Heritage Science Contribution to the Understanding of Meaningful Khipu Colours

Lucrezia Milillo [1,*], **Marei Hacke** [2], **Sara Norrehed** [2], **Ilaria Degano** [3], **Francesca Gherardi** [4] and **Ellinor Gunnarsson** [5]

1 Department of Social Anthropology, University of St Andrews, St Andrews KY16 9AJ, UK
2 Heritage Laboratory, Swedish National Heritage Board, 62122 Visby, Sweden
3 Department of Chemistry and Industrial Chemistry, University of Pisa, 56124 Pisa, Italy
4 Investigative Science, Fort Cumberland Laboratories Historic England, Portsmouth PO4 9LD, UK
5 Collections, National Museums of World Culture, 402 27 Gothenburg, Sweden
* Correspondence: lm335@st-andrews.ac.uk

Abstract: This work is the first scientific study of khipu dyes and inorganic mordants and auxiliaries, paving the way for a new approach to understanding khipus' meaningful materiality, technology, and colours. Khipus have usually been described as "Andean knotted records", but they are much more than complex knotted cords: a great part of the information encoded resides in khipus' incredible colours. The objects of this study are two Wari khipus, 1932.08.0001 and 1932.08.0002, now at the Museum of World Culture in Gothenburg, Sweden. After a morphological study of the khipus, the objects were imaged with multiband imaging (MBI) as an aid for the sampling decisional process. The khipus were then analysed non-invasively by X-ray fluorescence (XRF) spectroscopy on selected areas of particular interest. The khipus were consequently sampled for elemental characterisation by micro-XRF, and liquid chromatography coupled with high-resolution mass spectrometry (HPLC–HRMS) for characterising the organic dye composition. This paper presents a part of the results of the project "Meaningful materials in the khipu code", with the intent to shed light on the difficulties and possibilities of investigating khipu colours and dyestuffs. MBI and XRF revealed unforeseeable structural characteristics, such as remnants from a heavily degraded thread in an area of missing thread wrapping and a dual-coloured thread that was previously deemed single-coloured. The organic dyes identified by HPLC–HRMS comprised indigoids, cochineal, and an unknown flavonoid-based dyestuff. XRF of the inorganic components revealed associations of several elements with specific colours.

Keywords: khipu; Wari; textiles; dyestuffs; heritage science; multiband imaging; X-ray fluorescence; high-performance liquid chromatography; mass spectrometry

1. Introduction

Khipus are a uniquely Andean three-dimensional communication medium made of colourful knotted cords. Khipus were interpreted through sight and touch. The Incas used them to encode information regarding the census, calendars, and accounting [1] using a decimal positional system of knots [2–4].

What is still missing is a full understanding of how the qualitative information was encoded (that is, "what are these numbers counting?"). Moreover, there are extensive chroniclers' testimonies that khipus were used for narrating stories and Inca history too: how narrative information was registered in the khipu code is still obscure [5–8]. With the advent of data science, urgent calls for digitisation and computational analysis of khipus are raised in order to decipher khipu writing [9].

The way the cords were manufactured and organised in relation to each other would convey meaning through fibre type and colour [10], ply direction [11], and possibly also

knot attachment [12,13]. Recent research disclosed relational meanings of colourful cords [6(165-166)-14-16], yet, prior to this study, no physical–chemical analyses of colourants had been conducted on Andean khipus. Colour remains largely uncharted territory: taphonomic processes and conservation practices have surely altered the original khipu colours, and, with that, our possibility to understand a vast part of khipus' meaning. No standard protocol for recording khipu colours has been developed to date that meets the extreme variability in khipu colours.

Chroniclers from the 16th and 17th centuries also often stress the importance of colour for recording qualitative information on Inca khipus [6] (pp. 165–166), [14–16] (pp. 359–361; 152; 155). However, we have no information about the dyestuffs and mordants for conferring meaningful colours to Andean khipus. Given the lack of this knowledge, many issues can be raised regarding the understanding of colour patterns and colour meaning of archaeological khipus. Factors such as soil composition, archaeological context, and later treatments in collection contexts have surely affected dyestuffs in different ways.

Knowing more about khipu dyes will enhance our understanding of meaningful khipu colours. By extension, it will also open new ways to investigate the meaning that may emerge from the process of khipu-making. What is more, dyes might have been relevant to the khipu code not only for the colourful result they produced on khipu cords but also for the socially produced significance of the dyestuff material and/or the potentially meaningful way of handling or processing required for colouring the threads (an example of this type of study in a Mesoamerican context can be found in [17,18]).

In this work, we will present the analyses carried out on two Wari khipus at the Museum of World Culture, Gothenburg. The Wari were a south-central Andean and coastal civilisation that flourished from about 500 to 1000 AD. Wari khipus are today believed to be the forefathers of the Inca khipu technological tradition, and they display unique features, such as thicker cords and smaller dimensions, no decimal organisation of knots, and, most importantly, complex and colourful thread wrappings, which are believed to store most of the Wari khipus' information [19–22]. Future comparisons of Wari and Inca khipu dyestuffs might tell more regarding the shared cultural trait of thread wrapping and the evolution of khipu technology.

The organic and inorganic components of khipu 1932.08.0001 and 1932.08.0002 were characterised following an analytical approach that implemented multiband imaging (MBI), X-ray fluorescence spectroscopy (XRF), and liquid chromatography coupled with high-resolution mass spectrometry (HPLC–HRMS).

2. Provenance

A collection of objects, including khipus 1932.08.001 and 1932.08.0002, arrived in Gothenburg in 1932. It was donated by Swedish consul Sven Karrel to the ethnographic department of the Gothenburg Museum, which, at that moment, was directed by Erland Nordenskiöld. In the catalogue, the only reference to provenance is "Nasca" [23] so far.

3. Description of the Khipus

In this section, khipus will be briefly described in order to present the key that is used to identify the areas where the analyses have been carried out. In Table 1, the colours of khipu 1932.08.0001 and 1932.08.0002 are listed. A Pantone® extended gamut coated guide has been used to classify khipu colours by juxtaposition of the chart to the khipu. A descriptive colour name is listed from the closest hexadecimal colour identified through the Encycolorpedia search engine [24]. For ease of legibility, short colour terms will be used in this article. Representation of the khipu cord structures is provided in the Supplementary Materials (Tables S1 and S2).

Table 1. Table of colours found on khipu 1932.08.0001 and 1932.08.0002.

Pantone® Colour Code	Closely Related Descriptive Colour	Short Colour Term	Material	1932.08.0002	1932.08.0001
*	Ivory	Ivory	Cotton	x	x
468	Durian white	Ecru	Cotton	x	x
4685	Manila	Ecru	Cotton	x	x
463	Violin brown	Brown	Cotton	x	x
7505	Coyote brown	Brown	Cotton	x	x
464	Bronze	Light Brown	Cotton		x
465	Camel	Yellow	Cotton		x
467	Burlywood	Beige	Cotton		x
2311	Tumbleweed	Beige	Cotton		x
201	Royal red	Red	Wool	x	x
7613	Old rose	Pink	Cotton	x	x
7612	Tuscany-Antique brass	Pink	Cotton	x	x
442	Ash grey	Blue	Cotton		
5497	Morning blue	Blue	Cotton	x	x
7538	Dolphin grey	Blue	Cotton		
455	Antique bronze	Green	Cotton	x	
*	Black	Black	Cotton	x	x

* no correspondence found.

Registering visually perceived colours is a qualitative process subjected to several influential factors, such as light source, background, eye tiredness, and cultural conceptualisation of colour [25]. For this reason, the specific hues do not have to be considered absolute and are only meaningful in the context of this specific study. Even though the same colour chart was used to register colours of other khipus in different museums, visually perceived colours registered in different environmental conditions can only be compared on an approximate and qualitative level but not for quantitative or statistical studies. This is also why study of khipu colours needs to be sustained by dye analysis.

4. Khipu 1932.08.0002

Khipu 1932.08.0002 presents a loop and branched Wari khipu morphology (for a description of morphological Wari khipu types, see [21]) (Figure 1). To a primary cord looped in a circle, only one pendant (P_1) is attached. P_1 hosts eight subsidiary cords (P_1s_1–P_1s_8), the last of which also hosts eight subsidiary cords of second level ($P_1s_8s_1$–$P_1s_8s_8$). P_1s_6, P_1s_7, $P_1s_8s_7$, and $P_1s_8s_8$ also host one subsidiary cord each. All twenty-two cords are made of cotton except the red threads, which are made of animal fibre. Colour patterns on this khipu include solid (monochrome) cords, mottled, and typical Wari-style segmented cords—which means that one cord starts solid and ends barber pole [21].

Figure 1. Khipu 1932.08.0002. The scale bars in the colour chart are 5 cm. The khipu as laid out in this photograph is approximately 30 cm × 15 cm.

Similar colours are present on the thread wrappings around P_1 and P_1s_8. The wrapping on P_1 presents eight distinct bands, while the wrapping on P_1s_8 has six bands (Figure 2).

Figure 2. Left photo: wrapping on 1932.08.0002 P_1; **right photo**: wrapping on 1932.08.0002 P_1s_8.

5. Khipu 1932.08.0001

Khipu 1932.08.0001 also presents a loop and pendant Wari khipu morphology (Figure 3). To a primary cord looped in a circle, four pendants (P_1–P_4) are attached. While the first two pendants host, respectively, eight and nine subsidiaries, the last two pendants do not have any subsidiary cord at all.

Figure 3. Khipu 1932.08.0001. The scale bars in the colour chart are 5 cm. The khipu as laid out in this photograph is approximately 42 cm × 35 cm.

The khipu is made of a total of thirty-three cords (pendants and subsidiaries), all of which are made of cotton.

The colour patterns on this khipu include solid (monochrome), mottled, barber pole, and segmented cords.

Only two subsidiaries are segmented ($P_2s_8s_1$, $P_2s_9s_1$), and both are segmented in Wari style. The first one is highly deteriorated right after the segmentation, which makes it possible to see the structure of Wari segmentation's construction (Figure 4).

Figure 4. 1932.08.0001 $P_2s_8s_1$, segmentation structure visible due to cord deterioration. The upper half of the cord (right) is monochrome. Half-way through the cord, a brown thread is attached. This allows to obtain the lower half with barber pole pattern. The cord length in this photograph is approximately 2 cm.

On khipu 1932.08.0001, there are eight sequences of thread wrappings: four almost identical sequences are on top of the four pendant cords; two similar ones are on the second-to-last subsidiary cords of P_1 and P_2 (P_1s_7 and P_2s_8); another pair of similar thread wrappings are on P_1s_8 and P_2s_9; and one is on P_1s_5 in correspondence to the first simple knot (Figure 5).

Figure 5. 1932.08.0001 $P_1s_5K_1$, wrapped single knot with red dyed animal fibre thread. The knot is approximately 8 mm in length.

All the wrappings on cords begin right below the point of attachment of the cord, with two interesting exceptions: P_1s_7 and P_2s_8. The wrapping sequence is ecru–pink–ecru in the first one and ecru–pink in the latter. It is likely that the wrapping on P_2s_8 would have had the same sequence as P_1s_7 because the loose ends that both wrappings terminate with are a sign of unravelling. Careful morphological study noticed some black material at the top of

both thread wrappings (Figure 6). We hypothesise, and will confirm later in this article, that other extra bands were present at the top (and possibly also at the bottom) of these two thread wrappings so that the band sequence for each of them would originally have been black–ecru–pink–ecru–black. The questions raised by the observation of the black material at the top of these two thread wrappings guided the research team in mapping P_2s_8 by XRF.

Figure 6. Black material visible at the very top of 1932.08.0001 P_1s_7 (**left photo**) and 1932.08.0001 P_2s_8 (**right photo**). The wrapping sections in the photographs are approximately 1 cm to 1.5 cm long.

6. Sampling

Investigation of the thread wrappings, the most meaningful aspect of Wari khipus, was at the centre of this research. However, it was not possible to take samples from thread wrappings because this would have heavily compromised the structural stability of the khipus. Samples for colour analysis were only taken from khipu 1932.08.0002. This is because, compared to 1932.08.0001, 1932.08.0002 has more pendants presenting colours corresponding to those in the thread wrappings. The assumption is that, by studying the colours of the pendants in 1932.08.0002, we can also expand our knowledge about thread wrappings on the same khipu. Given the shared origin of the two khipus, the analyses of samples from pendants of 1932.08.0002 are also considered to shed light on the thread wrappings on pendants of khipu 1932.08.0001. See Supplementary Materials Table S3 for a list of the wrapping bands from the two khipus with colours corresponding to the samples taken in this study.

Samples were cut off by pendant cords in areas with loose ends. On some occasions, pendants can terminate both with loose ends and with loops, which are the result of the multiple plies of threads the cord has been manufactured with. Cutting out the looped segments of a khipu pendant end prevents observation and study of the cord structure, and, for this reason, this option is not desirable from a conservation ethics point of view. The sampling areas were selected based on the morphological study, as well as the observations we could make on account of what emerged from the technical photography executed on the whole khipu.

Seven samples were taken from khipu 1932.08.0002; each one is between 6 and 10 mm (Figure 7 and Supplementary Materials Table S3). These samples were then photographed again in comparison to other khipu samples within the broader research project "Meaningful materials in the khipu code".
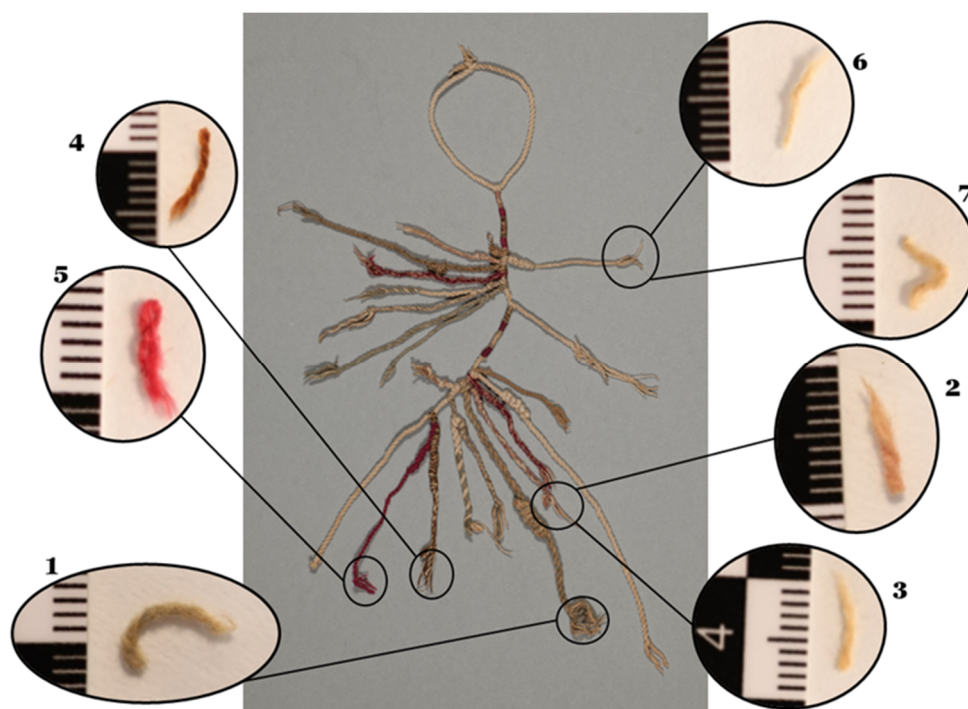
Figure 7. Samples (1–7) collected from khipu 1932.08.0002. The khipu as laid out in this photograph is approximately 30 cm × 15 cm.

Both khipus contained small areas or remnants of black threads, which could not be sampled. Furthermore, various shades of ecru, light brown, yellow, and green were also not sampled.

7. Materials and Methods

7.1. Multiband Imaging

Multiband imaging (MBI) is a photographic method that encompasses a set of light and filter combinations to spatially visualise the different components of an object. Depending on the chemical nature of a material, its reflection and luminescence behaviour will generate different responses in the created images. Use of MBI can be especially helpful when designing a sampling strategy. Other useful aspects can be visualising surface coatings, staining, restorations, or patterns. Multiband imaging is an indicative method, and interpretation should be completed with the knowledge that mixtures of dyes, different mordants, or residues from soil or dirt can affect the results. However, even if MBI needs to be accompanied by other analytical methods, it provides initial insight and imagery that can guide the sampling process and be of great use to communicate and visualise results from the accompanying methods.

A crucial part of creating photographic material that can be referenced and compared to other work outside this study was to employ a systematic approach. Therefore, the setup and postprocessing follow the guidelines described in "Multispectral Imaging in Reflectance and Photo-induced Luminescence modes: a User Manual" [26]. In this study, the khipus and samples were imaged with visible (VIS), infrared (IR), and ultraviolet (UV) light. Using filters, either UV or IR reflectance, and luminescence were registered (UVL, UVR, and IRR). By combining VIS with IRR, false colour (IRFC) images were created [26,27]. Multiband reflectance subtraction (MBR) was used to investigate the presence of indigo; the method encompasses capturing two images at narrow bandwidth and calculating the difference [28].

The main purpose of using MBI on the khipus was to investigate the different visual responses from different dyes and colourants. Using photographic techniques on archaeological or historical textiles has previously been shown to be successful [29–31].

The khipus were photographed in situ at the magazine storage area of the Museum of World Culture in Gothenburg to guide the sampling decisions. In order not to stress the junctions of the khipus, they were left on their original paperboard support. Unfortunately, the paperboard had inherent luminescence in the ultraviolet region, therefore not providing the best visualisation of the khipus in their entirety. A filter paper could be slid under a smaller specifically interesting area to improve visualisation. In addition, the khipus were not flat, but, again, the decision was made not to interfere with the khipu structure, for example, by using pins to flatten the objects, and, therefore, some shadows in the images had to be accepted. Despite these practical difficulties arising from the real-life situations that now and then appear in this field of research, imaging the objects provided useful guidance for the study.

All images were acquired using a modified full spectrum Nikon D810 camera (IR-UV-blocking filter removed) with a Nikon AF Nikkor 50 mm lens. The objects were placed approximately 100 cm (whole khipu) or 50 cm (sections and samples) from the camera. Two identical light sources were placed symmetrically at approximately 45 degree angle toward the objects. Depending on the image, different light sources were used, and filters were mounted on the camera lens (Table 2). All filters used were from the Cultural Heritage Science Open Source (CHSOS) Technical photography Robertina filter set [32] or Multispectral Antonello filter set [33]. An X-rite Macbeth chart and a white Spectralon diffuse reflectance standard (99%) were used for image calibration. A white non-luminescent homogeneous paper board was used for flat-fielding. Postprocessing was completed following the guidelines proposed in the user manual and by using nip2 software [26]. The method for infrared false colour was adapted to Adobe Photoshop from the principles in the manual [26] following personal communication with J. Dyer (12 October 2022).

Table 2. Summary of light sources and filters used for different images. See Supplementary Materials for specifications.

Image	Light Source (Set of Two)	Filter in Front of Camera
VIS	Godox LED1000 II	UV-IR blocking bandpass, c. 400–700 nm
UVL	Engelbrecht UVA-366 black lights	UV-IR blocking bandpass, c. 400–700 nm
IRR	IR LED Synergy 21 M30205	IR cut-on, c. 800 nm cut-on, 25% transmittance at 900 nm
MBR	Kaiser Studiolight 1000 with an Osram halogen Superphot 1000 W bulb	Bandpass, 630 ± 5 nm + 740 ± 5 nm

7.2. XRF

The two khipus, 1932.08.0001 and 1932.08.0002, were analysed at the magazine storage area of the Museum of World Culture in Gothenburg. XRF point analysis and maps were collected using a Bruker Artax 800 instrument with molybdenum tube at 50 kV and 600 µA in air atmosphere. A capillary lens provided a spot size of <100 µm. A stack of at least four pure cellulose filter papers was slid under each area of analysis, which provided a blank background of Bremsstrahlung. Software Bruker ARTAX version 7.8.2.0 was used to control the XRF instrument as well as to evaluate the data. Automatic corrections were performed for escape peaks and background using cycle setting 1. Details of the analysed areas, scan time per point, number of measurement points, spot distance, scan area, and total scan time are listed in Supplementary Materials Table S4. Selected results are discussed in this manuscript, and a report of all XRF results from the in-situ analyses is available from Zenodo [34].

The samples collected from khipu 1932.08.0002 were analysed at Fort Cumberland laboratories, Historic England (Portsmouth, UK). The samples were mounted on filtered paper and covered with a nylon net to fix them to the support. The area was mapped by micro-XRF using a Bruker M4 Tornado µ-XRF spectrometer. Elemental maps were collected

at 50 kV and 200 μA with a vacuum, spot distance 50 μm, measure time 5 mm/s for three cycles. Cotton fibres (samples 1, 2, 3, 4, 6, and 7) and animal fibre (sample 5) were analysed in 2 distinctive maps. Separate accumulated spectra were collected from each thread from an area of about 4.5 mm^2. The intensity and net area of the peaks and the maps provide semi-quantitative data about the elements detected, assuming constant thickness of the threads across the investigated areas. Software Bruker M4 Tornado was used for data processing.

7.3. HPLC–HRMS

HPLC-ESI-Q-ToF MS analyses were carried out using an Agilent 1200 Infinity HPLC with a Quadrupole-Time of Flight tandem mass spectrometer 6530 Infinity Q-ToF detector with a Jet Stream ESI interface (Agilent Technologies, Santa Clara, CA, USA). The ESI operating conditions were the following: drying gas (N2, purity >98%): 350 °C and 10 L/min; capillary voltage 4.5 KV; nebuliser gas 35 psig; sheath gas (N2, purity >98%): 375 °C and 11 L/min. The nozzle, skimmer, and octapole were set at 1000 V, 65 V, and 750 V, respectively. High-resolution MS and MS/MS spectra were acquired both in negative and positive mode in the range 100–1000 m/z with a scan rate of 1.04 spectra/sec, employing AutoMS/MS acquisition mode (1 precursor per cycle, CID voltage for tandem mass spectra 30 V, collision gas N2, purity 99.999%, FWHM (full width half maximum) of quadrupole mass bandpass used during MS/MS precursor isolation 4 m/z). The mass spectrometer was calibrated daily using Agilent tuning mix HP0321. MassHunter® Workstation Software (B.04.00) was used to carry out mass spectrometer control, data acquisition, and data analysis.

For the chromatographic separation, an Agilent Poroshell 120 EC-C18 was used (3.0 × 75 mm, particle size 2.7 μm,) with a pre-column Zorbax (4.6 × 12.5 mm, particle size 5 μm), both Agilent Technologies (Palo Alto, CA, USA). The injection volume was 5 μL for the extracts of samples 4 and 5 and 15 μL for the extracts of samples 1, 2, 3, 6, and 7.

The two eluent solutions were: A: formic acid (FA, 0.1% *v/v*) in bi-distilled water; B: FA (0.1% *v/v*) in acetonitrile (ACN, HPLC grade). The elution program started at 15% B, held for 2.6 min, and then a linear gradient to 50% B was applied in 13 min, then to 70% B in 5.2 min and to 100% B in 0.5 min and then held for 6.7 min; re-equilibration time took 11 min. The flow rate was 0.4 mL/min.

Details on chemicals and sample treatments can be found in the Supplementary Materials section on HPLC–HRMS.

Data interpretation was based on comparison with reference materials available in the laboratory and literature data on chromatographic analyses of Peruvian textiles and dyestuffs.

8. Results and Discussion

The results are organised showing first the results of the multiband photography, then discussing the XRF mapping and then the organic dyes.

A summary of the results is reported in Table 3.

Table 3. Summary of all results from XRF, MBI, and HPLC–HRMS analyses. XRF results in bold indicate significant levels of the element associated with the colourant and are confirmed by both XRF of samples and XRF in situ. All samples are made of cotton, with exception of sample 5, which is made of wool.

Colour	Sample	XRF Areas In Situ 1932.08.0002	XRF Areas In Situ 1932.08.0001	MBI Samples and In Situ	XRF Samples and In Situ	HPLC–HRMS Samples
blue	1	P_1s_8—Band 1, 3, 5	P_1_Band 3, 5, 6 P_2s_9—Band 1	UVL-dark MBR-bright IRRFC-red	**Ca**, **Cl**, Fe, Cu	Indigo
pink	2	P_1_Band 1, 2, 3 P_1s_8—Band 4	P_1_Band 1 P_2s_8_Band 2 P_2s_9_Band 3	UVL-dark IRRFC-orange–yellow	Fe, Cl, K	Cochineal
ivory	3	P_1_Band 2 P_1s_8—Band 4	P_1_Band 1, 3, 6 P_2s_8_Band 1 P_2s_9_Band 2	UVL-light IRRFC-white		Blank
brown	4	P_1s_8—Band 1		UVL-very dark IRRFC-orange	**K**	Cochineal (very weak)
red	5	P_1s_8—Band 2, 6	P_1_Band 4, 6	UVL-very dark purple IRRFC-orange UVFC-green	Ca	Cochineal (most intense) plus unknown anthraquinone
ivory	6	P_1_Band 2 P_1s_8—Band 4	P_1_Band 1, 3, 6 P_2s_8—Band 1 P_2s_9_Band 2	UVL-light IRRFC-white		Blank
ecru	7	Point analyses P_1s_2	\	UVL-dark IRRFC-yellow	Ca, Cl, Fe, K	Flavonoid dye based on rutin, luteolin, and luteolin-O-glucosides
black pink		P_1	P_2s_8 P_2s_8_Band 2		**Fe**, **Mn**, **K** S, Sn	

8.1. Multiband Imaging

Multiband imaging provided useful documentation of the objects. The techniques that specifically contributed to the sampling strategy and understanding of the khipus during this study were UVL, IRRFC, and MBR (for indigo). Some complementary images are included in the Supplementary Materials (Figures S1–S8).

In visible light, some cords of khipu 1932.08.0002 appear undyed. The UVL image, however, revealed P_1s_2 as a two-coloured cord (Figure 8). Consequently, XRF point analysis was performed on this cord and one sample from each of the two different coloured threads was taken. One of the threads (sample 7) was shown by HPLC to contain a flavonoid-based dye-stuff. The UVL image overall improved visualization of two-coloured threads, enhancing the difference between the colours.
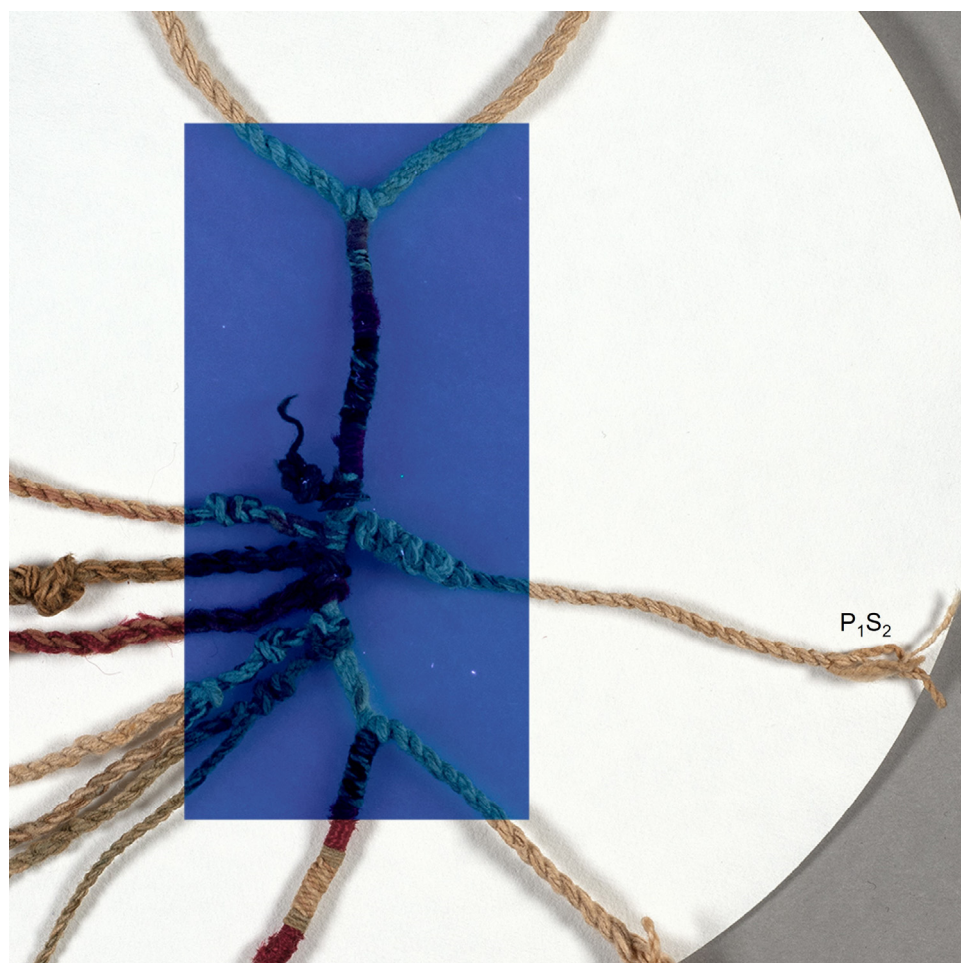
Figure 8. Section of khipu 1932.08.0002 in VIS and UVL (overlay) where UVL helps to visualise the differently coloured threads. Cord P_1s_2 appears undyed in visible light, but UV light clearly shows there are two different threads. The khipu section is approximately 11 cm × 11 cm.

The transparent and loose structure of the samples imposed some difficulty in interpretation as shadows and reflected luminescence in some respect hindered clear visualisation.

Infrared false colour images (IRRFC) can be helpful to differentiate colours that look similar in visible light (Figure 9). Samples 2, 3, 6, and 7 are all similar with different tints in the VIS image: 2 is slightly pink; 7 is slightly ecru. In the false colour image, these samples become more distinguishable. Sample 2 becomes light orange–yellow, 3 and 6 become white, and 7 becomes light yellow. Samples 3 and 6 had the same beige tint in the visible image and were rendered white in the false colour image. HPLC indicated that both samples were undyed. Samples 2, 4, and 5 were all found to be dyed with cochineal but have different colours in the visible image—pink, red, and brown. In the false colour image, sample 2 turns orange–yellow and 4 and 5 turn orange. Even though this might imply that these samples have something in common, this also highlights that one needs to be careful in interpretation. HPLC and XRF showed that, even though these samples all contain cochineal, there are differences in elemental composition. In addition, sample 5 contains an unknown anthraquinone. Sample 1 goes from blue to red in the infrared colour image, implying that it could have been dyed with indigo. Indigo can also be visualised in the MBR image, where the presence of indigo appears bright. In Figure 9, all samples that contain indigo are marked with "*" for clarity, but only samples 1–7 from khipu 1932.08.0002 are discussed in this manuscript. Indigo in sample 1 was also confirmed by HPLC.
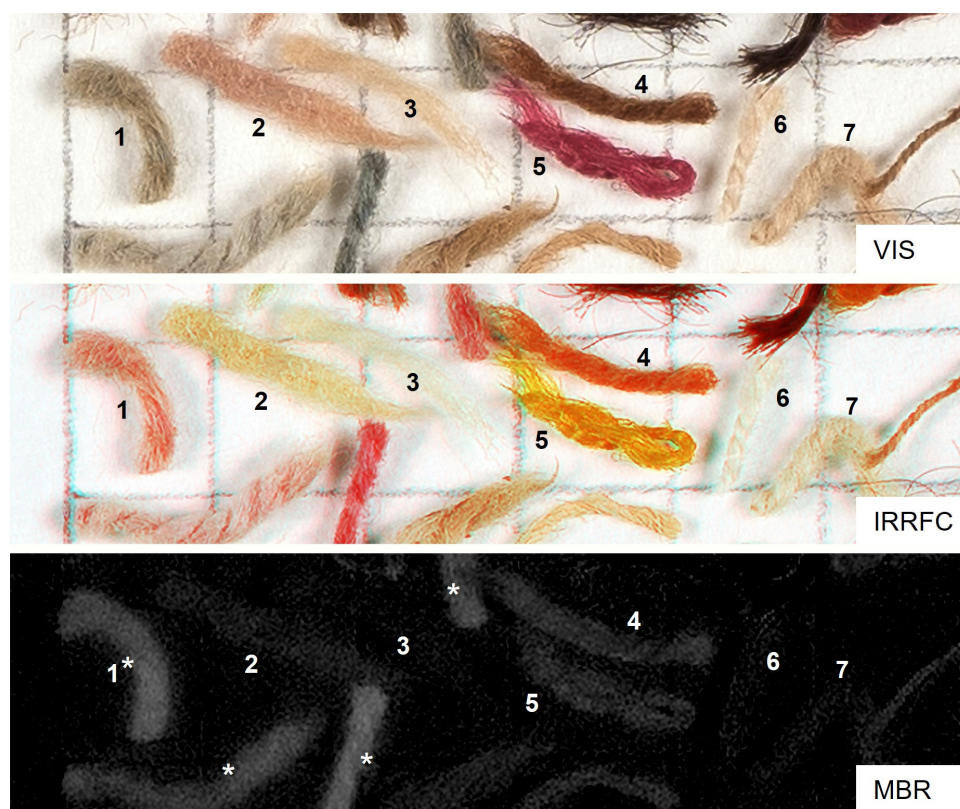
Figure 9. Samples 1–7 from khipu 1932.08.0002 in VIS, IRRFC, and MBR placed on a 5 × 5 mm grid. The MBR image shows the difference between reflectance at 630 and 740 nm; bright areas suggest the presence of indigo. Asterisk marks are placed on samples where indigo was identified by HPLC; for clarity, also samples not discussed in this manuscript are marked.

8.2. Analysis of the Inorganic Components

Elemental maps were acquired by portable micro-XRF to evaluate the distribution of inorganic components used as colorants, mordants, or dyeing auxiliaries in proteinaceous and cellulosic fibres. Evaluation of the presence of mordants and dyeing auxiliaries in archaeological textiles is complex as elemental contamination can generate misleading results.

The greyscale maps show the distribution of each element in the samples. The areas where an element is present in highest concentration (high peak area in the spectrum) are bright, while, in dark grey or black areas, the element is in low concentration or not present (low peak area in the spectrum or absence of the peak).

The XRF instrument used in the magazine storage area of the Museum of World Culture in Gothenburg for in situ mapping on khipu areas has the limitation that elements of relatively light atomic weight may be missed due to the air gap between the analysed area and the detector. This means that the instrument cannot reliably inform on the presence or absence of aluminium. Detection of alum, being historically the most important mordant, is of special interest. The benchtop micro-XRF instrument used for mapping khipu samples has the advantage that measurements can be carried out in a vacuum, thereby enabling detection of lighter elements.

XRF maps on the khipus in situ reveal the presence of several elements, such as S, Cl, K, Ca, Ti, Mn, Fe, Cu, Zn, Hg, and Br (Figure 10). The XRF spectra in vacuum show several additional elements of lighter atomic weight: Al, Si, and P (Figures 11–13). The following paragraphs discuss each of the detected elements to place the results obtained from XRF in situ on khipu areas and XRF of khipu samples in a context of possible auxiliaries and mordants used in conjunction with textile dyes.
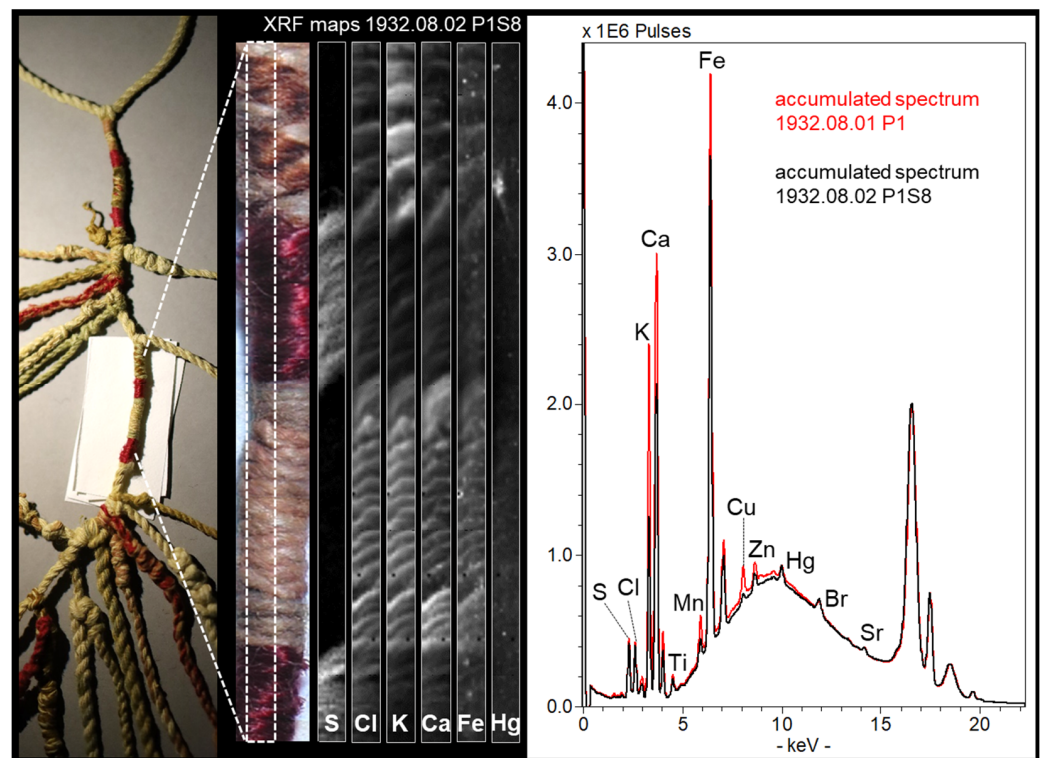
Figure 10. XRF maps of elements that showed differences in distribution from area P_1s_8 on khipu 1932.08.0002. Map size is 24 mm × 0.9 mm. Accumulated spectra of the XRF maps of the comparable wrappings (see Figure 2) from the two different khipus, 1932.08.0001 P_1 and 1932.08.0002 P_1s_8. The similarity between the two accumulated spectra provides further evidence that the two khipus are a pair and that results from one can inform on the other (see Supplementary Materials Table S3).
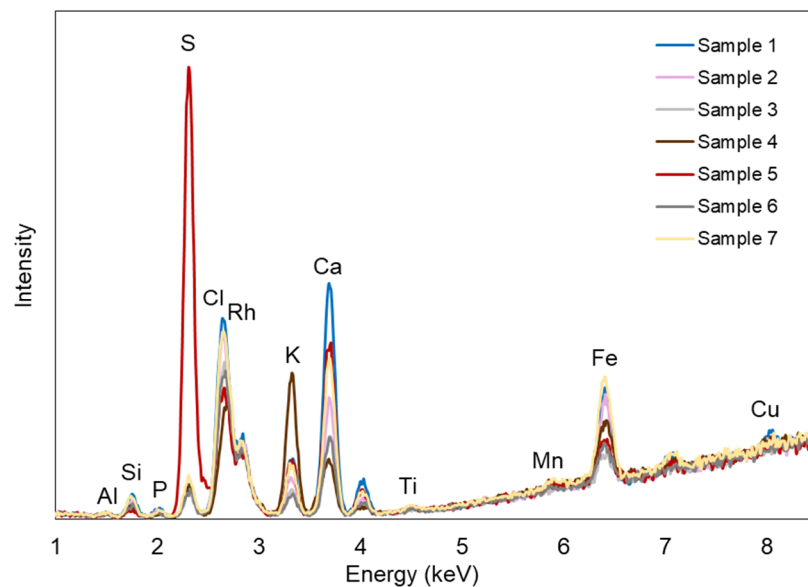
Figure 11. Accumulated spectra collected from an area of about 4.5 mm2 from each thread sample of khipu 1932.08.0002.
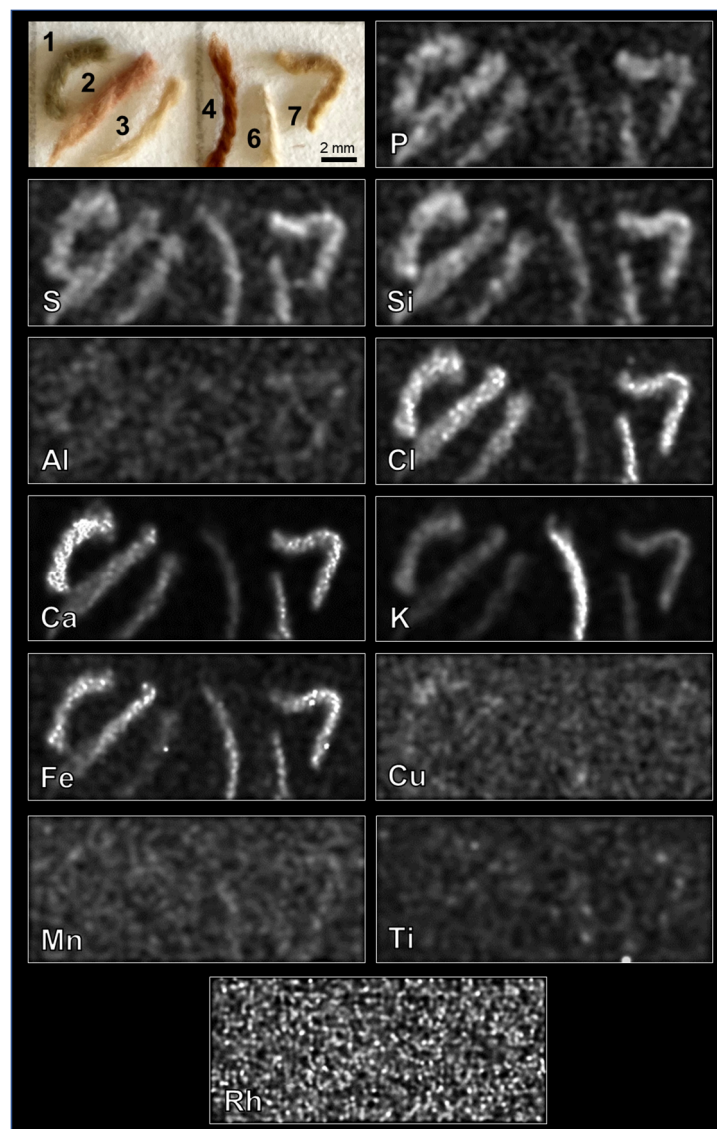
Figure 12. XRF maps of phosphorous, sulphur, silicon, aluminium, chlorine, calcium, potassium, iron, copper, manganese, titanium, and rhodium (rhodium anode of the X-ray tube) measured in the cotton samples (from left to right: sample 1–sample 7) from khipu 1932.08.0002.
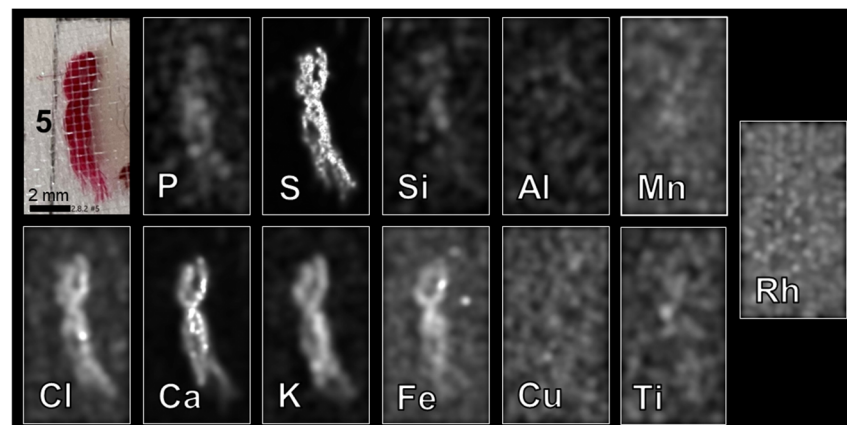
Figure 13. XRF maps of phosphorous, sulphur, silicon, aluminium, manganese, chlorine, calcium, potassium, iron, copper, titanium, and rhodium (rhodium anode of the X-ray tube) measured in the animal fibre sample from khipu 1932.08.0002.

8.2.1. Aluminium

Aluminium was not detected by XRF in situ on khipu areas, while the analysis in vacuum carried out on thread samples allowed its detection. The accumulated spectra of all the threads exhibit the peak for aluminium; however, no differences in intensities between the undyed and dyed threads were shown (Figure 11).

Aluminium is found in alum salts, which are the most common mordants for textiles. According to the literature, considering that silicon is the most common contaminant when the ratio between the heights of Al and Si signals is higher than 0.5, alum salts were probably used as mordants [35,36]. In these thread samples, the Al/Si ratio was too variable, preventing conclusive information from being obtained.

Peru has natural resources of alum salts (e.g., aluminium potassium sulphate) [37,38], and the hypothesis is that they were used as mordants in the highly advanced textile technologies of the pre-Columbian civilisations. This was proven analytically in 1913 by ashing samples of Paracas textiles [39]. Valette found silicates of lime and aluminium associated with cochineal dyed fibres. Other inorganic elements identified by Valette were significant amounts of lime in the ashes of blues and greens dyed with indigo, ferruginous clay (yellow ochre) for some yellow fibres, and notable amounts of iron in some browns. Valette suggested that the mordanting may have been carried out by suspension of aluminium-containing clay in water. Fester, instead, identified sulphate ions in the fibres (analytical method not stated), suggesting that the mordant was more likely in the form of aluminium sulphate or aluminium potassium sulphate [38].

It Is known that alum was used as a mordant In ancient Egyptian and also Roman textiles and the processes and effects of alum and other mordants were described by Pliny the Elder [40] (pp. 183–186). The history of alum in Europe, Asia Minor, and the ancient Roman and Egyptian empires has been described by several authors [41–44], but historical accounts of the use of alum in the Americas are scarce. Nevertheless, Fester [38] reports that 17th-century chronicler Bernabé Cobo provides an account of several kinds of alum present in Peru ([38] citing Antonio Raimondi, who wrote about alum sources in Minerales del Perú, Lima 1939, supplement to first edition 1878). However, contrary to what Fester reports, Cobo mentions use of alum for dyeing purposes only with reference to Mesoamerica: «De Piedra alumbre se hallan en estas Indias cuatro ó cinco especies. En la Nueva España lo hacen muy blanco, lúcido y trasparente, del cual usan los tintoreros en sus tintes [. . .] («In these Indies [Peru] there are four or five types of alum stones. In New Spain [which included contemporary central and south USA, Mexico and most of Mesoamerica until Costa Rica. The author most likely refers to the Mesoamerican area] they make it very white, shiny and transparent, [. . .]». Translation of the author) [45]. In the extensive work by Ana Roquero "Tintes y tintoreros de America", she mentions "kollpa", the Quechua name for aluminium potassium sulphate ("Piedra alumbre" in spoken Spanish), but there is no mention of chroniclers that refer to Viceroyalty of Peru ([46] (p. 91).

8.2.2. Silicon and Phosphorus

Silicon and phosphorus were not detected by XRF in situ on khipu areas. XRF in vacuum on khipu samples enabled detection of silicon and phosphorus, where the maps for each element showed comparable signal intensities among the samples.

8.2.3. Sulphur

Sulphur was detected by both XRF techniques and is always highest in animal fibres due to the presence of sulphur in the proteins of the fibres (red areas in Figure 10 and sample 5 in Figure 11)

XRF mapping of khipu areas or samples of cotton allowed some differences in sulphur content to be detected between differently coloured threads (Figure 12).

8.2.4. Chlorine

XRF maps of the khipu areas in situ indicate that chlorine is generally higher in cotton compared to wool and is often highest in blue areas. The chlorine signal in analysis of thread samples is also highest in the blue (sample 1) and significant in the pink (sample 2). The other dyed and undyed samples all contain chlorine but at lower levels (Figure 11).

Chlorine can be associated with use of a salt as mordant or alkaline additive to the dyeing bath. The fact that the undyed samples contain chlorine may indicate that they were treated with a salt but left undyed or that they were dyed with a fugitive dye or that chlorine is inherently present in the cotton source fibres.

8.2.5. Potassium

The signal for potassium was relatively high for all khipu areas and the khipu samples. Figure 14 shows that potassium was highest in the yellow and pink areas; the same observation was made for the ecru threads in the map on 1932.08.0001 P_2s_2 blue-ecru cord. Figure 15 shows that the pink threads in map 1932.08.0001 P_2s_8 ivory–pink wrapping are higher in potassium than the ivory threads. The pink threads are also higher in sulphur, tin, and manganese. No sample was available for dye analysis. Figure 10, the XRF map of 1932.08.0002 P_1s_8 multicoloured wrapping, shows potassium levels that are highest in the brown threads at the top of the map; these are equivalent to sample 4. Potassium levels were also slightly elevated in the pink when compared to the blue. Potassium was lowest in the red animal fibres in Figures 10 and 14.
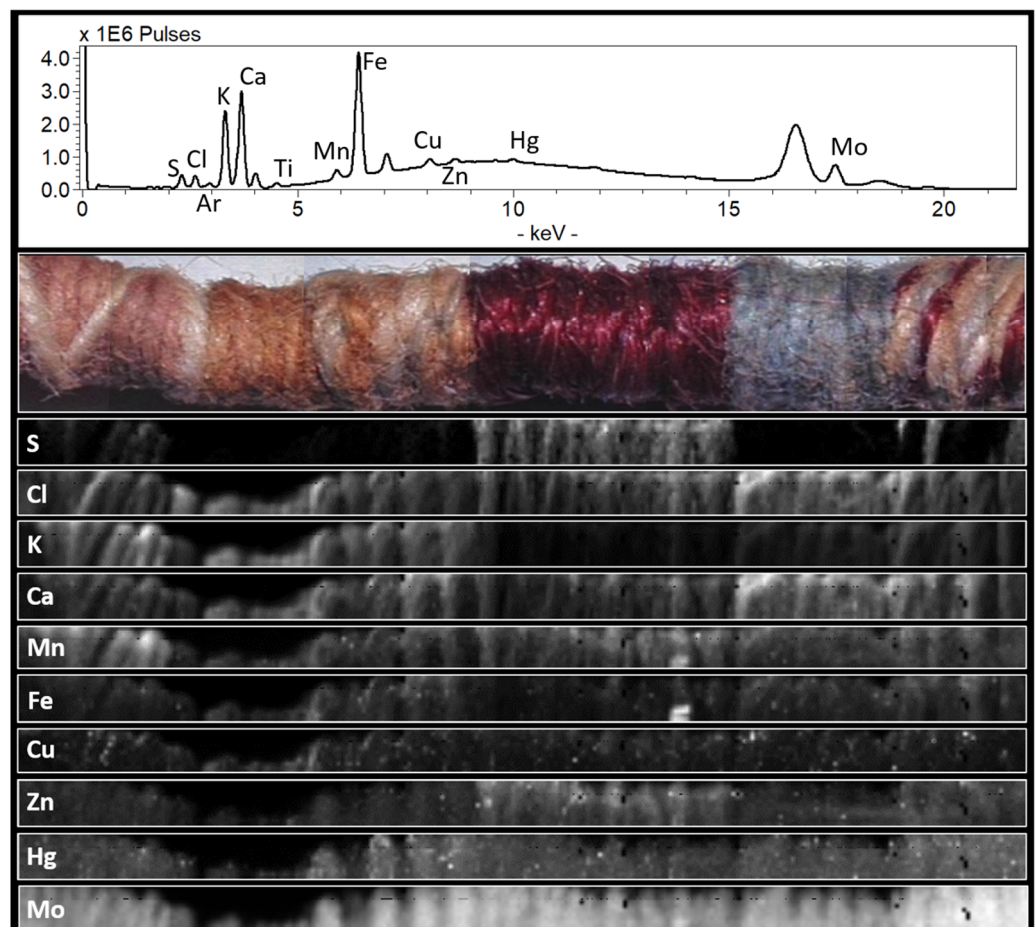
Figure 14. XRF maps from 1932.08.0001 P1 multicoloured wrapping bands 1–6. Map size is 1 mm × 22.95 mm.
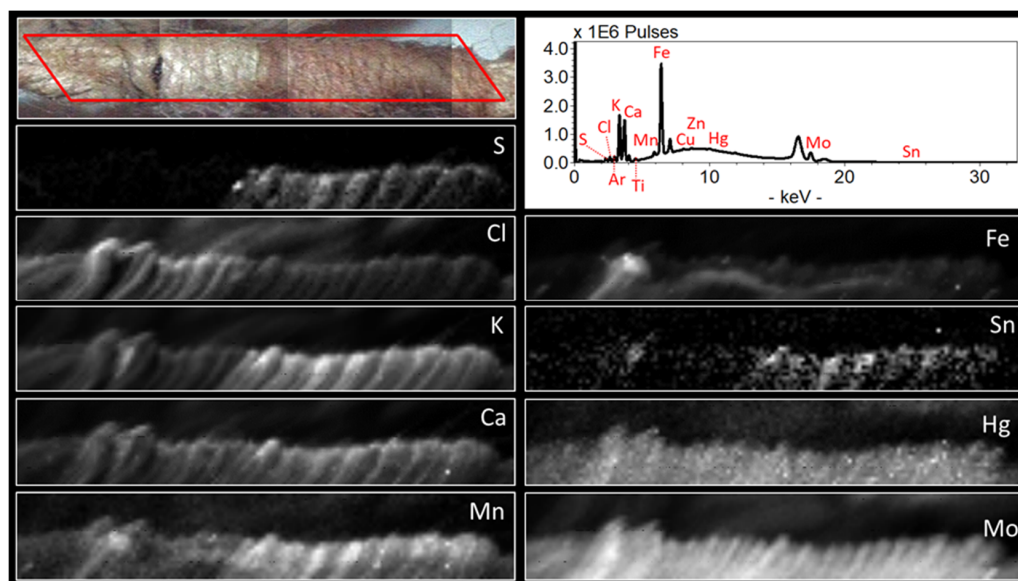
Figure 15. XRF maps from 1932.08.0001 P_2s_8 ivory-pink wrapping. Map size is 2 mm × 11.79 mm.

For the khipu samples, the potassium signal was significantly higher in brown sample 4 compared to any other sample (Figures 11 and 12). The intensity of the potassium signal was lowest in undyed samples 3 and 6.

The presence of potassium in dyed threads possibly indicates use of potassium carbonate, in the form of wood ash, to alkalise the bath. It is known that ashes were used as auxiliaries to darken the colour of dyebath in several American cultures, including the Navahos and many Native American peoples of the Wisconsin and Great Lakes region [47–49]. Although we are not aware of use of saltpetre for dyeing purposes, we know that Peruvian natives knew about this material since "zuca", the indigenous name of saltpetre, is reported by Bernabé Cobo [46] (p. 91).

Calcium was present at relatively high levels on all khipu area maps. Generally, the highest levels were associated with blue areas, as in 1932.08.0001 P_1 multicoloured wrapping band 1–6 map (Figure 14), 1932.08.0001 P_2s_2 blue–ecru cord map, 1932.08.0001 P_2s_9 blue–ivory–pink wrapping map, and 1932.08.0002 P_1s_8 multicoloured wrapping map (Figure 10).

The XRF map of thread samples also showed that calcium is present in every sample, especially in the blue one (sample 1). It is also distributed in the red (sample 5) and ecru (sample 7) samples, and it is lowest in the brown (sample 4) and undyed samples (samples 3 and 6) (Figures 11–13). The fact that calcium is highest in the blue sample, which was dyed with indigo, indicates that it was probably added as an auxiliary reagent to create an alkaline dyeing bath. Burned shells, limestones, or lime could have been used for this purpose. This result is consistent with data collected from Paracas textiles dyed with indigo [50,51] and confirms the early results obtained through ashing analysis [39].

8.2.6. Titanium

Titanium was present in all accumulated spectra from the XRF analysis on khipu areas but never showed a coherent distribution in the XRF maps. Titanium is most likely present as a contaminant from dust or sand.

8.2.7. Iron and Manganese

The XRF map on 1932.08.0001 P_2s_8 ivory–pink wrapping shows an iron-containing thread that is running under the ivory and pink wrapping (Figure 15). It emerges on the left side of the mapping area and becomes visible as the end of a black thread, indicating a now missing area of black wrapping from the top of the wrapping and possibly from the bottom too. A further thread that was high in iron was identified in the XRF map of

1932.08.0002 P_1 pink–ivory wrapping, where a black thread was interspersed in the ivory area, creating, therefore, a mottled band in the thread wrapping instead of a solid ivory colour band. The black threads from both khipus showed similar compositions as they also contain potassium, manganese, and possibly tin. XRF mapping of the areas that did not contain black threads showed that iron is distributed everywhere and is most likely present as a contaminant. However, some variations between colours were noted, and, in general, the undyed areas and the red animal fibres were lower in iron than any of the dyed cotton threads (Figure 10).

In the XRF analysis of thread samples, iron is distributed in every thread, especially in the coloured ones (Figures 11–13). The ecru (sample 7), blue (sample 1), and pink (sample 2) samples exhibit the highest iron content, suggesting use of iron mordants. Alternatively, an explanation for iron in the blue sample could be use of iron sulphate as a reducing agent in the indigo vat, as suggested by Fester [38]. The relatively high iron content in sample 7 is somewhat puzzling as an iron mordant on a yellow flavonoid dye would normally result in a darker shade than this very-light-coloured ecru sample. Alternatively, iron may be present as an ochre (iron oxide), as previously identified in Paracas textiles by Valette [39]. This was not investigated here. All three cochineal dyed samples, pink (sample 2), brown (sample 4), and red (sample 5), also contained iron, but the red animal fibre sample shows a lower iron signal, close to those of undyed samples 3 and 6. This may indicate that the detected iron is a general background contamination and not related to the dye. Alternatively, the varying iron levels in the three cochineal dyed samples could point to different recipes, in terms of source and concentration of iron in the bath, when dyeing animal or plant fibres. Iron was also found in red and yellow areas of Paracas textiles [50,51] and in purple Peruvian textiles [52]. Low signals of manganese are found in the samples, and they appear to be associated with iron. Manganese is a common impurity in sources of iron. Natural deposits of iron were mined in the Andean region, and stagnant mud was also used to turn textile dyes darker or even black [49]. Roquero reports that the Quechua term "kollpa" was used to indicate both iron sulphate and aluminium, with the only distinction that the first was "kollpa negra" and the second one was "kollpa blanca". She reports Cobo mentioning use of iron sulphate mixed with tannins to obtain black dyes [46(92)].

8.2.8. Copper and Zinc

Copper and zinc are present in every accumulated spectrum of the analysed areas but are not resolved in the XRF maps. For the XRF analysis of samples, copper was found only in the blue sample (sample 1) (Figures 11 and 12), but, in the map, its distribution is less clear (Figures 12 and 13). Whether copper or zinc were employed as mordants is, therefore, uncertain, although similar results were obtained in analysis of Peruvian textiles where copper was also detected in a blue indigo-dyed sample [50–52].

8.2.9. Mercury

Mercury is present in every accumulated spectrum of the analysed khipu areas, and an uneven, speckled distribution can be discerned on most maps. The presence of mercury on these objects is interpreted as originating from a biocide treatment, for example, from brushing with so-called sublimate, a solution of mercury (II) chloride [53]. Mercury was not present in the spectra of khipu samples, possibly due to uneven application where the ends of the cords were missed.

8.2.10. Tin

In Figure 15, the pink area was higher in sulphur than the white area. In this khipu area, the signal for tin coincides with the signal for sulphur and may indicate a tin mordant used on this pink thread. Unfortunately, a sample for dye analysis from this thread was not available, and the analyses of other pink areas did not show the same results. The only other colour where tin was also detected were the black threads mapped in situ.

8.3. Analysis of the Organic Dyes

All the samples collected from khipu 1932.08.0002 were treated for extraction and determination of organic dyes. The chromatographic profiles of the extracts of ivory cotton samples 3 and 6 were both superimposable to the procedural analytical blank and can thus be considered undyed. Blue cotton sample 1 features indigotin and indirubin as the colouring components (Figure S9), well-known molecular markers of indigo-producing species; in the Peruvian area, the most common are Indigofera suffruticosa and Cybistax antisyphilitica [54].

The extracts of pink cotton sample 2, brown cotton sample 4, and wool bright red sample 5 contained the main molecular marker of cochineal dyestuff, namely carminic acid (Figures S10, S11 and Figure 16, respectively). Cochineal dye extracted from Dactylopius coccus is a very well-known Peruvian dyestuff, which began to appear more frequently in ancient Peru in the Wari and Tiwanaku period (700–1100 AD) [54,55]. In addition, samples 2 and 5 also contained further molecular markers of cochineal dcII, kermesic acid, flavokermesic acid, dcIV, and dcVII. Sample 5 was indeed the most intensely coloured of the three yarns dyed with cochineal; besides the known markers of this dye, a further unknown anthraquinone was detected in its extract, eluting shortly after carminic acid, whose spectrum features maximise at 279, 515, and 551 nm (Figure 16, inset C).
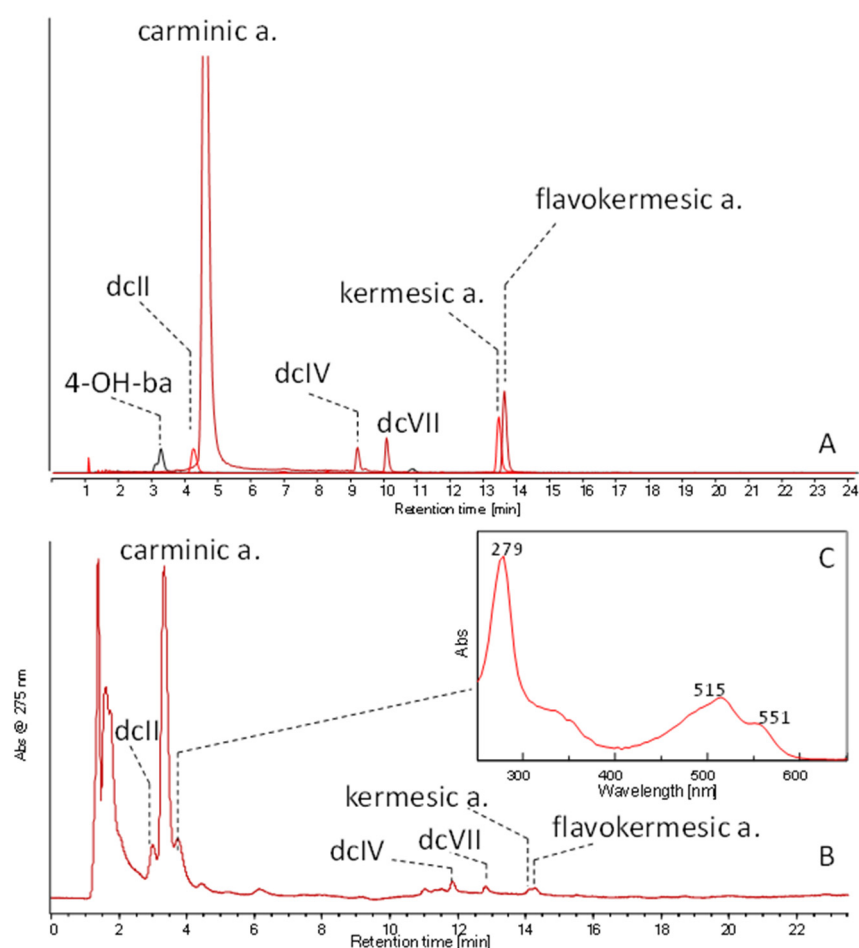
Figure 16. Chromatograms obtained for the extract of sample 5. (**A**): HPLC(-)ESI extracted ion chromatograms corresponding to the molecular ions of carminic, kermesic, and flavokermesic acids, Dactylopius coccus markers dcII, dcIV, and dcVII, and 4-hydroxybenzoic acid (4-OH-ba); (**B**): HPLC-DAD chromatogram extracted at 275 nm; (**C**): UV-Vis spectrum acquired in correspondence of the unknown peak at 3.9 min in the HPLC-DAD chromatogram.

The brown colour of sample 4 cannot be explained by the nature of the dyestuff, which is present in very low concentration (see the chromatogram in Figure S11); possible hypotheses entail use of mordants or modifiers in the dye bath, or an originally darker cotton, as discussed below.

Finally, the extract of ecru cotton sample 7 contained several flavonoids, such as rutin, luteolin, and two luteolin-O-glucosides. The detected profile, unfortunately, does not match any of the available reference materials or composition reported in the literature; moreover, both quercetin and luteolin are extremely common in plant extracts and thus quite unspecific. Nonetheless, their detection proves that the yarn was actually purposefully dyed in yellow with a flavonoid-based dyestuff (Figure S12).

All the samples were investigated for presence of polyphenols related to use of tannins as dyes or plant-based mordants. No specific marker for tannins was found (e.g., ellagic or gallic acid, or their glycosides). Sample 5 contained 4-hydroxybenzoic acid, which is a common plant metabolite but also a known molecular marker related to ageing of protein-based fibres, such as wool [56]. This compound is also present in the extract of sample 7 (Figure S12), most probably as a component of the flavonoid dyestuff or produced by photo-degradation [57]. Finally, 4-hydroxybenzoic was also unexpectedly detected in traces in the extract of sample 4 (Figure S11). Its presence may suggest that, in this case, the cochineal dye was applied to an originally brown cotton sample since 4-hydroxybenzoic acid was detected in the extract of Peruvian brown cotton reference material.

9. Conclusions

Multimodal analysis, where archival, morphological, non-invasive, and invasive/destructive scientific methods have been employed to examine the two Wari khipus, 1932.08.0001 and 1932.08.0002, proved to be effective. Provenance and meticulous morphological study enabled improved understanding of khipu structure and to select areas of interest for more detailed study. Together with multiband imaging, they provided essential guidance for designing an effective and ethical sampling strategy. XRF in situ was fundamental to disclose information on areas where sampling was not possible (thread wrappings). It also revealed unforeseeable structural characteristics that produced a new understanding of these khipus' structure and revolutionised our idea of the affordances of khipu morphological study. Identification of an iron-mordanted thread in the wrapping of 1932.08.0002 P_1 allowed us to label the second band as mottled and not solid. Mapping of an iron-mordanted thread running beneath 1932.08.0001 P_2s_8 thread wrapping allowed us to virtually reconstruct the pattern of the thread wrappings on P_2s_8 and, by analogy, P_1s_7. These were likely to present five bands instead of two and three, respectively. The extremities of these wrappings were constituted by an iron-mordanted and now lost thread.

XRF in a vacuum on khipu samples revealed mordants and other inorganic auxiliaries. Calcium- and potassium-based compounds were used as alkaline agents in the dyeing bath. The signal of calcium was particularly high in the sample dyed with indigo, and potassium compounds (such as ashes) were probably added to darken the colour of the dyebath. These results were entirely consistent with XRF results obtained in situ from various areas on the khipus. The results of XRF on samples also indicated use of iron mordants for variously dyed cotton samples, especially blue, pink, and ecru, while the distribution of iron in the XRF maps in situ was less clear and pointed mainly to general high background contamination with iron.

HPLC revealed that cochineal was used for dyeing pink (sample 2), brown (sample 4), and red (sample 5). It proved that samples 3 and 6 were undyed and that the blue (sample 1) was dyed with indigo. Additionally, it confirmed the hypothesis proposed based on multispectral imaging that sample 7 was indeed dyed in yellow. This implies that P_2 of 1932.08.0002 was not a solid colour cord but a mottled ivory-yellow cord. It is possible that this also applies to other cords on the same khipu, as Figure 8 suggests. This finding, together with the new structural understanding provided by XRF in situ, puts into question current practices of khipu morphological analysis and colour notation. It goes without

saying that it also undermines the affordances of using these data for statistical comparative purposes. By reporting the first dye analyses ever conducted on a khipu, as well as a new understanding of khipu morphological study, this research paves the way for deeper understanding of meaningful khipu colours and possibly to socially constructed meanings associated with the dyes and dyeing practices.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/heritage6030124/s1, Table S1: Representation of khipu 1932.08.01 cord structure [58], Table S2: Representation of khipu 1932.08.02 cord structure, Table S3: Samples, colours and corresponding areas of wrapping on both khipus, Figure S1 Overview VIS image of Khipu 1932.08.0002, Figure S2. Overview UVL of Khipu 1932.08.0002, Figure S3. Overview VIS image of samples from the "Meaningful materials in the khipu code" project, Figure S4. Irradiance of Godox LED 1000 II measured with an Ocean Optics Flame spectrometer at 100 cm distance, Figure S5. Irradiance of Godox LED 1000 II measured with an Ocean Optics Flame spectrometer at 100 cm, Figure S6. Irradiance of Kaiser Studiolight 1000 fitted with an Osram Halogen Superphot 1000W bulb measured with an Ocean Optics Flame spectrometer at 100 cm, Figure S7. Irradiance of Engelbretcht UVA-366 black lights measured with an Ocean Optics Flame spectrometer at 100 cm, Figure S8. Transmission of CHSOS Robertina Technical Photography filter set [32], Table S4. XRF positions and measurement details, Figure S9. Chromatograms obtained for the extract of sample 1, Figure S10. Chromatograms obtained for the extract of sample 2, Figure S11. Chromatograms obtained for the extract of sample 4, Figure S12. Chromatograms obtained for the extract of sample 7.

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