish substances.

FTIR-ATR spectroscopy

In order to collect information about sample composition, Fourier transform infrared-attenuated total reflectance (FTIR-ATR) was performed on the samples by an Agilent Cary 630 FTIR spectrophotometer coupled with a diamond ATR. Data acquisition was performed in the 4000–400 cm^{-1} range with a 4 cm^{-1} resolution. The identification of materials present in the bone matrix and in the red-painted layers was performed by comparison between literature data, reference compounds, spectra collected and database spectra.

Scanning electron microscopy (SEM)-energy dispersive X-ray (EDX) analysis

Both regions of the samples, with preserved pigment and without pigment, were observed and analysed using Hitachi S4800 scanning electron microscopy (SEM) coupled to an energy dispersive X-ray (EDX) probe. Elemental microanalysis information collected by EDX gave elemental information to identify the nature of pigments.

Due to their nature, i.e. not conductive materials, all the samples required a metallization process with Au–Pd to avoid conduction problem, and they were analysed using

low voltage (10 kV e 20 kV), selecting specific power for each measurement according to sample characteristics.

Gas chromatography-mass spectrometry (GC–MS) and pyrolysis gas chromatography-mass spectrometry (Py-GC/MS)

The nature of organic binders was investigated with a gas chromatograph Agilent Technologies 6890 N (Network GC system) coupled to a mass spectrometry Agilent 5973 Network Mass Selective Detector. GC separation was achieved in a 5% phenyl methylpolysiloxane DD-5MS capillary column (30 m × 0.25 mm). The organic fraction was extracted with a step-by-step procedure including four different solvents: cyclohexane, methanol, acetone and chloroform.

In order to identify oil, waxes and fat samples were derivatized using m-trifluoromethylphenyl trimethylammonium hydroxide, 5% in methanol, referring to a method already successfully tested on vegetable and drying oils, natural resins, waxes and balsams (Rigon et al. 2020; Izzo et al. 2013; Izzo et al. 2014; Fuster Lopez et al. 2016; Izzo et al. 2017; Caravà et al. 2020; Lodi et al. 2020).

The derivatized extracts were analysed with the following conditions. The initial temperature was 100 °C, and the oven temperature was programmed with a gradient of 10 °C/min up to 320 °C. Samples were injected (1µL) in split mode, and the injector temperature was set to 290 °C. The interface temperature of GC–MS was set at 250 °C. A continuous flow rate of 1.2 mL/min of helium was used as carrier gas. Ions were generated by electron ionization (70 eV), and the mass spectrometer was set to 50–650 m/z in full scan mode at a speed of 7 scans per second.

Selected samples were also investigated with thermally assisted hydrolysis and methylation Py-GCMS (THM-Py-GC–MS) (Van Keulen and Schilling 2019). The apparatus consists in a Frontier Lab 3030D pyrolyser was in combination with a Thermo Scientific Trace 1310 gas chromatograph and a Thermo Scientific ISQ mass spectrometer. The pyrolysis unit is directly linked to a Supelco SLB5 ms column (with a length of 20 m, an internal diameter of 0.18 mm and a film thickness of 0.18 µm) by a split connector. Helium with a programmed flow (0.5 to 1.2 ml/min) is used as carrier gas in combination with a temperature program was set from 35 °C (held 1 min) – 60 °C/min –110 °C – 14 °C/min – 240 °C – 5 °C/min – 315 °C (held 2 min). The column is directly coupled to the ion source of the mass spectrometer. The temperature of the interface was 270 °C, and the ion source temperature was 240 °C. Mass spectra were recorded in the range 29–600 m/z in full scan mode at a speed of 7 scans per second.

For collecting and processing mass spectral data, Xcalibur 2.1 software was used.

Interpretation of the GCMS and THM-Py-GC–MS results was made using the ESCAPE system, an expert system for characterizing Py-GCMS data using AMDIS & Excel (Van Keulen and Schilling 2019).

Results and discussion

The results collected by means of the multi-analytical approach are fully summarized in Table 2.

Not in all cases the employ of all analytical techniques was possible, mainly due to the morphology and size of samples. The following discussion based on the results obtained is focused on the most meaningful samples.

Macro- and micro-morphology of painted bones

The optical microscope images of the studied bone fragments and the description of their main features are reported in Table 3.

All the samples are characterized by red brownish shades of preserved coloured areas and just in X3-1 sample the coloured area (bright red) covers the whole surface. X2, X3-2, X5 and X7 show similar superficial features characterized by regions with not homogeneous preserved red brownish colour. The bone fragment X8 shows a black speckle pigmentation.

The sample X9 is the only one characterized by a particular and very fragile consistency. It presents a brownish painted area on the surface.

Most samples observed through scanning electron microscopy (SEM) are characterized by small rounded-like particles. They form a dense layer that give a regular aspect to the surface. The sample X1 was the only one that showed a particular and unique surface consisting of lamellar-shape structures. With higher magnification (Fig. 3) we could observe that these irregular-shape structures were perfectly interpenetrate one another to form a unique and compact layer. However, the origin of this peculiar structure needs further research.

The morphology of sample X9 was completely different from the other samples, since it was characterized by a "spongy" aspect. At higher magnification (Fig. 4), it was possible to disclose the typical structure of spongy tissue of bones, as also described in literature (Bosch et al. 2011; Fernández-Jalvo and Marín Monfort 2008; Papageorgopoulou et al. 2009).

The composition of red-painted areas

The red-painted areas were investigated by elemental, spectroscopic and gas chromatographic-mass spectrometric analysis.

Table 2 Summary of the results collected by multi-analytical approach

Sample name	SEM with secondary electron detector	EDX°	UV–VIS spectroscopy	FT-IR	GC–MS °° (Py-GC–MS)	Considerations
X1	Particular surface of lamellar-shape structure forming an single compact layer	Ca (***), P (*), C (*), O (**), Fe (tr), Hg (**), S (**), Al (*), Si (*), Mg (*)	Cinnabar	1789/712 cm^{-1} $CaCO_3$ 1394 cm^{-1} $\upsilon_3CO_3^{2-}$ 872 cm^{-1} $\upsilon_2CO_3^{2-}$ 1025 cm^{-1} (?) 1025 cm^{-1} ($\delta_3PO_4^{3-}$) 600/566 cm^{-1} ($\upsilon_4PO_4^{3-}$)	Monosaturated fatty (myristic, palmitic and stearic) acids Difatty (suberic and azelaic) acids P/S = 2.1 Phthalates	The painted fragment is likely a mixture of a vegetable drying oil (Chia seed oil?) and cinnabar (red pigment) Phthalates: products of oxidation of terpenic fraction or contamination?
X2	NP	NP	Red earth pigment (?)—interference from bone matrix	1439/1409 cm^{-1} $\upsilon_3CO_3^{2-}$ 868 cm^{-1} $\upsilon_2CO_3^{2-}$ 1018 cm^{-1} ($\delta_3PO_4^{3-}$) 958 cm^{-1} ($\delta_1PO_4^{3-}$) 600/559 cm^{-1} ($\upsilon_4PO_4^{3-}$)	Monosaturated fatty (myristic, palmitic and stearic) acids Difatty (suberic and azelaic) acids Aromatic compound (naphthalene and benzene) and *n*-alkanes ascribed to bitumen	The red colouration partially matches with red earth pigment but there is not enough data to support the hypothesis A mixture of a vegetable drying oil and bituminous material used as binder can be assumed
X3-1	Compact surface characterized by single rounded shape units that interpenetrate one another	Ca (***), P (**), O (**), C (*), Hg (***), S (***), Si (**), Al (*), Mg (*), Na (tr)	Cinnabar	1789/712 cm^{-1} $CaCO_3$ 1390 cm^{-1} $\upsilon_3CO_3^{2-}$ 872 cm^{-1} $\upsilon_2CO_3^{2-}$ 1021 cm^{-1} ($\delta_3PO_4^{3-}$)	NP	The data suggest the presence of cinnabar as the red pigment
X3-2	Irregular surface probably due to the presence of the pigment	Ca (***), P (**), C (*), O (**), Fe (**), Si (**), Al (*), Mg (*), Na (tr)	Red earth type pigment matching	1629 cm^{-1} (weak) amide I 1454/1405 cm^{-1} $\upsilon_3CO_3^{2-}$ 868 cm^{-1} $\upsilon_2CO_3^{2-}$	Monosaturated fatty (myristic, palmitic and stearic) acids P/S < 1 Phthalates	A red earth or ochre type pigment was likely employed. An animal fat used as binder can be assumed
X4	NP	NP	Red earth type pigment matching	2916 cm^{-1} stretching CH_2 CH_3 group 1443/1405 cm^{-1} $\upsilon_3CO_3^{2-}$ 868 cm^{-1} $\upsilon_2CO_3^{2-}$ 1018 cm^{-1} ($\delta_3PO_4^{3-}$) 958 cm^{-1} ($\delta_1PO_4^{3-}$) 600/539 cm^{-1} ($\upsilon_4PO_4^{3-}$)	Monosaturated fatty (myristic, palmitic and stearic) acids Difatty (suberic and azelaic) acids P/S = 2.2 Phthalates	The data suggest the presence of red earth pigment mixed with a vegetable drying oil (Chia seed oil?) used as binder Phthalates: products of oxidation of terpenic fraction or contamination?
X5	NP	NP	Red earth type pigment matching	1633 cm^{-1} amide I 1398 cm^{-1} $\upsilon_3CO_3^{2-}$ 872 cm^{-1} $\upsilon_2CO_3^{2-}$ 1003 cm^{-1} ($\delta_3PO_4^{3-}$) 708 (weak) cm^{-1} $CaCO_3$ (?) 596/555 cm^{-1} ($\upsilon_4PO_4^{3-}$)	NP	The data suggest the presence of red earth pigment

Table 2 (continued)

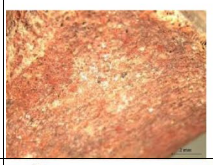
Sample name	SEM with secondary electron detector	EDX°	UV–VIS spectroscopy	FT-IR	GC–MS °° (Py-GC–MS)	Considerations
X6	NP	NP	Red earth pigment type (?)—interference from bone matrix	2919 cm^{-1} stretching CH_2 CH_3 group 1637 cm^{-1} amide I 1443/1409/872 cm^{-1} $_{\upsilon}CO_3$ 1014 cm^{-1} ($\delta_1 PO_4^{3-}$) 581/563 cm^{-1} ($\upsilon_4 PO_4^{3-}$)	Monosaturated fatty (myristic, palmitic and stearic) acids Difatty (suberic and azelaic) acids P/S = 2.5 Phthalates	The painted fragment likely contains red earth pigment mixed with a drying oil (Chia seed oil?) Phthalates: products of oxidation of terpenic fraction or contamination?
X7	NP	NP	Red earth pigment type (?)—interference from bone matrix	1625 cm^{-1} amide I 1405 cm^{-1} $_{\upsilon 3}CO_3^{2-}$ 872 cm^{-1} $_{\upsilon 2}CO_3^{2-}$ 1014 cm^{-1} ($\delta_1 PO_4^{3-}$) 708 cm^{-1} $CaCO_3$ (?) 600/559 cm^{-1} ($\upsilon_4 PO_4^{3-}$)	Monosaturated fatty (myristic, palmitic and stearic) acids P/S < 1 Phthalates	The data suggest the presence of red earth pigment mixed in a lipidic binder (animal fat?) Phthalates: products of oxidation of terpenic fraction or contamination?
X8	Compact surface made by rounded shape structure that interpenetrate one another to form an unique layer	Ca (***), P (***), C (*), O (**), Fe (*), Si (*), Al (*), Mg (*), Na (tr)	Reflectance spectra could match with red earth pigment	1402 cm^{-1} $_{\upsilon 3}CO_3^{2-}$ 868 cm^{-1} $_{\upsilon 2}CO_3^{2-}$ 1014 cm^{-1} ($\delta_1 PO_4^{3-}$) 600/559 cm^{-1} ($\upsilon_4 PO_4^{3-}$)	NP	The results suggest the presence of red earth or ochre pigment
X9	Smooth surface, (feather-like surface). It has been observed rounded shape structures	Ca (***), P (***), C (*), O (**), Fe (*), Si (**), Al (*), Mg (**), Na (tr), Cl (tr)	Reflectance spectra could match with red earth pigment	3688/3613 cm^{-1} OH/NH group 1625 cm^{-1} amide I 1409 cm^{-1} $_{\upsilon 3}CO_3^{2-}$ 872 c $_{\upsilon 2}CO_3^{2-}$ 1018 cm^{-1} ($\delta_1 PO_4^{3-}$) 906 cm^{-1} $\delta_1 PO_4^{3}$ (?) 581/559 cm^{-1} ($\upsilon_4 PO_4^{3-}$)	NP	The results suggest the presence of red earth pigment

°For EDX results: (***) very abundant; (**) abundant; (*) present; (tr) traces

°°Fatty acids as their corresponding methyl esters

NP not performed

Table 3 Features of all the samples from Xcambó archaeological site

Sample name	Picture	Description (by means of Optical Microscopy)
X1		Fragment of long bone (3mm). Quite homogeneous red pigmented layer.
X2		Fragment of skull (4mm) + smaller fragments. Surface covered with red-brown colour.
X3-1		Little fragment of long bone. Sample is entirely covered with a homogeneous red pigmented layer.
X3-2		Fragment of long bone (~7mm). Red-brownish colour on the surface.
X4		Fragment of rib (6mm) + one little fragment. Red-brownish colour on the surface with little dots of black colour in the backside
X5		Fragment of scapula (~1cm). Red-brownish colour on the surface.
X6		Fragment of long bone (6mm). Red layer on the surface.
X7		Fragment of distal radius (6mm) + smaller fragments. Samples are covered with little dots of red-brownish and black colour.
X8		Fragments of rib (5/6 mm). Samples are non-homogeneous covered with red-brownish colour.
X9		4 small fragments of left femur (the biggest one 3mm). "Spongy-like" aspect typical of bones tissue. Surfaces of the samples are covered with brownish colour.

The elemental composition of red pigments was performed by an energy dispersive X-ray probe (EDX) coupled with SEM. The obtained data are coherent with previous research outputs from the study of red-painted human bones, that is to say the use of red earth and/or ochre and cinnabar.

Figure 5 illustrates the EDX results of the samples X3-2 and X9, where the simultaneous presence of iron

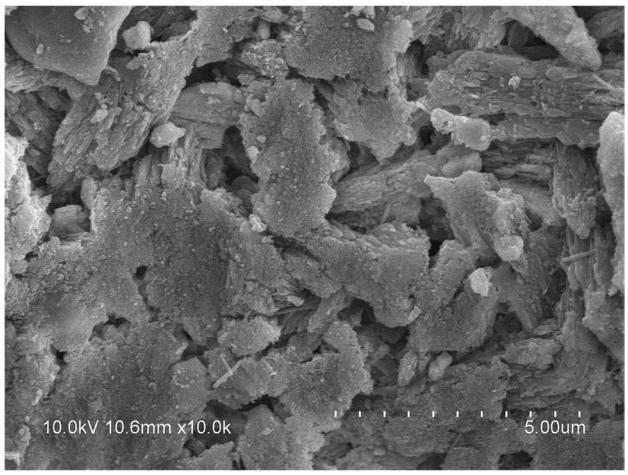


Fig. 3 SEM secondary electron image of sample X1 (10.0 kV 10000X). Surface is characterized by a compact layer consisting of lamellar-shape structures interpenetrate one another

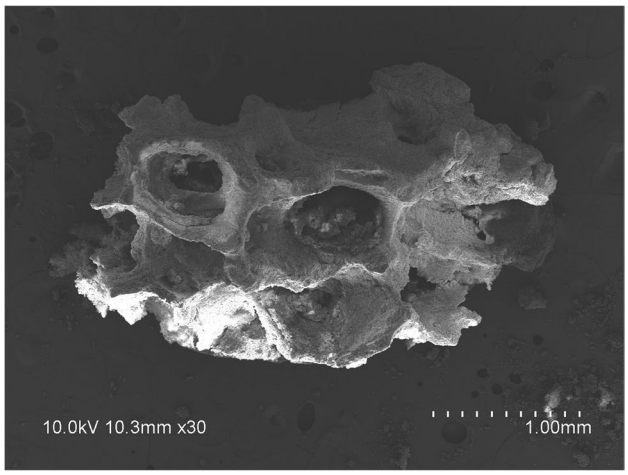


Fig. 4 SEM secondary electron image of sample X9 (10 kV 30X). Typical spongy aspect of bone tissue

(Fe) and oxygen (O) suggest the use of red earth type pigment (Fe_xO_x). The majority of the analysed samples were painted using iron-based red pigments.

Differently, elemental data registered on the samples X1 and X3-1 showed, among other elements, mercury (Hg) and sulphur (S), as depicted in Fig. 6. In the case of sample X1, a mixture of cinnabar (HgS) and iron-based red pigments was identified.

Furthermore, aluminium (Al) and silica (Si) were detected in almost all the samples, with a high amount in pigments classified as red earth ones, since Al and Si-containing compounds are part of the chemical composition itself, which suggests that probably pigments are more realistically ochre, i.e. phyllosilicates minerals, to be better defined by FT-IR analysis.

Similar and complementary results were obtained by UV–VIS spectroscopy. Although spectra acquisition was difficult due to the irregular and tridimensional shape of the samples, the small pigmented areas and the interference from the bone matrix, the results collected corroborate what was observed by SEM–EDX: two kinds of red pigments were identified, namely red earth/ochre and cinnabar.

Examples of both types are shown in Fig. 7. Samples X3-2, X5, X8 and X9 showed a typical spectrum of a red earth pigment; the sample X3-1 corresponds to a cinnabar instead (Picollo et al. 2018). As also found by EDX analysis, the sample X1 could be a mixture of both pigments.

These results, as also reported in the Introduction, are consistent with specific literature, which states that red colour types available to Maya were various, such as hematite (Fe_xO_x), ferruginous clays/ochre ($Al_2O_3SiO_2 \bullet {}_xH_2O$) and cinnabar (HgS), being the latter the rarest and most precious pigment because of its limited supply (Batta et al. 2012; Doménech-Carbó et al. (2012); Vázquez De Ágredos Pascual et al. 2016b; Ávila et al. 2014).

Most of the FTIR-ATR spectra showed the typical characteristic absorptions due to the bone matrix: hydroxyapatite ($Ca_{10}(PO4)_6(OH)_2$) and calcium carbonate($CaCO_3$) were identified, being the latter the most prevalent. Its occurrence can be explained as a residue on the surface pores and/or in the micro-cracks of the bone matrix but could be also due to ionic substitution phenomena (absorption or substitution via ionic-exchange) in the hydroxyapatite mineral ($Ca_5(PO_4)_3OH$). As reported in Lachowicz et al. (2017); Rajendran 2011; Lee-Thorp August 2002), diagenesis natural phenomena lead to the replacement of the ionic groups OH^- and $-PO_4^{3-}$ by another anionic compound, such as $-CO_3^{2-}$. This type of carbonate compound has a different chemical nature compared to carbonate compound deriving from deposit phenomena, so it can be distinguished by the presence of a specific peak at about 713 cm^{-1} in the FTIR spectra results (Lachowicz et al. 2017).

Figure 8 shows the FTIR-ATR spectra of X1 epifisis, X1 diafasis, X3-1 and X4 samples. They are all characterized by a the typical vibration at around 1390 cm^{-1} and 870 cm^{-1} due to the stretching of $-CO_3^{2-}$ ($_{\upsilon 2}CO_3$). Except for the sample X4, in all the IR spectra, we can observe the characteristic peak at 715 cm^{-1} of carbonate compound that is probably absorbed in the hydroxyapatite matrix as discussed above. The peaks at a ~ 1020 cm^{-1} (stretching (PO_4^{3-})), 600 cm^{-1} and 560 cm^{-1} (bending (PO_4^{3-})) are typical of phosphate-based compounds.

As regards the organic fraction, FT-IR results did not allow to identify any organic compounds. Except for the weak peaks at ~ 2919 cm^{-1} and 2849 cm^{-1} in IR spectra of the samples X6 and X4 (Fig. 9), no typical absorptions

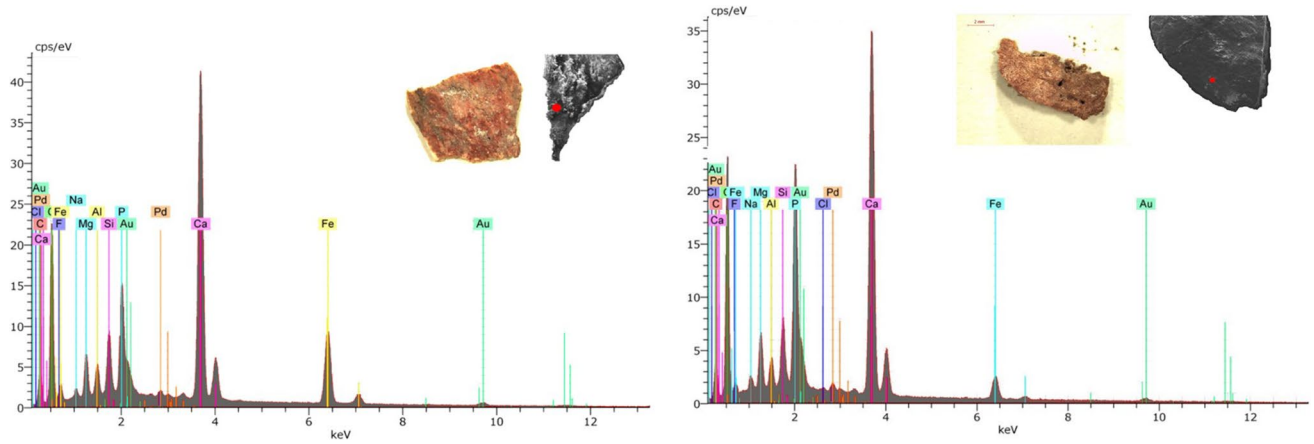


Fig. 5 EDX spectra of sample X3-2 on the left and X9 on the right: in both mainly Fe and O have been detected. It suggests the presence of red earth (or ochre) type pigment. Si and Al can be ascribed to the chemical composition of the pigment that more likely it is ochre-type one

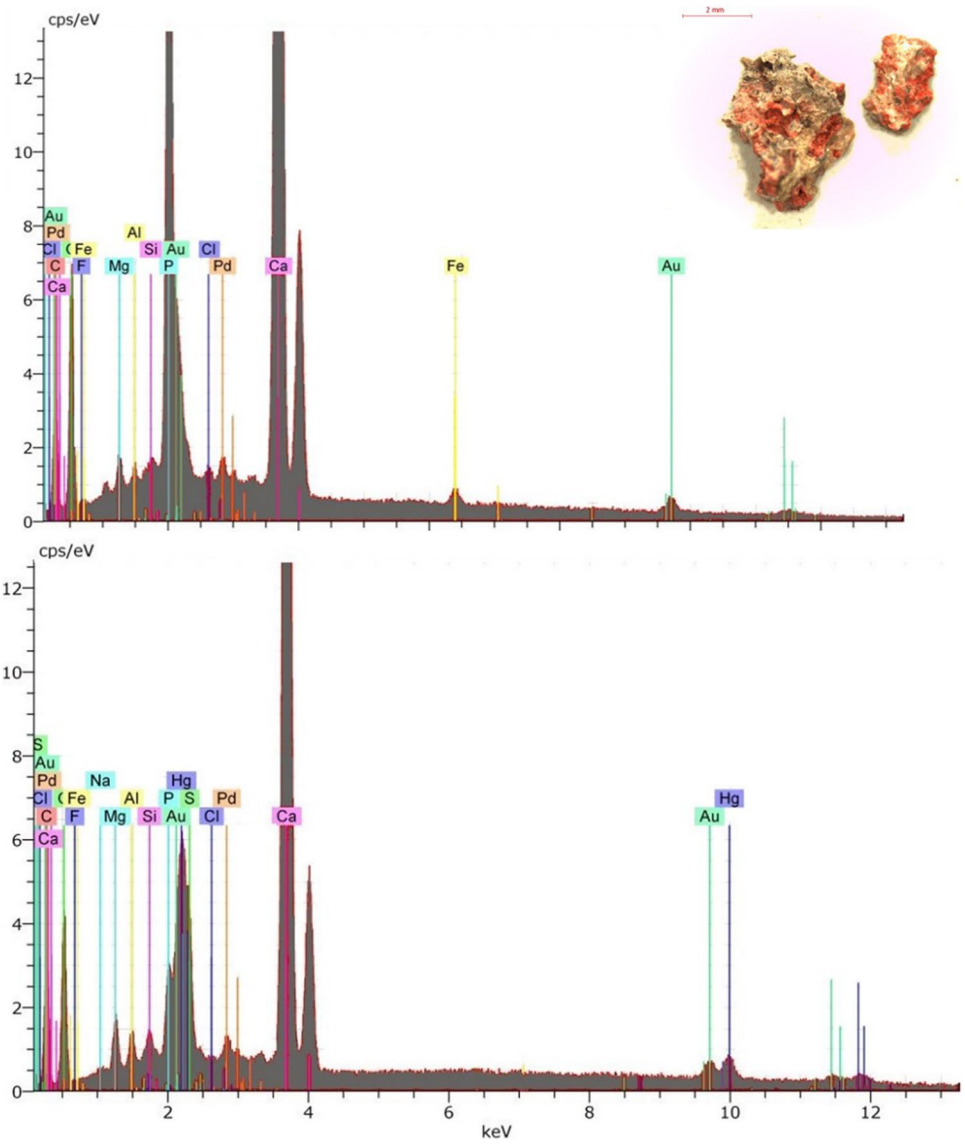


Fig. 6 EDX spectra of sample X1 epifisis: data show the simultaneously presence of oxygen, iron, mercury and sulphur. It could be explained considering a mixture of both pigments: red earth and cinnabar

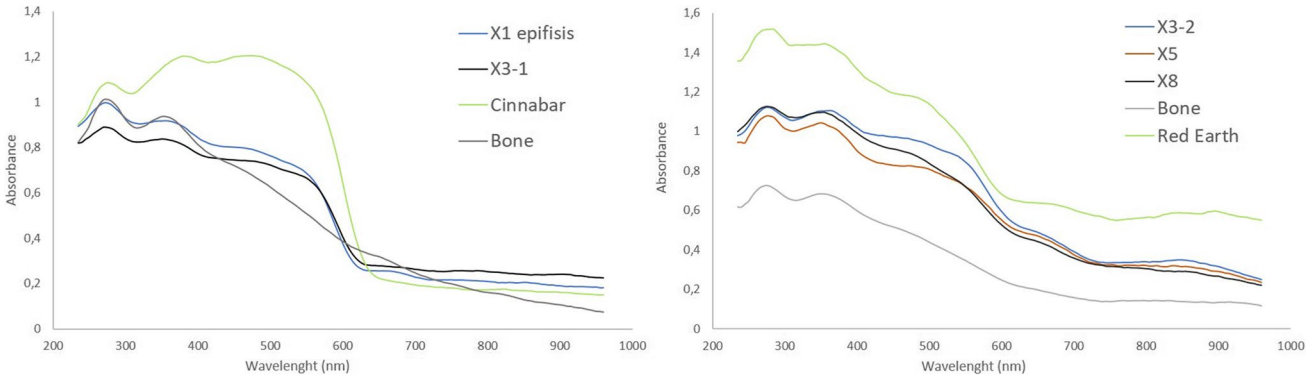


Fig. 7 Reflectance UV–VIS spectra of cinnabar and red earth-kind samples

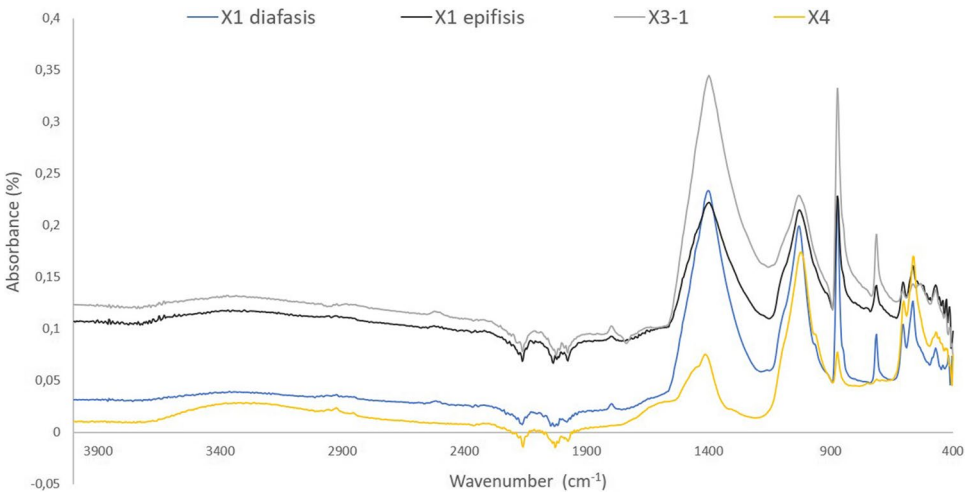


Fig. 8 FTIR-ATR spectra of samples X1 epifisis, X1 diafasis, X3-1 and X4. The typical peaks of carbonatic compound at 1394 cm^{-1} due to of the stretching of the $-CO_3^{2-}$ group and its vibration at ~ 872 cm^{-1} are visible

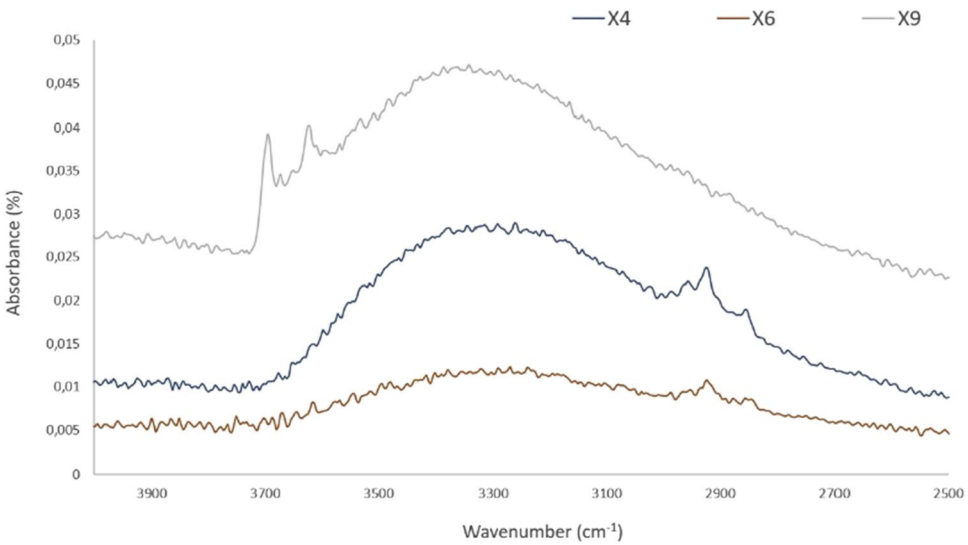


Fig. 9 FTIR-ATR spectra of samples X4, X6 and X9. In samples X4 and X6, a weak peak at ~ 2919 cm^{-1} of an organic fraction is observed. The X9 sample spectra show two peaks at 3688 and 3616 cm^{-1} typical of OH group

related to organic materials were detected, probably because their concentration was under the detection limit of this spectroscopic technique (ca 5%) and/or because of degradation processes during ageing in a burial context.

Only in the spectrum of the sample X9 we observed some peaks at 3688 and 3616 cm^{-1} due to the absorption of OH group. Hydroxyl group can be related to the presence of water (Bartošova et al. 2015) combined with the N–H stretching (amide A) (Sivakumar et al. 2014; Gu et al. 2013) and to the stretch vibration of hydroxyapatite although the presence of OH group in the bone material has been debated as Chunju Gu et al. (Gu et al. 2013) reported. Table 4 summarizes all the FTIR peak assignments observed in the samples.

To elucidate the presence of organic compound used as binding media and/or ointments in red paints, GC–MS and py-GC–MS analysis were performed.

It has to be said that the nature of the samples itself (burial conditions) compromised the GC–MS analysis performance, in particular considering their alteration due to natural processes of deterioration. In fact, during burial, bacterial action alters the condition of the original distribution of organic material (Dudd et al. 1998), making the scientific investigation process more complex and difficult. Furthermore, in many cases, the organic compounds identified fell below the limits of detection, although the GC–MS technique has been a very useful tool for the identification of the organic compound in low concentration—even in archaeological samples (Lucero Gómez et al. 2014) as it

Table 4 FTIR band observed in the spectra of the samples with peak assignment

X1	X2	X3-1	X3-2	X4	X5	X6	X7	X8	X9	Assignment bond	Source
					1633				3688 3616	Stretching NH-OH	Water or hydroxyapatite or protein
				2916		2919				Stretching CH_2-CH_3 group	Organic compound(s)
1789		1789								Stretching C = O	Carbonate or organic material
			1629			1637	1625		1625	Amide I	Collagen
	1439		1454	1443		1443				$CaCO_3$	Carbonate
1394	1409	1390	1405	1405	1398	1409	1405	1402	1409	$\upsilon_3CO_3^{2-}$	Carbonate
1025	1018	1021		1018	1003	1014	1014	1014	1018	$\delta\ {}_3PO_4^{3-}$	Phosphate
	958			958					906	$\delta\ {}_1PO_4^{3}$	Phosphate
872	868	872	868	868		872	872	868	872	$\upsilon_2CO_3^{2-}$	Carbonate
712		712			708 (?)		708 (?)			$CaCO_3$	Carbonate
600	600			600	596	581	600	600	581	$\upsilon_4PO_4^{3-}$	Phosphate
566	559			539	555	563	559	559	559	$\upsilon_4PO_4^{3-}$	Phosphate
										$\upsilon_4PO_4^{3-}$	Phosphate

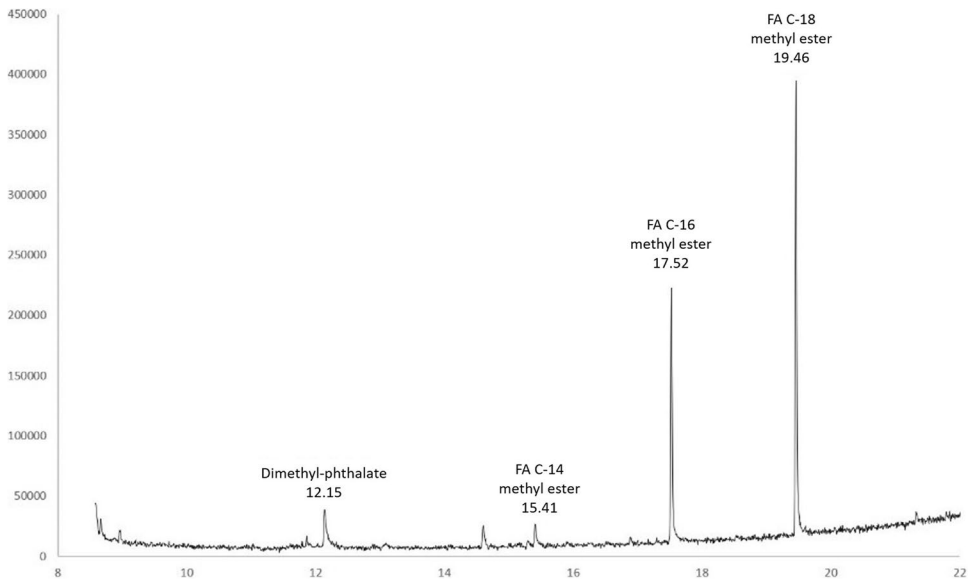


Fig. 10 GC–MS spectra of sample X1: linear monocarboxylic fatty acids were detected, in particular myristic acid (15.41 min), palmitic acid (17.52 min) and stearic acid (19.46 min)

was in the samples analysed in this research—thanks to its high sensitivity.

The GC–MS results obtained from extracted fractions of the painted area from sample X1 show the presence of a lipidic fraction. As illustrated by the chromatogram of Fig. 10, GC–MS analysis allows the detection of saturated monocarboxylic fatty acids, ranging from C9 to C18 carbon atoms, in particular myristic acid (C14:0), palmitic acid (hexadecenoic acid 16:0) and stearic acid (octadecanoic acid 18:0), being the latter two the most abundant.

Even though similar results were observed in Calakmul archaeological site (Rigon et al. 2020) (where the presence of animal fat was supposed), some important differences came to light, which are represented by traces of dicarboxylic acids, in particular suberic and azelaic acids, detected in samples X1, X2, X4 and X6. This evidence suggests the presence of vegetable drying oils as binding media for Xcambó Maya corporal paints: dicarboxylic acids, in fact, are the typical by-products of the oxidation of unsaturated fatty acids naturally occurring in fresh drying oils (Sarret et al. 2014). In burial condition, it is common to register groundwater leaching phenomena that affect the dicarboxylic fatty acid survival (Regert et al. 1998). Consequently, we think that GC–MS analysis reported the presence of the dicarboxylic acids just in traces most likely due to the leaching processes.

So, the origin of the detected fatty acids may be related to the use of a drying oil considering the presence of saturated and dicarboxylic fatty acids, being the latter the natural biomarkers of oils with drying properties. The calculated molar ratios between palmitic and stearic acid (P/S) ranged between 2.1 and 2.5. These values could refer to the use of the vegetable oil extracted from the Chia seeds, as reported in the specific literature (Kulczyński et al. 2019; Ciftci et al. 2012; Nitrayová et al. (2014); Ayerza 1995). This hypothesis is reinforced by some studies related to the use of this siccative oil by the Maya populations (Vázquez De Ágredos Pascual et al. 2016b). In addition, it is known that Chia grows in tropical and subtropical climates, such as the areas covered by this study in Mexico.

In samples X3 and X7, the dicarboxylic acids were not detected (maybe because of the groundwater leaching processes in burial context), and the calculated P/S were lower than 1, suggesting the presence of animal fats in the ointments (Dudd et al. 1998, Seher et al. 1980), as assumed in the case of Calakmul painted bones (Rigon et al. 2020).

Different results, compared to Calakmul site (Rigon et al. 2020), have been highlighted thanks to Py/GC–MS results

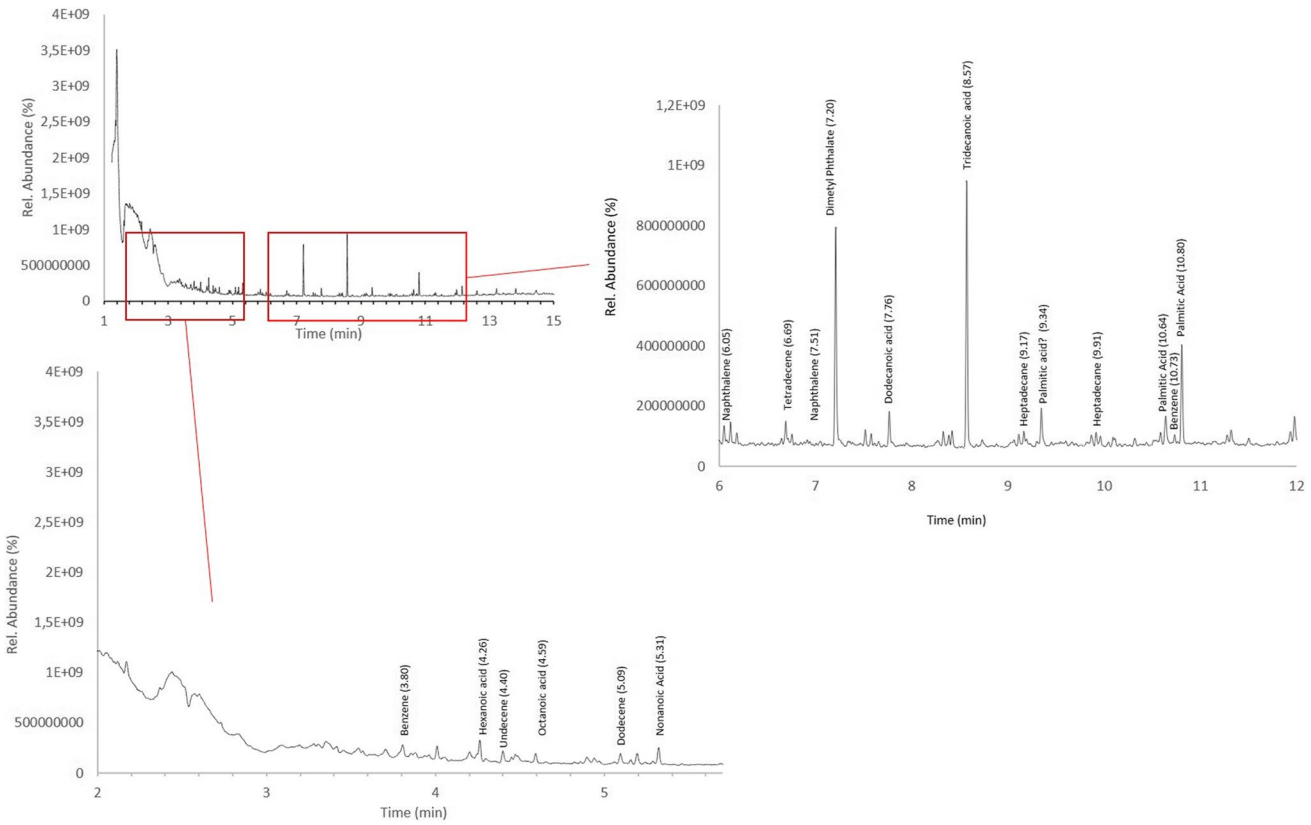


Fig. 11 Py-GC/MS spectra of sample X2: a meaningful aromatic fraction and a series of alkanes and alkenes were identified. They are the typical products of thermal dissociation of asphaltene. For this reason, it has been supposed the presence of bituminous material in the sample

obtained from selected "raw" samples (i.e. without any pre-treatments, as described in the experimental details).

In particular, the pyrogram of the sample X2 (Fig. 11) shows the presence of saturated monocarboxylic fatty acids (as pointed out by GC–MS in other samples), a series of alkanes and alkenes (C10-C25), naphthalene and benzene. This result could suggest the presence of bituminous material (also known as "asphalt" (Connan et al. 2006; Burger et al. 2016; Zhao et al. 2012)) associated with the aromatic fraction observed. Specifically, in the sample were identified naphthalene and benzene which can be considered some of the natural biomarker of asphalt (Languri et al. 2002). In fact, bituminous materials are made mainly by asphaltene fractions which, under pyrolysis thermal process, lead to their dissociation in alkanes and alkenes and a large number of aromatic compounds (Languri et al. 2002).

Phthalic acid derivates were observed in the samples. An instrumental contamination has been excluded because of the high concentration of the signals (Fig. 10).

In nature, phthalates are present in environmental soils, water and atmosphere (Toshinari et al. 2001) as contaminants, so archaeological samples can be easily contaminated by these products via burial soil contact.

However, phthalates are widely used as plasticizers in industrial polymeric product formulation, but this assumption, of course, does not fit with the archaeological origin of the samples, so the hypothesis of a contamination with phthalate plasticizers, probably due to the storage condition, could be more plausible. In addition, common consolidation treatments in archaeological conservation practices involve the use of polymeric products in order to preserve fragile materials (i.e. poly-acrylic and poly-vinylic polymers). Although these treatments should be reversible and conservative methodology must not damage the artefacts, they often interfere with raw materials of the samples (López-Polín n.d.).

Apart from being a problem of material contamination, these practices can completely interfere with diagnostic investigation results which report altered information. In particular, most of the polymeric products use for previous treatments in conservation protocols can change their own characteristic of stability, reversibility and effectiveness during aging so for example they can change their physical–chemical properties and became no longer soluble in original solvent so they transform themselves in no longer removable materials from porous objects (López-Polín n.d.).

However, such a presence in archaeological objects could be explained also as the result of auto-oxidation phenomena of terpenic structure that leads to poly-carboxylic acids compound such as phthalic acid observed by Sarret et al. (Sarret et al. 2014). Terpenic compounds can be ascribed to a natural resin used by Maya in body ointment formulation as a binder material (Carmen et al. 2018). They widely used natural resins in handcraft object manufacturing for many purposes including body ointment formulation in burial ritual context.

Following what has been discussed so far and considering the Py-GC/MS results that have not reported the presence of synthetic polymers, we can state that there is no strong evidence for the use of an industrial product on the samples. The hypothesis that assumes the presence of the phthalic acid as the result of auto-oxidation phenomena of terpenic fraction seems to be more plausible even if further investigation is needed to deepen this hypothesis.

Considerations

The knowledge achieved during last decades of studies concerning the Maya culture and, in particular, the information gained through the study of the samples of painted bones from Xcambò and Calakmul has given a significant contribution to reconstruct the cultural habitat and social customs of this pre-Hispanic culture.

Some of these ancient rituals and beliefs are the same ones on which modern Maya rites are based on. Ancient beliefs survive in modern ceremonies by blending traditions typical of Christianity with ancestors Maya deities worship, as can be seen, for example, on the Camino Real de Campeche Route (Mexico) between October 30 and 31 and November 1, on the occasion of the ceremonies related to the Day of the Dead. In this same sense, among the grave goods with which the current Mayans are buried, aromas, in the form of incense, food, flowers and aromatic plants, are of great importance (Maldonado Cano 2003). On the other hand, in current Mayan communities, there is an "odoriferous and visual memory" (the latter associated with colour (Vázquez de Ágredos Pascual et al. 2009)) to remember the deceased, which, once again (Ruz Sosa 2003), confirms that aroma and colour continue to play a relevant role among the current Mayans. The scent memory in these contemporary ceremonies is spread through the fragrances of various flowers; but it also impregnates foods that come from the same flowers (for example, the izote flower, which the Mayan Achí ethnic group tastes at the Day of the Dead). An odoriferous universe that invites the deceased to enter our homes to taste, olfactory, "the grace" of the food they knew in their earthly life. They are current beliefs in which pre-Hispanic tradition and renewal (in line with the new Christian practices) intermingle, which materialize in practices in which colours and smells evoke the ancient traditions of the pre-Hispanic Mayans, giving the current practice of cultural identity.

Therefore, in this perspective, the deep awareness of the ancient is necessary and essential to better understand the nowadays.

Conclusions

Since the second half of the twentieth century, the study of the pigments that were used in the funeral rituals of the Mayan culture has been increasingly frequent. However, there are few, if not almost absent, analyses focused on the identification of organic compounds used for this same purpose. Its "invisibility" has not favoured its study until very recent dates, and its physicochemical identification, the few occasions that it has been possible, has contributed to a better understanding of the burial customs of this ancient culture of America.

In this sense, this study is one of the few researches related to organic materials employed as binding media by ancient Maya for body painting purposes. In fact, ointments were made by a mixture of pigments and organic binders.

Previous investigation done by the same research group in Calakmul archaeological site (Rigon et al. 2020), just as others results (Batta et al. 2012; Cervini-Silva et al. 2012; Tiesler et al. 2013; Vázquez De Ágredos Pascual et al. 2016b), had shown that red pigments used by Maya were mainly red earth and cinnabar. This evidence has been confirmed also for the samples described in this paper, but meaningful differences were observed concerning the organic fraction as discussed in the paper.

The detection by GC–MS and Py-GC/MS of saturated linear monocarboxylic fatty acids and di-carboxylic fatty acids especially azelaic and suberic (typical oxidation products of unsaturated fatty acids especially vegetal oil) suggests the use of a vegetable drying oil. In one sample, some alkanes and alkenes derivates and aromatic compounds (i.e. naphthalene and benzene) were observed. They are the typical products of thermal dissociation during pyrolysis analysis of bituminous materials.

Based on these results, we could affirm that realistically Maya used mixing pigments, above all red ones, with an organic matrix consisting in vegetable drying oil (most likely Chia seed oil) with probably addition of bituminous materials to manufacture body coloured ointments used in burial ceremonies.

These colour and scent shrouds must have served at least two purposes. On a practical level, alleviate the stench that the posthumous treatment of the human body should produce in a context as humid and favourable to the decomposition of the body such as the jungle, especially considering that the funeral rituals among the Mayan royalty could extend up to 10 days. On the other hand, they fulfilled a symbolic purpose, since these shrouds of colour (a metaphor of blood, that is, of the vital liquid) and aroma (a fragrant matrix that favours rebirth) would guarantee life beyond physical death. Both elements, colour and aroma, as previously expressed, continue to be essential in the funeral customs of the current Mayans.

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Declarations

Conflict of interest The authors declare no competing interests.

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