Journal of Archaeological Science: Reports 67 (2025) 105337

Contents lists available at ScienceDirect

Journal of Archaeological Science: Reports

journal homepage: www.elsevier.com/locate/jasrep

The application of fragrant ointments and pigments as a funeral practice in the Guatemalan Petén

Inés Zazueta de la Toba[a], Montserrat Soria Castro[b], Patricia Quintana[b], Vera Tiesler[a], Jarosław Źrałka[c], Juan Luis Velásquez[d], Mara Reyes[e], Lilian Corzo[e], Gloria I. Hernández-Bolio[b,*]

[a] *Facultad de Ciencias Antropológicas, Universidad Autónoma de Yucatán, Mexico*
[b] *Departamento de Física Aplicada, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Unidad Mérida, Mexico*
[c] *Institute of Archaeology, Jagiellonian University, Krakow, Poland*
[d] *Nakum Archaeological Project, Flores Island, Guatemala*
[e] *Instituto de Antropología e Historia de Guatemala, Ciudad de Guatemala, Guatemala*

A R T I C L E I N F O

Keywords:
Archaeometry
Body pigments
Maya
Ointments

A B S T R A C T

This paper studies thirteen bone samples from six sites in the Guatemalan Petén, whose surface was pigmented with various reddish hues as one of the activities of mortuary ceremonies. Archaeometric analyses as X-ray fluorescence and scanning electron microscopy disclosed the presence of pigments including iron oxide and cinnabar. Likewise, organic components were revealed by gas chromatography coupled to mass spectrometry as terpenes of the abietane type, confirming the application of fragrant funeral ointments. The set of inorganic and organic analyses contributed to the understanding of a sensorially related funerary practice of the ancient Maya from Guatemalan Petén.

1. Introduction

While some modern societies view death as the poignant and inevitable culmination of the human existence, the ancient Maya believed in symbolic continuity between life and death (De la Garza, 1999). For them, it was a continuous cycle of generation, destruction, and renewal. Thus, once the person died, their disembodied form retained certain powers. The body was considered a relic, being the enclave point between life on earth and at the underworld, between the living and the dead (Tiesler, 2008:49). For these reasons, the body of the deceased underwent a series of posthumous treatments both to confront and accept death and to receive support from the mourners. Among these treatments was the use of colors and fragrances, which carried specific cultural meanings, directly related to the animic forces and entities (López Austin, 2004).

The application of red pigments and fragrant ointments to their deceased may be based on Mesoamerican beliefs that considered the human body as an amalgam of diverse, heterogeneous substances, mirroring the structure and organization of the cosmos. These materials, tissues (including bones), and viscera, are composed of dense matter that, despite its solidity, was subject to wear, aging, and inevitable degradation over time. These organic remains are part of the *ecumene,* the shared space–time inhabited by humans and other living beings (Velásquez García, 2011, 2022). Similarly, parts of the body were believed to be composed of ethereal, vaporous substances, perceptible only in dreamlike or trance-like states. These substances either resided within dense anatomical structures or moved in and out of the body. They have been described as entities or animic forces, as they were thought to dwell in the *anecumene*, the cosmic space–time inhabited by gods and ancestors (López Austin and López Luján, 1997, 1999; Velásquez and Tiesler, 2018:88).

Considering this, *itz* was a substance that flows through plants, trees, and flowers, being an essential matter of creation, fertility and life. It was a nectar or resin that flowed through every living thing, and not only within organic organisms but also within minerals. Just like the life-giving blood that ran through the veins and arteries of humans and animals, it had the property of being viscous and additive (Vázquez de Ágredos et al., 2018:56). The application of reddish pigments and plants that provided scents and textures was a way to retain such ethereal substances that the deceased would need on their journey to the

* Corresponding author.
E-mail address: gloria.hernandez@cinvestav.mx (G.I. Hernández-Bolio).

https://doi.org/10.1016/j.jasrep.2025.105337
Received 14 November 2024; Received in revised form 20 June 2025; Accepted 25 July 2025
Available online 11 August 2025

afterlife. In turn, it was a way to remember those colors and smells that constantly filled them with emotions in daily life (Houston and Newman, 2015; Velasco Lozano and Nagao, 2006:43).

Regarding the application of scents and colors on the body, the main evidence is found in the skeletal remains, and so far, studies have been hampered by a lack of reference data, technical limitations, and the deterioration (or scarcity) of bone elements. Epigraphic and historical evidence suggests that funerary practices involved sprinkling pigments, smearing ointments, or applying painted wrappings to the bodies or skeletal remains of the deceased (Sahagún, 1981). However, it is difficult to determine whether these substances were applied directly to the skin or to the bones (Fitzsimmons et al., 2006). In both cases, the red coloring minerals—and any associated organic components—are found adhered to the bone surface. This occurs because bone hydroxyapatite serves as a matrix that adsorbs molecules from the ointments (Dong et al., 2007). Consequently, archaeology has turned to increasingly precise techniques to identify these ephemeral materials (Ghisalberti and Godfrey, 1998; Lambert et al., 2000). Although it is difficult to clarify the precise recipe of the ancient fragrant ointments, some researchers have proposed the use of five essential ingredients: fragrance, aromatic excipient, astringent, preservative, and pigment (Vázquez de Ágredos et al., 2015). However, to date, chemical-physical analyses have only allowed for the identification of two of the ingredients: the aromatic excipient and the mineral pigment/organic colorant in the blends. It is possible that all these ingredients were mixed due to the lack of knowledge of additional application methods, such as alcohol distillation. Thus, the only way to apply the fragrances to the skin may have been by incorporating a support substance (Vázquez de Ágredos et al., 2018:58).

Typically, research has focused on higher-status burials, as these sites yield the most substantial evidence of pigmentation. Moreover, most of these studies do not integrate more than two physicochemical characterization techniques, such as those described below. For example, the study carried out on Burial 1 at Tak'alik Ab'aj, Tomb 19 at Rio Azul, Burial III-9 at Calakmul, and Burial XIII-3 and Burial XIII-sub at Palenque, the so-called Red Queen, from which two inorganic compounds were distinguished in the funerary red cover: a red pigment, composed primarily of cinnabar (HgS), and calcium apatite $Ca_5(PO_4)_3(OH)$, as well as an organic layer composed of a burseraceous-type resin identified as white copal; it was the fusion of cinnabar with white copal that allowed the creation of an aromatized body pigment (Tiesler et al., 2013; Vázquez de Ágredos et al., 2012, 2018). The study of Batta et al. (2013) focusing on the yellow and red-pigmented bones from Jaina analyzes at least eight high-ranking individuals, identified by their dental decorations with stones such as pyrite, hematite, and jadeite, as well as by intentional skull modeling and their grave goods. The yellow pigment on the bones is goethite (an iron hydroxide), which can be obtained from hematite. They suggest that the yellow was originally red (hematite) and that environmental conditions transformed the color. Another case was presented by Cheung et al. (2013), who discovered the royal tomb of a Maya king beneath the El Diablo pyramid in El Zotz, El Petén. The undisturbed tomb contained the remains of an adult male, possibly Chak' "Fish-Dog" Ahk, along with six child sacrifices. There was evidence of pigmentation on the bones, and after the application of non-destructive technologies, the team determined the use of hematite and cinnabar both on the wrapping of the deceased (as well as the stucco placed underneath), on the body, and inside one of the shells offered.

One of the largest studies was that by Bolio (2009; Bolio Zapata et al., 2012), who analyzed 72 individuals from the sites of Sihó, Xcambó, Oxkintok, Ek Balam, Isla Cerritos and Uaymil, Dzibanché, and Palenque, using XRD and SEM techniques; the study revealed that hematite was more commonly used in coastal and smaller-scale sites, while cinnabar, a highly valued mineral, was found in royal burials such as those of Yuknom Yuch'ak K'ak in Calakmul, the probable ruler Testigo Cielo of Dzibanché, and K'inich Janaab' Pakal and the Red Queen in Palenque.

Regarding the characterization of organic compounds, research has been scarce. Some exemplary studies have been carried out by Izzo et al. (2022) when analyzing 10 bone elements from Xcambó by gas chromatography-mass spectrometry (GC–MS), in which they recorded the presence of dicarboxylic fatty acids, particularly suberic and azelaic acid. These dicarboxylic acids are typical by-products of the oxidation of unsaturated fatty acids, which are commonly present in a wide range of organic substances and are a common characteristic of oils. By using GC–MS, Rigon et al. (2020) identified several monocarboxylic fatty acids ranging from C9 to C18 carbon atoms. The source of these molecules may be related to animal fats, a hypothesis that authors consider plausible given that stearic acid was found in higher concentrations than palmitic acid, a condition typically observed in animal fat samples. A study using pyrolysis–gas chromatography and mass spectrometry on bone fragments from Calakmul identified the presence of isoprene, a polymeric component of the natural latex. This finding aligns with the characterized gum extracted from *C. elastica* tree (Rigon et al., 2020:9). Finally, Pérez López et al. (2021:4) were able to identify, in samples from Xcambó, the presence of abietane-type diterpenoids, including abietic, pimaric, and isopimaric acids – main components of the resins of Pinaceae species–, suggesting the use of *Pinus* spp. resins for the formulation of ointments applied in funerary contexts. Likewise, the presence of beeswax was reported for the first time due to the presence of four signals corresponding to methyl groups, methylenes, a vinyl proton, and an esterified hydroxymethylene, distinctive of waxes.

Given this perspective and the opportunity to analyze two sets of samples from sites with different levels of social complexity and organization in the Guatemalan Petén, an area that has not yet been explored, this study applies new protocols and cutting-edge archaeometric techniques to integrate the organic and inorganic results into the corpus and complement the information on mortuary fragrances and pigments. The primary aim of this research is to investigate the use of scented-pigmented ointments by characterizing the mineral and organic components that may have been part of fragrant mixtures used on the deceased in El Petén. Chemical identification by non-destructive XRF was applied to discern whether the red color of the macroscopically observable pigmentation was due to the presence of iron oxides (Fe), cinnabar (HgS), or another mineral. Furthermore, SEM-EDS analyses led to the morphological and elemental characterization of the pigments. Finally, the organic fraction of the ointments was studied by GC–MS to detect fragrant molecules or excipients and identify their possible source. This multidisciplinary approach aims to offer a comprehensive understanding of one of the biocultural and funerary customs of the ancient Maya.

2. Materials and methods

2.1. Archaeological samples

The samples analyzed in this study come from six archaeological sites located in the Guatemalan Petén, as shown in Fig. 1. One of them is Nakum, a major site in the Northern Petén with evidence of occupation spanning from the Middle Preclassic (1000–400 BCE) to the Late Classic (600–800 CE). In Southeastern Petén, five *señoríos*—individual and hierarchical political entities comprising interdependent communities (Laporte and Torres, 1994)—were examined:

Ixtonton, occupied from the Late Preclassic (400 BCE – 100 CE) through the Terminal Classic (800–900 CE; the cave of Ixkun, mainly visited during the Late Preclassic (400 BCE–100 CE) and the Early Classic (250–550 CE); Sacul, which shows notable development during the Late Classic (600–800 CE); and finally, El Chal, a site with occupation spanning the Late Classic (600–800 CE) through the Postclassic period (900–1520 CE) (Chocón, 2008; Laporte and Torres, 1994; Sharer, 1994; Tiesler et al., 2024). Only bone remains with a visible presence of pigment on the surface were selected, as in most of the burials, only a few bone pieces were pigmented. In total, 12 bones and 1 amorphous material from 11 burials were analyzed (Fig. S1); most of the remains

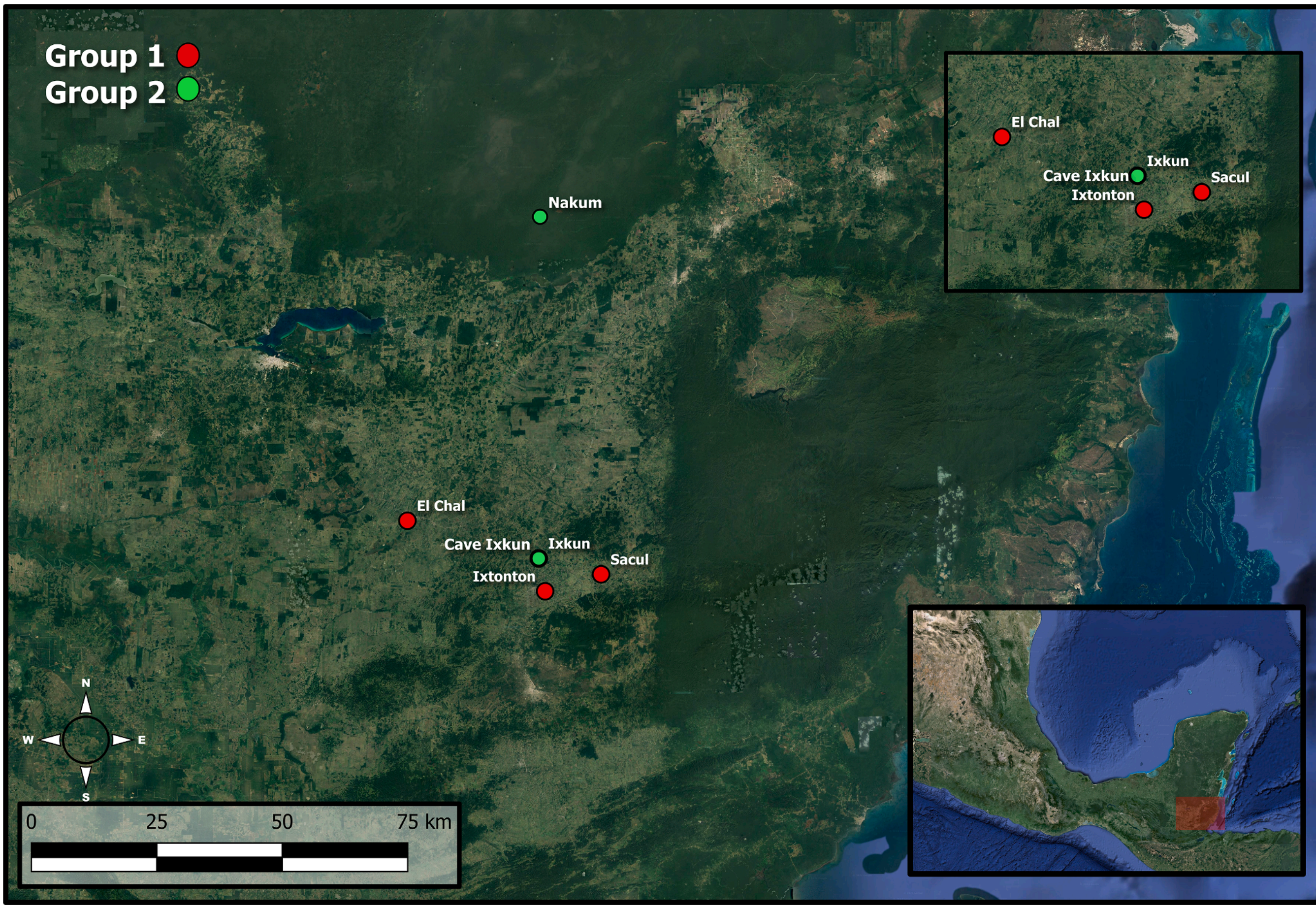

Fig. 1. Map showing the sites included in this study. Map by Inés Zazueta.

correspond to long bones of the extremities, with the exception of one skull and one rib sample. The amorphous material studied was found among the offerings. The distribution of the samples regarding burials and sites, as well as a brief description (type, status markers, offerings, and further information), is presented in Table 1.

It is of utmost importance to note that all the contexts described here, as well as the samples analyzed, are the result of several field seasons conducted by the Proyecto Atlas Nacional de Guatemala of the Instituto de Antropología e Historia –IDAEH (today DECORSIAP-IDAEH; Reyes, 2015) and the Nakum Archaeological Project (DEMOPRE; Koszkul et al., 2007:793), as well as the archaeologists, researchers, and field personnel involved in these efforts.

2.2. Sample preparation and analysis

In the initial stage of analysis, a detailed record was created for each sample, including measurements of weight and length, along with the assignment of a Munsell color code to document the red hues present on the bone surfaces. The Munsell color system served as a standardized reference tool to ensure consistency in color classification across the dataset. This method enables the visual categorization of tonal variations based on three properties of color: hue (basic color), value (lightness), and chroma (color intensity). For this study, the approach outlined by Quintana et al. (2015) was applied. Photographs were subsequently taken of the anterior and posterior views, as well as close-ups of the pigmented areas. To assess ritual patterns among the sampled burials and to identify differences and similarities related to social status and the organic and mineral origin of the burial ointments, a classification relying on the work of Krejci and Culbert (1995) and Price et al. (2014)was performed (see Table S1 and Table S2 for scoring criteria). This classification is based on the understanding that the characteristics of the mortuary site (form, arrangement, and offerings) can be correlated with social and subsistence practices (Parker Pearson, 2000). The goal is to identify those elements of the grave goods that were restricted to a very limited segment of the funerary population; to achieve this, specific indicators are summed to generate a score for each individual. Notably, red pigmentation is considered one of the indicators associated with high-status burial.

2.2.1. Elemental composition and morphology of the ointments

The elemental composition of the pigments was analyzed by X-ray fluorescence spectroscopy (XRF) at the Centro de Investigación en Corrosión (CICORR, Campeche, Mexico). A portable SANDRA (Non-Destructive X-Ray Analysis System), developed at the Institute of Physics of Universidad Autónoma de México (Ruvalcaba et al., 2010), was employed. The detector used was a 6 mm^2/500 µm/0.5 mm Be X-123Si-PIN from Ampetek. The X-ray tube has a molybdenum anode, Spellman™ XLG50P100/FL voltage source and the analysis conditions were 30 kV, 0.300 mA with an analysis time of 180 s. Spectral data were processed using PYMCA® software to quantify the area of the characteristic peaks.

Morphological and chemical analyses were performed using a JEOL JSM-7600F high resolution scanning electron microscope (SEM) coupled with an energy dispersive spectrometer (EDS), operating at 25 Kv.

2.2.2. Analysis of the organic fraction

Following a modified protocol from Pérez-López (2021), chloroform ($CHCl_3$), a medium polarity solvent, was used to extract the organic components from the bone samples. Of 13 initial samples, only 10 bone

Table 1
Samples with funerary pigments and ointments analyzed in this study.

Site	Lab ID	Burial	Skeletal segment (ID)	Description of the Burial
Nakum	NK 1	Burial 1	Long bone fragment	A tomb built within the unfilled chamber, oriented to the west of a temple located on the pyramid base of structure 15–1, belonging to the remains of a dynast. The skeletal remains found correspond to an adult of unknown sex.
Nakum	NK 2	Burial 2	Rib fragment	A crypt was found beneath the floor of the platform of Building 15 Sub.1, measuring 1.55 to 1.74 m long, 0.22 to 0.56 m wide, and 0.70 m high. To build it, two previous floors were cut, and large slabs were used as walls and roofing. The crypt was covered by a floor and corresponds to an individual burial of a middle-aged to older woman.
Nakum	NK 8	Burial 8	Amorphous material	Burial 8 was found in a tomb measuring 2 m by 1.30 m by 1.32 m at the top of Building X, within the fill of the pyramid. The skeletal remains were poorly preserved due to water infiltration and exposure to the fill. It probably corresponds to an adult individual.
Nakum	NK 10	Burial 10	Rib fragment	A crypt placed in the fill of the platform of the North Group (south side of the Complex). It is a young individual, estimated to be between 20 to 25 years old. The individual is male, as indicated by the prominent supraorbital arches and chin.
Nakum	NK 13	Burial 13	Tibia fragment	It was found in the filling of Building X and consists of a concentration of dispersed bone elements that are not anatomically articulated. It is a secondary collective burial, with the presence of at least four individuals recorded.
El Chal	CHAL 272	Burial 272	Femoral epiphysis	It was found in the Acropolis, South Structure, in a cyst (41–497-1) measuring 1.50 m long by 0.90 m wide. It is a multiple burial of at least five individuals. The group designated as Individuals B corresponds to the remains of four adults, two of whom are male and one female. All indications suggest that no bone segments were deposited, but rather parts of human bodies after maceration
Ixtonton	IT 288A	Burial 288	Femoral fragment	Burial 288 was found in Patio B of the Northeast Plaza, in front of the North Structure, dating to the Terminal Classic. Incomplete remains (15 % of the skeleton) of an adult were discovered, with parts covered in red and yellow pigment (femurs). There are also remnants of a fabric on the right ulna and radius, as well as on the femurs; these are cotton threads with traces of red and yellow.
Ixtonton	IT 288B	Burial 288	Radius fragment	
Ixkun	IK 27	Burial 27	Tibia fragment	An individual was found inside cave NE 2, which has a narrow entrance (1.50 m by 0.70 m) and a spacious interior with passages and stalactites. Bone remains are reported to be in a very heterogeneous state of preservation and not in anatomical position, belonging to an adult individual and possibly female.
Ixkun cave	IK 312–12	Burial 312	Skull fragment	This is a multiple burial located in Chamber 2 of Cave 2 in Ixkun, where 19 female individuals were identified, ranging in age from adults to infants, and showing significant evidence of disturbance and reduction.
Ixkun cave	IK 312–17	Burial 312	Metatarsal	
Sacul	SAC 183	Burial 183	Femoral fragment	An indirect primary burial was located in a funerary chamber, in a cyst with a slab roof, beneath the site known as PSP-46, at a depth of 1.20 m. It likely corresponds to an individual of early childhood or adolescence, with an approximate height of 1.20 m.
Sacul	SAC 204	Burial 204A	Hand phalange	This is a collective deposit that includes at least five adult individuals. It was found inside a funerary chamber measuring 3 m from northwest to southeast, 1.30 m from east to west, and 1.60 m high; the chamber was located 1.15 m below the surface, covered by limestone and black soil that is part of the structure's fill. The first skeleton (204a) corresponds to an adult individual.

fragments were selected for GC–MS analysis since in three cases, the bone size was not suitable for organic extraction (Nakum Burial 8 and Burial 10, and Ixkun Burial 312–17). Each selected fragment was placed in a beaker, and a volume ranging from 50 to 100 ml of $CHCl_3$ was added depending on the size of the bone. The maceration was carried by ultrasonic bath for 15 min and evaporated at room temperature. Subsequently, the concentrated fractions were resuspended in 600 μL of deuterated chloroform, and then placed in chromatography vials (1 mL). The organic analysis was performed in an Agilent gas chromatograph (model 5890) coupled to a mass detector (model 5970) for molecular characterization. Chromatographic runs were performed under the following conditions: injector at 250 °C, in splitless mode with a flow rate of 50 mL/min, while the column (HP-5MS 30 m x 250 μm x 0.25 μm) was operated with a flow rate of 1 mL/min. The starting temperature was 50 °C for one minute, then the temperature was raised with a gradient of 5 °C/min up to 300 °C. The chromatographic run time was one hour. Detection was carried in SCAN mode with an applied voltage of 1046 V and and m/z ranged from 50 to 650. The results were plotted in Origin 9 software. Each chromatogram was manually inspected, and the mass spectra of each peak were search against the NIST 2016 database (Wiley, Golm) (Supplementary material 2). Agilent GCMS Data analysis was used to integrate the area of each compound to allow a comparison between samples. The relative intensity of each identified metabolite was used to build a matrix (Supplementary material 3) to perform a comprehensive analysis of the organic components in the ointments by means of hierarchical cluster analysis (HCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) (https://www.metaboanalyst.ca/). It is important to note that phthalates, common contaminants associated with industrial polymers and organic solvents, were detected in all samples. As these are considered impurities, they were exclude from further characterization (Sarret et al., 2017:427) (Table S3).

2.2.3. Soil analysis

To rule out that the minerals detected in the pigments originated from soil composition, X-ray diffraction analysis (XRD) was conducted on a soil sample from Burial 8, Nakum, and a sample from Burial 312, Ixkun. The soil samples were ground and analyzed using a Bruker D-8 Advance diffractometer with a step size of 0.02 degrees, a pass time of 0.5 s, and operating parameters of 40kv 30 ma. Using Cu Kα1 tube.

3. Results and discussion

3.1. The mineral composition of the funerary pigments

By applying XRF, it was possible to identify iron (Fe) in all the samples (Table S4). However, since oxygen is not detectable with this technique, it was not possible to determine the specific type of Fe oxide present. Nonetheless, this method could help to understand if these minerals were used as a mixture or as simple components (Quintana et al., 2014). Calcium was also detected in all samples, attributed to the absorption from the bone matrix, specifically in the form of hydroxyapatite ($Ca_{10}(PO4)_6(OH)_2$) and calcium carbonate ($CaCO_3$) from the context. These findings align with those reported by Izzo et al. (2022), who observed similar calcium compounds in comparable contexts. Since iron oxides could also be due to soil (Arizmendi-Galicia et al., 2009), XRD analyses of representative soil samples were carried. In both cases, Nakum and Ixkun, the main mineralogical phases detected were calcite ($CaCO_3$), dolomite ($CaMg(CO_3)_2$), and quartz (SiO_2) (Fig. S2), thereby ruling out the soil as a potential source of the iron oxides found on the bones.

An exceptional case was presented by Burial 1 of Nakum (Fig. 4a), where the fluorescence spectrum detected mercury (Hg), sulfur (S), and a low signal corresponding to iron (Fe), suggesting the presence of iron oxide (Fig. 4b). To confirm a likely intentional mixture of cinnabar and iron oxide, SEM analysis using a LABE detector was carried (Fig. 2). Elemental mapping by EDS of bright particles, revealed the presence of Hg and S. Since these elements were found in an atomic ratio of 1:1, they were assigned as cinnabar (Fig. 3 and Table S4). The amount and uniformity observed for Fe suggest that a perfumed and pigmented ointment, made mainly of iron oxide, was applied. Once the iron oxide-impregnated ointment was applied to the body of the individual, as a base, the powdered cinnabar was sprinkled and adhered well to the body

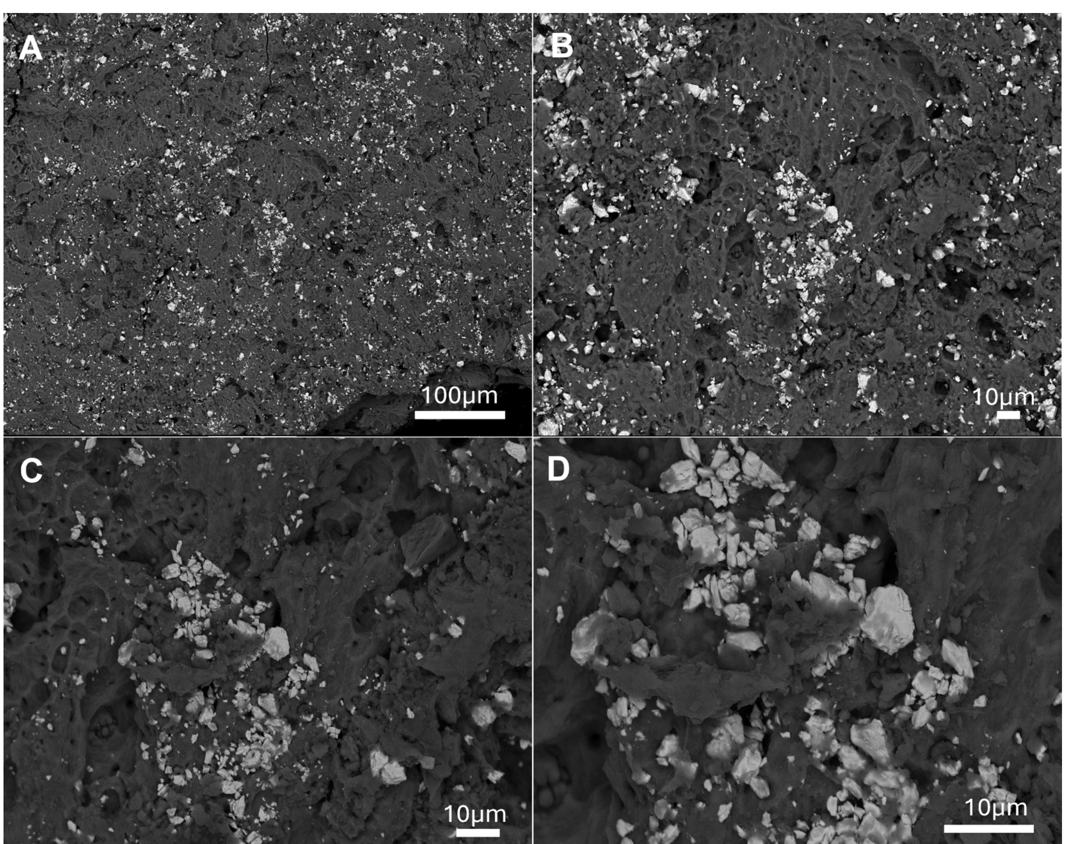

Fig. 2. SEM-LABE scanning images taken at different sections of Burial 1, Nakum. The bright particles correspond to powdered cinnabar.

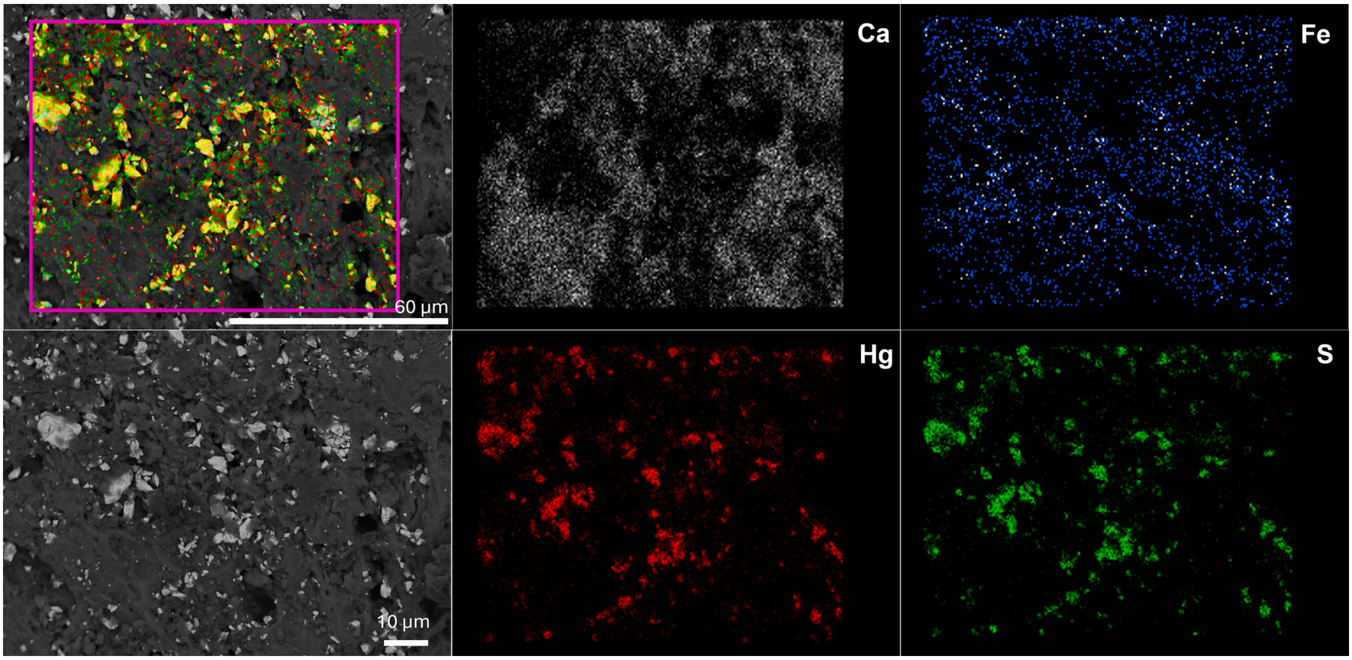

Fig. 3. EDS elemental mapping of Burial 1, Nakum. A homogeneous layer corresponding to that of bone Ca is observed with Fe, while particles composed by HgS show a powdered application of cinnabar.

due to the binding properties of the organic compounds in the ointment.

Another representative case is Burial 2 from Nakum, a rib head fragment exhibited a brownish hue in the anterior view, which was identified as sediment apposition (Fig. 4b). The XRF spectrum (Fig. 4c) showed the characteristic Fe signal, suggesting the presence of an iron oxide compound, although it was observed in low abundance. Below the adhered sediment, a reddish coloration was detected and further analyzed using the LABE detector, revealing small bright particles which became more visible as the magnification increased (Fig. S3; X2000, WD.8 mm). The EDS quantification showed the presence of Hg and S, in a 1:1 ratio (Table S5), confirming cinnabar as a component of the ointment which also contained low proportions of iron (Fe). Although the same components were identified in Burial 1, the composition and application appear to differ, as Burial 2 contained a lower proportion of Fe. The sample from Nakum Burial 13 exhibited a sporadic reddish coloration on the surface visible macroscopically (Fig. 4d), which corresponded to the presence of Fe and Hg in the XRF spectrum (Fig. 4e), as well as in the analysis by SEM through LABE detector. In Fig. S4, a regular surface of the sample showed some conglomerations of bright particles (X300, WD4.4 mm) exhibiting heavier elements; the elemental mapping indicated the same area for Hg and S, while EDS revealed a 1:1 ratio, so the presence of cinnabar was confirmed (Table S5).

Concerning the funerary contexts and pigments examined here, the “SECIHTI Ciencia de Frontera Project” has studied approximately 25 burials from the sites of the Guatemalan Archaeological Atlas and the Nakum Archaeological Project (Zkalka et al., 2012), reporting that red was the only color used for bone pigmentation. Other colors, such as green (green stones in the offerings) and black, are also often present in the mortuary context, but not on the bones. Regarding the presence of iron oxide in all site preparations, its occurrence is feasible because of their proximity to the well-known Maya Mountains, which have massive deposits of hematite, goethite, limonite and manganese oxide, all of them used for red, orange, yellow and black pigments despite their hardness (Dunham et al., 1989:271; Laporte Mejía, 2005:10). Cinnabar was identified only in samples from the Nakum site, and its origin may come from several locations (Zrałka et al., 2008). Although the pre-Hispanic exploitation of mercury and cinnabar mines has not been an extremely explored topic, it has been reported that the Guatemalan Highlands could have been a favorable region for the presence of volcanoes and the geothermal system (Gazzola, 2022:106). These two points would be the closest sources for Nakum, however it cannot be ruled out that the mineral may have come from México: in the Sierra Gorda, a region that includes the states of Guanajuato, Hidalgo, San Luis Potosí and Querétaro, 11 deposits have been archaeologically documented for cinnabar extraction sites (178 mines in total), with archaeological zones such as Ranas and Toluquilla, positioning the region as the primary cinnabar producer (Herrera, 2003:213); in Guanajuato, cultures such as Chupícuaro settled in the hills where cinnabar deposits were found in the surrounding volcanoes.

The mixture of several minerals did occur, as evidenced in the NK 1 sample that had a homogeneous layer of iron on the bone support, and with cinnabar applied in a sprinkled form. This method of combining minerals has been reported in other contexts. For example, in Teopancazco, miniature vessels were recorded in burials that contained mixtures of cinnabar with 2 to 5 other components, among them hematite, galena, mica and jarosite, as well as pine resin. All of them were combined to form body paints, which not only had symbolic and/or cosmetic functions, but also medicinal ones (Vázquez de Ágredos Pascual and Manzanilla, 2016:9; Ejarque Gallardo et al., 2018). In a study conducted on Preclassic samples from the Chupícuaro culture, the mixture of iron oxide with dispersed, scarce, and small-sized particles of cinnabar was identified on a funerary mask painted in red and white, presenting a case very similar to Nakum Burial 1 (Vázquez de Ágredos Pascual et al., 2019:104101).

Authors such as Vázquez de Ágredos Pascual and Vidal Lorenzo (2017:160) mention that cinnabar was applied in very small quantities and combined with other less toxic elements to mitigate the health risks associated with handling the raw material. From a hygienic perspective, this approach seems a consistent response, since constant application to the skin can lead to effects such as gingivitis, severe anemia, and other gastrointestinal and neurological disorders (Ávila et al., 2014:54). However, cinnabar is present across time and throughout Mesoamerica, as in the case of architecture in Teotihuacan (Doménech-Carbó et al., 2012; Gazzola, 2009, 2022; López-Puértolas et al., 2023; Martínez García et al., 2012) and as part of the mural painting of Palenque, Río Azul, Bolonkin, and other sites in the Maya area (Acuña, 2015; Couoh

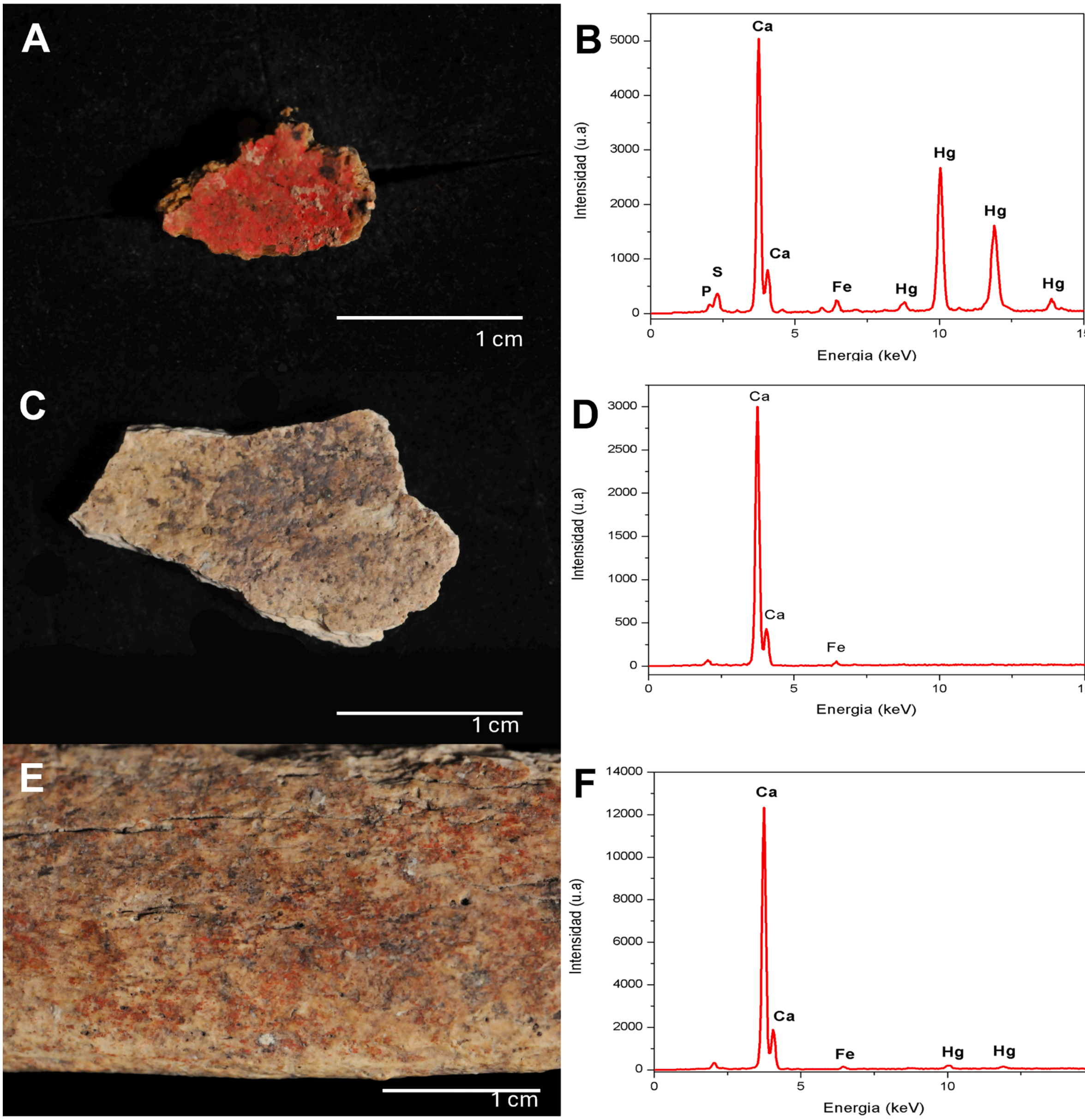

Fig. 4. Samples from Nakum with red pigment on the surface. A) Sample from Burial 1. B) XRF spectrum of Burial 1. C) Sample from the Burial 2. D) XRF spectrum of Burial 2. E) Sample from Burial 13. F) XRF spectrum of Burial 13. Photos by LBA/UADY.

Hernanández and Cuevas García, 2015; González Austria, 2024; Sheseña y Tovalín, 2021), also in axes (Vela, 2018), sculptures (Juárez-Rodríguez, 2017:5), ceramic vessels (Martínez García, 2012:187) and, of course, in burials (Ejarque-Gallardo, 2017; Gazzola, 2003). Therefore, it is possible that any potential health risks were consciously accepted to apply such a coveted and sacred color. It is a scarce pigment and complicated to obtain, and the evidence suggests that the circumstances in which it was used were highly specific, while maintaining a continuum through time and geographic space, as it has been identified at multiple sites.

The predominance of an iron oxide compound in these studied cases may be related to practical considerations, particularly given the abundant resources of this material in the vicinity. Although more difficult to process due to its greater hardness compared to cinnabar, its accessibility could have influenced its selection. However, this does not rule out cultural or symbolic factors that might also have shaped pigment choices. Cinnabar, which yields brighter reddish hues, was more sought but typically reserved for elite individuals. In the case of Nakum, with its dynastic burials of higher status, cinnabar was chosen but also mixed with a form of iron oxide, likely hematite, given the proximity of hematite (Fe_2O_3) deposits near the studied sites. From this, we can hypothesize that this was a way to economize on materials and facilitate grinding and mixing with other organic ingredients.

Regarding the relationship between the absence/presence of pigment by segment, Pérez-López (2022:73), in their review of 42 burials from the site of Xcambó, report that once the pigment is applied, there are various ways it can settle on the bone. It may concentrate on the joints of long bones and especially on the small joints of bones and feet, due to their flexible nature that creates a narrow pathway for the sliding of liquids, such as pigmented ointments. This predisposition aligns with the pigmented bone elements found in the studied burials.

3.2. *Organic components in the funerary ointments*

The analysis of the medium-polarity organic fraction of the ointments by GC–MS led to the identification of a variety of organic molecules, some of which were consistent across the samples, particularly abietane diterpenes, the main components of conifer resins typically found in Pinaceae species, although they can also occur in other plant families (González et al., 2010:811). Similarly, the monoterpene limonene was detected in several samples, suggesting the possibility of different unguent recipes. A total of 18 compounds were identified in the analyzed samples based on their presence/absence and concentration (relative intensity), and this information was used to build a matrix for performing a chemometric study. The dendrogram obtained by HCA, using the agglomerative Ward algorithm and the Spearman distance measurement method, revealed two groups of samples. One group consisted of the Nakum and Ixkun Cave samples, and the second, formed by the remaining samples from Atlas sites (Fig. 5). The grouping was confirmed through an additional measurement method, such as Pearson (Fig. S5).

To identify the metabolites responsible for this classification, and thus representative of the ingredients of each recipe, an OPLS-DA was performed using the groups obtained in the HCA as classes. The Score plot (44 % variance explained) showed a clear separation of the previously reported groups (Fig. 6).

Upon initial inspection, it was possible to distinguish in the chromatograms that those samples from the Atlas sites, displayed a high number of low molecular weight metabolites, reflected as signals having minor retention time (located between 8 and 25 min, Fig. 7); the second group, consisting of samples from Nakum and Ixkun Cave, exhibited a greater number of signals corresponding to higher molecular weight metabolites (located between 25 and 40 min, Fig. 8), thus suggesting differential recipes between these two groups. As observed in the VIP plot, the main metabolites influencing the clustering of samples from the Atlas sites, were terpenes (Fig. 6b). The limonene ($t_R = 8.75$ min), found in all the preparations from the Atlas sites, is a natural metabolite belonging to the group of monoterpenes, the limonoids. It is a major component of the aromatic essences and resins of numerous coniferous trees, such as pines, firs, cedars, larches, maples, among others (Filipsson et al., 1998). Additionally, in citrus fruits, it imparts the characteristic odor to the peels of fruits such as orange (Vieira et al., 2018). It possesses medicinal properties as an antioxidant, antibacterial, antiseptic, and as a natural insecticide (CheBi, 2022). Furthermore, depending on the isomer present, it can give a woody odor, as in pine, or fresh, as in citrus fruits. The limonene was the most important terpene in the first group.

Two additional sesquiterpenes were identified as γ-terpinene ($t_R = 9.57$ min) and cadalene ($t_R = 24.42$ min), which are present at 100 and 67 %, respectively, in the ointments from this group. γ-Terpinene is one of three isomeric monoterpenes, a volatile oily compound produced during a metabolic reaction in plants, particularly in conifers and gymnosperms (CheBi, 2022). Cadalene is a common component of essential oils of various species with cosmetic properties by providing color, a pleasant aroma, and antimicrobial, soothing and antioxidant effects (Dob et al., 2005:545, National Center for Biotechnology Information, 2023; CheBi, 2022). This metabolite is usually found in essential oils of species of the *Asteraceae* family, such as chamomile, wormwood

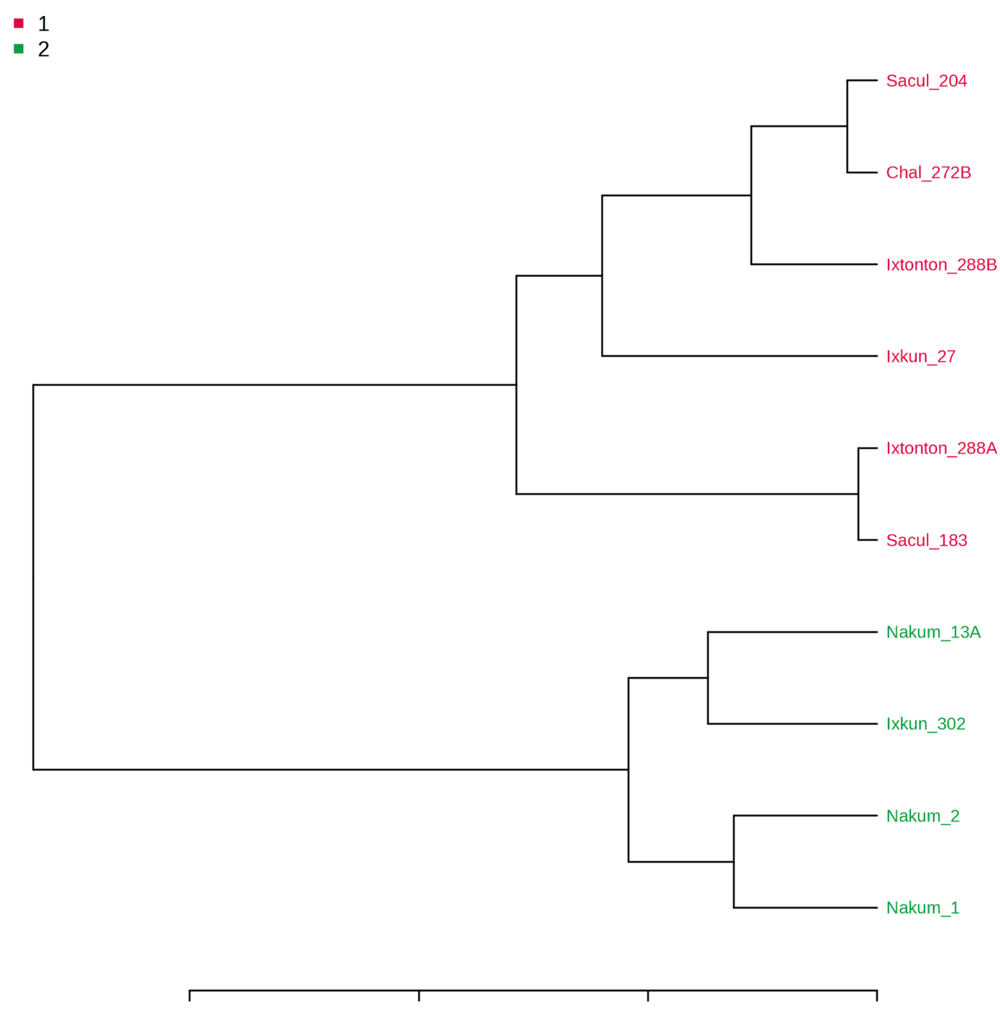

Fig. 5. Dendrogram of the organic fraction from funerary ointments obtained by HCA (Ward algorithm, Spearman measuring method).

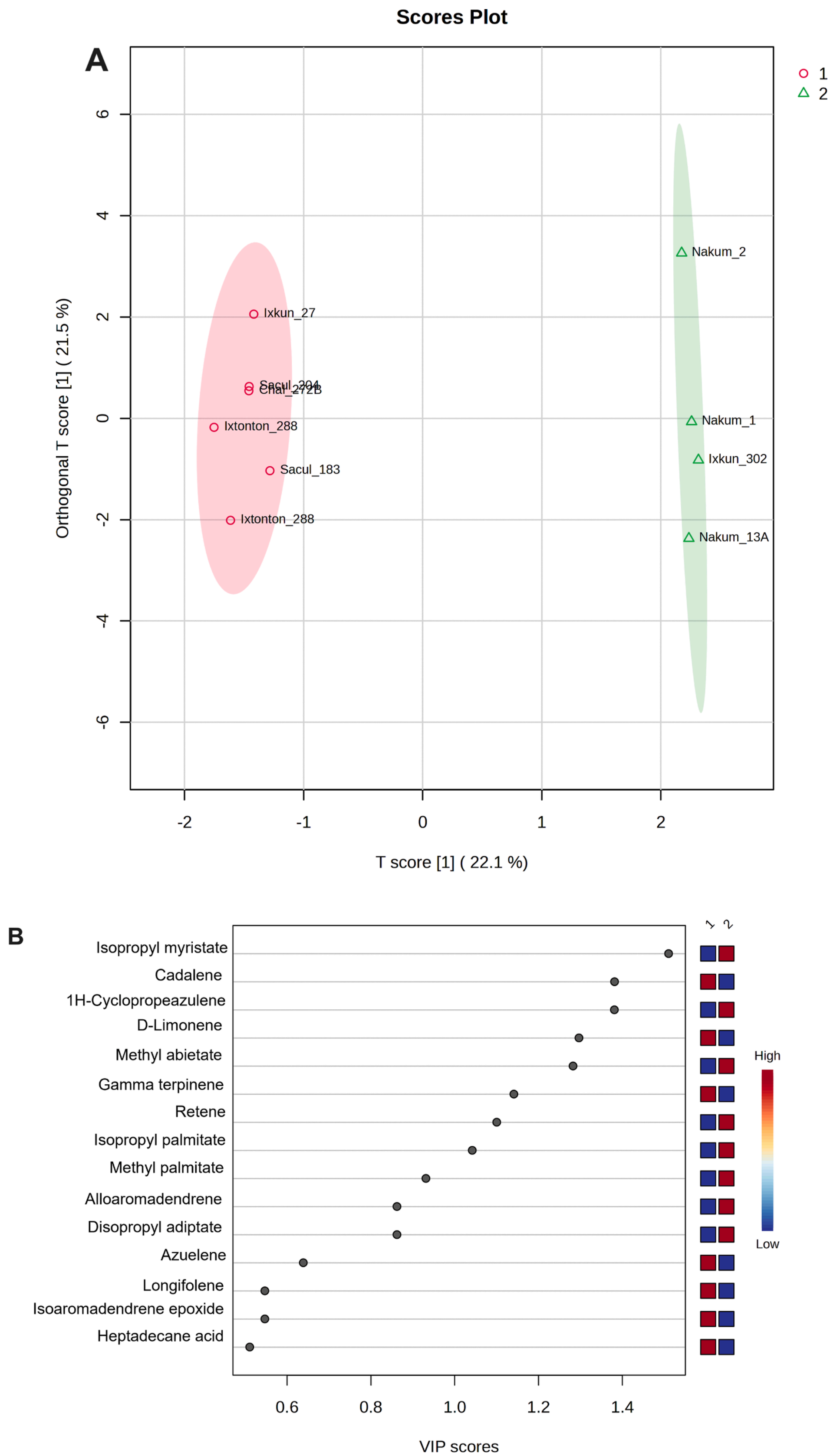

Fig. 6. A. OPLS-DA Score plot (44% variance explained) of the metabolites identified of the organic fraction of the funerary ointments. B. VIP plot showing the main metabolites contributing to the separation of the groups.

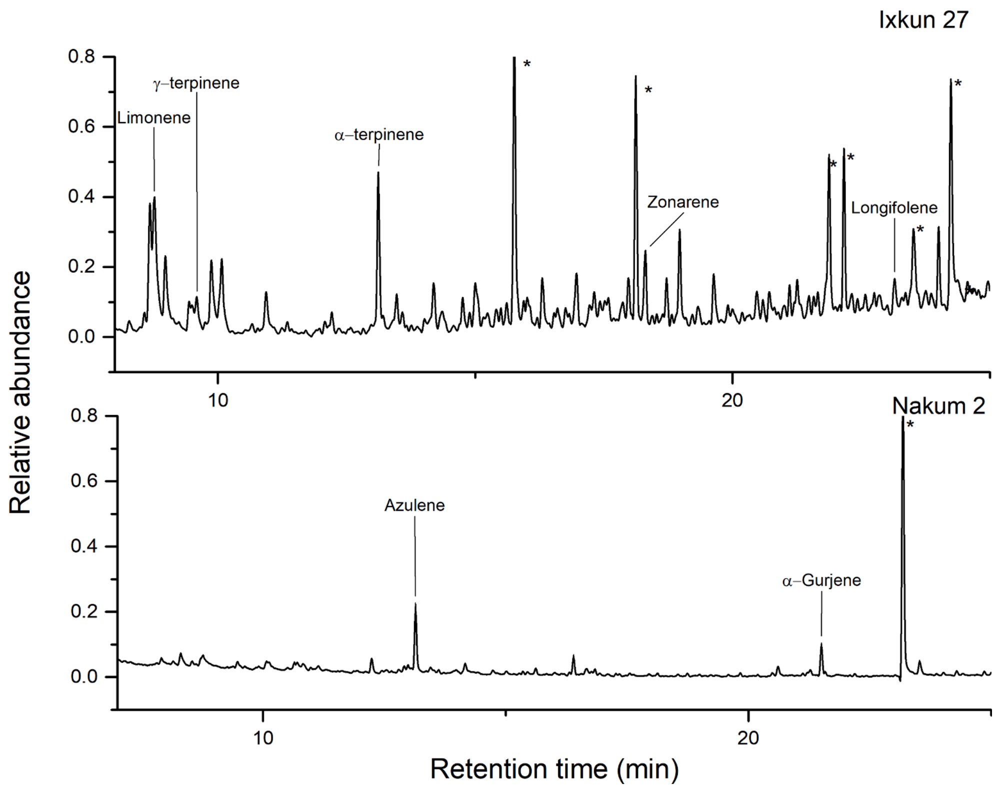

Fig. 7. Gas chromatogram of the organic fraction of Burial 27 from Ixkun and Burial 2 from Nakum, representative from Group 1 and 1 respectively, showing the names and retention times of some identified compounds in the region from 8 to 25 min. *The most common contaminants were: 3-Penten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-, (E)- (t_R = 15.7); 3-*tert*-Butyl-5-ethyltoluene (t_R = 18.11); 2-Ethyl-5-isobutylthiophen (t_R = 21.87); 4,5,6-Pyrimidinetriamine (t_R = 22.17); Diethyl Phthalate (t_R = 23.51); Spiro[cyclopropane-1,2′(1′H,4′H)-naphthalen-3′-one], 1′,1′-dimethyl-4′-phenyl- (t_R = 24.04); 2-Aminosulfonyltoluene (t_R = 24.23).

and yarrow, as well as in other fruits, barks and woods of other genera and species, including cotton seeds and members of the Pinaceae family (CheBi, 2022; Gordon, 1952).

A diversity of organic metabolites was identified as influencing the grouping of the Nakum and Ixkun Cave samples (Fig. 6b). α-gurjenene (t_R = 21.51 min) was the only sesquiterpene detected in this group, and it was present among all the samples; this metabolite is naturally found in Plagiochilaceae, Cannabaceae, and Lamiaceae, particularly in the inflorescences and leaves volatile oils (Sedano-Partida, 2018). α-gurjene acts as an antibacterial agent (CheBi Database, 2024). Likewise, this compound has been reported in plants of the genus *Protium,* also known as Burseraceae, such as *Protium neglectum* (Suárez et al., 2007). Retene (t_R = 35.8 min) is a polycyclic aromatic hydrocarbon that forms naturally through the degradation of specific bioditerpenoids when resinous woods are heated and distilled at a minimum temperature of 150 °C (Hautevelle et al., 2005; Davara et al., 2023).

Abietane-type diterpenes were also found in esterified form as methyl dehydroabietate (t_R = 37.967 min) and methyl abietate (t_R = 38.687 min; Hernández-Bolio et al., 2022:103435). These metabolites derive from dehydroabietic and abietic acid, which are characteristic of the Pinaceae family. They are produced through a particular method in which the wood is heated to obtain a resin, resulting in a syrup-like texture and high adhesion properties, which may have facilitated pigment attachment to the body (Izzo et al., 2013:597; Hernández-Bolio et al., 2023). Furthermore, these compounds have been reported as biomarkers of pinaceous resins (Hautevelle et al., 2005).

Isopropyl palmitate (t_R = 32.36) and methyl stearate (t_R = 34. 227 min) are fatty acid esters of plant origin (National Center for Biotechnology Information, 2023), which have been reported in genus like *Camellia* and *Solanum* (Torres et al. 2007); in the formulation of funerary ointments, they can be responsible of providing an oily texture, applicable to the skin (Ramírez and Vishnupad, 1993). However, given its widespread presence in modern skin products, its identification in archaeological samples should be interpreted with caution, as it may also result from contamination introduced during previous handling or conservation processes.

In general, the multiple molecules detected point to the use of two or more plant species for the formulation of fragrant ointments; particularly, it is possible to identify ingredients with adhesive properties (e.g., pine tar), which allowed for more efficient handling of the mixture, in addition to conferring odorous and coloring attributes. A significant number of metabolites with these properties have been reported in the *Pinaceae* family in botanical and ecological studies specializing in the chemical characterization of different taxa and *Pinus* organs (Coppen et al., 1988; Barnola et al., 1994; Dagne et al., 1999; Barnola and Cedaño, 2000; Uddin et al., 2008). One example is the study conducted by Coppen and collaborators (1993) on the *Pinus hondurensis* variety from the Mountain Pine Ridge region in Belize and the Poptún region in Guatemala, where several of the main compounds coincide with those reported in this study (as shown in Supplementary material 2) such as α-terpinene, methyl dehydroabietate, limonene, and trace amounts of longifolene and myrcene.

The closest source of Pinaceae is located in the Maya Mountains of Belize: *Pinus caribaea* var. Hondurensis has managed to migrate eastward from the Ejido Caoba in Quintana Roo to Central America, reaching Nicaragua and the dry central valleys of Honduras and El Salvador (Kappelle, 2006). For the Guatemalan sites, from which the samples of this study were obtained, researchers have proposed that the ancient Maya planted pines, with sites such as El Pinal in Petén serving as the primary suppliers for large cities like Tikal (Dvorak et al., 2005). Other sites reported for their pine production include the secondary centers Curucuitz and Ix Ak, which were under the influence of the

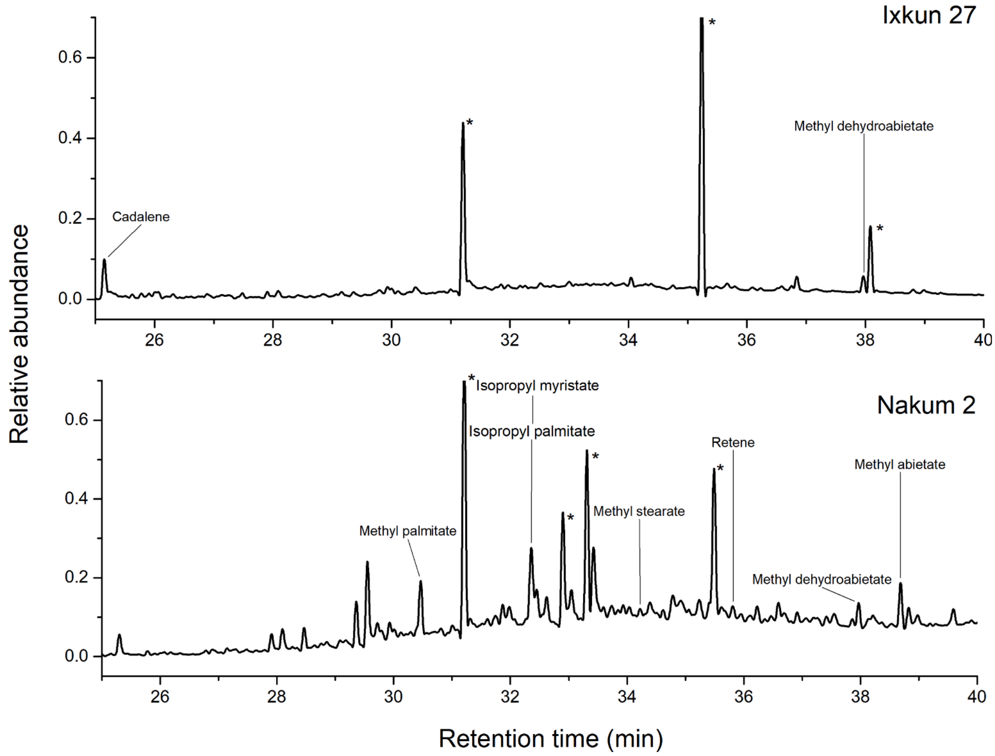

Fig. 8. Gas chromatogram of the organic fraction of Burial 27 from Ixkun and Burial 2 from Nakum, representative from Group 1 and 2, respectively, showing the names and retention times of some identified compounds in the region from 25 to 40 min. *The most common contaminants were: Phthalic acid, isobutyl octyl ester (t_R = 29.3); Carbonic acid, eicosyl vinyl ester (t_R = 31.07); Bis(acetylacetonate)iron (t_R = 32.9); p,p'-DDE (t_R = 35.2); 2-*tert*-butyl-9-amino-1,2,3,4-tetrahydroacridine (t_R = 35.4); m,p'-DDD (t_R = 38.08).

ruling center of Ixtonton (Laporte and Morales, 1994). This hypothesis is supported by the area's topography, which consists of the sediments composed of deep sticky clays where broad-leaved species proliferate, but pines do not. Thus, the presence of pine in this environment is likely the result of human intervention, although other possibilities have been explored by Harvey et al., (2019). The use of pine among the Maya is well documented. As a construction material, its use has been reported by authors such as Lentz (1991), who described a dependence on arboriculture in Copán, finding large particles of charcoal and hardwoods of *Pinus*, Angiosperms, and Arecaceae; the proportions of pine charcoal, discovered are across all areas and phases (Lentz, 1991:180). At the sites of Xunantunich, San Lorenzo, and Chan Hool in Belize, pine was used in the construction of ceremonial and residential structures (Lentz et al., 2005:574). In the Northern Lowlands, its presence was also reported by Castillo Acal et al. (2023:206), who identified pine charcoal in the refuse dump of a structure of Isla Cerritos.

Additionally, the use of Pinaceae seems to have had ritualistic connotations, serving as a fundamental element in establishing connections and exemplifying cosmological beliefs, this has been observed in the high concentrations of pine remains in the Actun Polbilche caves, Pook's Hill, Cave's Branch Cave, Actun Nak Beh, and others in the Upper Belize Valley (Morehart, 2001). The depositional characteristics of these remains are more consistent with torch debris. The use of pine as torches is logical due to the high flammability of its compounds and the volatile fractions, which make it an excellent source of light, odor, and smoke. The high concentrations lead the author to suggest that pine burning played a central role in cave use (Morehart, 2011:106). Dussol et al. (2016:54) reported 10 burials from Río Bec that were covered with a thick layer of ash composed of *Pinus*. Additionally, Chase and Chase (1998:317) found pine needles inside caches at Caracol, Belize. This confirms the presence of Pinaceae in the area, with material remains documented at various archaeological sites.

The use of pines continues today, as evidenced by ethnographic work conducted by researchers like Breedlove and Laughlin (1993), who documented the Tzotzil people of the Highlands of Chiapas and their intricate classification of pines, based on their bark, needles, and fruits. These pines have diverse uses, including construction materials, sacred purposes (such as making crosses that adorn tombs during funerals and for use in churches), and medicinal uses, such as treating spider bites, loosening teeth, and are placed around a sick person's bed to prevent chafing (Breedlove and Laughlin, 1993:182–184). Another study that discusses its current uses was conducted by Watanabe (1992) on the people of Santiago Chimaltenango, in the western Guatemalan Highlands, where the connection between lighting white candles (burning and releasing odors) and their association with corn and tortillas is mentioned, symbolizing sacrificial food for the gods. According to Houston and Taube (2000:267-273), burning and releasing smoke is linked to the fragrances of flowers in ancient times. Burning pine as a torch can be interpreted as a symbolic offering of the "breath of the soul," transforming it into a spiritual and fragrant essence, allowing the deities to consume that soul. In other words, the use of pine in the past is equivalent to the use of candles in contemporary times.

3.3. *Pigment – unguent blends for elite and commoners of the Guatemalan Petén*

Returning to status markers, it was assumed that since cinnabar is a scarce material and associated with burials of considerable richness, it would be found in those contexts with higher scores (Krejci and Culbert, 1995) than those with iron oxide. The results indicate that those samples to which cinnabar was applied have an average score of 3.75 (with 5 as the highest attainable score), while those with iron oxide had an average score of around 2.7. It is noteworthy that those with the highest scores in terms of social status come from the Nakum site (Table S2).

Thus, by integrating the results obtained from the status markers and the mineral and organic characterization, it is possible to identify two groups, as the chemometric study pointed out: the first, with higher status burials (Contexts including dynasts such as the case of burial 1, 2 and 8 of Nakum) and a geographically more remote sample belonging to Ixkun cave, where the recipe of ointments was composed of cinnabar as pigment, and plant species whose compounds are low volatile and, some of them, derived from the heating of coniferous resins thus giving it high adhesion properties. The case of Ixkun cave is particular due to its composition of iron oxide with similar low volatile molecules; and the second group, consisting of the nearby sites of Ixkun, Sacul, Ixtonton and El Chal, whose middle status burials had exclusively iron oxide and highly volatile organic molecules that, according to the literature, are present in greater abundance in the needles and inflorescences of Pinaceae (Barnola et al., 1994; Barnola and Cedaño, 2000; Kirima et al., 2020), thus suggesting the incorporation of these additional plant components to increase the aroma of the preparations. Additional molecules as sesquiterpenes and fatty acids, could indicate the presence of further plant ingredients in order to confer odor properties and increase the fluidity of the ointment (Hernández-Bolio et al., 2022). It could be observed that in all the cases the ointments, same as the human body, are constituted by heavy matter, e.g. a pigment, which might represent the *ecumene*, while the "ethereal" component or *itz,* is constituted by aromatic resins, which previously flowing through trees and flowers, is now impregnating life to the mixture and thus to the individual being embalmed.

4. Conclusions

In summary, it was documented that covering of body parts with red pigment occurred in funerary contexts whose offerings were more elaborate, and it was found in selected, high status types of burials. In the thirteen burials discussed here, a variety of cases were observed regarding the funerary context, ranging from the presence of pigmentation in caves, crypts, and burial chambers to simple cysts and garbage pits. There does not appear to be a clear pattern concerning the use of color and fragrances in relation to the type of burial, or additional characteristics such as age or gender. However, we refrain from drawing further conclusions on this matter due to the limited number of burials studied.

Regarding the relationship between the absence/presence of pigment by segment, our findings align with those reported by Pérez-López et al. (2022) as a result of pigment settling. Cinnabar was only identified at Nakum, while iron oxide was used at southeastern sites, possibly due to local availability or lower-status contexts. Organic analysis revealed two groups of metabolites—volatile and stable—indicating different preservation and application pathways. Resins from Pinaceae, confirmed by regional archaeological evidence, were central ingredients, along with compounds from at least three other plant species, suggesting complex vegetal mixtures. The multifunctionality of these substances, serving as pigment, fragrance, and excipient, reflects a sophisticated knowledge of their properties. It is proposed that if the other ingredients have not been identified, we limit ourselves to talking about the testable ingredients, and for future research, we propose the multifunctionality of an ingredient, such as Pinaceae resins, which could provide a very pleasant resinous odor and, at the same time, the flexibility to be applied to the skin. These were not static formulas with ingredients that fulfilled an exclusive function, but rather, they took advantage of the multifaceted characteristics that some organic components had, from antifungal, antibacterial to anti-inflammatory activity, of which the ancient Maya were probably aware (Hernández-Bolio et al., 2022).

In closing, we consider that the major contribution of this article lies in combining organic and inorganic characterization of ancient recipes of fragrant and pigmented ointments used by the Maya of the Petén, Guatemala, whereas previous research has focused either on the mineral or the organic characterization. In this work, we have attempted to offer a more integrated approximation to the study of colored funerary blends. We therefore hope to have broken new ground and with this more holistic approximation towards a more systematic scrutiny of sacred and intimate practices of the Maya, such as the coloring and ointments of their deceased.

CRediT authorship contribution statement

Inés Zazueta de la Toba: Formal analysis, Investigation, Writing – original draft. **Montserrat Soria Castro:** Supervision, Software, Methodology. **Patricia Quintana:** Writing – review & editing, Funding acquisition. **Vera Tiesler:** Supervision, Project administration, Funding acquisition. **Jarosław Źrałka:** Project administration. **Juan Luis Velásquez:** Project administration. **Mara Reyes:** Project administration. **Lilian Corzo:** Project administration. **Gloria I. Hernández-Bolio:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Vera Tiesler reports financial support was provided by Secretaría de Ciencia, Humanidades, Tecnología e Innovación (SECIHTI). Gloria Ivonne Hernandez-Bolio reports financial support was provided by Secretaría de Ciencia, Humanidades, Tecnología e Innovación (SECIHTI). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank to CICORR, Dr. Javier Reyes, and Dr. Mayra Manrique for the access to the XRF system, as well to M. Sc. Karla Hernández for XRF data processing. Also, to Laboratorio de Patología Acuática, at Cinvestav Unidad Mérida, in particular Dr. Leopoldina Aguirre and Dr. Víctor Vidal, who facilitated access to the laboratory and the use of the gas chromatograph, as well to Rosa Canul for her technical support. We are grateful also to Laboratorio Nacional de Nano y Biomateriales from Cinvestav Unidad Mérida for the facilities and laboratory equipment, as well as Dr. Victor Rejón for SEM analysis, M. Sc. Daniel Aguilar for XRD analysis, and M. Sc. Mario Herrera for preventive maintenance of the diffractometer. Also, thanks to Secretaría de Ciencia, Humanidades, Tecnología e Innovación (SECIHTI), which through the program "Estancias Posdoctorales por México 2022 (1), supports the Postdoctoral Position of GIHB. This research has been supported by SECIHTI through the Convocatoria Ciencia de Frontera 2019, Project number 61526 "*Understanding the sensory experience of the ancient and modern Maya. New archaeometric studies of organic food and fragrant compounds*".

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jasrep.2025.105337.

Data availability

Data will be made available on request.

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