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STUDY AND ANALYSIS OF PLANT REMAINS AND ORGANIC RESIDUES FROM THE TOMB OF TUTANKHAMUN

Nagmeldeen Morshed HAMZA

nagmeldeenmorshed.hamza@uniroma1.it

BIO/02

Tutor: Prof. Laura Sadori

Co-tutor: Prof. Gabriele Favero

External tutor: Prof. Eltayeb Sayed Abbas

Abstract (ENG)

The present PhD thesis concerns the study and conservation of the Tutankhamun collection, with particular emphasis on the analysis of plant remains and textiles swept from the rooms ground surface of Tutankhamun tomb.

The arid environmental conditions present in Egypt have allowed to perfectly preserve desiccated botanical materials from a wide range of contexts and time periods, spanning from the Predynastic Period to the Ottoman one. An interesting case study in this sense is represented by the tomb of the 18th Dynasty pharaoh Tutankhamun, who reigned between 1334 and 1325 BC. Other than the golden objects and the rich funerary assemblage, the tomb was also full of plant remains. The ancient Egyptians stored food crops in Tutankhamun's tomb for his afterlife. The food crops and plant remain were found in the form of already well documented flower garlands and stored in numerous containers as funerary offerings. An immense “natural” treasure was recently recovered from the storerooms of the Luxor Museum. Once moved to the new seat and museum, the Grand Egyptian Museum, it was time to start studying this precious “rubbish” recovered one century ago, at the end of the archaeological excavation of the tomb of Pharaoh Tutankhamun by Howard Carter’s team.

In fact, after cataloguing the most interesting findings, Howard Carter and his collaborators swept the remaining material from the surfaces of the tomb, including numerous plant parts, and stored them in a wooden box, initially found in the Luxor Museum in Luxor and recently moved to the Grand Egyptian Museum in Giza. The carpological remains deposited in this wooden box in 1933 are still in excellent condition and allowed identification at a species level. Over 9000 plant remains were identified, including food, medicinal and ornamental plants. These include taxa not previously recorded in the tomb (such as *Beta vulgaris* L.). Although plant remains do not carry the same monetary value as the golden artefacts, they can impart incredible knowledge about Tutankhamun and his time. They can provide us with clues for anything from the pharaoh’s life and health to greater socio-political issues like who Ancient Egypt traded with and how the well-to-do upper classes lived.

Plant remains also include reeds, which were used in ancient Egypt for the construction of wide variety of objects, such as baskets, ropes, nets, sandals, fishing rods, walking sticks, arrows, and other objects. In this study, μ-MRI was used for the identification of an archaeological reed

allowing not only to identify a species used in the tomb of Tutankhamun, but also proposing a new tool for archaeobotanical and archaeological analysis.

Organic remains in the studied archaeological context are not only represented by archaeobotanical evidence, but also by wooden artifacts, textile fragments and organic dyes. These were also analyzed within the present thesis, providing complementary information to the carpological study. The definition of clear, novel protocols for scientific analyses represents a strength of this thesis, which goes beyond the sole identification of materials.

When dealing with such archaeological artifacts, conservation issues should not be marginalized. In fact, these are also discussed in the present thesis, proposing preservation protocols to ensure the materials' accessibility by future generations.

Keywords: Tutankhamun, plant identification, organic remains, archaeobotany, textiles

Riassunto (ITA)

La presente tesi di dottorato riguarda lo studio e la conservazione della collezione di Tutankhamun, con particolare enfasi sull'analisi dei resti vegetali e tessuti raccolti dalla superficie del terreno delle stanze della tomba di Tutankhamun.

Le condizioni ambientali aride presenti in Egitto hanno permesso di conservare perfettamente materiali botanici essiccati provenienti da una vasta gamma di contesti e periodi storici, che vanno dal Periodo Predinastico a quello Ottomano. Un interessante caso di studio in questo senso è rappresentato dalla tomba del faraone della XVIII dinastia Tutankhamun, che regnò tra il 1334 e il 1325 a.C. Oltre agli oggetti d'oro e al ricco corredo funebre, la tomba era anche piena di resti vegetali. Gli antichi Egizi conservavano colture alimentari nella tomba di Tutankhamun per la sua vita nell'aldilà. Le colture alimentari e i resti vegetali sono stati trovati sotto forma di ghirlande di fiori già ben documentate e conservate in numerosi contenitori come offerte funebri. Un immenso "tesoro naturale" è stato recentemente recuperato dai depositi del Museo di Luxor. Una volta spostato nella nuova sede e museo, il Grand Egyptian Museum, è stato il momento di iniziare lo studio di questo prezioso "rifiuto" recuperato un secolo fa, alla fine dell'escavazione archeologica della tomba del faraone Tutankhamun da parte della squadra di Howard Carter.

Infatti, dopo aver catalogato le scoperte più interessanti, Howard Carter e i suoi collaboratori hanno raccolto il materiale rimanente dalle superfici della tomba, comprese numerose parti di piante, e le hanno conservate in una scatola di legno, inizialmente trovata al Museo di Luxor a Luxor e recentemente trasferita al Grand Egyptian Museum a Giza. I resti carpologici depositati in questa scatola di legno nel 1933 sono ancora in ottimo stato e hanno permesso l'identificazione a livello di specie. Sono stati identificati oltre 9000 resti vegetali, tra cui piante alimentari, medicinali ed ornamentali. Questi includono taxa precedentemente non registrati nella tomba (come *Beta vulgaris* L.). Sebbene i resti vegetali non abbiano lo stesso valore monetario degli artefatti d'oro, possono fornire conoscenze incredibili su Tutankhamun e il suo tempo. Possono fornirci indizi su qualsiasi cosa, dalla vita e dalla salute del faraone a questioni socio-politiche più ampie come con chi l'Antico Egitto commerciava e come vivevano le classi agiate.

I resti vegetali includono anche canne, che venivano utilizzate nell'antico Egitto per la costruzione di una vasta gamma di oggetti, come cesti, corde, reti, sandali, canne da pesca, bastoni da passeggio, frecce e altri oggetti. In questo studio, è stato utilizzato il μ-MRI per l'identificazione di una canna archeologica, consentendo non solo di identificare una specie

utilizzata nella tomba di Tutankhamun, ma anche proponendo un nuovo strumento per l'analisi archeobotanica e archeologica.

I resti organici nel contesto archeologico studiato non sono rappresentati solo da prove archeobotaniche, ma anche da manufatti di legno, frammenti di tessuto e coloranti organici. Anche questi sono stati analizzati all'interno della presente tesi, fornendo informazioni complementari allo studio carpologico.

La definizione di protocolli chiari e innovativi per le analisi scientifiche rappresenta un punto di forza di questa tesi, che va oltre la sola identificazione dei materiali.

Quando si tratta di tali manufatti archeologici, le questioni legate alla conservazione non dovrebbero essere marginalizzate. Infatti, queste sono discusse anche nella presente tesi, proponendo protocolli di conservazione per garantire l'accessibilità dei materiali alle future generazioni.

Parole chiave: Tutankhamun, identificazione delle piante, resti organici, archeobotanica, tessuti archeologici

INDEX

INTRODUCTION

Archaeobotany, sometimes referred to as palaeoethnobotany, is the field of study that examines plant remains found in archaeological sites. It delves into the historical interactions between humans and plants, with a particular focus on activities related to food and changes in ancient landscapes (Branch, 2014). Within this discipline, researchers analyze different types of plant fossils, categorizing them based on their size into two main groups: macro-remains and micro-remains. Macro-remains encompass items like seeds, fruits, wood, and basketry, while micro-remains include pollen, non-pollen palynomorphs (NPPs), phytoliths, and starch grains (Mercuri et al., 2010).

Although the origins of this field of research can be traced back to the 19th century, it was properly introduced in the 1960s (Fuller et al., 2014). Before then, plant remains were commonly discarded as their importance was not acknowledged. Fossilization of plant remains, and their retrieval in archaeological layers, can occur through different modalities. These include charring, waterlogging, mineralization, and desiccation (Pearsall, 2015). The peculiar environmental conditions found in Northern Africa, with very low humidity, make it possible for plant material to be found in a wide range of periods and contexts. This also allowed for the preservation of flowers and leaves comprising ornamental garlands and bouquets in Pharaonic sites (Fahmy, 1997).

The botanical elements in ancient Egypt were not just included for their aesthetic attributes, but also for their symbolism, and on account of their usefulness. Many plants are distinguished through depictions, drawings, and engravings on walls of tombs and temples (Barakat and Aziz, 2010). Iconographic evidence of plants in ancient Egypt is also present in other forms of art, including tomb paintings, temple reliefs, statues, jewelry, and hieroglyphs, confirming that plants that played a significant role in people's beliefs. Plants were an essential part of ancient Egyptian life and culture, serving as symbols of life, fertility, and the natural world (McDonald et al., 2018).

The artistic evidence from tombs, the lexicographic data from texts, the archaeobotanical and artefactual evidence from the excavation of tombs and settlements, along with data from ethnographic and ethnohistoric observations have been precious for gathering information on crop diversity, crop husbandry and the history of many species (Nicholson et al., 2000).

A world known case study is represented by the tomb of Pharaoh Tutankhamun (18th Dynasty, c. 1336-1327 BC; Uda et al., 2007). Its discovery by Howard Carter in 1922 remains one of

the most spectacular archaeological finds, having occurred almost accidentally (Allen, 2006). Tutankhamun's fame is ironically due to his former general, Horemheb, the last pharaoh of the 18th dynasty. He made sure that all references to his predecessor were destroyed, erasing him from history. Although the lack of textual evidence caused Tutankhamun to be forgotten, this omission allowed for him to be preserved for posterity, surviving the dismantling of royal tombs following the abandonment of the Valley of the Kings (Allen, 2006).

Tutankhamun's tomb is famous for the presence of prestigious artifacts, including the remarkable golden funerary mask (Uda et al., 2007). Nonetheless, Howard Carter managed to acknowledge the potential value of less prestigious findings, including plant remains. The abundance of foodstuffs clearly confirmed the believe of Ancient Egyptians in afterlife. A total of 116 baskets containing desiccated offerings of seeds and foodstuffs were recovered (Herselman, 2013). These were later subjected to archaeobotanical analyses (Germer, 1989; de Vartavan, 1990). Nonetheless, there are still some organic materials that were left unexplored. These are represented by the plant remains left on the tomb surfaces after cataloguing and removing all the most important artifacts. The floors were then swept, and all the residues were deposited in a wooden box (200 x 81 x 56 cm ca.). This was closed in 1933 and was stored in the Luxor Museum in Luxor until 2017. In 2018 it was moved to the Grand Egyptian Museum, where its contents started being subjected to multidisciplinary analyses, being the focus of the present thesis. Through their archaeobotanical study, it was not only possible to reconstruct plant availability and funerary offerings, confirming and updating past results, but also to apply and test novel diagnostic techniques. These include micro-magnetic resonance imaging (μ-MRI), an imaging technique based on the study of the magnetic properties of hydrogen nuclei and on the resonance phenomenon. This method has so far been tested against light microscopy for the study of archaeological wood, giving promising results (Stagno et al., 2023).

Desiccation not only favoured the preservation and subsequent analysis of plant remains but was also crucial the conservation of archaeological textiles. While textiles from Tutankhamun's tomb have been previously investigated in terms of woven patterns (Hoskins, 2011) and identification of fibres (Abdrabou et al., 2018), no study was so far aimed at the identification of dyes used to color them.

The present thesis is divided in three sections based on the type of materials analyzed or approach used. **SECTION 1** concerns the conservation strategy of the Tutankhamun collection, with particular reference to organic remains, including textiles, leather and reeds. This section is divided in three chapters, centered around different typologies of materials. In particular, chapter 3 considers materials used during another phase of live of the tomb: its

discovery in 1922. **SECTION 2** focuses on archaeobotanical remains, which allowed me to gather information concerning plant availability during the 18th Dynasty in Ancient Egypt, food plants and ritual uses. This section is divided in two chapters, based on the analytical approach used. **SECTION 3**, in contrast, is related to the study of textiles, with a focus on the extraction and analysis of colorants.

This manuscript is a collection of papers concerning materials collected in the tomb of Tutankhamun (Hamza, Ishii and Shaheen, 2021; Hamza and Shaheen, 2021; Hamza, 2021; Hamza et al., in preparation; Moricca et al., in review; Peruzzi et al., 2023; Serafini et al., in preparation) written by or in collaboration with the candidate. Most of the papers have been published in (or will soon be submitted to) peer-reviewed journals indexed in Scopus and Web of Science.

SECTION 1 focuses on the conservation of the world-famous Tutankhamun collection, which includes numerous organic artifacts, such as textiles and plant remains. A proper conservation strategy is crucial to ensure the collection's preservation in time and its accessibility to future generation. In particular, **Chapter 1** focuses on five artifacts from Tutankhamun's tomb: a hassock (or footstool) and four textile objects. They are taken as case studies to advance a proposal for a strategy that would ensure that present-day conservation decisions are integrated with those from the past. This research resulted in a publication authored by Hamza, N. M., Mie, I., & Eslam, S., titled "Conservation between scientific methodology and laboratory application: An integrated approach to past and present challenges" and published in the proceedings of the *ICOM-CC 19th Triennial Conference 2021 Beijing*.

This topic was further investigated in **Chapter 2**, where other artifacts, such as basket-work sandals and reed arrows are analyzed. The post excavation conservation strategy reported by Howard Carter is evaluated thoroughly. Other than a detailed process of documentation and cataloguing, boiling paraffin wax was poured on several artifacts. The proposed conservation strategy favors an approach of minimal intervention. It focuses on stabilization and consolidation. This chapter consists of a paper by Hamza, N. M. & Shaheen E. (2021), titled "Conservation of the Tutankhamun collection: strategy of conservation decision-making in the Grand Egyptian Museum (GEM) in Giza", published in *Colloque APROA-BRK 2021 – Conservatie-restauratie in context*.

Conservation is also the subject of **Chapter 3**. Here, an aspect usually considered as secondary in archaeological excavations is evaluated: the material evidence left by excavators. This consists of food boxes used to pack samples, newspapers used to wrap textiles, and the numbering cards made by Howard Carter. This study, authored by Hamza, N. M. under the

title “Conservation of evidence: the significance of objects biography from Tutankhamun tomb”, is published in *Young Professionals Forum Proceedings 2021.*

SECTION 2, Chapter 4 concerns the analysis of carpological remains collected from the surfaces of the tomb of Pharaoh Tutankhamun and stored in a wooden box until 2017. Plant remains, preserved mostly by desiccation, provide information about plant availability in ancient Egypt, food plants and ritual offerings. Due to their modern-looking appearance, ^{14}C dates were crucial to identift modern contaminations. This chapter consists of the paper titled “Trash or treasure? Plant remains from the tomb of Tutankhamun” authored by Hamza N. M., Moricca C., Sadori L., in preparation to be submitted to *Plants*.

Plant macro-remains are also studied in **SECTION 2, Chapter 5**. This chapter focuses on the application of µ-MRI for the identification of archaeological reeds from the same context. The potential of this technique for the analysis of reed culm anatomy is highlighted. This manuscript, titled “The high potential of micro-Magnetic Resonance Imaging for the identification of archaeological reeds: the case study of Tutankhamun”, is authored by Moricca C., Stagno V., Hamza N. M., Favero G., Sadori L., Capuani S., and in review at *Heritage.*

In **SECTION 3** the focus shift to pigments and dyes, present on several organic artifacts (such as wooden shawabtis and textiles) preserved in the tomb of Tutankhamun.

In **Chapter 6** pigment identification and gilding characterization were carried out on Tutankhamun’s polychrome wooden shawabtis. This study was performed both by imaging techniques and single-spot analyses, allowing to not only characterize the materials making up the shawabtis, but also to map the previous restorations on the original surface, providing crucial information for follow-up treatments and conservation works. This study, titled “Tutankhamun’s polychrome wooden shawabtis: Preliminary investigation for pigments and gilding characterization and indirect dating of previous restorations by the combined use of imaging and spectroscopic techniques” and authored by Abdrabou, A., Abdallah, M., Sultan, G. M., Mostafa, M., Bayoumi, H., Magdy, R., Abd El Kader, M. A., Hamza, N. M., Mamdouh, D., Elsayed, H. M., Abbas, E. & Kamal, H. M. was published in *Open Archaeology*.

Chapter 7 concerns the identification of dyes present on a linen fragment. This was performed through the innovative gel-supported microextraction with agar gel and the Nanorestore Gel® High Water Retention (HWR), and a combination of Fiber Optic Reflectance Spectroscopy (FORS) and Surface Enhanced Raman Scattering (SERS). This research was published under the title “Applying Gel-Supported Liquid Extraction to Tutankhamun’s Textiles for the Identification of Ancient Colorants: A Case Study” and authorship of Peruzzi G., Ciccola A.,

Bosi A., Serafini I., Negozio M., Hamza N. M., Moricca C., Sadori L., Favero G., Nigro V., Postorino P., Curini R. in *Gels*.

Dyes and textiles are also the focus of **Chapter 8**. Here, a textile fragment from Tutankhamun's tomb is used as a case study for the presentation of an innovative approach for the extraction and clean-up of natural dyes. The approach involves the use of an in-situ gel supported liquid micro-extraction (InGEL) combined with a dispersive liquid-Liquid MicroExtraction (dLLME) clean-up methodology, novel to heritage science, to purify and preconcentrate the dye molecules from both in-solution and gel extraction systems. The method was validated using targeted UHPLC-MS/MS (Ultra-high performance liquid chromatography-MS/MS**).** The result of this research is a manuscript titled "Overcoming the limit of in situ gel supported liquid microextraction: development of the new InGeL-LC-MS analyses, a smart methodology for the identification of natural dyes from Tutankhamun tomb relics", authored by Serafini I., Bosi A., Vincenti F., Peruzzi G., McClure K. R., Ciccola A., Hamza N. M., Moricca C., Sadori L., Montesano C., Sergi M., Favero G., Curini R. and soon to be submitted to *Analytica Chimica Acta*.

In the final chapter (**Chapter 9**) a summary of the data provided in **SECTIONS 1, 2** and **3** is presented. The conclusions represent the effort of the candidate to reconstruct the organic component of the funerary assemblage of Tutankhamun's tomb. Through this research, the candidate not only gathered information about plant availability and choice, but also characterized the dyes used to color textiles deposited in the famous tomb.

Innovation is a characterizing element of the present thesis, as the studied materials were used as case studies to support the development of novel techniques and methods for the study of cultural heritage materials.

References

1. Abdrabou, A., El Hadidi, N. M., Hamed, S., & Abdallah, M. (2018). Multidisciplinary approach for the investigation and analysis of a gilded wooden bed of King Tutankhamun. *Journal of Archaeological Science: Reports*, *21*, 553-564.
2. Allen, S. J. (2006). *Tutankhamun's tomb: The thrill of discovery*. Metropolitan Museum of Art.
3. Barakat, H. N., & Aziz, A. (2010). *Guide to plants of ancient Egypt*. Bibliotheca Alexandrina.
4. Branch, N. (2014). Agrarian landscapes: Environmental archaeological studies. In C. Smith (Ed.), *Encyclopedia of Global Archaeology* (pp. 84-90). Springer.

5. Carter, H. (1972). *The tomb of Tutankhamun*. Barrie & Jenkins Ltd.
6. Fahmy, A. G. E. D. (1997). Evaluation of the weed flora of Egypt from Predynastic to Graeco-Roman times. *Vegetation History and Archaeobotany*, 6, 241-247.
7. Fuller, D. (2020). *Archaeobotany*. Walter de Gruyter GmbH & Co KG.
8. Hepper, F. N. (2009). *Pharaoh's flowers: The botanical treasures of Tutankhamun*. Kws Publishers.
9. Herselman, C. (2013). *The Botanical Treasures of Tutankhamun*.
10. Hoskins, N. A. (2011). Woven Patterns on Tutankhamun Textiles. *JOURNAL of the American Research Center in Egypt*, 199-215.
11. McDonald, J. A. (2018). Influences of Egyptian Lotus symbolism and ritualistic practices on sacral tree worship in the fertile crescent from 1500 BCE to 200 CE. *Religions*, 9(9), 256.
12. Mercuri, A. M., Sadori, L., & Blasi, C. (2010). Editorial: Archaeobotany for cultural landscape and human impact reconstructions. *Plant Biosystems*, 144(4), 860–864.
13. Nicholson, P. T., & Shaw, I. (Eds.). (2000). *Ancient Egyptian materials and technology*. Cambridge University Press.
14. Pearsall, D. M. (2015). *Paleoethnobotany: A handbook of procedures*. Routledge.
15. Stagno, V., Moricca, C., Sadori, L., Dell'Aglio, E., Reale, R., & Capuani, S. (2023). Evaluation of the efficacy of micro-Magnetic Resonance Imaging compared with light microscopy to investigate the anatomy of modern and ancient waterlogged wood. *Magnetic Resonance Imaging*.
16. Uda, M., Yoshimura, S., Ishizaki, A., Yamashita, D., & Sakuraba, Y. (2007). Tutankhamun's golden mask investigated with XRDF. *International Journal of PIXE*, 17(01n02), 65-76.

SECTION 1

Conservation

CHAPTER 1

Conservation between scientific methodology and laboratory application: an integrated approach to past and present challenges

Hamza N. M., Ishii M., Shaheen E.

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THEORY, HISTORY, AND ETHICS OF CONSERVATION

Conservation between scientific methodology and laboratory application: An integrated approach to past and present challenges

Nagm Eldeen Morshed Hamza*
Grand Egyptian Museum – Conservation Center
Giza, Egypt
nagmhamza@gmail.com
negm_morshed@gem.gov.eg
Mie Ishii
Saga University
Saga, Japan
mitextileconservation@gmail.com
mishii@cc.saga-u.ac.jp
Eslam Shaheen
Grand Egyptian Museum – Conservation Center
Giza, Egypt
Eslam_shaheen@hotmail.co.uk
*Author for correspondence

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conservation, integration, Tutankhamun, decision-making, scientific methodology, Grand Egyptian Museum – Conservation Center (GEM–CC)

Abstract
King Tutankhamun's 18th-dynasty tomb collection is expected to be displayed at the newly built Grand Egyptian Museum in Giza. The collection is one of the earliest attempts at conservation in modern terms, integrating a scientific approach to its long-term preservation and providing firsthand information about changes and developments in conservation practices from the time of its discovery in 1922 to the present day. One of the conservator's main roles is to investigate the causes of the changes that occur in objects and to find a methodology to minimize these changes for future generations. Hence, understanding an object's composite materials and past conservation treatments is necessary to develop a methodology for present-day conservation work. By examining four organic artifacts from Tutankhamun's tomb—a hassock or "footstool" (Carter No. 034) and four textile objects (Carter No. 054f, 044t, 021cc, and 044r)—in four case studies, a hypothesis was put forward that present-day conservation decisions are integrated with those from the past. Such past decisions, in combination with the condition or state and scientific approach at the

INTRODUCTION

The tomb of King Tutankhamun from the 18th dynasty, or New Kingdom (ca. 1342–1325 BC), was discovered on November 4, 1922, in the Valley of the Kings in Luxor by a British archaeologist and Egyptologist, Howard H. Carter (1874–1939). His team spent ten years removing and recording all of the objects from the tomb, a total of 5,398 items (Carter 1972, Reeves 1990). Around 15,000 documents from this extraction are now held by the Griffith Institute, Oxford University, and can be consulted online.[1]

The British chemist Alfred Lucas (1867–1945) acted as scientist-conservator for the Tutankhamun collections. Lucas's *Antiquities: Their restoration and preservation* (Lucas 1924) is a firsthand account of the aims and methods behind the conservation of the artifacts from the famous excavation, although it also deals with a wider spectrum of materials. The objects were moved to the Egyptian Museum, where they were displayed in the galleries or put into storage, many remaining untouched. In 2002, the Egyptian government laid the first stone for a new museum—the Grand Egyptian Museum (GEM) in Giza—where the entire Tutankhamun collection was expected to be moved and displayed in state-of-the-art galleries. The GEM's mission is "to preserve, document, conserve, research, and exhibit its collections, and to educate and entertain its visitors, whether adult or child."[2] It has adopted preventive conservation as its primary policy (Kamal et al. 2018), conserving the Tutankhamun artifacts in the adjacent GEM Conservation Center (GEM–CC) in continuous regard for the excavator's intentions and the Egyptian Museum's activities: to preserve them for future generations. Japan has provided Egypt with support to implement the GEM–CC, and since 2007 continuous development programs have been run for conservation professionals, this paper being the outcome of one such program of international cooperation.[3]

Conservation of the Tutankhamun collection is a responsibility of utmost importance. Faced with a historical challenge, approaches to conserving ancient Egyptian artifacts were studied through integrating scientific methodology and laboratory work to generate a specific solution. This paper is the result of a discussion among members of the Organic Materials Laboratory at GEM–CC on the need to examine past and present conservation methods—specifically how a decision impacts on an object's long-term preservation. By looking at four organic artifacts from Tutankhamun's tomb in four case studies, the hypothesis is presented that modern-day

time, affect the object's preservation and influence current conservation decision-making. The authors compared the similarities and differences between the four artifacts in the case studies and concluded that changes in conservation thinking and methodology had become part of each object's characteristics, and that conservation decisions taken in the past had been integrated into the objects. Similarly, conservators need to be aware that present-day conservation decisions involving advanced scientific methods and laboratory applications also affect objects and must proceed with caution, i.e., remember that less is more and that challenging situations are also an integral part of conservation.

Figure 1. Hassock (cloth, bran, and beadwork; Carter No. 034, Sr. No. 4441, GEM No. 15971). Photo: Eslam Shaheen, GEM–CC

Figure 2. Hassock after conservation. Photo: NagmEldeen Hamza, GEM–CC

conservation decisions are integrated with past decisions and, furthermore, that the present condition or state of an object affects current conservation decision-making.

CONSERVATION RECORDS AT THE TIME OF EXCAVATION

Carter and his team developed an impressive system for documenting the condition and treatment applied to individual artifacts that used cross-referenced index cards. The artifacts were exposed to two conservation interventions. The first was applied in situ—to protect them from handling while being removed from the tomb—and the second took place at a conservation laboratory set up by Lucas at the nearby tomb of Seti II (Carter and Mace 2004). The treatment involved two stages: cleaning and consolidation. Cleaning was performed using soft bristle brushes and bellows, followed by consolidation to coat the artifacts with paraffin, beeswax, or some kind of synthetic adhesive such as cellulose nitrate (celluloid) dissolved in acetone, Duroprene dissolved in xylol, or Canada balsam dissolved in xylene (Gilberg 1997). As the use of synthetic polymers was new in the 1920s, it can be said that Carter and Lucas endeavored to preserve the treasures by using advanced scientific methods for the time. Even so, when the object was "too far gone for measurement," it was "not kept."[4] It is important to note that the idea of keeping deteriorated objects in the hope that conservation techniques might be developed in the future was not envisaged. What if a suitable preservation or restoration technique is developed a hundred years from now? This is reminiscent of our current position. Case studies on the conservation of the Tutankhamun artifacts are among the earliest records of integrating today's scientific methodologies, and yet these will not be the last in the relics' lives.

OBJECT CASE STUDIES

Four case studies are discussed. The object identification numbers for the Tutankhamun collection show how the objects have changed hands and these are the key to cross-referencing the records.

Case study 1: Hassock, cloth filled with bran and decorated with beadwork (Carter No. 034, Sr. 4441, GEM No. 15971)

Carter's object cards are firsthand records that identify an object (location, size, and description) and its conservation treatment. In this case (Card No. 34-1), boiling wax was poured over the beaded object as a conservation treatment. Carter took extra notes on the materials and construction of the hassock (Card No. 34-2) in which he reconfigures the sequence of the beading techniques. On its arrival at the Egyptian Museum in 1933, the hassock was stored in a wooden box inside storage room no. 55 until it was moved to GEM–CC in 2014. Inside this box were two other cardboard boxes, one containing detached beads and the other a carbonized textile with gold leaf (Figure 1).

Upon examining the hassock at the Organic Laboratory, a large number of detached beads were observed. Much of the bran stuffing had fallen out, and the condition appeared to have worsened. Although the wax was not functioning as a consolidator, the conservator decided not to remove

Figure 3. Tapestry woven garment as stored at the Egyptian Museum (Carter No. 054f, Sr. No. 3934, GEM No. 16017). Photo: GEM–CC

Figure 4. Separating the layers of the textiles during conservation at the Organic Lab, GEM–CC. Photo: NagmEldeen Hamza, GEM–CC

it; in some places, the wax was holding the beads together even after the threads had deteriorated and any mechanical action would have destroyed the object. A conservation decision was taken to support the deformation and reconstruct some parts of the bead decoration. This was possible due to Carter's notes being informative enough to understand the beading technique and their arrangement (Carter No. 34-2), and that the object was intended for display at the GEM. In summary, the conservation involved reshaping and re-constructing the object into a "footstool" by re-threading the bead decoration (Figure 2).

Case study 2: Tapestry woven garment (Carter No. 054f, Sr. No. 3934, GEM No. 16017)

Carter's record for this object indicates that the garment was entirely bunched together and sprayed with a solution of cellulose nitrate (recorded as celluloid by Carter) in acetone. From its arrival at the Egyptian Museum in 1933, the garment was kept inside storage room no. 55 until 2015, when it was moved to GEM–CC (Figure 3).

The synthetic polymers held the fragmented pieces together and it was difficult to separate them, as they had consolidated into a mass. Our first decision was not to risk opening the fabric. However, after carefully experimenting with humidification using acetone and water vapors with an ultrasonic humidifier (Tímár-Balázsy and Eastop 1998b), the cellulose nitrate was found to soften and the fragments could be successfully separated (Selwitz 1988). This treatment led to a surprising discovery on the inscriptions woven into the garment that opened up a new interpretation of King Tutankhamun's familial relationships, in that the cartouche did not belong to him but perhaps to another king or even a queen, providing new evidence concerning his parents (Tawfik et al. 2018). This demonstrated that records of past treatments can help construct a conservation methodology. Although the cellulose nitrate could not be removed from the object, it played a certain role in its preservation. Our current approach used this past treatment and devised a laboratory practice using modern tools and scientific principles in which the cellulose nitrate could be reactivated by acetone. Hence, this case study showed that past conservation activity is integrated into the present approach, and through experimentation challenges can open new doors to better understand our heritage (Figure 4).

Case study 3: Ornamental garment (Carter No. 044t, Sr. No. 4357, GEM No. 14695)

The excavation notes indicated that the ornamental garment, made from linen, had many metal sequences and was in very bad condition. Carter decided to spray it with a "strong solution of cellulose acetate in acetone."[5] Next, the garment was placed on a glass sheet covered with fabric, packed in a wooden box, and transferred to the EM in Cairo in 1933, where it was stored (Figure 5). In 2014, the garment was moved to the GEM–CC.

The garment was in extremely poor condition: the fabric and embroidery thread, which appeared to be linen, had turned completely dark brown, revealing an accelerated state of cellulose hydrolysis and carbonization. The metal sequins on the surface were in good condition, indicating a

Figure 5. Ornamental garment, before re-mounting (Carter No. 044t, Sr. No. 4357, GEM No. 14695). Photo: Eslam Shaheen, GEM–CC

Figure 6. The garment after mounting in 2019 by the staff of the Organic Lab, GEM–CC (Carter No. 044t, Sr. No. 4357, GEM No. 14695; the small piece: Sr. No. 3920, GEM No. 14061). Photo: Mohamed Ragab, GEM–CC

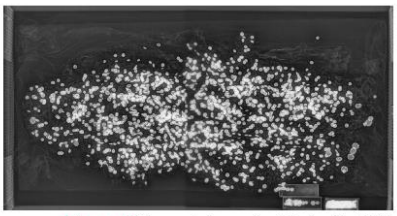

Figure 7. X-ray photograph of Carter No. 044t, Sr. No. 4357, GEM No. 14695. Photo: Hidetomo Mastushima, GEM–CC/GEM–JC

high content of gold. The object was under contract with the GEM–JC Project (see Note 3), and after discussion with the Japanese team, the decision was made not to treat it. It was placed in a concave mount made of archival material constructed by the members of the organic lab (Figure 6). It was then placed in a UV-cut, low reflectance, and antistatic case that was intended to minimize human and environmental hazards. X-ray photography revealed many underlying metal sequences that surface examination alone could not detect (Figure 7). This technology was not available to Carter nor was it present at the Egyptian Museum.

The case study demonstrated that scientific technology is becoming increasingly integrated into laboratory application. Advancements in new technology will reveal more opportunities to learn about the object—if preserved in a stable environment.

Case study 4: Decorated garments (Carter No. 021cc, Sr. No. 3274, GEM No. 15924 and Carter No. 044r, Sr. No. 3264, GEM No. 14060)

This case study consisted of two garments. Carter No. 021cc is a head covering representing a protective bird, made with colored linen thread in a tapestry technique. After excavation, it was "sprayed with a celluloid in amyl acetate" solution.[6] Carter No. 044r is an appliquéd garment with alternating blue, red, and green fabric strips. It was sprayed with cellulose acetate in amyl acetate (Figure 8). Since arriving at the Egyptian Museum in 1933, the garments have been kept in storage room no. 55.

The artifacts underwent a further intervention as part of a doctoral study on the conservation and restoration of antiquities at Cairo University (Abla 2001). Abla conducted experiments on mock samples and subsequently treated the two artifacts. One of the aims of the study was to investigate conservation methods for ancient Egyptian archaeological textiles, which involved experimental study and the development of a conservation methodology (Abdel-Kareem 2001).

Fifteen years after their second intervention, the two objects arrived at GEM–CC (Figure 9). The treatment carried out in 2001 had changed the artifacts dramatically and was non-reversible (Tétreault 1999). An active decision was taken not to perform any further treatment and to leave the object as it was (Ashley-Smith 2018). However, plans exist to produce a digital reconstruction of the object to help with its fuller interpretation.

Table 1. Case studies

Object type	Object registration number	Object material	Previous intervention
Hassock: cloth, bran, and beadwork	Carter No. 034	Beads, plant remains, textiles, leather	Boiling wax
Textile (tunic): tapestry woven garment in colors	Carter No. 054f	Linen, dyes	Sprayed with solution of celluloid in acetone
Textile: ornamental garment	Carter No. 044t	Linen, gold	Sprayed with strong solution of cellulose acetate in acetone
Textile: Tapestry woven garment	Carter No. 021cc	Linen, gold	Gold tarnished. Sprayed with strong solution of celluloid in amyl acetate
Textile: decorated garment	Carter No. 044r	Linen, gold	Sprayed with celluloid in amyl acetate

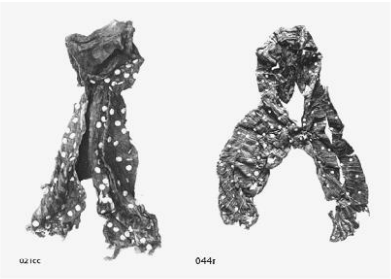

Figure 8. Decorated garments (Carter No. 021cc, Sr. No. 3247; GEM No. 15924; and Carter No. 044r, Sr. No. 3264, GEM No. 14060). Photo: Harry Burton. Courtesy of The Griffith Institute

Figure 9. Decorated garments, treated in 2001 (Carter No. 021cc, Sr. No. 3274, GEM No. 15924 and Carter No. 044r, Sr. No. 3264, GEM No. 14060). Conserved in 2001 (Abla 2001). Photo: GEM–CC

DISCUSSION

In the four case studies described, all of the objects shared the fact that they were found in the "antechamber" of Tomb 62 in the Valley of the Kings. They were all similar in that they were made of organic matter, mainly linen, and in very poor condition, showing a serious degree of cellulose degradation, darkening, and embrittlement of the fibers (Tímár-Balázsy and Eastop 1998a). The aim was to preserve the objects by means of a scientific conservation treatment at the time of excavation. This was conducted by Carter and Lucas and documented in the written notes, drawings, and photographs by Harry Barton. They were moved for storage to the Egyptian Museum around 1933 and then to the GEM–CC around 2014. The four objects, with seemingly similar conditions, showed differences in how they were recorded, treated, stored, and re-treated before coming to the GEM–CC. Our observation and decision about their conservation also varied, from reconstructive treatment to remounting, despite the institutional mandate to choose primarily preventive conservation. What were the reasons behind these decisions?

In the first case study on the hassock (Carter No. 034), the conservation decision depended on the availability of past written records, drawings, and photographs of the object. As the secondary material was informative, the conservation took a more restorative direction aimed at the object's display at the GEM.

In the second case study on the tapestry woven garment (Carter No. 054f), at the time of excavation, it was sprayed with cellulose nitrate, which did not keep the fibers together as intended, hence the aim of the treatment had been unsuccessful. It was decided not to take any further action but to place the object in a new mount and cover instead. The object was examined with X-ray photography, which revealed metal decorations that could not be discovered by surface examination alone. This case study supported the idea of keeping options open for future developments in conservation.

In the third case study on the ornamental garment (Carter No. 044t), there was very little information recorded by Carter. The conservation approach was decided after considering the chemical information on past conservation treatments and laboratory experiments. The conservator's training and the availability of equipment influenced the decision to challenge the status quo and unfold the pieces. This led to new archaeological insights and interpretation of the garment, and exemplified the need to adopt a proactive approach.

In the fourth case study on the decorated garments (Carter No. 021cc and 044r), the objects in question were educational in purpose, and the experimental nature of the study would have changed their characteristics to a great extent. Recognizing this, it was decided not to undertake any conservation treatment.

This collective study taught us that an integrated approach exploring past records, publications, and scientific and practical conservation methodology is fundamental in deciding how to proceed in a present-day context, and

that there is no single formula that applies to all conservation. It is possible that future developments may change every aspect of conservation, and that our treatment may not necessarily be the last. Thinking about future effects when planning conservation is necessary because, as these case studies demonstrate, developments will be integrated into an object and become part of its characteristics. In other words, what has been done and what is being done now can have multiple impacts on the future preservation of our heritage.

CONCLUSION

The preservation of Tutankhamun's tomb collection is a huge challenge. Working in direct contact with the objects and studying the debates and practices of the past while reviewing our own practices revealed that present-day conservation decisions integrate decisions from the past more comprehensively than initially realized. It was discovered that past decisions, in combination with the condition or state of an object and past trends in scientific approach, affect an object's characteristics, even becoming part of it. It is necessary to be aware that our present-day conservation decisions will affect the object in the same way and to keep in mind that scientific methodologies and laboratory applications are both advancing. Conservators must take care not to be heavy-handed and to remind themselves that less is more—that the challenge to discover the best conservation approach is integral to their efforts.

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NOTES

[1] For more information, see "Tutankhamun: Anatomy of an Excavation": http://www.griffith.ox.ac.uk/discoveringTut/ (accessed 5 May 2020).

[2] The Grand Egyptian Museum: http://gem.gov.eg/index/AboutGEM%20-Vision&Mission.htm (accessed 5 May 2020).

[3] The Grand Egyptian Museum Joint Conservation Project (GEM–JC Project), a joint conservation project between the Japan International Cooperation Agency (JICA) and the GEM. For more information, see https://www.jicagem.com/ (accessed 5 May 2020).

[4] Carter No. 050k: "(k) Remains of the shirt or shawl, too far gone for measurement: not kept." http://www.griffith.ox.ac.uk/gri/carter/050k.html (accessed 5 May 2020).

[5] Carter No. 044t: "Sprayed with strong solution of cellulose acetate in acetone." http://www.griffith.ox.ac.uk/gri/carter/044t.html (accessed 5 May 2020).

[6] Carter No. 044r: "Treatment sprayed with celluloid in amyl acetate." http://www.griffith.ox.ac.uk/gri/carter/044r-c044r-3.html (accessed 5 May 2020).

REFERENCES

Abdel-Kareem, O. 2001. Monitoring, controlling and prevention of the fungal deterioration of textile artifacts in the Museum of Jordanian Heritage. *Mediterranean Archaeology and Archaeometry* 10(2): 85–96.

Abla, O. 2001. Conservation and restoration studies of the Ancient Textile Collection in the Egyptian Museum, as applied on chosen pieces. Ph.D. thesis, Conservation Department, Faculty of Archaeology, Cairo University, Egypt, 210–18.

Ashley-Smith, J. 2018. The ethics of doing nothing. *Journal of the Institute of Conservation* 41(1): 6–15.

Carter, H. 1972. *The tomb of Tutankhamen*, 53–70. London: Barrie & Jenkins.

Carter, H. and A.C. Mace. [1923] 2004. *The tomb of Tut.ankh.Amen. Search, discovery and clearance of the antechamber*, vol. I. London: Duckworth Publishers.

Gilberg, M. 1997. Alfred Lucas: Egypt's Sherlock Holmes. *Journal for the American Institute for Conservation* 36(1): 31–48. https://cool.culturalheritage.org/jaic/articles/jaic36-01-003_indx.html

Kamal, H., M. Elkial, and S.T. Tawfik. 2018. The role of preventive conservation in designing King Tutankhamun galleries in the Grand Egyptian Museum. *Studies in Conservation* 63(sup. 1): 138–45.

Lucas, A. 1924. *Antiques: Their restoration and preservation,* rpt. 1974. Portway, Bath: Cedric Chivers.

Reeves, N. 1990. *The complete Tutankhamun: The king, the tomb, the royal treasure,* 56–70. Cairo: American University in Cairo Press.

Selwitz, C. 1988. *Cellulose nitrate in conservation*, Research in Conservation series, vol. 2, 15–30. Los Angeles: The Getty Conservation Institute.

Tawfik, T., S. Thomas, and I. Hegenbarth-Reichard. 2018. New evidence for Tutankhamun's parents – Revelations from the Grand Egyptian Museum. *Sonderdruck aus Mitteilungen des Deutschen Archäologischen Instituts Abteilung Kairo* 74: 177–92.

Tétreault, J. 1999. *Coatings for display and storage in museums,* CCI Technical Bulletin 21. Ottawa: Canadian Conservation Institute.

Tímár-Balázsy, A. and D. Eastop. 1998a. Fibres. In *Chemical principles of textile conservation*, chap. 1, 3–66. London: Butterworth-Heinemann.

Tímár-Balázsy, A. and D. Eastop. 1998b. Humidification. In *Chemical principles of textile conservation*, chap. 16, 275–83. London: Butterworth-Heinemann.

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CHAPTER 2

Conservation of the Tutankhamun collection: strategy of conservation decision-making in the Grand Egyptian Museum (GEM) in Giza

Hamza N. M., Shaheen E.

CONSERVATION OF THE TUTANKHAMUN COLLECTION: STRATEGY OF CONSERVATION DECISION-MAKING IN THE GRAND EGYPTIAN MUSEUM (GEM) IN GIZA

NAGMELDEEN MORSHED HAMZA & ESLAM SHAHEEN

Hassock Cart No. 034, JE 62751, bran and beadwork
(photograph by Harry Burton, Courtesy of the Griffith Institute, Oxford, UK)

Carter No. 620 (119 examples from 32 pairs of basket-work sandals
(photograph by Harry Burton, Courtesy of the Griffith Institute, Oxford, UK)

INTRODUCTION

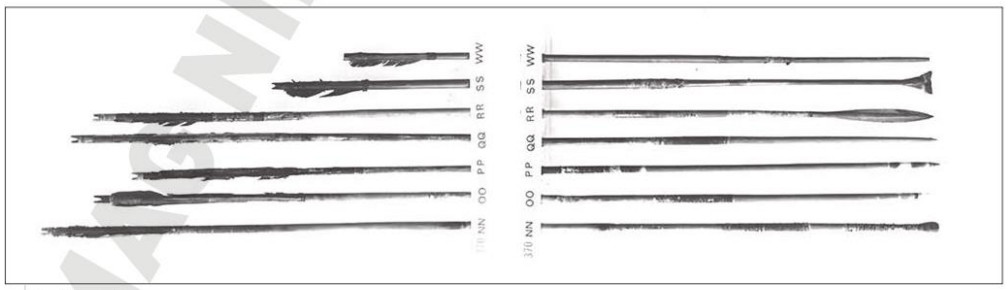
The discovery of the tomb of Tutankhamun in 1922 by Howard Carter is celebrated as one of the biggest archeological discoveries of all times. For the first time, humankind could see the wealth Egyptian kings were buried with. Among the many thousands of finds, were the Arrows, 'sewn sandals' and footstool (hassock) of the king.

MATERIALS

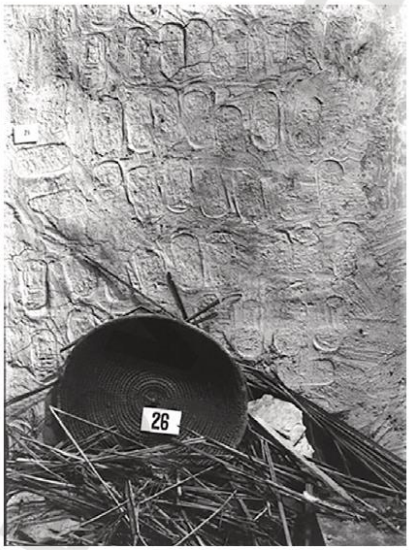
Sandals

The sole of 'sewn sandals' is made of transverse bundles of vegetable materials (usually halfa grass, *Desmostachya bipinnata* or *Imperata cylindrica*, but other grasses were sometimes used too), which are secured with a strip of doum palm leaf (*Hyphaene thebaica*) that wraps the grass bundle and secures it by the sewing. The stitches of the subsequent rows are sewn through the previous row, either through (part of) the bundle, or mainly through its winder. The transverse rows are finished with an edge, which is made in the same technique, i.e. a fibre core, which is wrapped and sewn with palm leaf strips. An edge can have two or three rows. There is considerable variety in the tightness and refinement of the sewing; the expensive C-type, however, distinguishes itself by the default high quality of manufacturing: all finds from Tutankhamun's tomb are of this type (cfr. publications of André Veldmeijer).

The straps consist of various parts. At the back, a pre-strap, consisting of a strong loop of palm leaf, is clad and secured to the edge of the sandal by additional cladding, which is stitched through the sole. The loop points upwards. The pre-strap serves as an attachment and reinforcement of the back strap, which is fixed to it with a papyrus strip (*Cyperus papyrus*). Usually, the wrapping extends a few centimetres high and includes the pre- and back strap. Although the pre-strap is made of palm, the back strap is made of various layers of papyrus, which results in an aesthetic pleasing effect. The back strap is rather thick and widens dorsally of the foot.



Tutankhamun's arrows
(photograph by Harry Burton, Courtesy of the Griffith Institute, Oxford, UK)



Arrow inside the tomb of King Tutankhamun
(photograph by Harry Burton, Courtesy of the Griffith Institute, Oxford, UK)

The front strap is attached at the centre of the back strap by means of looping. It usually consists of a core that is clad transversely with strips of palm leaf; in Tutankhamun's sandals they are clad twice. This cladding tightly secures the looping to the back strap. The lower part of the front strap is inserted in the sole off-centre to fit better between the first and second toe. At the ventral surface of the sole, the strap is secured with a crown sinnet.

Arrow
A considerable number of bows and arrows were found in the 'Annex' to the tomb of Tutankhamun (106 composite bows and 278 arrows). The arrows vary in length between 250 mm and 914 mm (10ins and 36ins) but one example is only 150 mm (6ins) long. Carter makes reference to sixteen different classes of arrow varying in detail and size. He describes only a few attributes but illustrates a selection. A number have heads of bronze, both foliate and «bullet» shaped i.e. with blunt, conical head. A generic form is the traditional, chisel-ended arrow with wooden foreshaft tipped with a single, transversely mounted microlith which, in these examples, is made of «glass» (sic), probably obsidian. They belong with our Type Al arrows. Ivory/bone pointed arrows are represented as well as others with wooden points and wooden bumper arrows with «flared» heads. In the collection are thirteen examples in which there is apparently no separate foreshaft; the shaft being made from a single, parallel- sided length of wood; these arrows carry four vanes on the shaftment. In general, the arrows have four feathers, more rarely three and have a separate nock-piece of bone, wood or ivory.

Hassock
Four hassocks and eleven footstools were discovered in the tomb of King Tutankhamun. Howard Carter preserved three from the hassock (*Cart No. 034, 354 and 361*) and did not preserve one (*Cart No.431b*) because of its bad condition. The hassocks normally have a circle shape and are made of leather or cloth and filled with bran and decorated with a pattern from beadwork. The footstool has a rectangle shape and is made normally from wood decorated in different paint and gilt. The hassock and footstool were distributed inside Tutankhamun's tomb between the Antechamber and the Annex. Carter discovered four footstools and one hassock in the Antechamber while seven footstools and three hassocks were discovered in the Annex. We can note from the description by Howard Carter that some of the footstools belong to chairs or were found with them. The diagram shows the distribution of both the hassock and the footstool inside

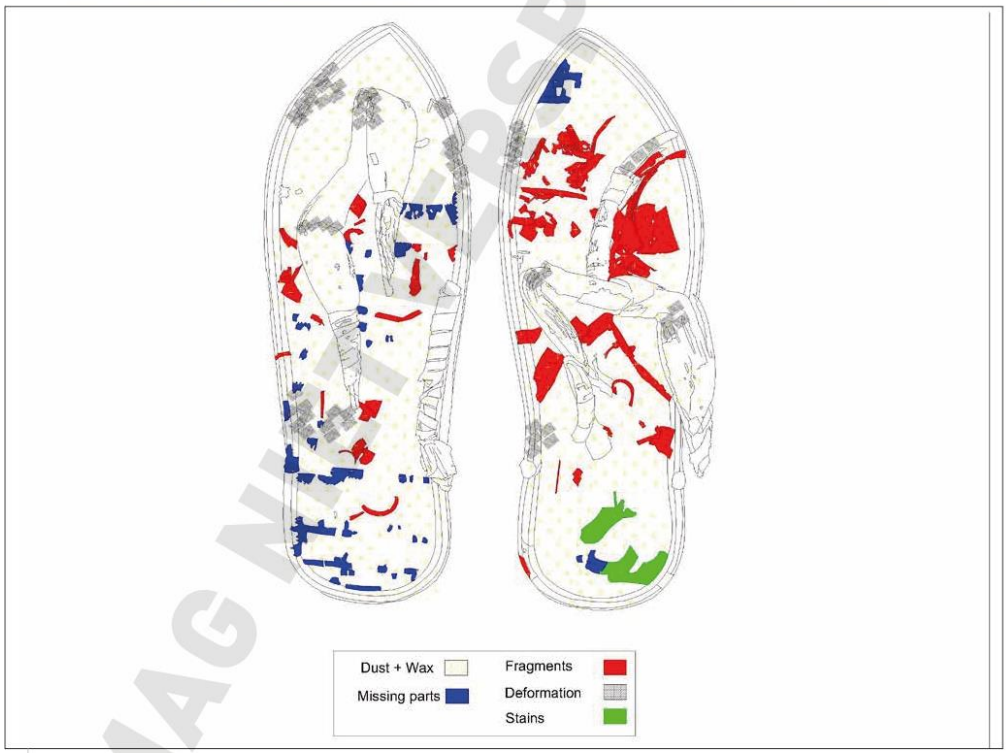
the tomb of Tutankhamun. The hassock on which our case studies was the only one, was discovered in the Antechamber *Cart No.34*. This hassock was kept inside the storeroom of Cairo Egyptian Museum from 1933 till 2014 in a wooden box from the time of its discovery.

POST-EXCAVATION TREATMENT

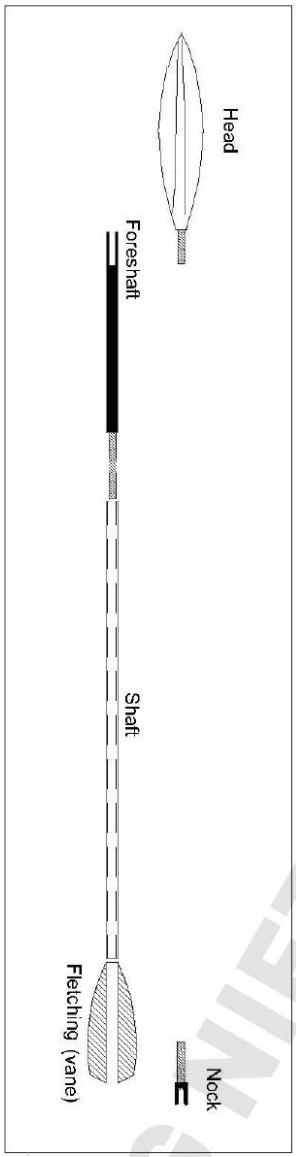
The only previous intervention made in the tomb in 1922 was boiling wax poured over the objects, Carter writes in his notes "Poured on melted paraffin wax". The objects were stored in the Egyptian museum Cairo where they were kept inside the storage No. 55 from 1933 until 2014. After 2014 the objects were moved to the conservation center of the Grand Egyptian Museum to conserve and prepare for the first-time display in the new museum. After 80 years in storage without any intervention.

Pre-Conservation
First Examination & Documentation
Preparing objects for display in the GEM-CC consists of various steps, which are documented visually (photography, annotated Auto-Cad diagrams, i.e. a visual document of the condition of the object in which the different types of deterioration and damage are marked in different colours and/or codes, sketches) as well as in a narrative format. The first step is manifold, involving the macroscopic study of the objects and a literature review of the object and object type, as well as discussions with specialists to understand the manufacturing technology and to identify the materials used (see above) as these determine the conservation strategy. Furthermore, areas of damage are registered and interpreted: whether they are ancient or more recent, as well as the type of damage. Previous interventions influence the conservation strategy, which means that, simultaneously to the aforementioned study, excavation records, the archives of the museums and conservation reports (if they exist) are consulted to get insight into previous treatment. The GEM-CC has a wide range of equipment, varying from plain magnifiers and stereo- and optical light microscopes to Scanning Electron Microscope (SEM) and Fourier Transformation Infra-Red (FT-IR), but before using these, careful consideration of what is needed is paramount. For example, if the material at hand is in such a good state, identification of the material can be done with less expensive and complicated means, such as magnifying glass or stereomicroscope. Consulting other specialists, such as archaeobotanists, secures adequate identification.

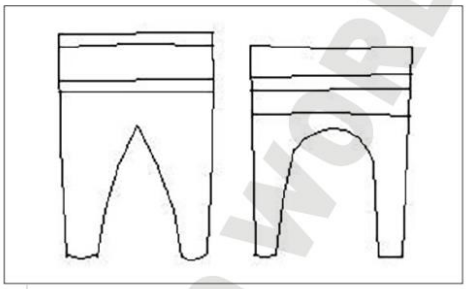
Documentation and Condition assessment
Photographic and digital document of the condition
Several methods of documentation were applied as photographic documentation, archaeological documentation and description of the objects (dimensions and deterioration aspects, composition), as well as digital documentation by computer software as AutoCAD 2d. Visual assessment by naked eye was used to evaluate the changes of the objects, deterioration aspects and the factor of deterioration. The afore-mentioned techniques allow us to make a detailed condition assessment.
In the case of chosen objects several types of damage that were recorded can be summarised here:
- Mechanical damage, caused by use, rough handling (e.g. by the robbers and/or the priests alike).
- The condition of the objects suggests that fluctuations in rh post-excavation and while in the tomb over an extended period have had a negative impact on the organic objects as they are hygroscopic materials which lose water easily in a dry environment, making the fibres extremely brittle. Moreover, if humidity rises, it absorbs water too easily, which leads to the most important aspects of damage and deformation on the shape of the sandals. These rather specific features, therefore, might indicate different areas within the tomb with different environmental conditions.
- The wax, used by the excavators, protected the objects after they were excavated but also resulted in accumulated dirt.



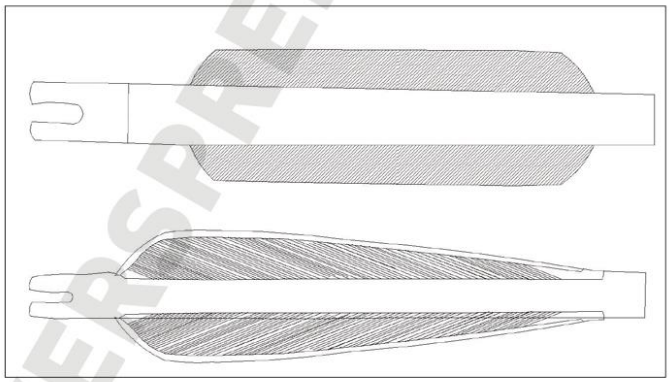
Photographic documentation, AutoCAD diagrams
(photographs by Eslam Shaheen, © diagram by NagmEldeen Hamza)

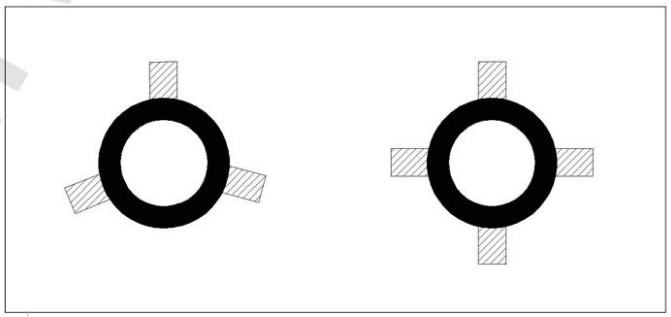


AutoCAD 2D drawing shows the construction of the arrow
(© drawing by NagmEldeen Hamza)

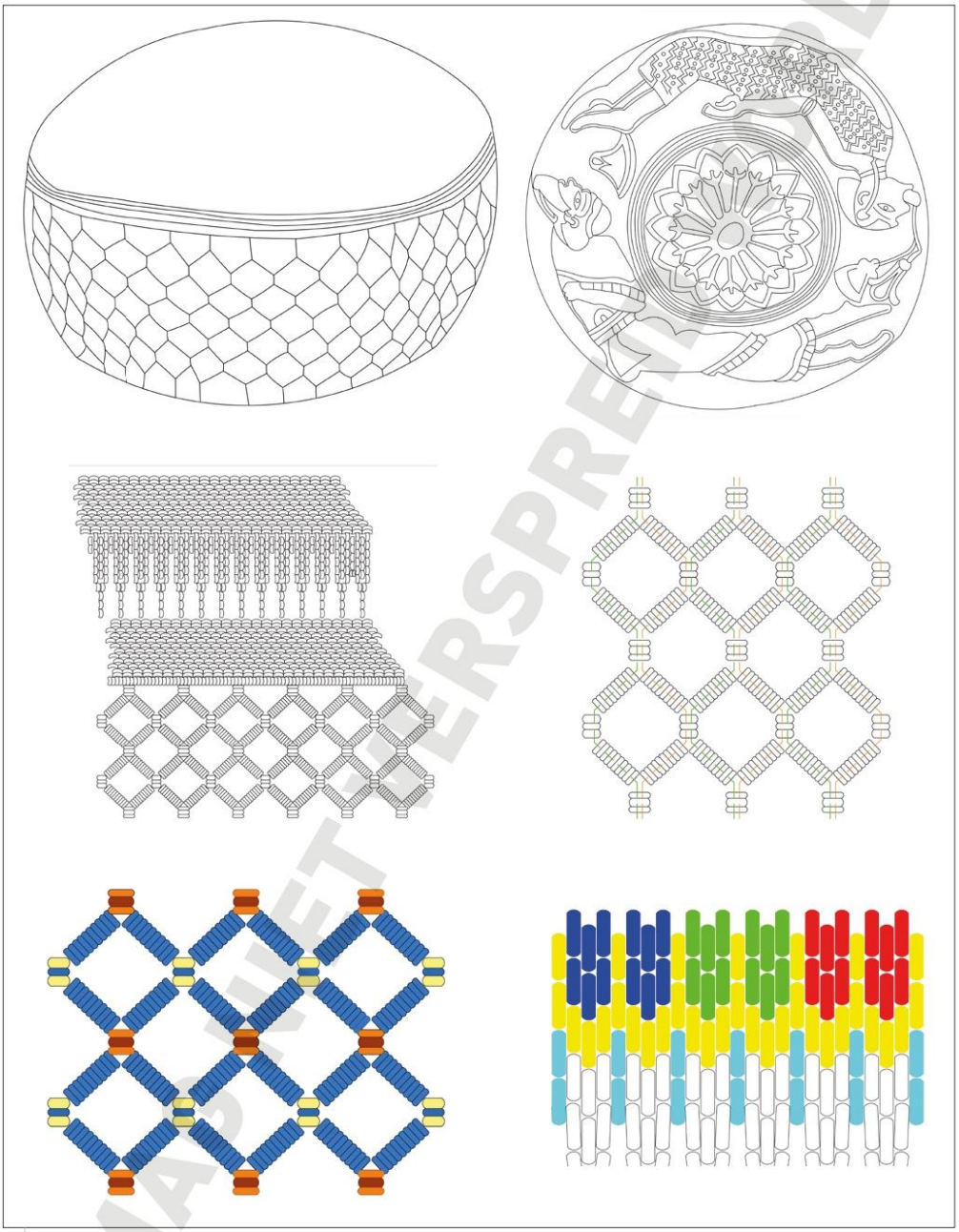


AutoCAD 2D drawing shows the U- and V-shape of the nocks
(© drawing by NagmEldeen Hamza)





AutoCAD 2D drawing shows the shape and number of the feathers on the arrow
(© drawing by NagmEldeen Hamza)



AutoCAD 2D drawing shows the shape of the hassocks and the beads arrangement
(© drawing by NagmEldeen Hamza)

CONSERVATION OF HASSOCK, CLOTH, BRAN, AND BEADWORK (CARTER NO. 034, SR. 4441, GEM NO. 15971)

Carter's object cards are firsthand records that identify an object (location, size and description) and its conservation treatment. In this case (Card No. 34-1), boiling wax was poured over the beaded object as a conservation treatment. Carter took extra notes on the materials and construction of the hassock (Card No. 34-2) in which he reconfigures the sequence of the beading techniques. On its arrival at the Egyptian Museum in 1933, the hassock was stored in a wooden box inside storage room no. 55 until it was moved to GEM–CC in 2014. Inside this box were two other cardboard boxes, one containing detached beads and the other a carbonised textile with gold leaf.

Hassock (cloth, bran and beadwork; Carter No. 034, Sr. No. 4441, GEM No. 15971)
(photograph by Eslam Shaheen, GEM-CC)

Upon examining the hassock at the Organic Laboratory, a large number of detached beads were observed. Much of the bran stuffing had fallen out, and the condition had worsened. Although the wax was not functioning as a consolidator, the conservator decided not to remove it; in some places, the wax was holding the beads together even after the threads had deteriorated and any mechanical action would have destroyed the object. A conservation decision was taken to support the deformation and reconstruct some parts of the bead decoration.
This was possible due to Carter's notes being informative enough to understand the beading technique and their arrangement (Carter No. 34-2), and that the object was intended for display at the GEM. In summary, the conservation involved reshaping and re-constructing the object into a 'footstool' by re-threading the bead decoration.

Hassock after conservation (Carter No. 034)
(photograph by NagmEldeen Hamza, GEM-CC)

POST-CONSERVATION

After conservation and reconstruction, preparations were made for the proper display or long-term storage of the objects.

Long-term storage / display
The organic objects are stored in special rooms designated for Tutankhamun's antiquities, with suitable conditions for the preservation of organic materials, most notably a regulated climate (temperature and relative humidity). The relative humidity remains between 45-55%, with a temperature between 18-22°C. and the light levels are kept at a level less than 50 lux, without ultraviolet.

Discussion
The GEM-CC procedure is one of minimal intervention for several reasons: stabilising and Consolidation is the main goal of the conservation strategy, particularly with the view in mind that new techniques in the future might have better results. The most important principles that must be considered during the conservation is the object should be re-treatable, because if a new material is developed in the future which is more effective, the old material should be easy to replace or at least not damage the original material in choosing materials to be used, these should be clearly distinguishable from the original, in order to allow future conservators to easily recognise this intervention. The use of fresh plant fibre is avoided because in due course aging may occur for these new fibres as well, making them indistinguishable from the old fibre, which will mislead the scientific research or a conservator in the future not putting any ancient fibre from fragments of sandals back without making absolutely sure that this is the right place; if there is the slightest doubt, placing fragments back should not be undertaken. The whole procedure is monitored in detail and an extensive file is made with all relevant information. As a rule, conservation intervention has both positive and negative effects. Thus, before starting the work, a strategy should be made based on the study of the condition of each piece at hand

in order to decide on the type of interventions. Furthermore, the aim of conservation is to show the object as much as possible in its pre-damaged state and prevent future deterioration, with the minimum of intervention, and in such a way that additional future investigation is not prohibited by it.

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BIBLIOGRAPHY

CLARK John Desmond, PHILIPS James & PRESTON Staley, *Interpretations of Prehistoric Technology from Ancient Egyptian and other Sources. Part I: Ancient Egyptian Bows and Arrows and their relevance for African Prehistory*, in *Paléorient*, vol. 2, nr. 2, 1974, p. 323-388; DERRICK Michele, STULIK Dusan & LAUNDRY James, *Infrared spectroscopy in Conservation Science*, Los Angeles, 1999; EL HADIDI Nesrin & HAMDY Rim, *Basketry accessories: Footwear, bags and fans in ancient Egypt*, in *Journal of archaeological science*, 38, 2011, p. 1050-1061; FLORIAN Mary-Lou, KONKRIGHT Dale & NORTON Ruth, *The conservation of artifacts made from plant materials*, Los Angeles, 1990; GOURLAY Yvon, *Les sparteries de Deir el-Médineh. XVIIIe-XXe dynasties. I. Catalogue des techniques de sparterie*, Paris, 1981; ID., *Les sparteries de Deir el-Médineh. XVIIIe-XXe dynasties. II. Catalogue des objets de sparterie*, Paris, 1981; GREIS Elhami, *Anatomical identification of plant material from ancient Egypt*, in *Bulletin de l'Institut d'Egypte*, 31, 1949, p. 249-283; HAMZA NagmEldeen Morshed & VELDMEIJER André, *Conserving, reconstructing and displaying Tutankhamon's sandals: the GEM-CC's procedure*, in *Jaarbericht Ex oriente lux*, 45, 2014-2015, p. 93-107;
McLEOD Wallace, *Composite bows from the tomb of Tutankhamun (Tutankhamun Tomb series, 3)*, Oxford, 1970; VELDMEIJER André, *Studies of ancient Egyptian footwear. Technological aspects. Part VI. Sewn sandals*, in IKRAM Salima & DODSON Aidan (eds.), *Beyond the horizon: Studies in Egyptian art, archaeology and history in honour of Barry J. Kemp*, Cairo, 2009, p. 554-580; ID., *Tutankhamon's footwear. Studies of ancient Egyptian footwear* (with contributions by Clapham Alan, Endenburg Erno, Gräzer Aude, Hagen Fredrik, *et al.*), Leiden, 2011; [http://www.sidestone.com/library/tutankhamun-s-footwear].

CONSERVATION OF THE TUTANKHAMUN COLLECTION: STRATEGY OF CONSERVATION DECISION-MAKING IN THE GRAND EGYPTIAN MUSEUM (GEM) IN GIZA

King Tutankhamun's 18th-dynasty tomb collection is expected to be displayed at the newly built Grand Egyptian Museum in Giza. The collection is one of the earliest attempts at conservation in modern terms, integrating a scientific approach to its long-term preservation and providing first-hand information about changes and developments in conservation practices from the time of its discovery in 1922 to the present day. One of the conservator's main roles is to investigate the causes of the changes that occur in objects and to find a methodology to minimise these changes for future generations. Hence, understanding an object's composite materials and past conservation treatments is necessary to develop a methodology for present-day conservation work. By examining four organic artifacts from Tutankhamun's tomb – a hassock or 'footstool' (Carter No. 034) and four textile objects (Carter No. 054f, 044t, 021cc and 044r) – in four case studies, a hypothesis was put forward that present-day conservation decisions are integrated with those from the past. Such past decisions, in combination with the condition or state and scientific approach at the time, affect the object's preservation and influence current conservation decision-making. The authors compared the similarities and differences between the four artifacts in the case studies and concluded that changes in conservation thinking and methodology had become part of each object's characteristics, and that conservation decisions taken in the past had been integrated into the objects. Similarly, conservators need to be aware that present-day conservation decisions involving advanced scientific methods and laboratory applications also affect objects and must proceed with caution, i.e. remember that less is more and that challenging situations are also an integral part of conservation.

CHAPTER 3

Conservation of evidence: the significance of objects

Biography From Tutankhamun Tomb

Hamza N. M.

CONSERVATION OF EVIDENCE:
THE SIGNIFICANCE OF OBJECTS BIOGRAPHY FROM TUTANKHAMUN TOMB

Nagmeldeen Hamza
Conservator Grand Egyptian Museum-Conservation Center, Giza, Egypt

ABSTRACT

Tutankhamun is famous throughout the world for the wealth of the objects found in his tomb. Numerous books have been written about the golden treasure, but no study writes about the materials used during the discovery. Howard Carter who discovered the tomb used in his everyday life inside the tomb some materials such as boxes, paper, cards and pieces of newspapers for packing and transportation of the objects from the tomb to Cairo Egyptian museum, the research focus on these materials which have a historical context with the objects and consist its biography.

The research focus on the packing materials used with the objects discovered in Tutankhamun tomb, not as usual on the objects itself. Howard Carter used with the objects newspaper such as the Times, Morning Post and Egyptian Gazette which date back to 1922 the time of discovery of Tutankhamun's tomb, photography and food boxes which he bring from outside Egypt and other related packing materials.

The aim of our research is to present a new ways of thinking about conservation of museum objects, thinking more about the objects biography. Despite the objects is the main concern of museum conservators but some time the packing

Nagmeldeen Hamza
nagmhamza@gmail.com
Egypt

fig. 1

1. Newspaper Used during restoration process. (Courtesy of the Griffith Institute, Oxford, UK).

materials can add more significance value to objects. The research present a study of object's life through the materials used within, Analyze the social lives of objects (Object 'biographies'), to understand what stage in an object's life we are studying, recording, representing and conserving. Know more about everyday life in the tomb and excavator, not only this but also the evidence around the world during this time. Thinking about how, where and why things are made (materials and technique), discussing their condition and significance. The research open discussion engage community with people from different nationality about different articles and evidence related to their country in these pieces of newspaper. Thinking about how to display these pieces within the objects to engage the community with preserving not only the objects but also the evidence of the history will be very interesting to complete the story about Tutankhamun's tomb and to be attractive for visitor in our new museum GEM-CC.

INTRODUCTION

Biography is the life story: help us to understand what stage in an object's life we are studying, recording, representing and conserving. Analysis of objects is useful because they help us to think about: how things are made (materials and technique), where they were made and used, why they are in poor or good condition, how their significance varies.

2a-b. Food boxes and box for glass plate negatives used as packing boxes.

fig. 2

MATERIALS

H. Carter used in his everyday life inside the tomb of Tutankhamun some materials such as boxes, paper, cards and pieces of newspaper for packing and transportation of the object from the tomb to the Egyptian museum at Cairo (fig. 1 e fig. 2).

All these materials have a historical context with the archaeological objects from the tomb; these materials can tell rich stories about the everyday life of the excavators. The idea from this case study is to study biographies of objects with these pieces of newspapers with the everyday life of the excavator, making link between the dates of the newspaper and what happened inside the tomb in this date with the aim to preserve them as a part of the history of the objects.

3. The Times Newspaper used as a packing material for Tutankhamun textiles in 1922.

fig. 3

4. Newspaper used as a packing material for Tutankhamun textiles in 1922.

fig. 4

In this case study conservation focus on preserving the materials used with the objects as packing and storing as they have social lives. Looking into newspaper which Carter used with the objects such as the Times, Morning Post and Egyptian Gazette, as packing materials for textiles from the tomb to the Egyptian museum at Cairo (fig. 3 e fig. 4). Conservation decided to preserve the biography of objects and the diaries of excavators.

Conservation in this case not an intervention but extraction of data from these materials. documenting the articles in this newspaper records that some of them talk about the discovery of the tomb, other article talk about the fleet's tour London fire visitors to Luxor and other one talk about American scientists from Metropolitan at the tomb, a lot of articles have interesting evidence . Also we will give a hint about the letter of excavator to their friend such as the letter from Mace to his wife.

fig. 5

5. Simulation of display Design for newspaper pieces.(Author design).

Documentation for the food and photographic boxes which Carter brings it from London and used it as storage boxes for objects, also numbering cards made by Carter. Packing paper which have found with objects contain different number than Carter number gives more details about the development of numbering of Tutankhamun's collection by the time.
Two popular objects were received in these materials the textiles and the hassock. Textiles were packed in both cardboard and wooden boxes inside these boxes were the pieces of the newspaper. The cardboard box with the hassock used to preserve the beads, this box was a French box of gelatin silver bromide photographic plates, marketed at the beginning of the 20th century by the Lumière company "anonymous society of photographic plates and papers Antoine Lumière and his sons", which thought to be brought by Hurry Burton the photographer of the tomb. A nice story about this box is that the arrival of gelatin silver bromide plates changed the history of photography at this time. Also give more details about why Hurry Burton chooses this type of plates which allow producing in advance large quantities that can be preserved before and after the shooting.
Thinking about how to display these pieces with the objects to engage the community with preserving not only the objects but also the evidence of the history will be very interesting to complete the story about Tutankhamun's tomb and to be attractive for visitor in our new museum GEM-CC (fig. 5).

CONCLUSION

The preservation of Tutankhamun's tomb collection is a huge challenge. Working in direct contact with the objects and studying the debates and practices of the past while reviewing our own practices revealed that present-day conservation decisions integrate decisions from the past more comprehensively than initially realized.

All conservators must accept that communicating with the public is part of their role Public engagement will be the way forward. In building a strong profession conservation has a future if the wider community feel and thinks it is important, this come out when conservators engage the community about their work to make the case that the work they do is important. Conservators need to convince the general public that conservation matters. Conservation is not only the intervention or treatment we applied to our collections, conservation can apply to preserve the materials around the objects which maybe data, storage materials and registration cards which we can call it objects biography.

BIBLIOGRAPHY

H. Carter, *The tomb of Tutankhamen*, London 1972

H. Carter, and A.C. Mace. *The tomb of Tut.ankh.Amen. Search, discovery and clearance of the antechamber*, London [1923] 2004, voll. 1

M. Gilberg, *Alfred Lucas: Egypt's Sherlock Holmes*, in Journal for the American Institute for Conservation, 36(1), 1997, pp. 31-48 https://cool.culturalheritage.org/jaic/articles/jaic36-01-003_indx.html.

A. Lucas, *Antiques: Their restoration and preservation*, Bath rpt. 1974

N. Reeves, *The complete Tutankhamun: The king, the tomb, the royal* treasure, Cairo 1990

R. Hall , Egyptian Textiles - Osprey Publishing, 1986

http://www.griffith.ox.ac.uk/discoveringTut/.

https://www.newspapers.com/paper/the-morning-post/7847/.

https://egyptian-gazette.com/.

https://en.wikipedia.org/wiki/The_Egyptian_Gazette.

https://www.mortonsflour.com/history/.

http://www.collections.musee-bretagne.fr/ark:/83011/FLMj0272407.

SECTION 2

Archaeobotany

CHAPTER 4

Trash or treasure? Plant remains from the tomb of Tutankhamun

Hamza, N. M., Moricca, C., Sadori L.

Trash or treasure? Plant remains from the tomb of Tutankhamun

Nagmeldeen Morshed Hamza[1,2,3], Claudia Moricca[2], Laura Sadori[2]

[1] Department of Earth Sciences, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy

[2] Department of Environmental Sciences, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy

[3] Grand Egyptian Museum, Conservation Center, Al Remaya Square, Al Haram, Giza Governorate, 3513204, Egypt

Introduction

While thinking about Pharaohs, Tutankhamun (his reign 1334-1325 BC, 18th Dynasty) immediately comes to mind (Uda et al., 2007). The discovery of his tomb (KV 62) in the Valley of Kings (Luxor, Egypt) by Howard Carter in 1922 remains to this day one of the most spectacular archaeological finds of all times. After spending thousands of pounds to finance the excavation works, Lord Carnarvon was ready to give up the concession, but was persuaded by Howard Carter to continue for one last field season. This decision was worth the extra costs, as the flight of steps leading to the famous tomb was uncovered on the second day of the extension (Edwards, 1972). While the tomb had been violated during the reign of Ramses VI (1141-1133 BC), it was clear that valuable contents survived the robbery, as its entrance was sealed by the necropolis staff (Edwards, 1976). Its treasure included numerous golden objects, amongst which was the funerary mask of Tutankhamun, currently stored at the Egyptian Museum of Cairo and representing the top technology in the production of mummy masks (Hassan, 2016).

Unlike commonly done by other archaeologists of the time, Howard Carter managed to acknowledge the potential value of less prestigious findings, including plant remains. These include funerary garlands (Hamdy, 2007) and the contents of 20 containers stored in the annex of the tomb (de Vartavan, 1990). All the remaining plant material was swept from the surfaces of the tomb and deposited in a wooden box (200 x 81 x 56 cm). The box was closed in 1933 and was stored in the Egyptian Museum in Cairo until 2017. In 2018 it was moved to the Grand Egyptian Museum, where scientists started working on it in 2019.

The present study aims at analysing the archaeobotanical contents of this wooden box, until recently considered as “trash”, but which has proven to be a treasure of its own.

Materials and Methods

A volume of approximately 80 litres of filling was processed for this archaeobotanical study. Plant remains were separated through dry sieving, using piled up sieves with 10- and 2-mm meshes. Remains from each fraction and from the residue were then hand-picked. Macro-remains were observed under a digital Keyence microscope HX-950F (magnification between 0.1x to 1000×) equipped with a camera, which allowed photographic acquisition. Morphological identification was performed by comparing the samples against several atlases (de Vartavan and Amorós, 1997; Cappers, Neef and Bekker, 2009; Neef et al., 2012; Cappers and Bekker, 2013; Jacomet, 2006; Sabato and Pena-Chocarro 2021) and modern reference samples.

Considering the type of context and the dry environmental conditions which led to the preservation of plant remains, it was necessary to perform high precision ^{14}C AMS dating. Selected remains were sent to the CIRCE (Center for Isotopic Research on the Cultural and Environmental heritage) laboratory in Caserta (Italy), which provided us with calibrated dates (Terrasi et al., 2008).

Results

Almost 9000 well-preserved fragments of seeds and fruits, belonging to 41 taxa, mostly identified at species level, and attributed to 21 different plant families were found (Table 1). Botanical nomenclature follows World Flora Online (2023).

	Family	**Species**	**Common name**	**Plant part**	**Number**
GYMNOSPERMS	Cupressaceae	*Cupressus sempervirens* L.	cypress	twig	1
		Juniperus sp.	juniper	seed cone	2180
				seed cone scale	1
				underdeveloped cone	1
ANGIOSPERMS	Amaryllidaceae	*Allium cepa* L.	onion	tunic fragment	11
				basal plate	9
		Allium sativum L.	garlic	tunic	17
		Allium cepa/sativum	onion/garlic	flowering stem	3
	Amaranthaceae	*Beta vulgaris* L.	beet	perianth	1
	Apiaceae	*Coriandrum sativum* L.	coriander	mericarp	125
				pedicel	2
	Arecaceae	*Hyphaene thebaica* Mart.	doum palm	mesocarp	5

		Medemia argun Württemb. ex H.Wendl.	Argun palm	fruit	13
		Phoenix dactylifera L.	date palm	seed	97
				seed fragment	74
				fruit	7
				fruit fragment	13
				fruit cap	13
				undeveloped seed	5
	Asteraceae	*Carthamus tinctorius* L.	safflower	fruit	2
		Centaurea depressa M. Bieb.	centaury	fruit	3
	Boraginaceae	*Cordia myxa* L.	Assyrian plum	achene	1
	Brassicaceae	*Raphanus raphanistrum* L.	wild radish	pedicel	1
	Cucurbitaceae	*Citrullus lanatus* (Thunb.) Matsum. & Nakai	watermelon	seed	1586
	Euphorbiaceae	*Ricinus communis* L.	castor bean	seed	3
				capsule fragment	5
	Fabaceae	*Cicer arietinum* L.	chickpea	pedicel	1
		Ceratonia siliqua L.	carob	seed	1
		Lathyrus oleraceus Lam.	green pea	seed	4
		Medicago polymorpha L.	burr medick	fruit	2
		Vicia faba L.	broad bean	tegument	3
		Vicia/Lathyrus sp.	-	seed	1
		Trigonella foenum-graecum L.	fenugreek	seed	35
		Pulses indet	-	pod fragment	1
	Lythraceae	*Punica granatum* L.	pomegranate	exocarp	7
	Malvaceae	*Grewia tenax* (Forskk.) Fiori.	grewia	endocarp	83
				fruit	2
		Gossypium sp.	cotton	seed	1
		Malva sp.	malva	seed	16
				fruit	2
	Myrtaceae	*Myrtus communis* L.	myrtle	fruit	1
	Poaceae	*Eragrostis* sp.	lovegrass	spikelet	2
		Hordeum vulgare L.	barley	spikelet	18
				rachis fragment	1

		Triticum aestivum/durum	naked wheat	spikelet	1
		Triticum turgidum subsp. *dicoccon* (Schrank ex Schübl.) Thell.	emmer	caryopsis	15
				husk	108
				rachis fragment	9
		Sorghum bicolor (L.) Moench	sorghum	husk	7
		Cereals indet.	-	caryopsis	1
	Oleaceae	*Olea europaea* L.	olive	endocarp	613
				endocarp with pedicel	1
	Polygonaceae	*Persicaria* sp.	-	fruit	1
	Rhamnaceae	*Ziziphus spina-christi* (L.) Mill.	Christ's thorn	endocarp	3287
				fruit	1
	Rosaceae	*Prunus dulcis* D.A. Webb	almond	endocarp	49
				half endocarp	1
	Sapotaceae	*Mimusops laurifolia* (Forssk.) Friis	Egyptian persea tree	seed	3
		cf. *Mimusops laurifolia* (Forssk.) Friis		Floral gem	10
	Vitaceae	*Vitis vinifera* L.	grapevine	seed	345
				pedicel	7
	Indet.	-	-		5
				TOTAL	8823

Table 1. List of identified of taxa and relative counts.

The botanical remains were preserved by desiccation, favoured by the dry and constant environmental conditions present in the tomb of the pharaoh. This allowed for them to retain a modern looking appearance, in terms of both size and colour, also preserving some of the most fragile vegetative parts, such as *Allium cepa* L. (onion) and *Allium sativum* L. (garlic) tunics (Fig. 1a), and complete fruits (e.g., *Phoenix dactylifera* L.). Some of the remains have acquired a darker brown colour (e.g., *Lathyrus oleraceus* Lam.; Fig. 1i).

Food plants, including cereals, pulses, and fruit crops, constitute a relevant part of the assemblage. Fabaceae and Poaceae are qualitatively the most represented families, with four taxa each. From a quantitative perspective, the most abundant taxa are represented by *Ziziphus spina-christi* (L.) Mill. (Christ's thorn; 3287 endocarps; Fig. 1n), *Juniperus* sp. (juniper; 2180 seed cones), and *Citrullus lanatus* (Thunb.) Matsum. & Nakai (watermelon; 1586 seeds; Fig. 1b).

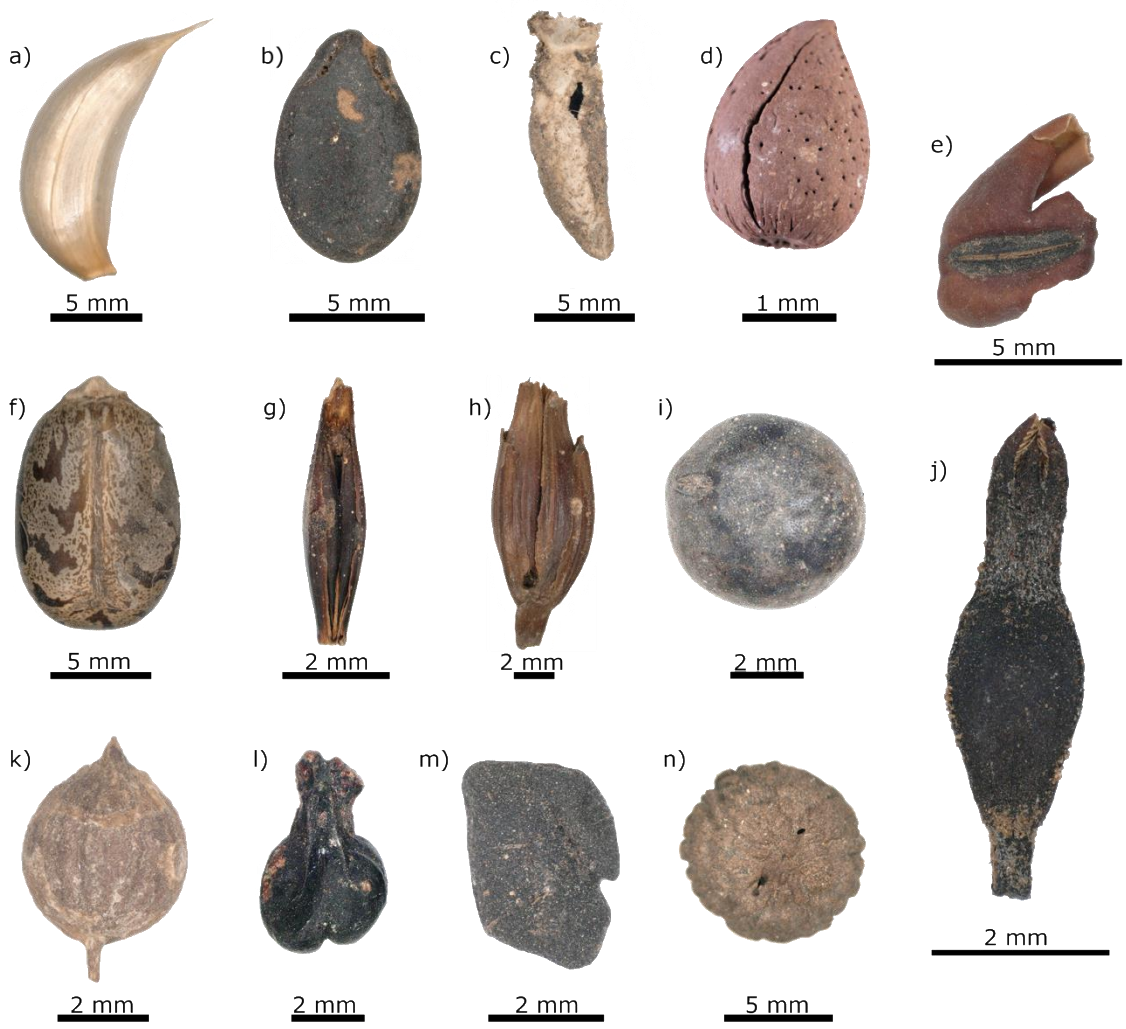

Figure 1. Selected plant remains from the wooden box: a) *Allium sativum*; b) *Citrullus lanatus*; c) *Centaurea depressa*; d) *Prunus dulcis*; e) *Vicia faba*; f) *Ricinus communis*; g) *Hordeum vulgare*; h) *Triticum turgidum* subsp. *dicoccon*; i) *Lathyrus oleraceus*; j) cf. *Mimusops laurifolia*; k) *Coriandrum sativum*; j) *Vitis vinifera*; m) *Trigonella foenum-graecum*; n) *Ziziphus spina-christi*.

^{14}C AMS dating allowed to confirm the coherence of a *C. lanatus* seeds with the closing of the tomb (Fig. 2). However, it also led to the identification of modern contaminations (Tab. 2).

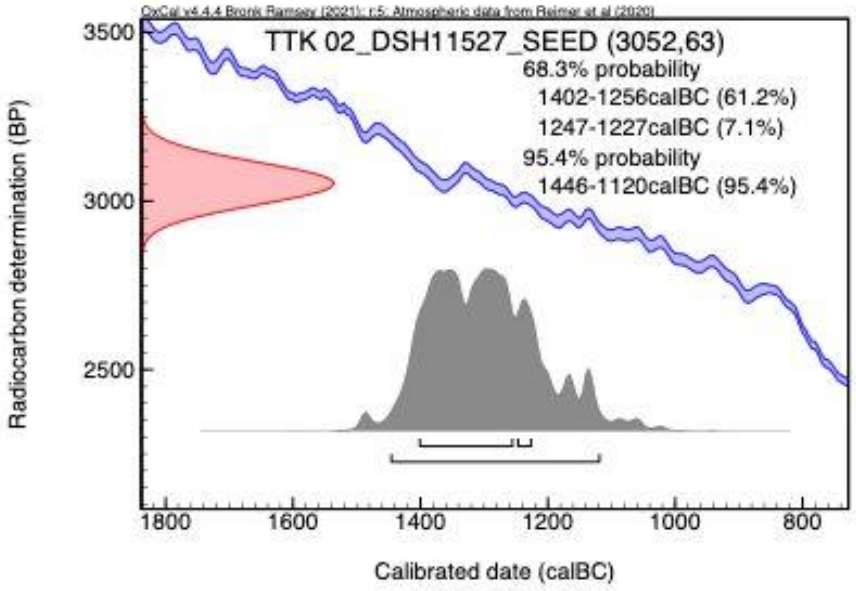

Figure 2. Calibrated ^{14}C AMS date of a *Citrullus lanatus* seed.

Taxon	**Plant part**	**Calibrated date (2σ)**
Luffa aegyptiaca Mill.	seed	1672 – post-1902 AD
Citrus arantium L.	seed	1675 – 1942 cal. AD
Citrus sp.	pericarp fragment	1675 – 1942 cal. AD

Table 2. Calibrated ^{14}C AMS dates identifying modern contaminations.

Discussion

The arid environmental conditions present in Egypt have allowed to preserve desiccated materials from a wide range of context and time periods, spanning from the Predynastic Period (e.g. Fahmy, 2005) to the Ottoman Period (Palmer et al., 2009). An attempt to summarize all archaeobotanical studies performed on Egyptian materials was done by de Vartavan and Asensi Amorós, who first published the Codex of Ancient Egyptian Plant Remains in 1997, then updating it in 2010 (de Vartavan, Arakelyan and Asensi Amorós, 2010).

Our knowledge of plants in ancient Egypt is also greatly favored by iconography. In fact, plants are included in various forms of ancient art, such as mural paintings in tombs, temple reliefs, statues, and hieroglyphs, providing us with valuable evidence of their significance in ancient Egypt (Cornelius, 1989).

The present study reveals a vast assemblage of perfectly preserved plant remains from the tomb of the pharaoh Tutankhamun. While this context has been subject of previous archaeobotanical studies (eg. Germer, 1989; de Vartavan, 1990), new data is presented in this article, including species not previously recorded.

These are largely represented by vegetative plant parts, such as teguments of fava beans (*Vicia faba* L.; Fig. 1e), fragile pedicels (of chickpea – *Cicer arietinum*, and wild radish – *Raphanus raphanistrum*) and perianths (*Beta vulgaris* – beet). These plant parts, along with fragile papery tunics of onion and garlic, are usually not preserved in archaeobotanical assemblages due to tissue softness and can be included in the so-called category of "missing plants" (Fahmy, 2005). Other plants not previously recorded in the deposit are *Cordia myxa* (Assyrian plum) and *Eragrostis* sp.

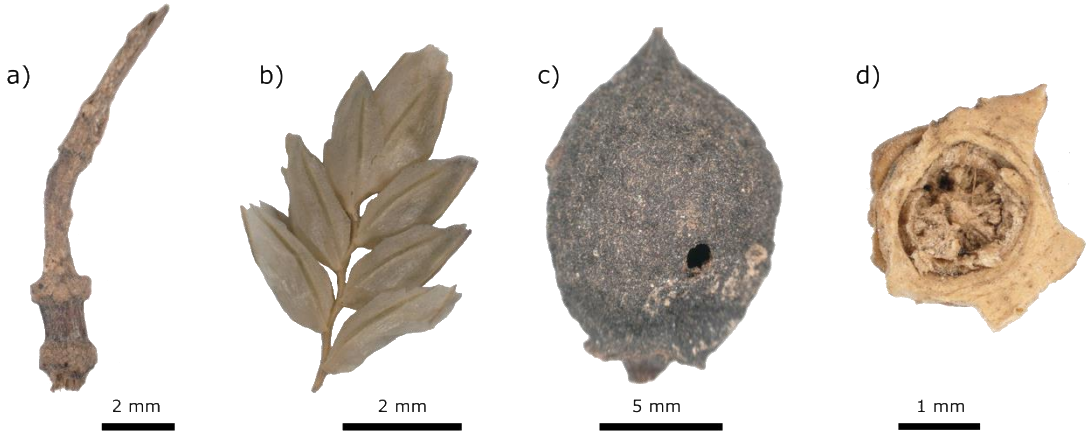

Figure 3. Plant taxa not previously recorded in the tomb of Tutankhamun: a) *Raphanus raphanistrum*; b) *Eragrostis* sp.; c) *Cordia myxa*; d) *Beta vulgaris*

Our analysis confirms some of the data reported in previous studies in the tomb of Tutankhamun and other contemporary contexts, such as the nearby tomb KV63 (Hamdy and Fahmy, 2018). Not surprising is the concentration of juniper seed cones. While *Juniperus* sp. is not native of Egypt, it was found already in Predynastic contexts (Hamdy, 2005), where it was interpreted as being imported from longer distances (such as Sinai, Libya, Levant, East Africa, or Arabia).

Not only have they been previously found in the tomb of the king (de Vartavan, 1990), but they are common in other ancient Egyptian graves. It is believed that both juniper seed cones and juniper resin were used during the embalming process (Abdel-Maksoud and El-Amin, 2011). Furthermore, juniper is mentioned in the Ebers Papyrus (17^{th}-16^{th} centuries BC) as a medicinal plant, along with other species found in the tomb, such as garlic, castor oil plant (*Ricinus*

communis; Fig. 1f), coriander (*Coriandrum sativum*; Fig. 1k), fenugreek (*Trigonella foenum-graecum*; Fig. 1m), dates (*Phoenix dactylifera*), and pomegranate (*Punica granatum*; Aboelsoud, 2010). Egyptians believed that garlic and onion favored endurance, with the former also being used as a treatment for asthma, for sore throats and toothache.

Coriander was also used as a medicinal herb, appreciated for its digestive properties, while dates and castor oil served as laxatives. The finding of *R. communis* in the studied "box" represents the first attestation of this species in the tomb of Tutankhamun. Interesting is also the association of wine, stored in the tomb (Guasch-Jané et al., 2006), with medicinal plants, as it is believed that many herbs were steeped in wine and drunk as an oral medicine (McGovern, Mirzoian and Hall, 2009).

The finding of numerous medicinal plants is particularly relevant in the tomb of Tutankhamun, as genetic studies performed on his mummy revealed several pathologies including Köhler disease II and indications of malaria tropica (Hawass et al., 2010).

While fenugreek is known to have been used in Ancient Egypt as a medicinal plant to treat respiratory disorders, cleanse the stomach, calm the liver, soothe pancreas, and reduce swellings (Verna et al., 2020), it could have had a different use in the tomb of Tutankhamun. A hypothesis can be advanced following the modern use of fenugreek seeds in Egypt, with them being toasted, grind and added to dough to make softer bread. Previously interpreted in the tomb of the king as an insect repellent in grain storage (Panagiotakopulu et al., 1995), it seems more likely that it was used as a fumigant in incense burning following the Egyptian tradition of the "Holy Smoke" (Hiles and Mahmood, 2021).

Another class of remains widely represented in the archaeobotanical assemblage is that of food plants. This is not surprising, as funerary offerings in the New Kingdom Egypt were meant to reflect the special role of the deceased, providing him with anything that he might need during the afterlife (Smith, 1992). The plant families that show the biggest variety, especially in terms of food plants, are Fabaceae and Poaceae (Fig. 1g and Fig. 1h). Nonetheless, this class of remains also includes numerous fruit plants, such as *Punica granatum*, *Prunus dulcis* (Fig. 1d) and *Citrullus lanatus*. The latter is native to Africa and has been cultivated since ancient times. Its depictions are common also in ancient Egyptian tomb (Paris, 2015). Nonetheless, its presence in the studied context could be overrepresented, as a single watermelon can contain hundreds of seeds.

The presence of numerous *Vitis vinifera* pips (Fig. 1l) and 7 pedicels in the studied sediment is not surprising, as it is also present in wall paintings of other Egyptian tombs, such as the tomb of Nakht at Thebes (ca. 1425–1350 BC, 18th Dynasty; Wills, 2018). Grape remains were also

recorded in previous archaeobotanical studies concerning the tomb of Tutankhamun (eg. De Vartavan, 1990) and the study of dry residue samples from the same context highlighted the offering of both red and white wines (Guasch-Jané et al., 2006).

Modern contaminations

The presence of modern contaminations, in the form of *Luffa aegyptiaca* (luffa) seeds, *Citrus arantium* (mandarin orange) seeds and *Citrus* sp. pericarp fragments, was assessed through high precision ^{14}C AMS dates. The dates are coherent with the phase of discovery the tomb and documentation of its contents by Howard Carter and his team (1922-1933). These plant remains represent evidence of the life of the workers responsible for these operations, who probably ate mandarin oranges on the site and used luffa sponges to clean the tomb surfaces. Material evidence of this "phase of life" of the tomb is also found in the form of newspapers and food boxes used to pack samples and wrap archaeological textiles (Hamza, 2021). Nevertheless, it is undeniable that the presence of modern contaminations makes it harder to assess the authenticity of the remaining portion of the assemblage. For this reason, new ^{14}C AMS dates need to be carried out on an array of other samples.

Discussion

Ziziphus spina-christi (L.) Willd. fruits are eaten by natives in the region of the Upper Nile, and the immature fruits are said to be medicinally useful as a laxative and febrifuge (Hepper, 2009).

The Eber papyrus indicates that Christ's thorn fruits and leaves were used frequently in ancient Egyptian medicine for a variety of foods and the species continues to be used in folk medicine today. In ancient Egyptian prescriptions, it was used in remedies against swellings, pain, and heat, and thus should have anti-inflammatory effects (Kadioglu, 2016).

Z. spina-christi occurs as an ingredient of 33 ancient Egyptian prescriptions in the papyri Ramesseum V, Edwin Smith (Sm), Ebers (Eb), Hearst (H), Berlin 3038 (Bln), and Brooklyn 47.218.48+85 (Brk) (Kadioglu, 2016). Besides that, fruits and a kind of bread made of it are some of the main items in the food offering rituals for the dead written down in tombs (CITATION). It occurs among the typical cereals, fruits, and drinks of the ancient Egyptians, and, thus, seems to have played an important role in the diet of the living as well. The tree was a typical part of the indigenous ancient Egyptian wild flora and was also planted in ancient Egyptian gardens (Kadioglu, 2016; Nicholson and Shaw, 2000).

The earliest recorded medicinal use of juniper berries occurs in ancient Egypt. A papyrus dating back to 1500 BC contains a recipe to cure tapeworm infestations, as well as for mummification

processes. Juniper oil was used for anointing the body; juniper berries were also incorporated between the layers of linen bandages around mummies and combined with natron, which preserved the flesh. Egyptian medical texts described it as a diuretic and laxative. The juniper berries were used also in Egyptian cosmetics and medicine (Hepper, 2009).

De Vartavan and Asensi Amoros (1997) listed ten sites in Egypt, dating back to the pre-dynastic and dynastic eras, containing remains of watermelon (Paris, 2016). Watermelon is native of tropical and subtropical Africa (Charles, 1987, Bates et al., 1995), widely cultivated in the Mediterranean Region and Sudan. Oil and tar are obtained from the seeds in Upper Egypt. Domestication of the watermelon (*Citrullus lanatus*) has alternatively been placed in South Africa, in the Nile valley, or, more recently, in West Africa, with the oldest archaeological evidence coming from Libya and Egypt (Renner et al., 2019). In ancient Egypt, however, the watermelon may have been cultivated primarily for its seed (Täckholm, 1961; Germer, 1985), as is the case in many parts of Africa today (FAO, 1988). A 3500-year-old leaf from an Eighteen Dynasty Pharaonic tomb revealed that Egyptians in the New Kingdom were cultivating domesticated watermelon with red-fleshed, non-bitter domesticated form (Renner et al., 2019).

Conclusions

The study of archaeobotanical materials recovered from Tutankhamun tomb the pharaoh of the Eighteen Dynasty provide not only new elements on funerary royal equipment, but also to know more on daily life in Ancient Egypt. The vast botanical treasure from Tutankhamun tomb is not less valuable than any of the artefacts discovered in the tomb. The value of these botanical treasures lies precisely in the knowledge gathered from them, considering the obvious absence of textual evidence of Tutankhamun.

Thanks to the extremely dry environmental conditions present in the tomb, which allowed a near-perfect state of preservation of even the most fragile plant remains, and to the exceptional caution of Howard Carter and his team, the plant remains from the sweepings of the tomb surfaces turned out to be a real treasure for archaeobotanists. In other situations, they would have been discarded as garbage.

The present study highlights how the Egyptian custom of placing everyday items in tombs has increased the chances of preserving remains of plants (Manniche, 1989), allowing entire plant assemblages to remain intact for thousands of years.

It was thus possible to characterize the archaeobotanical assemblage of the tomb of Tutankhamun, comprised mostly of funerary offerings, represented by both food and medicinal plants, necessary to accompany the pharaoh in his afterlife. Furthermore, this study made it

possible to identify the presence of not previously recorded species in the tomb of Tutankhamun, such as *Beta vulgaris*, *Cordia myxa*, *Eragrostis* sp. and *Raphanus raphanistrum*. Other than performing ^{14}C AMS dates on a wider array of botanical remains, future perspectives include morphometric analyses of the retrieved grape pips, and a comparison with selected modern cultivars. This would allow us to obtain more precise information about the fruits selected as an offering for the king, and the heritage they left. Furthermore, aDNA analyses could be attempted.

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Bibliography

Abdel-Maksoud, G., & El-Amin, A. R. (2011). A review on the materials used during the mummification processes in ancient Egypt. *Mediterranean Archaeology & Archaeometry*, *11*(2).

Cappers, R. T., & Bekker, R. M. (2013). *A manual for the identification of plant seeds and fruits* (Vol. 23). Barkhuis.

Cappers, R. T., Neef, R., & Bekker, R. M. (2009). *Digital atlas of economic plants* (Vol. 1). Barkhuis.

Cornelius, I. (1989). The garden in the iconography of the Ancient Near East: a study of selected material from Egypt. *Journal for Semitics*, *1*(2), 204-228.

de Vartavan, C. (1990). Contaminated plant-foods from the tomb of Tutankhamun: A new interpretive system. *Journal of Archaeological Science*, *17*(5), 473-494.

de Vartavan, C., Arakelyan, A., Amorós, V. A. (2010). *Codex of ancient Egyptian plant remains*. Second edition. Sais Academic Books: London.

Edwards, I. E. S. (1976). *Tutankhamun, his Tomb and its Treasures*. Metropolitan Museum of Art.

Edwards, I. E. S. (1972). Some reflections on the Tutankhamun exhibition. *The Burlington Magazine*, *114*(829), 202-208.

Fahmy, A.G.E.D.(2005). Missing plant macro remains as indicators of plant exploitation in Predynastic Egypt. *Vegetation history and archaeobotany*, *14*(4), 287-294.

Germer, R. (1989). *Die Pflanzenmaterialien aus dem Grab des Tutanchamun.* Hildesheim : Gerstenberg.

Germer, R. (1985). Flora des pharaonischen Ägypten. von Zabern.

Guasch-Jané, M. R., Andrés-Lacueva, C., Jáuregui, O., & Lamuela-Raventós, R. M. (2006). First evidence of white wine in ancient Egypt from Tutankhamun's tomb. *Journal of archaeological science*, *33*(8), 1075-1080.

Hamdy, R. (2007). Plant remains from the intact garlands present at the Egyptian museum in Cairo. *Fields of Change. Progress in African Archaeobotany. Groningen: Groningen Archaeological Studies, Barkhuis & Groningen University Library*, 115-126.

Hamdy, R., & Fahmy, A. G. (2018). Study of plant remains from the embalming cache KV63 at Luxor, Egypt. *Plants and People in the African Past: Progress in African Archaeobotany*, 40-56.

Hamza, N. M. (2021). Conservation of evidence: the significance of objects. Biography from Tutankhamun tomb. In: Young Professionals Forum Proceedings 2021 (pp. 121-125). Centro Conservazione “Restauro La Venaria Reale”.

Hawass, Z., Gad, Y. Z., Ismail, S., Khairat, R., Fathalla, D., Hasan, N., Ahmed, A., Elleithy, H., Ball, M., Gaballah, F., Wasef, S., Fateen, M., Amer, H., Gostner, P., Selim, A., Zink, A., & Pusch, C. M. (2010). Ancestry and pathology in King Tutankhamun's family. *Jama*, *303*(7), 638-647.

Hepper, F. N. (2009). Pharaoh's flowers: the botanical treasures of Tutankhamun. Kws Publishers.

Hilles, A. R., & Mahmood, S. (2021). Historical background, origin, distribution, and economic importance of fenugreek. *Fenugreek: Biology and Applications*, 3-11.

Jacomet, S. (2006). Identification of cereal remains from archaeological sites. *Basel University, Basel*.

Kadioglu, O., Jacob, S., Bohnert, S., Naß, J., Saeed, M. E., Khalid, H., ... & Efferth, T. (2016). Evaluating ancient Egyptian prescriptions today: anti-inflammatory activity of Ziziphus spina-christi. Phytomedicine, 23(3), 293-306.

Manniche, L. (1989). An ancient Egyptian herbal. University of Texas Press.

Neef, R., Cappers, R. T., & Bekker, R. M. (2012). *Digital atlas of economic plants in archaeology* (Vol. 17). Barkhuis.

Ogden, J., Nicholson, P. T., & Shaw, I. (2000). Ancient Egyptian materials and technology.

Pagnoux, C., Celant, A., Coubray, S., Fiorentino, G., & Zech-Matterne, V. (2013). The introduction of Citrus to Italy, with reference to the identification problems of seed remains. *Vegetation History and Archaeobotany*, *22*(5), 421-438.

Palmer, S. A., Moore, J. D., Clapham, A. J., Rose, P., & Allaby, R. G. (2009). Archaeogenetic evidence of ancient Nubian barley evolution from six to two-row indicates local adaptation. *PLoS One*, *4*(7), e6301.

Panagiotakopulu, E., Buckland, P. C., Day, P. M., & Doumas, C. (1995). Natural insecticides and insect repellents in antiquity: a review of the evidence. *Journal of Archaeological Science*, *22*(5), 705-710.

Paris, H. S. (2015). Origin and emergence of the sweet dessert watermelon, *Citrullus lanatus*. *Annals of Botany*, *116*(2), 133-148.

Patrick, E.M., Mirzoian, A., Gretchen, R.H. (2009). Ancient Egyptian herbal wines. *PNAS*, *106*(18), 7361-7366.

Renner, S. S., Pérez-Escobar, O. A., Silber, M. V., Nesbitt, M., Preick, M., Hofreiter, M., & Chomicki, G. (2019). A 3500-year-old leaf from a Pharaonic tomb reveals that New Kingdom Egyptians were cultivating domesticated watermelon. bioRxiv, 642785.

Sabato, D., & Peña-Chocarro, L. (2021). *Maris Nostri Novus Atlas. Seeds and fruits from the Mediterranean Basin*. Ediciones Doce Calles.

Smith, S. T. (1992). Intact tombs of the Seventeenth and Eighteenth Dynasties from Thebes and the New Kingdom burial system. *Mitteilungen des Deutschen Archäologischen Instituts, Abteilung Kairo*, *48*, 193-231.

Täckholm, V. (1961). Botanical identification of the plants found at the Monastery of Phoebammon.

Terrasi, F., De Cesare, N., D'Onofrio, A., Lubritto, C., Marzaioli, F., Passariello, I., ... & Palmieri, A. (2008). High precision 14C AMS at CIRCE. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*, *266*(10), 2221-2224.

Uda, M., Yoshimura, S., Ishizaki, A., Yamashita, D., & Sakuraba, Y. (2007). Tutankhamun's golden mask investigated with XRDF. *International Journal of PIXE*, *17*(01n02), 65-76.

Verna, N., Verna, N., Goyal, M. Rawat, I. (2019). *Trigonella foenum-graecum* L. – Fenugreek: S.N. Ghosh, P. Jain, A. Nath (eds.). *Medicinal Plants in India: Importance & Cultivation* Vol. 2. New Dehli: Jaya Publishing House

Wills, H.L. (2018). A Fine Vintage: Grapes and Wine in Ancient Egypt. UCL: Researchers in Museum. Online. Accessed December 6th, 2022. https://blogs.ucl.ac.uk/researchers-in-museums/2018/03/20/a-fine-vintage-grapes-and-wine-in-ancient-egypt/

WFO (2023). *World Flora Online*. Published on the Internet; http://www.worldfloraonline.org. Accessed on: 15 Oct 2023.

CHAPTER 5

The high potential of micro-Magnetic Resonance Imaging for the identification of archaeological reeds: the case study of Tutankhamun

Moricca C., Stagno V., Hamza, N. M., Favero, G., Sadori L., Capuani, S.

heritage

MDPI

Article

The high potential of micro-Magnetic Resonance Imaging for the identification of archaeological reeds: the case study of Tutankhamun

Claudia Moricca [1], Valeria Stagno [2,*], Nagmeldeen Morshed Hamza [1,3,4], Gabriele Favero [1], Laura Sadori [1] and Silvia Capuani [2]

[1] Department of Environmental Biology, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy; claudia.moricca@uniroma1.it; nagmeldeenmorshed.hamza@uniroma1.it; gabriele.favero@uniroma1.it; laura.sadori@uniroma1.it

[2] National Research Council – Institute for Complex Systems (CNR-ISC) c/o Department of Physics, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy; valeria.stagno@uniroma1.it; silvia.capuani@isc.cnr.it

[3] Department of Earth Sciences, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy

[4] Grand Egyptian Museum, Conservation Center, Al Remaya Square, Al Haram, Giza Governorate, 3513204, Egypt

* Correspondence: valeria.stagno@uniroma1.it; laura.sadori@uniroma1.it

Abstract: This study explores the potential of micro-Magnetic Resonance Imaging (μ-MRI) for identifying archaeological reeds from the tomb of Tutankhamun. Reed plants have had various historical uses in the past, with ancient Egyptians extensively employing them for crafting a wide range of items. The distinct cross-sectional characteristics of *Arundo donax* (giant reed) and *Phragmites australis* (common reed) are observed and described via optical microscopy and μ-MRI. While optical microscopy offers higher resolution, μ-MRI provides advantages for studying archaeobotanical specimens, as it eliminates the need for mechanical sectioning and potentially damaging fragile samples. The application of μ-MRI on a selected archaeological reed allowed to identify it as *Phragmites australis*, shows that μ-MRI can yield clear images, maintaining the integrity of the sample. In contrast, diagnostic features appeared greatly deformed on the thin section observed via optical microscopy. Despite of the limitations related to sample size and the need for sample soaking, μ-MRI presents a valuable tool for analyzing archaeological remains in the field of cultural heritage, with the potential for broader applications. Overall, this study contributes to the expanding toolkit available to researchers studying plant remains, providing insights into reed identification and preservation in archaeological contexts.

Keywords: optical microscopy; wood imaging; reed anatomy; ancient Egypt; archaeobotany; desiccated plant remains; diagnostic technique.

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1. Introduction

Reeds are herbaceous, rhizomatous, perennial plants belonging to the Poaceae family. They typically grow along rivers, or on the edges of ponds, in shallow water [1]. They are characterized by robust and lightweight cylindrical stems. The mechanical properties of the fibres they are composed of make reeds strong and elastic [2]. For this reason, reeds have found various purposes throughout human history, for example for the construction of huts [3], rafts and boats [4], tools and common objects [5]. Reeds have played an important role in the development of music and continue to be used for producing woodwind instruments such as clarinets [6] and the Japanese hichikiri [7]. While it is difficult to estimate when reeds were first used for this purpose, single-toned pipes made of bone

(a material less subjected to biodeterioration) have been attested since the early Stone Age [8].

Archaeobotany, the study of plant remains found in archaeological contexts, is of great help in reconstructing past human-plant relationships. Humans have always exploited natural resources, not only to obtain food, but also other raw materials such as ropes or fabric, and wood. They have shaped and modified the surrounding environment by domesticating crop plants, using land for pasture and agriculture, generally leading to a decline of forest cover [9]. Plant remains can be classified based on size, with macroremains (e.g., seeds, fruits, and wood) being visible by naked eye. Their preservation in archaeological layers can occur following different modalities, which include charring and waterlogging [10]. The arid climate, which characterizes Egypt, has favoured the preservation of numerous plant materials by desiccation, leaving direct evidence of the past application of reeds. In the Predynastic and Pharaonic periods, *Phragmites australis* (Cav.) Steud. (common reed), *Arundo donax* L. (giant reed) and *Saccharum spontaneum* L. (wild sugarcane) were used to make baskets, ropes, nets, sandals, fishing rods, walking sticks, arrows, and other objects [11-12]. Writing tools were also obtained from culms, by cutting obliquely and splitting like a quill pen. Culms were not the only plant part being used, as leaves found an application for making mats and wrapping the bodies of the deceased, while rhizomes were used in traditional medicine due to their diuretic and diaphoretic properties [9]. Amongst the three mentioned species, *Phragmites australis* is reported to have been used the most in the Predynastic and Pharaonic periods in Ancient Egypt [11].

While material evidence already provides a lot of information about the use of reeds by ancient Egyptians, iconography can give an extra insight. *Phragmites* was used as a hieroglyphic sign [13], and is illustrated on wall paintings, such as those of the Tomb of Puyemre, the Temple of King Sethos I at Abydos [14], and the temple of Ramses III (20th Dynasty) in Medinet Habu [15]. In contrast, the mural paintings in the tomb of Nakht (18th Dynasty) are believed to depict *Arundo* along with papyrus [14].

In this context, identifying the reed plants preserved in Egyptian tombs is of particular interest, allowing us to assess plant availability and choice of materials. An interesting case study is represented by the famous tomb of Tutankhamun (1334-1325 BC – 18th Dynasty), discovered by the archaeologist Howard Carter in 1922, where several reed objects were recovered. These include reed arrows (inventory numbers 046a, 048y, 050yy), baskets (042, 093, 097, 117a, 129, 146), a kohl tube (044y), reed pad (052), sandals (094a, 104b), tray with partitions (119), a cane decorated with gold at top and bottom (229), bundle of reeds (249), a torch upon sun-dried brick (263), a pen case (271e), two statuettes of the King upon a float, and a mat (442d) [16]. Reed fragments were also recently recovered in a wooden box containing all the remains that were swept from the tomb surfaces after all the other objects were photographed (Fig. 1). The box was closed in 1933 and was stored in the Egyptian Museum in Cairo until 2017. In 2018 it was moved to the Grand Egyptian Museum, where its contents were subjected to scientific analyses [17-19].

Figure 1. Some of the reed fragments found in the wooden box containing the remains swept from the surfaces of Tutankhamun's tomb.

1.1. Reed anatomy and its study

Reeds are monocotyledons, flowering plants whose seed germinates from one embryonic leaf. This means that, unlike wood, they lack the tissues that form new (secondary) growth in thickness, as well as the vascular cambium, and a long-lived primary root [20]. The different layers with different functions can be observed by looking at the stem cross-section, which can be used to identify the plant taxon. Reeds present a circular cross-section outline [21], but a square or triangular outline can be of diagnostic value for specific plant taxa, such as Lamiaceae or *Carex* respectively [20].

The outermost layer is called the epidermis and can be one or more layered. This is followed by the hypodermis, which is often defined as absent if it does not show significant differences from the adjacent cortical layer, the cortex. The latter can be very narrow, or wide and multi-layered. It is delimited on the inner side by the endodermis, and sometimes contains some vascular bundles itself. Vascular bundles, the conducting vessels of the plant, are usually embedded in the sclerenchyma. They can be surrounded by a cap of fibres [20]. Reed stems have vascular bundles with two metaxylem vessels and one phloem pole [22].

Unlike wood, whose diagnostic features are observed along three anatomical sections (transverse/cross, tangential, and radial), reed stem identification is performed by solely observing the cross-section [22]. This is typically done via optical microscopy (e.g., [22]), although scanning electron microscopy (SEM) can also be used (e.g., [24]). Anatomical differences between *Arundo donax* and *Phragmites australis* stem anatomy are slight [22]. For this reason, discerning between the two in archaeological contexts is problematic (e.g., [23, 25]).

A recent advance in the study of plant stem anatomy is represented by micro-Magnetic Resonance Imaging (μ-MRI), based on the analysis of magnetic properties of hydrogen nuclei and on the resonance phenomenon [26]. μ-MRI allows the observation of the internal anatomy of biological materials and tissues through images representative of the spatial distribution of mobile protons (mainly from water). μ-MRI is a multiparametric investigation. It provides, superimposed to anatomical information, chemical-physical and physiological features obtained by MR images "weighted" with different MRI parameters, such as the relaxation times T_1, T_2 and T_2^* of water protons surrounding the environment [27]. The μ-MRI technique allows to acquire an infinite number of images by virtually slicing the sample with an extremely variable orientation, and currently reaches an in-plane resolution of 8x8 μm^2 [28]. This method has already been used to investigate

archaeological wood [26, 28-33], demonstrating the advantage of not needing to mechanically section the sample, in contrast to the transmitted light microscopy technique. It was also tested against the latter technique, proving to be a promising and complementary wood diagnostic method [26]. In this paper, we aim to test, for the first time, the potential of μ-MRI for the study of the anatomy of monocotyledon stems. While it is important from a methodological point of view, our study also contributes to the knowledge of plant exploitation in the 18th Dynasty of Egypt, shedding some light on the mystery of pharaoh Tutankhamun. In fact, in Germer's study of plant remains from the tomb of the king [34], numerous reed artifacts were identified as being made of either *Arundo donax* or *Phragmites australis*.

2. Materials and Methods

2.1. Sample preparation

A total of three reed samples were analysed in the present work. These are represented by two fragments of about 1.5 cm in height and 2 mm in thickness of modern reference samples of reed stems (*Arundo donax* and *Phragmites australis*) collected along the shores of the Aniene river, in Rome (RM), outside the *Grande Raccordo Anulare*. The third sample is an archaeological fragment of an unknown reed (size ca. 1 x 0.5 cm). *A. donax* and *P. australis* were selected due to their hollow stems [22, 35], unlike *Saccharum spontaneum*, also used in ancient Egypt [36]. The third chosen sample is an archaeological fragment of an unknown reed (size ca. 1 x 0.5 cm), found inside the box containing the sweepings from the tomb of Tutankhamun.

A stereomicroscopic observation was performed prior to this study, in the attempt of identifying the sample without soaking or cutting. However, it was inconclusive for the identification of the specimen. Modern sample size was chosen to fit the NMR capillary tube, which has a diameter of 10 mm. Sample preparation (of both archaeological and reference specimens) consisted in soaking them in distilled water until they sank, indicating their complete saturation. This was necessary to exploit most of the potential offered by NMR by increasing the signal-to-noise ratio (SNR) to improve the MR image quality. Sample soaking was also essential for the preparation of thin sections to be observed via optical microscopy.

2.2. Optical microscopy (OM)

Thin cross-sections of the soaked samples were later obtained by mechanical cut using a razor blade, perpendicurarly to the growth direction of each reed. These were then mounted on a slide and observed using a transmitted light Leica DM750 microscope (magnification: 40x, 100x, 200x). A Leica ICC50W camera and the Leica Application Suite (version 4.13.0) were then used to acquire pictures.

2.3. Micro-magnetic resonance imaging (μ-MRI)

MR images were obtained by virtually cutting the waterlogged samples acquired using a Bruker Avance-400 spectrometer operating at 9.4T with a 10 mm micro-imaging probe equipped with a high performance and high strength magnetic field gradient unit characterized by a maximum gradient strength of 1200 mT/m and a rise time of 100 μ. A FLASH-type sequence, with parameters optimized for the different samples, was used to obtain T_2*-weighted images. All the parameters used, such as echo time (TE), repetition time (TR), in-plane resolution (R), number of slices (N° slices), slice thickness (STK), number of scans (NS), matrix size (MTX), field of view (FOV) and acquisition time (AT) are reported in Table 1.

Table 1. Optimized μ-MRI acquisition parameters.

Parameters	Archaeological unknown reed	*Arundo donax*	*Phragmites australis*

N° slices	4	3	4
TR (ms)	450	800	1000
TE (ms)	4.7	2.8	3.0
STK (μm)	500	500	500
FOV (cm)	0.85	0.90	0.95
MTX (mm^2)	512	512	512
NS	128	256	128
AT (h)	8	29	18
R (μm^2)	17 x 17	17 x 17	18 x 18

3. Results

3.1. Arundo donax

A thin section of *Arundo donax* observed via optical microscopy is shown in Fig. 2a, allowing us to assess the stem anatomy of the plant. A rather thick epidermis (outer layer) can be observed. This is followed by two layers of small cells, and 8 layers of larger cells. These are all thick-walled and rounded. No vascular bundles can be observed within the cortex. The sclerenchyma cylinder has a thickness of 6-8 cells and is interspersed throughout with vascular bundles. The vascular bundles are comprised of two very large metaxylem vessels, protophloem of approximately the same size as the metaxylem vessels, and 1 or 2 rather thick-walled, small vessels. Vascular bundles are surrounded by sclerenchyma sheaths, which are thicker on the side of the protophloem and often discontinuous in the proximity of the sieve tubes. These characteristics are in accordance with the description provided by Schweingruber [22].

The T_2*-weighted MR image (Fig. 2b) makes it possible to see the entire section, 4.3 mm thick from the epidermis to the inner surface. Most of the features can be observed, with different contrast. Indeed, T_2*-weighted images are characterized by a contrast that depends both on the T_2 value and the differences in the magnetic susceptibility between different tissues, which highlights the paramagnetic impurities that may be present in the sample [26]. Although the lower resolution compared to optical microscopy does not allow counting the number of cells making up each layer, the rather thick epidermis is clearly visible. The thin layer made of rather dark voxels suggests the presence of layers of small cells, with very small lumens characterized by very short T_2. This area is followed by a thicker layer of larger cells, which is characterized by lighter voxels due to their bigger lumen size which causes a slower T_2 relaxation. This is due to water filling the elongated cells. Vascular bundles can be observed in the sclerenchyma cylinder, while eight rows of vascular bundles are visible in the remaining part of the cross-section (density ~ 389 vascular bundles/cm^2). Vascular bundles appear to be slightly taller than wide and have a width of approximately 250 μm. The MR image also allows to characterize them as composed of three large cavities (two metaxylem vessels and the protophloem). In some of them, the presence of one or two sieve tubes can also be noticed. The sclerenchyma surrounding the vascular bundles provides dark voxels in the proximity of the protophloem, due to the difference in the magnetic susceptibility in the different tissues. Moreover, the sclerenchyma cells have a smaller lumen, leading to water being more hindered and characterized by shorter T_2 values compared to the water stored in the metaxylem vessels. Finally, in the MR image it is possible to see the inner surface of the stem.

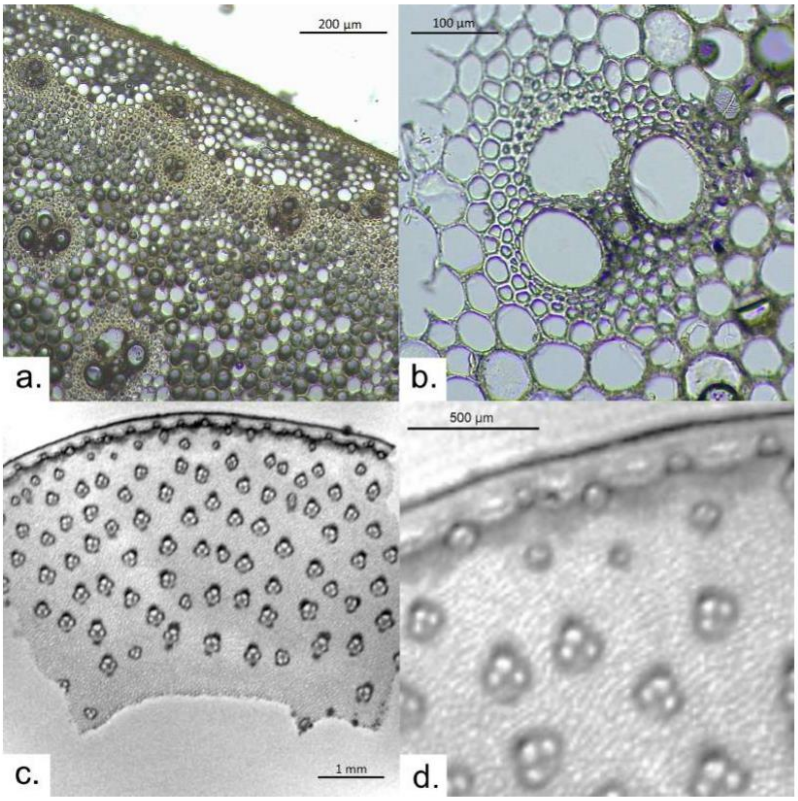

Figure 2. *Arundo donax*: a. thin section observed via optical microscopy; b. detail on a vascular bundle observed via OM; c. µ-MRI image of a sample slice; d. a zoomed part of the µ-MRI image .

3.2. Phragmites australis

A thin section of *Phragmites australis* observed by optical microscopy is pictured in Fig. 3a, making it possible to describe its stem anatomy. In this case, the epidermis is of medium thickness and is followed inwards by 3-4 layers of very thick-walled small cells. No vascular bundles are embedded in the cortex, while they can be observed in the sclerenchyma cylinder. The vascular bundles resemble those of *Arundo donax*, except the sclerenchyma sheaths are thinner. This description is in accordance with that provided by Schweingruber [22].

The T_2*-weighted MR image of *P. australis* (Fig. 3b) makes it possible to appreciate most of the stem features with different contrasts. The thickness of the stem is of ca. 1.8 mm. The epidermis, slightly thinner than that of *A. donax*, is characterized by very dark voxels due to the presence of cells with a very small lumen. This layer is followed by a thin cortex. Vascular bundles (with light voxels due to the bigger diameter of the cells they are made of) are visible in the sclerenchyma, while five other rows can be observed in the remaining part of the cross-section (density ~ 900 vascular bundles/cm^2). Vascular bundles appear to be slightly wider than tall and have a width of ca. 200 µm. They are comprised of three large cells (two metaxylem vessels and the protophloem). The vascular bundles are made up of two very large metaxylem vessels, protophloem of approximately the same size as the metaxylem vessels, and 1 or 2 rather thick-walled, small vessels. In some cases, it is also possible to appreciate the presence of one or two smaller cells, the sieve

tubes. A thin layer of sclerenchyma surrounding the vascular bundles appears with darker voxels, due to the difference in the magnetic susceptibility between different tissues. Moreover, sclerenchyma is also characterized by a shorter T_2 due to the smaller lumen of its cells. The MR image also shows the inner surface of the stem. The cells that make it up have lighter voxels due to the thinner-walled cells that make it up.

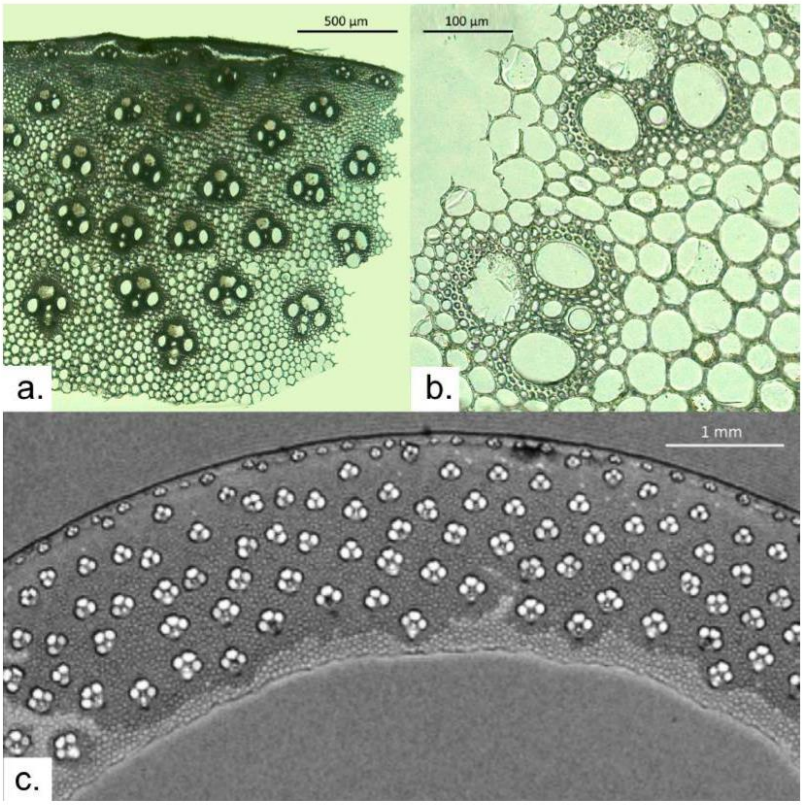

Figure 3. *Phragmites australis*: a. thin section observed via optical microscopy; b. close-up on two vascular bundles observed via OM; c. µ-MRI image of a sample slice.

3.3. Archaeological sample

The thin section of the archaeological sample (Fig. 4a) allows us to observe an outer layer composed of approximately six rows of cells with a very small lumen. Another feature that can be seen is vascular bundles. These appear deeply deformed. It is possible to characterize only one of them, at a higher magnification (Fig. 4b), as composed of three large cavities and a sieve tube. This is surrounded by sclerenchymatous fibres.

The MR image allows us to obtain an overall view of the archaeological sample (2 mm thick), starting from the outer layer (the epidermis), of medium thickness. This is characterized by black voxels due to the smaller lumen of its cells, which hinder water more than the subepidermal cells. The subepidermal tissue is followed by large longitudinal aerenchyma channels, whose function is to facilitate oxygen diffusion from the

shoots to the root tips [37]. Going inwards, a dark layer of cortical sclerenchyma is observed. In this case, the darker colour is due to the accumulation of paramagnetic substances, which likely indicates an advanced degradation of this area compared to the others. Three vascular bundles, composed of three large cavities each, are embedded in the cortical sclerenchyma. Four rows of vascular bundles, of increasing size going inwards, are also found in the cortex. These are wider than tall and have an average width of 300 μm. They are composed of three large cavities each: two large metaxylem vessels and the protophloem. At least one smaller vessel can be observed in most of the vascular bundles. Vascular bundles are surrounded by bundle sheath sclerenchyma, which appears thicker going outwards. The overall density of vascular bundles is approximately 465 vascular bundles/cm^2. Finally, a thick black layer delimits the inner surface of the stem. The black line indicates dense non-porous tissue where water has not penetrated, with a possible presence of paramagnetic substances which, by producing a local inhomogeneity of the magnetic field, drastically reduce the signal intensity [38].

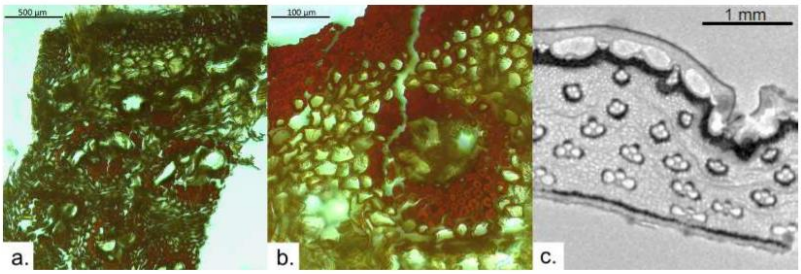

Figure 4. Archaeological reed fragment, preserved by desiccation, swept from the surfaces of the tomb of Tutankhamun in 1933, and preserved in a box in the Grand Egyptian Museum; a. thin section observed via optical microscopy; b. detail of vascular bundle observed via OM; c. μ-MRI image.

The μ-MRI images, obtained by virtually cutting the sample, allow us to observe the whole reed fragment, retaining its diagnostic features. Thus, it was possible to identify the archaeological fragment as *Phragmites australis*. The identification was based on a series of factors, including the overall thickness of the sample (from the epidermis to the inner surface of the stem). Another feature was represented by the clearly lined inner surface and rather thin sclerenchyma sheaths surrounding the vessels. Furthermore, vascular bundles appear wider than tall in the archaeological sample, as in the reference sample of *P. australis*. However, the most striking character is represented by the presence of large aerenchyma channels. These were not observed in either of the selected modern reference samples that come from the emergent part of reeds. Aerenchyma channels are completely absent in *Arundo donax* and present in submerged culms of *Phragmites australis* [39]. The presence of aerenchyma channels appears in fact to be related to the water table level, as emergent parts appear to lack aerenchyma, while this is evident in the waterlogged parts [40]. This difference can also be observed in samples analysed by Kawasaki et al. [7]. This leads us to believe that the studied archaeological sample corresponds to a submerged reed part.

4. Discussion

The aim of this paper was to test the application of micro-magnetic resonance imaging for the examination of plant stems. To this end, we included a comparison of optical microscopy and μ-MRI showing that the first technique attains a higher image resolution compared to the second, as already reported by Stagno et al. [26]. However, the image resolution in MRI is not strictly limited by the technique itself; instead, it depends on the

instrumentation used, particularly the strength of magnetic gradients. In fact, spectrometers that feature more powerful magnetic gradients can achieve resolutions of approximately 1 μm [26]. Despite having a lower resolution compared to optical microscopy and scanning electron microscopy, the application of μ-MRI in this specific context, involving an archaeological mummified/desiccated sample, allowed us to obtain considerably clearer images of the studied archaeological specimen. While the obtained image retains a high resolution, the studied sample lost integrity upon cutting with a razor blade and could no longer be used for diagnostic purposes. This problem was not faced with μ-MRI, in which all the images were obtained by virtual cut of the sample allowing to observe the whole reed fragment and retaining its diagnostic features.

Although numerous parameters, such as the thickness of the cross-section, the shape and size of the vascular bundles, and the number of rows of vascular bundles in the cortex, were used for the identification of the archaeological sample, the presence of aerenchyma channels was a crucial diagnostic criterion. They are air-storing tissues at the rhizome periphery of *Phragmites australis*, typically rectangular in this species [39]. These are not present in *Arundo* species [39]. The existence of well-developed aerenchyma systems is a significant anatomical adaptation that facilitates the transfer of oxygen from above-ground organs to rhizomes and roots [41]. However, this type of structures is only present in the submerged parts of reeds [40]. It is for this reason, that aerenchyma channels were not observed in the modern reference sample of *P. australis*.

When evaluating the proposed method it is worth considering the necessity of soaking a sample for μ-MRI analysis. While this does not represent an issue for waterlogged archaeobotanical remains, this problem cannot be omitted when it comes to evaluating the advantages and disadvantages of applying μ-MRI for the study of desiccated and charred material. Compared to optical microscopy – which requires a fresh or soaked sample for the preparation of thin sections, or even embedding in resin when detailing with particularly fragile or degraded plant material [41] – μ-MRI proves to be a less invasive method. This technique, in fact, only requires soaking the sample and placing it in the NMR capillary tube. Images are then acquired by virtually orienting and slicing the specimen [26], avoiding manipulation and limiting the required sample size. On the other hand, variable pressure scanning electron microscopy (VP-SEM), which has been used in the past for the study of archaeological reeds (e.g., [23]) and is still the preferred method for non-invasive and non-destructive analysis, has the advantage of not requiring sample preparation, including soaking [42]. However, it is preferable for observed wood/stem surfaces to be rather even, which can be achieved in different ways, such as fracturing charcoals [43] or preparing thin sections of waterlogged wood and treating them with albumin [44]. Another issue relates to sample size limitations, which are determined by the diameter of the NMR tube [26] or by the VP-SEM chamber [42]. For this reason, when dealing with bigger samples (such as the ones subject of the present study), subsampling can prove to be necessary for all the discussed techniques. Nevertheless, it is important to highlight that sample size limitations are not dependent on either of the techniques, but rather on the available instrument model [33].

5. Conclusions

In the present paper, we evaluated the application of μ-MRI for the identification of archaeological reeds, and it proved to be highly beneficial. In fact, in this specific case, it allowed us to clearly observe the transversal section of the studied sample, which, in contrast, disintegrated while cutting with a razor blade to obtain a thin section for the conventional optical microscopy inspection. While μ-MRI requires soaking a sample in water, unless it is already preserved by waterlogging, it offers the advantage of providing a great variety of image contrasts associated with various chemical, physical, and physiological features. To obtain high-resolution MR images, other than high magnetic gradient strength, a small-bore magnet and a small field of view are required. Therefore, μ-MRI is suitable for analysing small samples, unless a larger-bore instrument, such as those used

for the investigation of small animals, is employed. Therefore, it is possible to evaluate carrying this type of analysis on a subsample of the studied object. From a methodological perspective, this study introduces a novel application of nuclear magnetic resonance in the field of cultural heritage, which has the potential to be tested on a wider range of materials. Increasing the number of analytical approaches available for the study of cultural heritage objects, with a focus on plant remains, will have several positive implications. Firstly, this would make scientific analyses more widespread and accessible, thus providing valuable information about numerous artifacts, contributing to the knowledge about plant exploitation by past communities. Secondly, this could represent an additional stimulus for technological development, which we are confident could lead to higher resolution NMR images, further improving the quality of this type of analysis.

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References

1. Liu, J., Kopold, P., van Aken, P. A., Maier, J., & Yu, Y. Energy storage materials from nature through nanotechnology: a sustainable route from reed plants to a silicon anode for lithium-ion batteries. *Angewandte Chemie* **2015**, *127*, 9768-9772.
2. Enciclopedia Treccani. Canna. Enciclopedia online. Available online: https://www.treccani.it/enciclopedia/canna#:~:text=Nome%20genericamente%20attribuito%20a%20numerose,che%20coniugano%20resistenza%20ed%20elasticit%C3%A0 (accessed on 10 September 2023).
3. Ramsey, M. N., Maher, L. A., Macdonald, D. A., Nadel, D., & Rosen, A. M. Sheltered by reeds and settled on sedges: Construction and use of a twenty-thousand-year-old hut according to phytolith analysis from Kharaneh IV, Jordan. *Journal of Anthropological Archaeology* **2018**, *50*, 85-97.
4. Oron, A., Galili, E., Hadas, G., & Klein, M. Early maritime activity on the Dead Sea: bitumen harvesting and the possible use of reed watercraft. *Journal of Maritime Archaeology* **2015**, *10*, 65-88.
5. Romano, L., Celant, A., & Montorfani, M. V. Reed-swamps in the Sumerian material culture: archaeological, archaeobotanical, and experimental insights from the Abu Tbeirah excavations. In *Southern Iraq's Marshes: Their Environment and Conservation*; Jawad, L. A, Ed; Springer: Cham, Switzerland, 2021; pp. 33-54.
6. Kolesik, P., Mills, A., & Sedgley, M. Anatomical characteristics affecting the musical performance of clarinet reeds made from *Arundo donax* L. (Gramineae). *Annals of Botany* **1998**, *81*(1), 151-155.
7. Kawasaki, M., Nobuchi, T., Nakafushi, Y., Nose, M., & Shiojiri, M. Structure and biomechanics of culms of *Phragmites australis* used for reeds of Japanese wind instrument "hichiriki". *Microscopy Research and Technique* **2015**, *78*(4), 260-267.
8. Perdue, R. E. *Arundo donax* — source of musical reeds and industrial cellulose. *Economic Botany* **1958**, *12*, 368-404.

9. Mercuri, A. M., Sadori, L., & Blasi, C. Editorial: Archaeobotany for cultural landscape and human impact reconstructions. *Plant Biosystems* **2010**, *144*(4), 860-864.
10. Miksicek, C. H. Formation processes of the archaeobotanical record. *Advances in archaeological method and theory* **1987**, *10*, 211-247.
11. Boulos, L., & Fahmy, A. G. E. D. Grasses in ancient Egypt. *Kew Bulletin* **2007**, 507-511.
12. Noaman, S. Usage of *Arundo donax* L. as a sustainable material in interior design and architecture. In *Cities' Identity Through Architecture and Arts: Proceedings of the International Conference on Cities' Identity through Architecture and Arts (CITAA 2017), May 11-13, 2017, Cairo, Egypt*; Catalani, A., Nour, Z., Versaci, A., Hawkes, D., Bougdah, H., Sotoca, A., Ghoneem, M., Trapani, F., Eds.; Routledge: London, United Kingdom, 2018; p. 201.
13. Kiviat, E. What reed (*Phragmites*) ecology tells us about reed management. Part 1. Confronting reed's lurid reputation. *News from Hudsonia* **2005**, *20*(1), 1-6.
14. Thomas, E. Terrestrial marsh and solar mat. *The Journal of Egyptian Archaeology* **1959**, *45*(1), 38-51.
15. Barakat, H. N., & Aziz, A. *Guide to Plants of Ancient Egypt*. Bibliotheca Alexandrina: Alexandria, Egypt, 2010.
16. Malek, J., Moffett, J., Hutchison, S., Miles, E., Magee, D., & Rawlinson, K. *Tutankhamun: Anatomy of an Excavation*. Carter Archives - Main Object List: 001-049. Available online: http://www.griffith.ox.ac.uk/gri/carter/HomePage.html#042 (accessed 15 September 2023).
17. Abdrabou, A., Abdallah, M., Sultan, G. M., Mostafa, M., Bayoumi, H., Magdy, R., ... & Kamal, H. M. Tutankhamun's polychrome wooden shawabtis: Preliminary investigation for pigments and gilding characterization and indirect dating of previous restorations by the combined use of imaging and spectroscopic techniques. *Open Archaeology* **2022**, *8*(1), 30-54.
18. Peruzzi, G., Ciccola, A., Bosi, A., Serafini, I., Negozio, M., Hamza, N. M., ... & Curini, R. Applying Gel-Supported Liquid Extraction to Tutankhamun's Textiles for the Identification of Ancient Colorants: A Case Study. *Gels* **2023**, *9*(7), 514.
19. Serafini, I., Bosi, A., Vincenti, F., McClure, K. R., Ciccola, A., Hamza, N. M., Moricca, C., Sadori, L., Montesano, C., Sergi, M., Favero, G. Overcoming the limit of in situ gel supported liquid microextraction: development of the new. InGeL-LC-MS analyses, a smart methodology for the identification of natural dyes from Tutankhamun tomb relics. *Talanta* (in preparation).
20. Cutler, D. F., Botha, C. E. J., & Stevenson, D. W. *Plant Anatomy: An Applied Approach*. Blackwell Publishing: Malden, United States, 2008.
21. Tian, S., Yang, R., Pan, Z., Su, X., Li, S., Wang, P., & Huang, X. Anisotropic reed-stem-derived hierarchical porous biochars supported paraffin wax for efficient solar-thermal energy conversion and storage. *Journal of Energy Storage* **2022**, *56*, 106153.
22. Schweingruber, F. H. *Anatomy of European Woods*. Haupt: Stuttgart, Germany, 1990.
23. Figueiral, I., Bouby, L., Buffat, L., Petitot, H., & Terral, J. F. Archaeobotany, vine growing, and wine producing in Roman Southern France: the site of Gasquinoy (Béziers, Hérault). *Journal of Archaeological Science* **2010**, *37*(1), 139-149.
24. Martín-Seijo, M., Teira-Brión, A., Currás, A., & Rodríguez-Rellán, C. After the fire: the end of a house life-cycle at the Iron Age site of Nabás (North-western Iberia). *Vegetation History and Archaeobotany* **2020**, *29*, 427-446.
25. Baruch, U. Identification of Charred Wood Remains from 'En Gedi. *Atiquot* **2005**, *49*, 43-48.
26. Stagno, V., Moricca, C., Sadori, L., Dell'Aglio, E., Reale, R., & Capuani, S. Evaluation of the efficacy of micro-Magnetic Resonance Imaging compared with light microscopy to investigate the anatomy of modern and ancient waterlogged wood. *Magnetic Resonance Imaging* **2023**, *102*, 164-178.
27. Callaghan, P.T. *Principles of Nuclear Magnetic Resonance Microscopy*. Oxford University Press Inc: New York, United States, 1991.
28. Capuani, S., Stagno, V., Missori, M., Sadori, L., & Longo, S. High-resolution multiparametric MRI of contemporary and waterlogged archaeological wood. *Magnetic Resonance in Chemistry* **2020**, *58*(9), 860-869.
29. Cole-Hamilton, D. I., Kaye, B., Chudek, I. A., & Hunter, G. Nuclear magnetic resonance imaging of waterlogged wood. *Studies in Conservation* **1995**, *40*(1), 41-50.
30. Telkki, V. V. Wood Characterization by NMR & MRI of Fluids. *eMagRes* **2012**, *1*(1).
31. Stagno, V., Mailhiot, S., Capuani, S., Galotta, G., & Telkki, V. V. Testing 1D and 2D single-sided NMR on Roman age waterlogged woods. *Journal of Cultural Heritage* **2021**, *50*, 95-105.
32. Stagno, V., & Capuani, S. Decay of a Roman age pine wood studied by micro magnetic resonance imaging, diffusion nuclear magnetic resonance and portable nuclear magnetic resonance. *ACTA IMEKO* **2022**, *11*(1), 10.
33. Longo, S., Egizi, F., Stagno, V., Di Trani, M. G., Marchelletta, G., Gili, T., ... & Capuani, S. A multi-parametric investigation on waterlogged wood using a magnetic resonance imaging clinical scanner. *Forests* **2023**, *14*(2), 276.
34. Germer, R. *Die Pflanzenmaterialien aus dem grab des Tutanchamun*. Gerstenberg Verlag: Hildesheim, Germany, 1989.
35. Spatz, H. C., Beismann, H., Brüchert, F., Emanns, A., & Speck, T. Biomechanics of the giant reed *Arundo donax*. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **1997**, *352*(1349), 1-10.
36. El-Gawad, A. M. A., & El-Amier, Y. A. Anatomical features of three perennial swampy plants of Poaceae, grown on the water stream banks in Nile Delta, Egypt. *Journal of Medicinal Botany* **2017**, *1*, 58-64.
37. Yamauchi, T., Shimamura, S., Nakazono, M., & Mochizuki, T. Aerenchyma formation in crop species: a review. *Field Crops Research* **2013**, *152*, 8-16.

38. Capuani, S., Gambarini, G., Guarnieri, R., Di Pietro, G., Testarelli, L., & Di Nardo, D. Nuclear magnetic resonance microimaging for the qualitative assessment of root canal treatment: an ex vivo preliminary study. *Diagnostics* **2021**, *11*(6), 1012.
39. Danin, A., & Naenny, W. Contribution to the recognition of reeds by their rhizome anatomy. *Flora Mediterranea* **2008**, *18*, 385-392.
40. Armstrong, J., Afreen-Zobayed, F., Blyth, S., & Armstrong, W. *Phragmites australis*: effects of shoot submergence on seedling growth and survival and radial oxygen loss from roots. *Aquatic Botany* **1999**, *64*(3-4), 275-289.
41. Fogli, S., Marchesini, R., & Gerdol, R. Reed (*Phragmites australis*) decline in a brackish wetland in Italy. *Marine environmental research* **2002**, *53*(5), 465-479.
42. Bleicher, N. An easy low-budget method to produce thin-sections of heavily decayed archaeological wood. *Dendrochronologia* **2008**, *26*(1), 9-11.
43. Stokes, D. J. *Principles and Practice of Variable Pressure/Environmental Scanning Electron Microscopy (VP-ESEM)*. John Wiley and Sons: West Sussex, United Kingdom, 2008.
44. Cartwright, C. R. Identifying the woody resources of Diepkloof Rock Shelter (South Africa) using scanning electron microscopy of the MSA wood charcoal assemblages. *Journal of Archaeological Science* **2013**, *40*(9), 3463-3474.
45. Balzano, A., Merela, M., & Čufar, K. Scanning electron microscopy protocol for studying anatomy of highly degraded waterlogged archaeological wood. *Forests* **2022**, *13*(2), 161.

SECTION 3

Pigments and dyes

CHAPTER 6

Tutankhamun's Polychrome Wooden Shawabtis: Preliminary Investigation for Pigments and Gilding Characterization and Indirect Dating of Previous Restorations by the Combined Use of Imaging and Spectroscopic Techniques

Abdrabou A., Abdallah M., Sultan G. M., Mostafa M., Bayoumi H., Magdy R., El Kader M. A. A., Hamza N. M., Mamdouh D., Elsayed H. M., Abbas E., Kamal H. M.

Research Article

Ahmed Abdrabou*, Medhat Abdallah, Gilan M. Sultan, Mohamed Mostafa, Hind Bayoumi, Ramy Magdy, Mohamed A. Abd El Kader, Nagmeldeen M. Hamza, Dina Mamdouh, Hassan M. Elsayed, Eltayeb Abbas, Hussein M. Kamal

Tutankhamun's Polychrome Wooden Shawabtis: Preliminary Investigation for Pigments and Gilding Characterization and Indirect Dating of Previous Restorations by the Combined Use of Imaging and Spectroscopic Techniques

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Abstract: To the best of our knowledge, such a detailed study on polychrome wooden shawabtis of King Tutankhamun (18th Dynasty in ancient Egypt) has not been reported in the literature, so the purpose of our study is to noninvasively identify the polychrome layers and previously applied materials for a number of wooden shawabtis that belong to King Tutankhamun through a protocol based on imaging techniques integrated with single-spot spectroscopic techniques. In the first step, imaging techniques (visible, ultraviolet induced visible luminescence, ultraviolet reflected, visible-induced infrared luminescence, infrared reflected, and infrared false color) and optical microscopy were applied to gather information and provide evidence on the distribution of original and previously applied materials on the polychrome surfaces. In the second step of our work, we analyzed the selected areas with single-spot analyses (handheld X-ray fluorescence spectroscopy and visible reflectance spectroscopy) and X-ray diffraction analysis. The materials of the previous restoration interventions were studied by Fourier transform infrared spectroscopy. The application of a protocol based on imaging techniques integrated with data obtained from single-spot spectroscopic techniques allowed the characterization of a remarkable number of polychrome layers and some previous restoration materials and mapping of their distribution on the original surface, which provides not only essential data for the follow-up treatment and conservation works but also offers important information for the study of polychrome wooden shawabtis of other periods in ancient Egypt.

Keywords: shawabtis, Tutankhamun, multispectral imaging, orpiment, handheld XRF

* **Corresponding author: Ahmed Abdrabou,** Wood Conservation Laboratory, Grand Egyptian Museum – Conservation Center (GEM.CC), Giza, Egypt, e-mail: ahmed_abdrabou87@yahoo.com
Medhat Abdallah: Directorate of Conservation, Saqqara Store Rooms, Giza, Egypt
Gilan M. Sultan, Mohamed Mostafa, Hind Bayoumi, Ramy Magdy, Mohamed A. Abd El Kader: Wood Conservation Laboratory, Grand Egyptian Museum – Conservation Center, Giza, Egypt
Nagmeldeen M. Hamza: Organic Conservation Laboratory, Grand Egyptian Museum-Conservation Center, Giza, Egypt
Dina Mamdouh: FTIR Laboratory, Grand Egyptian Museum-Conservation Center, Giza, Egypt
Hassan M. Elsayed: Department of Tutankhamun Collection, Grand Egyptian Museum, Giza, Egypt
Eltayeb Abbas: Directorate of Archaeological Affairs, Grand Egyptian Museum, Giza, Egypt
Hussein M. Kamal: Directorate of Conservation Affairs, Grand Egyptian Museum-Conservation Center, Giza, Egypt
ORCID: Ahmed Abdrabou 0000-0003-3423-3884

1 Introduction

Shawabtis (also called shabtis or ushabtis) are funerary statuettes found in ancient Egyptian burial assemblages that have been traditionally interpreted as workers enlisted by their owners to conduct work on their behalf in the afterlife. They became popular in the Middle Kingdom (2055–1650 BCE) and were abundantly produced until they decreased in usage toward the end of the Ptolemaic Period (332–30 BC) (Hoving, 1978; Schneider, 1977).

Shawabtis found in King Tutankhamun's tomb come in a variety of styles and materials. The tomb contains 413 shawabtis of which 365 are workmen, 36 are overseers, and 12 are directors. These shawabtis were housed in 24 boxes, 10 of which were recovered from the northeast corner of the treasury and 14 from the annex. They were also made of a variety of materials, including 189 shawabtis of wood, 121 of faience, 42 of alabaster, 8 of limestone, 3 of black granite, and 50 of quartzite (Carter, 1933; Reeves, 1990).

The multidisciplinary analytical techniques of polychrome artifacts, belonging to museum collections, particularly unique objects, which emphasize the necessity of working with noninvasive techniques, have gained much interest in recent years and have been reported by many authors to study the original polychrome layers as well as modern restoration interventions in order to yield more information useful for conservation processes (Abdallah, Abdrabou, & Kamal, 2020; Abdrabou, Abdallah, Shaheen, & Kamal, 2018; Abdrabou, El Hadidi, Hamed, & Abdallah, 2018; Abdrabou, Abdallah, Nabil, Matsuda, & Kamal, 2019; Bonizzoni et al., 2018; Bracci et al., 2015; Brunel-Duverger, Laval, Lemasson, Brodie-Linder, & Pagès-Camagna, 2019; Dyer & Sotiropoulou, 2017; Gard et al., 2020; Ismail, Abdrabou, & Abdallah, 2016).

Like most of the objects in the tomb of Tutankhamun, some shawabtis were treated by Lucas before being transferred to the Egyptian Museum, where they were displayed. In 2017, 105 wooden shawabtis were transported to the Grand Egyptian Museum's Conservation Center for investigation and conservation works in preparation for the exhibition at the new museum. Twelve shawabtis were the subject of the first targeted diagnostic study inside the Wood Conservation Laboratory at the Grand Egyptian Museum's Conservation Center (GEM CC). So, the primary objective of our current study is to identify the chemical composition of the pictorial layers used to decorate the shawabtis as well as to present a panoramic view of the previous restoration materials and their distribution on the original surface via a wide array of noninvasive analytical techniques, which provides not only essential data for the study of polychrome wooden shawabtis of King Tutankhamun with those from different periods in ancient Egypt, but also important information for the follow-up treatment and conservation works. The selected analytical protocol aimed to limit the sampling as much as possible, while at the same time obtaining a large set of significant data. The analytical investigations that have been designed for this type of object represent the first step in the characterization of polychrome wooden shawabtis that belong to King Tutankhamun.

2 Materials and Methods

2.1 The Studied Artifacts

In this work were investigated, by means of imaging and spectroscopic techniques, 12 polychrome wooden shawabtis from the collection of King Tutankhamun (Figure 1). The numbers, locations inside the tomb, dimensions, and descriptions of these shawabtis are summarized in Table 1.

2.2 Methodology

In our work, the sequence of investigation and analysis began with multispectral imaging followed by closer inspection under a microscope to characterize the spatial distribution of pigments and later applied materials.

Figure 1: The selected shawabtis from King Tutankhamun collection as numbered in Table 1, and the spots marking the locations listed in Table 3 from where analysis by single-spot spectroscopic techniques were performed (indicated by English numbers); a) Shawabti GEM GEM 3163; b) Shawabti GEM 3414; c) Shawabti GEM 3429; d) Shawabti GEM 3173; e) Shawabti GEM 3511; f) Shawabti GEM 3503; g) Shawabti GEM 3528; h) Shawabti GEM 3499; i) Shawabti GEM 3491; j) Shawabti GEM 3651; k) Shawabti GEM 3467; l) Shawabti GEM 3452.

Based on the data from the technical imaging and detailed examination, spots of each area of interest were then analyzed using hand held X-ray fluorescence (XRF) and Vis-RS. After this, the measurements by X-ray diffraction (XRD) were directly performed on the same spots (made by XRF) in a nondestructive mode without any sample preparation. Finally, four samples from the previous restoration materials were carefully scraped off with a metallic scalpel for analysis by Fourier transform infrared spectroscopy (FTIR).

2.2.1 Multispectral Imaging (MSI)

Technical images were captured with a Nikon D90 DSLR (CMOS sensor) digital camera that had been modified for "full spectrum (c. 350–1,000 nm)" by removing the inbuilt UV-infrared reflected (IR) blocking filter and fitted with a Tokina Macro 35 mm F/2.8D DX lens that was used at its widest F-stop setting (2.8) to capture all luminescence images with shutter speeds ranging from 0.8 to 8 s. The camera was operated fully

Table 1: A summary of the shawabtis showing the numbers, dimensions, location inside the tomb, and description

Image no.	Object no.	Length (cm)	Location in the tomb	Carter description [The Griffith institute (2000–2004)]
a	Carter No: 611a JE 60914 GEM 3163	23.5	with group of shawabtis inside a kiosk (Carter no. 611)	Made of wood wearing wig striated with blue, beard, eyes, and eyebrows black, eyeballs white, holding in hands the pick, hoe, and two baskets, and the inscription incised and filled in with blue
b	Carter No: 323j JE 60864 GEM 3414	25.5	With a group of shawabtis inside a kiosk (Carter no. 323) Center of chamber	Made of wood covered with red painted layers, details of face and beard black; text blue, holding the hoe in R. hand, pick in L. hand, and a basket in both hands
c	Carter No: 319c JE 60895 GEM 3429	27	With a group of shawabtis inside a kiosk (Carter no. 319)	Made of wood covered with red painted layers, details of face and beard black, text blue, and holding an ankh sign in both hands. Treated with dilute celluloid solution
d	Carter No: 324a JE 60918 GEM 3173	23	With group of shawabtis inside a kiosk (Carter no. 324)	Made of wood smeared with yellow; wearing wig striated with blue, while beard, eyes, and eyebrows black, eyeballs white, and holding a pick, hoe, and two baskets in hands. The inscription incised and filled in with blue. Treated with dilute celluloid solution
e	Carter No: 323n JE 60851 GEM 3511	24.5	With a group of shawabtis inside a kiosk (Carter no. 323) Center of chamber	Made of wood and covered with red painted layers in the shape of a mummy; wearing a round wig painted with blue, beard and eyebrows with black, and eyeballs and collarettes with white
f	Carter No: 418e JE 60848 GEM 3503	22.5	With a group of shawabtis inside a kiosk (Carter no. 418) Center of chamber	Made of wood and covered with red painted layers in the shape of a mummy; wearing a round wig painted with dark blue, beard and eyebrows with black, and eyeballs and collarettes with white
g	Carter No: 323b JE 60856 GEM 3528	27.3	With a group of shawabtis inside a kiosk (Carter no. 323)	Made of wood and covered with gesso painted layers (red–yellow–black), holding a pick in the left hand and a basket in both hands. Treated with dilute celluloid solution
h	Carter No: 602b JE 60846 GEM 3499	29.5	With a group of shawabtis inside a kiosk (Carter no. 602b) Center of chamber	Made of wood and covered with painted light red layers, wearing a painted black round wig with gilded uraeus, gilded temple band, and bead collarette round the neck, holding a gilded band of linen in the left hand and a gilded flagellum, and round the wrists gilded bracelets in the right hand. Eyes and eyebrows black and eyeballs white
i	Carter No: 602d JE 60844 GEM 3491	26.4	With a group of shawabtis inside a kiosk (Carter no. 602)	Made of wood and covered with painted light red layers, wearing h3t head-dress with gilded uraeus, gilded temple band, and bead collarette round the neck, holding a gilded band of linen in the left hand and a gilded flagellum, and round the wrists gilded bracelets in the right hand. Eyes and eyebrows black and eyeballs white
j	Carter No: 512a JE 60783 GEM 3651	23.6	With a group of shawabtis inside a kiosk (Carter no. 512)	Made of wood and covered with gilded gesso layers, wearing a head-dress and beard; holding a pick and a hoe in hands. The eyes and eyebrows painted black

(*Continued*)

Table 1: *Continued*

Image no.	Object no.	Length (cm)	Location in the tomb	Carter description [The Griffith institute (2000–2004)]
k	Carter No: 608d JE 60813 GEM 3467	24.2	With a group of shawabtis inside a kiosk (Carter no. 608)	Made of wood and covered with gilded gesso layers wearing a round wig and beard. The eyes and eyebrows painted black. Text and wig filled with blue
l	Carter No: 512c JE 60807 GEM 3452	23.4	With a group of shawabtis inside a kiosk (Carter no. 512)	Made of wood and covered with gilded gesso layers, wearing a round wig and beard; the eyes and eyebrows painted black

manually and tethered to a computer to allow sharp focusing in nonvisible modes (IR and UV) using live view mode with the picture style set to "Neutral" and an ISO speed of 100. Except for the acquisition of UV-induced visible luminescence images, where it was set to a color temperature of 6,500 K, white balance was set to "Custom." To calibrate the camera, an X-Rite ColorChecker Passport and a reference gray scale were used (Abdrabou, Hussein, Sultan, & Kamal, 2022; Cosentino, 2015; Dyer, Verri, & Cupitt, 2013).

In each case, the shawabti was illuminated by two radiation sources that were symmetrically positioned at approximately 45° with respect to the camera's focal axis and at approximately the same height. To select the wavelength range of interest, a filter or combination of filters was placed in front of the camera lens. Table 2 summarizes the filters and radiation sources used for imaging techniques. All images were captured as RAW files and converted to TIF (tagged image file) format and a set of recommended presets which turn-off all enhancements (e.g., recovery, fill light, blacks, contrast, brightness, clarity, saturation, as well as setting the tone-curve to linear) were applied using Adobe Photoshop. Post-processing procedures for the calibration of the visible (VIS), ultraviolet induced visible luminescence (UVL), ultraviolet reflected (UVR), visible-induced luminescence (VIL), and IR images as well as the creation of infrared false color (IRFC) images are then carried out using the "British Museum (BM) workspace" and nip2 software (Martinez & Cupitt, 2005) as described by Dyer et al. (2013).

2.2.2 Optical Microscopy (OM)

For detailed observations of the polychrome layers, we used a Zeiss Stereo DV 20 (portable stereomicroscope) equipped with an Axio Cam MRC5 with an optical zoom of 28 up to 560×.

2.2.3 XRF

The painted preparation layers were nondestructively analyzed by a handheld XRF (HH-XRF) spectrophotometer using a Thermo Scientific Niton XL3t with a "GOLDD" detector. It was placed in contact with the selected area, and the X-ray spot size was 3 mm in diameter. The X-ray tube has a Ag anode of 50 kV and 200 μA. The standardization modes selected are the "Cu/Zn Mining", which includes the elements of interest for the analysis of painted preparation layers (e.g., Ca, S, Si, Cu, Fe, As, Pb, Sn, Cl, and P) and the "precious metal" mode, which includes the elements of interest for the analysis of gilding (e.g., Au, Ag, and Cu). This analysis uses four separate filters to determine the concentrations in percentage of elements: a high filter (30 s counting time), a main filter (30 s), a low filter (30 s), and a light filter (30 s), leading to a total measurement time of 120 s per analysis. The software utilizes the Fundamental Parameters algorithm to determine the concentrations of each element. The spectra obtained from the XL3t were downloaded to a computer for analysis by the Thermo Scientific NDT program. The total points considered for HH-XRF analyses were chosen for different colors and conservation situations following suggestions from the results of imaging analyses.

Table 2: A summary of the filters and radiation sources used for imaging techniques

Imaging techniques	Filters	Radiation sources
VIS	X-Nite CCI	White fluorescent lamps
UVL	UV/IR Baader filter + X-Nite CCI	Ultraviolet LED lamps (365 nm)
UVR	A Schneider B + W 403 + X-NiteCC1	Ultraviolet LED lamps (365 nm)
VIL	A Schott RG840 cut-on filter	White LED lamps
IR	A Schott RG840 cut-on filter	Infrared LED lamps (900 nm)
IRFC	Made by digitally editing the VIS and IR images in Adobe Photoshop	

2.2.4 Vis-RS

A Konica Minolta Spectrophotometer CM-2600d was used to record the reflectance spectra of the painted layers under standard illuminant d65 (the di:8°/de:8° geometry (diffused illumination, 8° viewing angle), including the specular component (measuring area: circular area with a diameter of 5 mm). Spectra are collected along a 400–700 nm wavelength range every 10 nm. The light diffused in the integrating sphere is received by the illumination-monitoring optical system and guided to a sensor. The light reflected from the painted surfaces and the diffused light are divided into each wavelength component by a measuring optical system and illumination-monitoring optical sensor, respectively, and the signals proportional to the light intensity of each component are output to the analog processing circuit.

2.2.5 XRD

PANalytical pro model PW3040 XRD with a Cu anode at 30 mA/40 kV was directly performed on the shawabtis. Each shawabti was placed inside an XRD apparatus on a sample holder, and an approximately flat surface of each color was exposed to the X-ray beam. Diffraction patterns were interpreted using X'Pert HighScore software.

2.2.6 FTIR

FTIR measurements were conducted using an FTIR spectrometer (IRPrestige-21, Shimadzu) in the 400–4,000 cm^{-1} range, with a resolution of 8 cm^{-1}. Samples of the previous restoration materials were identified by comparing the obtained spectra with literature data (Derrik, Stulik, & Landy, 1999) and standards created in the FTIR laboratory at the GEM CC.

3 Results and Discussion

3.1 Analysis of Polychrome Layers

The chromatic palette of these shawabtis includes blue, yellow, red, white, black, and gilding. Table 3 summarizes imaging, OM, HH-XRF, and XRD analysis results for the different polychrome layers of these shawabtis.

3.1.1 Blue and Greenish Hue Painted Layers

In the VIL images (Figures 2b and 3b), the blue and greenish blue painted layers appeared as bright white, while all other materials appeared dark. The luminescence of such areas indicates the presence of Egyptian blue (Accorsi et al., 2009; Amenta, 2014; Abdallah & Abdrabou 2018). According to XRF spectra of the blue painted layer (Figure 2c), the elements that presented the highest concentrations were silicon (Si), copper (Cu), calcium (Ca), and sulfur (S). This result provides strong evidence for the presence of Egyptian blue pigments, confirming the corresponding visible reflectance spectrum (Figure 2d), which matched published spectra of Egyptian blue with an inflection point at about 630 nm (Abdrabou, et al., 2022; Aceto et al., 2014; Edreira, Feliu, Fernández-Lorenzo, & Martín, 2001). The presence of S in the blue regions would suggest the presence of gypsum, whereas the presence of tin (Sn) impurities in the XRF analysis (Table 1) allowed us to assume that a bronze scrap was used to produce the Egyptian blue pigment (Abdrabou et al., 2018; Jaksch,

Table 3: A summary of imaging, OM, HH-XRF, and XRD analysis results for the painted layers

Object no.	Spot no.	Color	Imaging				OM	Elements detected by XRF	XRD results
			UVR	UVL	IR	VIL			
GEM 3163JE 60914	1	Blue	Dark	None	Dark	Bright white	Blue particles	**Cu, Ca, Si**, Fe, S, Sn, K, Cl, Ti, Sr, As	Cuprorivaite-Gypsum
	2	Black	Dark	None	Black	None	Fine black particles	**Ca**, Fe, S, Si, Cl, K, As, Ti	—
GEM 3414JE 60864	3	Blue	Dark	None	Dark	Bright white	Blue particles	**Cu, Ca, Si, Fe, As, S**, Al, K, Ti, Cl, Sn,	Cuprorivaite-Gypsum
	4	Red	Dark	None	Bright	None	Red particles with some single yellow particles	**Fe, As, Ca, S, Si**, Al, Cl, K, Ti	Hematite-Quartz
	5	Black	Dark	None	Black	None	Fine black particles	**Fe, Ca, As, S, Si**, Al, Cl, K, Ti	—
GEM 3429JE 60895	6	Greenish Blue	Dark	None	Dark	Bright white	Blue crystalline matrix with green tone	**Cu, Ca, Si**, Fe, S, As, Sn, Cl, K, Sr	—
	7	Red	Dark	None	Bright	None	Red particles with some single yellow particles	**Ca, Fe, As, S, Si**, Al, Cl, K, Ti	Hematite-Quartz
	8	Black	Dark	None	Black	None	Fine black particles	**Ca, Fe, As, S, Si**, Al, Cl, K, Ti	—
GEM 3173JE 60918	9	Greenish Blue	Dark	None	Dark	Bright white	Blue crystalline matrix with green tone	**Cu, Ca, Si**, Fe, Cl, Sn, S, As, K, Sr,	Cuprorivaite-Calcite-Quartz
	10	Yellow	Dark	Yellow	Bright	None	yellow particles	**As, Ca, Fe, S, Si**, K, Ti, Sr	—
	11	Black	Dark	None	Black	None	Fine black particles	**Ca, Fe**, S, Si, Cl, K, P, As, Ti	—
GEM 3511JE 60851	12	Blue	Dark	None	Dark	Bright white	Blue particles	**Cu, Ca, Si**, Fe, S, As, Sn, Cl, K, Sr,	Cuprorivaite-Gypsum
	13	Dark blue	Dark	None	Dark	Bright white	Dark blue particles	**Cu, Ca, Si**, Fe, S, As, Sn, Cl, K, Sr,	—
	14	Red	Dark	None	Bright	None	Red particles with some single yellow particles	**Fe, Ca, As, Si, S**, Al, Cl, K, Ti	Hematite-Quartz
	15	White	Bright	White	Bright	None	White particles with single yellow and blue particles	**Ca, As, Fe, Si, S**, Al, Cl, K, Ti, Cu	Gypsum-Weddellite-Quartz
	16	Black	Dark	None	Black	None	Fine black particles with some single yellow particles	**Fe, Ca, As, S**, Si, Al, Cl, K, Ti, Cu	—
GEM 3503JE 60848	17	Greenish blue	Dark	None	Dark	Bright white	Blue crystalline matrix with a green tone	**Cu, Ca, Si**, Fe, S, As, Sn, Cl, K, Sr,	Cuprorivaite-Calcite-Quartz

(*Continued*)

Table 3: *Continued*

Object no.	Spot no.	Color	Imaging				OM	Elements detected by XRF	XRD results
			UVR	UVL	IR	VIL			
	18	Red	Dark	None	Bright	None	Red particles with some single yellow particles	**Fe, Ca, As, Si, S**, Al, Cl, K, Ti	Hematite-Quartz
	19	White	Bright	White	Bright	None	White particles with some single yellow and blue particles	**Ca, As, Fe, Si, S**, Al, Cl, K, Ti, Cu	Gypsum-Weddellite-Quartz
	20	Black	Dark	None	Black	None	Fine black particles with some single yellow particles	**Fe, Ca, As, Si, S**, Al, Cl, K, Ti	—
GEM 3528JE 60856	21	Yellow	Dark	Yellow	Bright	Some particles appeared as white bright	Yellow particles with some blue particles	**Ca, As, Fe, S, Si, Cu**, K, Ti, Sr	—
	22	Red	Dark	None	Bright	None	Red particles	**Ca, Fe**, Si, S, Cl, K, Ti, As	Hematite-Calcite
	23	Black	Dark	None	Black	None	Fine black particles	**Ca, Fe**, Si, S, Cl, K, Ti, As, Sr	—
GEM 3499JE60846	24	Red	Dark	None	Bright	None	Red particles with some single yellow particles	**Fe, Ca, As, S, Si**, Al, K, Ti	Hematite-Quartz
	25	Black	Dark	None	Black	None	Black particles	**Ca, Fe, S, Si, As**, P, K, Ti	
	26	Yellow gilding	—	None	—	—		Au, Ag, Cu	—
	27	Dark gilding	—	None	—	—		Au, Ag, Cu	—
JE60844	28	Red	Dark	None	Bright	None	Red particles with some single yellow particles	**Fe, Ca, As, S, Si**, Al, K, Ti	Hematite-Quartz
	29	Yellow gilding	—	None	—	—		**Au, Ag, Cu**	—
	30	Dark gilding	—	None	—	—		**Au, Ag, Cu**	—
GEM 3651JE 60783	31	Yellow gilding	—	None	—	—		Au, Ag, Cu	—
	32	Dark gilding	—	None	—	—		Au, Ag, Cu	—
	33	Dark gilding	—	None	—	—		Au, Ag, Cu	
	34	Yellow previous material	Dark	Very dark	—	—		**Fe**, S, Zn, Ba, Ca, Sr	—
GEM 3467JE60813	35	Blue	Dark	None	Dark	Bright white	Blue particles	**Cu, Ca, Au, Si**, Fe, Ti, Sn, Ag, Cl, As	—
	36	Yellow gilding	—	Green	—	—		Au, Ag, Cu	—
GEM 3452JE 60807	37	Yellow gilding	—	Yellow	—	—		Au, Ag, Cu	—
	38	Yellow gilding	—	Green	—	—		Au, Ag, Cu	—

Note: Elements in boldface are correlated to the main pigment mineral.

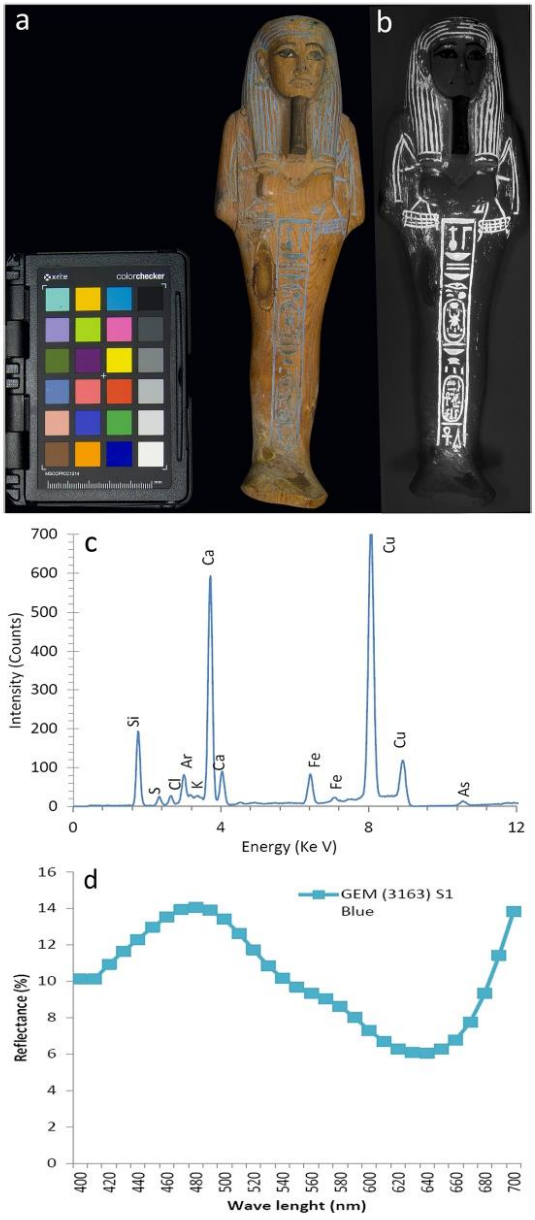

Figure 2: Characterization of the blue painted layer (Object GEM 3163, Spot 1): (a) visible image; (b) VIL image; (c) XRF spectrum; and (d) visible reflectance spectrum.

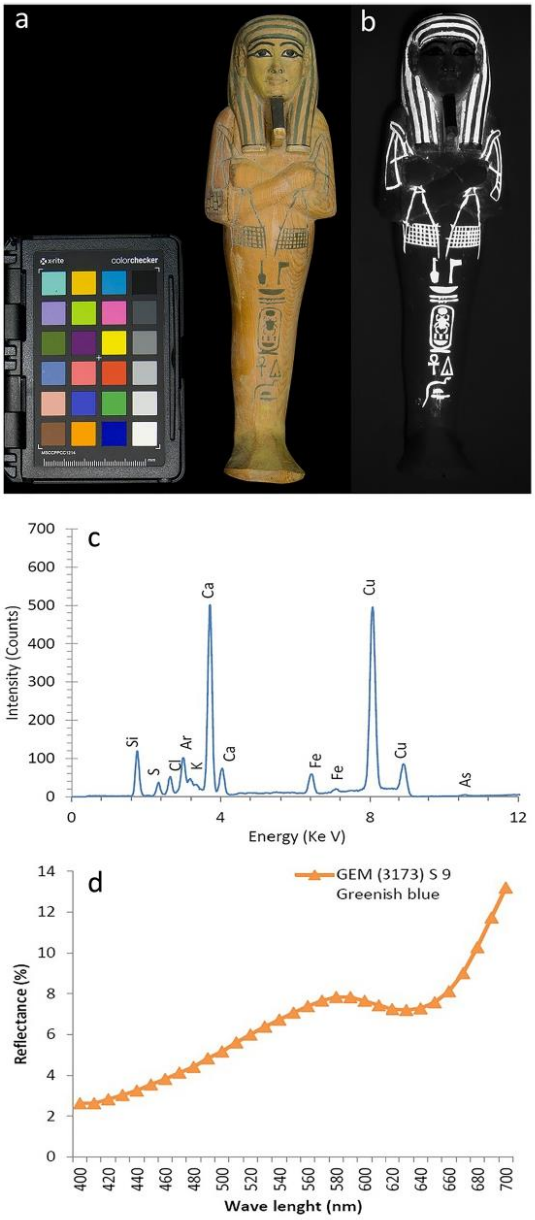

Figure 3: Characterization of the greenish blue painted layer (Object GEM 3173, Spot 9): (a) visible image; (a) VIL image; (c) XRF spectrum; and (d) visible reflectance spectrum.

Seipel, Weiner, & El Goresy, 1983). Applying XRD (Figure 4a) confirmed the presence of gypsum and cuprorivaite (the main component of Egyptian blue) for the blue painted layers in accordance with the attribution made after the VIL and XRF spectra. The Egyptian blue pigment was the first synthetic pigment ever produced by man and dates to the Protodynastic period in Egypt (around 3200–3000 BC). It was found on a Protodynastic period bowl with markings attributed to the Scorpion King and was extensively used from the 4th Dynasty in Egypt until the end of the Roman period (Abdrabou, Abdallah, & Kamal, 2017; Corcoran, 2016; Ganio et al., 2015; Mirti et al., 1995). Egyptian blue is a multicomponent pigment made from locally derived component minerals of copper, calcium, silica (in the form of quartz, SiO_2), and about 1% flux, usually comprising lime (CaO) from calcium carbonate to form calcium copper tetrasilicate crystals (cuprorivaite: $CaCuSi_4O_{10}$), the chromophore in the Egyptian blue. This material can be characterized by its blue tabular crystals, which have been fritted in an oxidizing atmosphere at 850–1,100°C (Hatton, Shortland, & Tite, 2008; Ismail et al., 2016; Lee, 1997; Pradell, Salvado, Hatton, & Tite, 2006).

In general, Egyptian blue is a very stable pigment, and there are examples to be seen in Egypt that have been exposed for thousands of years without the loss of color, as seen in Figure 1. However, some objects painted with the Egyptian blue pigment show some discoloration that varies considerably, from a relatively greenish hue to black (Daniels, Stacey, & Middleton, 2004). In the studied shawabtis GEM no. 3429, 3173, and 3503, the microscopic investigation reveals that the greenish hue is a surface phenomenon, with what appears to be fresh, bright blue pigment lying beneath the greenish layer. Moreover, the microscopic investigation did not show any yellow particles in the greenish hue areas, so the hypothesis of a mixture

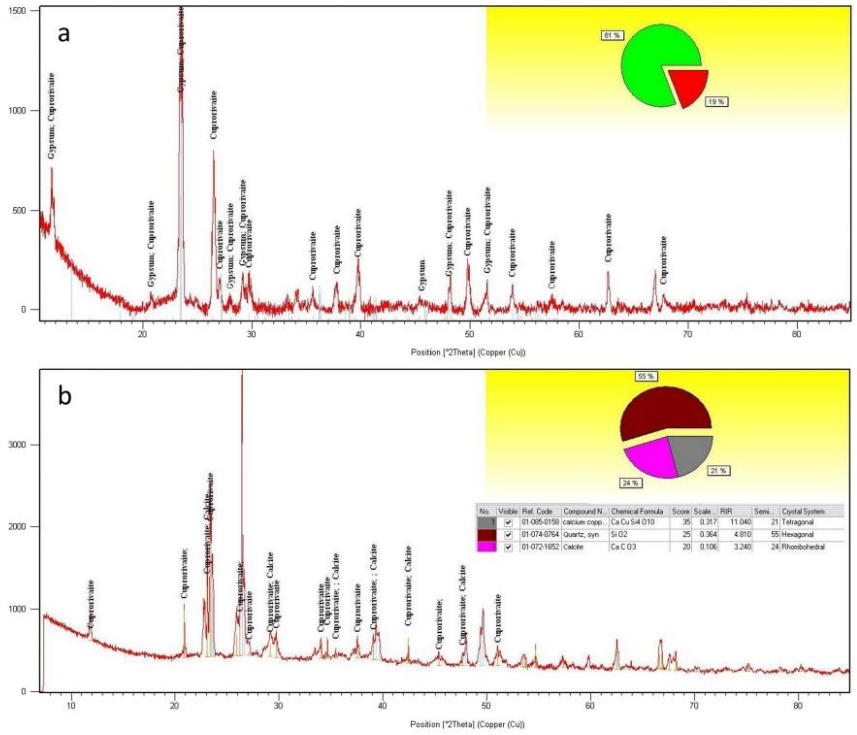

Figure 4: XRD patterns of: (a) the blue painted layer (Object GEM 3163, Spot 1) and (b) the greenish blue painted layer (Object GEM 3173, Spot 9).

of Egyptian blue and yellow pigments can be discarded. It is possible that the greenish hue is due to the degradation of the Egyptian blue pigment to atacamite or its polymorphs through the process known as "copper chloride cancer" (Abadir, 2014) or due to the darkening of organic materials used as binders or previous consolidation materials (Daniels et al., 2004). According to the XRF results of the greenish hue areas (Figure 3c), the elements that presented the highest intensities were Si, Cu, and Ca, with a significant amount of chlorine (Cl). This result provides strong evidence for the presence of the Egyptian blue pigment, confirming the corresponding visible reflectance spectrum (Figure 3d), which matched published spectra of Egyptian blue, with shifting in the reflectance ranging between 400 and 600 nm in the greenish blue painted layer due to its discoloration. The presence of Cl in the greenish hue areas would probably point to the presence of halite or a chlorine-containing solid phase and, considering the chemical reactivity of cuprorivaite, this solid phase could be atacamite, $Cu_2(OH)_3Cl$, or one of its polymorphs, paratacamite or clinoatacamite, which are products of the degradation of Egyptian blue when in contact with high-chloride concentration solutions. By applying XRD to the greenish hue areas (Figure 4b), apart from calcite and quartz, cuprorivaite (the main component of Egyptian blue) was detected. The absence of atacamite or its polymorphs in the diffractogram could be due to a lower percentage of atacamite or that the new phase formed on the surface of the blue pigment is amorphous. Consequently, the XRD technique is not able to detect it. Therefore, further analysis using Raman spectroscopy and gas chromatography/mass spectrometry will be necessary to determine its identity more precisely. This phenomenon (the coexistence of Egyptian blue and atacamite on blue-green colors) has already been reported by many authors (Giménez, 2015; Green, 2001; Lee, 1997; Riederer, 1977).

3.1.2 Yellow Painted Layer

The yellow fluorescence of yellow areas of shawabti no. 3528 under UV (Figure 5b) suggests that a yellow painted layer was probably made of arsenic (As)-based pigments such as orpiment since it had fluorescence properties (Abdrabou et al., 2018; Stuart, 2007). In addition, the microscopic examination revealed large yellow particles in a granular form, as well as the presence of some single particles of the blue pigment in the yellow painted layer (Figure 5d). These single particles appeared as bright white in the VIL image (Figure 5c), which refers to the Egyptian blue pigment. Moreover, comparing the VIS and VIL images (Figure 5a and c) allowed for the surviving Egyptian blue pigment to be mapped, and it is clear that its presence is much more extensive than what was discernible with the naked eye. In Figure 5e, the XRF spectrum of the yellow painted layer is shown. It shows a high intensity of As, Ca, Cu, S, Si, and Fe. It is interesting to note that Cu, Ca, and Si are detected in the yellow areas, clearly related to the single particles of Egyptian blue in accordance with the attribution obtained by VIL imaging. The presence of these blue single particles in the yellow paint layer could be accidental impurities due to the use of a dirty brush or could be added to achieve a higher intensity of the yellow sensation. The high intensity of As and S provides strong evidence for the presence of an arsenic sulfide pigment that is most likely orpiment (As_2S_3) in a nearly pure form, as confirmed by Vis-RS. In Figure 5f, the inflection point at 480 nm and the slope of the reflectance curve of the yellow layer fit with published reference spectra for orpiment is shown (Aceto et al., 2014; Cavaleri, Giovagnoli, & Nervo, 2013). Orpiment is found locally in Egypt associated with gold and silver ores (in Edfu and the Eastern Desert) or in copper ores (Saini mines), in hot-spring deposits, and volcanic sublimation, but others have reported that orpiment was imported from Syria or Asia Minor (Abdrabou et al., 2017; Bonizzoni et al., 2011; Bonizzoni et al., 2018; Bracci et al., 2015; Davies, 1995; Gard et al., 2020; Sakr, Ghaly, Abdulla, Edwards, & Elbashar, 2020; Scott et al., 2004; Scott, Warmlander, Mazurek, & Quirke, 2009). Historically, its earliest findings are dated to the 2nd Dynasty (Colinart, 2001) and several later findings in Egyptian artifacts have been reported (Abdrabou et al., 2017; Abdrabou, et al., 2022; Ambers, 2004; Amenta, 2014; Bonizzoni et al., 2011; Brunel-Duverger et al., 2019; Colinart, 2001) particularly during the 18th Dynasty, when it was extensively used to give a brilliant yellow, gold-like color with a shiny glaze, so it has been described as royal yellow (Sakr et al., 2020).

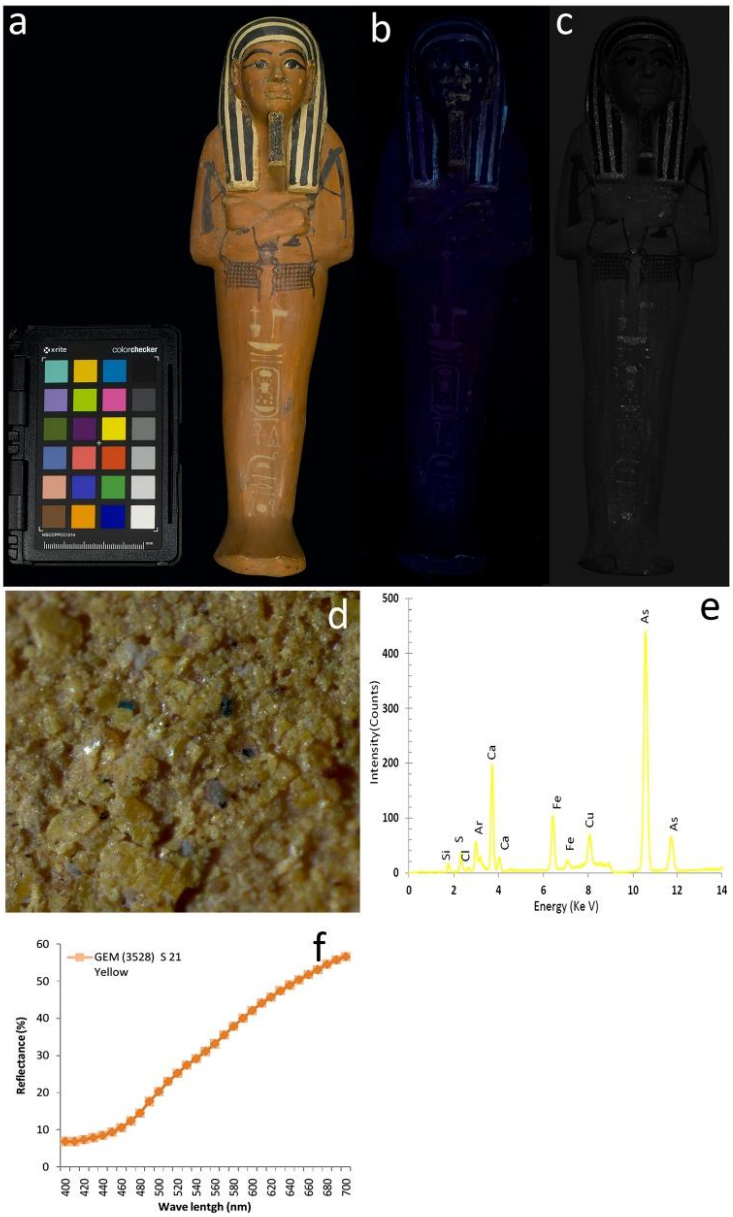

Figure 5: Analytical characterization of the yellow painted layer (Object GEM 3528, Spot 21): (a) visible image; (b) UV luminescence image; (c) VIL image; (d) an optical micrograph; (e) XRF spectrum; and (f) visible reflectance spectrum.

3.1.3 Red Painted Layers

All the red painted layers appeared darker in the UV-induced luminescence (UVL) images, which may suggest that the red pigment is red ochre, consistent with the strong quenching properties of the iron-based pigment (McCarthy, 2001). The microscopic examination showed the presence of some single particles of yellow pigment in some red paint areas (Figure 6a), which may be due to the use of dirty brushes or could be added to achieve a higher intensity of the red sensation. The results of XRF analysis for some points on the red painted layers (Figure 6b) showed the presence of Fe, Ca, As, Si, and S with a high intensity, in addition to a small amount of aluminum (Al). The presence of As and S is clearly related to the single grains of orpiment that are shown by the microscopic examination. The presence of Fe, Ca, Si, and Al provides strong evidence for the presence of red ochre, as confirmed by Vis-RS (Figure 5c), which displayed a sigmoid shape with an absorption band between 400 and 500 nm, a sharp positive slope between 550 and 600 nm, and an inflection point at 580 nm, which are indicative of red ochre (Abdrabou et al., 2022; Aceto et al., 2014; Cavaleri et al., 2013; Edreira et al., 2001; Guglielmi, Andreoli, Comite, Baroni, & Fermo, 2021; Miriello et al., 2018). Finally, applying XRD to the red painted layers showed that, apart from quartz, the presence of hematite is detected, in accordance with the attribution made by Vis-RS and XRF. The red areas are all colored with hematite (α Fe_2O_3), the main chromophore found in red ochres, and they contain a small proportion of other crystals, including quartz, and are better described as ochres rather than as pure hematite. This is in keeping with the other evidence from other Egyptian contexts, where red ochres are by far the most commonly reported red pigment. Red ochre was used to create a red color on wooden artifacts in ancient Egypt, according to many works (Bonizzoni et al., 2018; Lee & Quirke, 2000; Pagès-Camagna & Guichard, 2010).

3.1.4 White Painted Layers

The microscopic examination revealed the presence of some single particles of the blue and yellow pigments in the white painted layer (Figure 7a). The blue single particles appeared as bright white in the VIL image, which refers to the Egyptian blue pigment. The results of XRF analysis for some points on the white painted layers (Figure 7b) showed the presence of Ca and S with a high intensity, in addition to a small amount of Si, As, Fe, and Cu. The presence of Ca and S provides strong evidence for the presence of calcium-based pigments such as calcium sulfate. The presence of As and S is clearly related to the single grains of orpiment that are shown by the microscopic examination, while silicon and copper are detected in the white areas, clearly related to the single particles of Egyptian blue that are shown by VIL imaging. At some times, the ancient Egyptians added Egyptian blue to the white colors to achieve a higher intensity of the white sensation (Edreira, Feliu, Fernández-Lorenzo, & Martín, 2003). Finally, applying XRD to the white painted layers (Figure 7c) showed that, apart from quartz and weddellite (calcium oxalate), the presence of gypsum is detected, in accordance with the attribution made by XRF. Calcium-based pigments such as calcite, gypsum, and huntite were the most commonly used white pigments in Egyptian painting and have been reported in many works to create a white color on wooden artifacts (Ambers, 2004; Bonizzoni et al., 2011; Heywood, 2001).

3.1.5 Black Painted Layers

IR images strongly suggest the presence of carbon-based black in the black paint areas, as carbon is opaque under infrared emission and appears dark (Abdrabou et al., 2017). The microscopic investigation of the black pigment indicated the fineness and evenness of particles and did not show any fibrous structure, so it is possible to exclude the burnt vegetable origin of the black pigment (Abdrabou et al., 2018). In addition, some shawabtis (GEM 3503–3511) showed some single particles of orpiment pigments in the black painted layer, which may be due to the use of dirty brushes. In XRF analysis, phosphorus was detected in the black

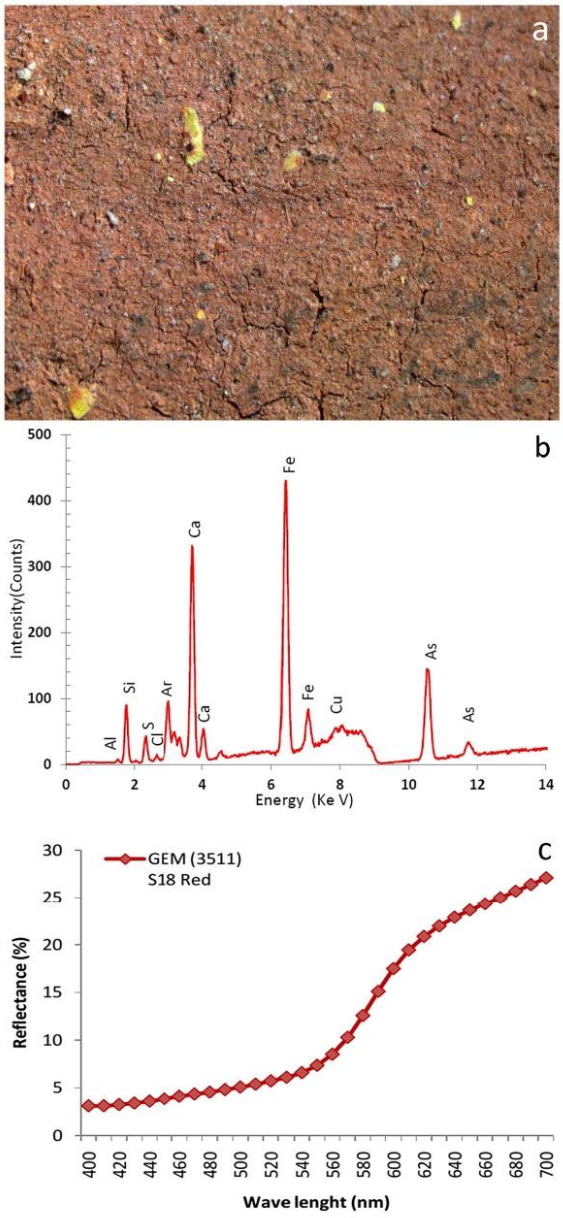

Figure 6: Characterization of the red painted layer (Object GEM 3511, Spot 18): (a) An optical micrograph; (b) XRF spectrum; and (c) visible reflectance spectrum.

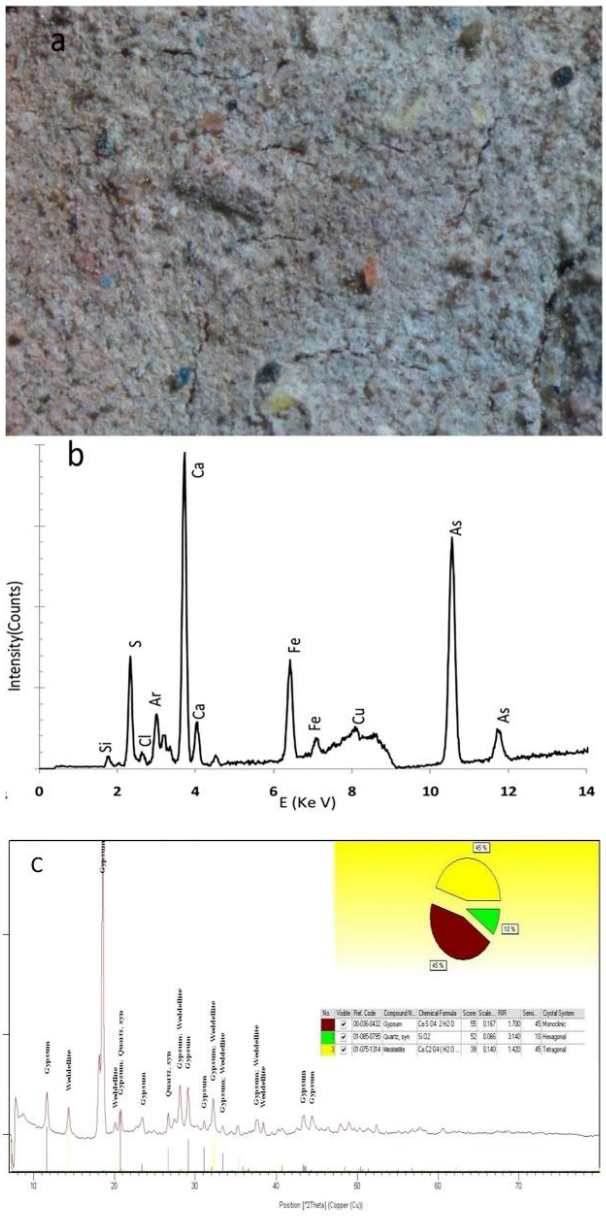

Figure 7: Characterization of the white painted layer (Object GEM 3503): (a) An optical micrograph; (b) XRF spectrum; and (c) XRD pattern.

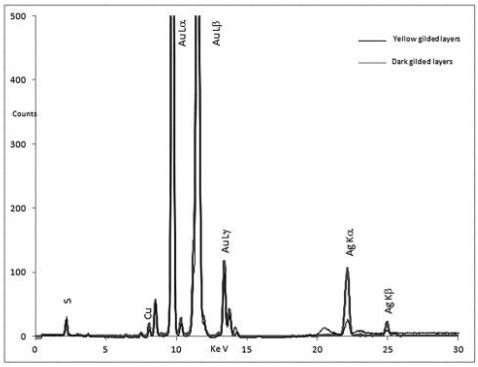

Figure 8: Comparing the obtained XRF spectra for yellow and dark areas of gilding.

painted layers of shawabtis GEM no. 3503 and 3511. This result provides evidence for the presence of carbon obtained from animal origins that is most likely bone black, which is one of the oldest pigments known to humans and was originally made by charring animal bones (Abdrabou et al., 2018; Mahmoud, 2014), while phosphorus was not detected in the XRF results from the black painted layers of the other shawabtis, making it most likely that they are of lamp black or soot, produced by the combustion of vegetable matter or oil (Ambers, 2004; Lee & Quirke, 2000).

3.1.6 Gilded Layers

The majority of the gold used in ancient Egypt was obtained from alluvial deposits and from quartz rock found between the Nile and the Red Sea (Colinart, 2001). Their composition ranged from very pure to those containing at least 40% of silver (electrum) and copper, with percentages not exceeding 1.5%. The majority of Tutankhamun's golden objects are gilded, either with gold foil or gold leaf applied over a firm support to achieve the rich appearance of solid gold (Abdallah, Abdrabou, & Kamal, 2018; Abdrabou et al., 2018; James, 1972). There are some gilded wooden shawabtis among them, with varying degrees of color ranging from bright yellow to reddish brown. According to the semiquantitative XRF results, the gilded layers with a bright yellow color have a similar composition and are composed of pure gold (Au) ranging from 99.2 to 98.5%, with less than 1% of Ag and Cu. The gilded layers with a reddish brown color exhibit a high Ag content that reaches 9%. The XRF spectra obtained for the yellow and dark colors were compared. XRF spectra (Figure 8) showed an increase in the Ag intensity in the dark areas of the gilded layers, with no significant change in the S and Cu intensities. The increase of Ag on the dark color of the gilded layers with the presence of S suggests the formation of distinct silver sulfide complexes, namely, AgAuS and Ag_3AuS_2, which correspond with previously published data for some dark gold Egyptian leafs/foils (Abdrabou, El Hadidi, Hamed, & Abdallah, 2018; Frantz & Schorsch, 1990; Hatchfield & Newman, 1991; Rifai & El Hadidi, 2010; Tissot, et al., 2015).

3.2 Analysis of Previous Restoration Materials

Table 4 shows a summary of the UVL imaging, XRF, and FTIR analysis results of the previous restoration material samples.

Table 4: : Summary for UVL imaging, XRF and FTIR analysis results of four samples from the previous restoration materials

Sample no	Object. no	UVL imaging	Elements detected by XRF	IR bands (cm^{-1})
1	GEM 3452JE 60807	Yellow	—	3,437, 2,970, 1,732, 1,653, 1,454, 1,278, 839
2		Green	—	3,427, 2,916, 1,695, 1,462, 1,379, 1,273, 1,173, 1,051, 728
3	GEM 3467JE 60813	Green	—	3,427, 2,916, 1,695, 1,462, 1,379, 1,273, 1,173, 1,051, 728
4	GEM 3651JE 60783	Very dark	**Fe, S, Zn, Ba, Ca**, Sr	2,916, 1,463, 1,124, 1,080, 904, 794, 728, 669, 609

Note: Elements in boldface are correlated to the main pigment mineral.

A UVL image (Figure 9b) revealed the presence of a yellowish emission from luminescent materials on the gilded surface (Object GEM 3452). This luminescence probably relates to cellulose nitrate (Grant, 2000) used in the previous restoration interventions made by Lucas during the discovery of the tomb, as Carter mentioned in his handwritten object cards. As shown in Figure 8c, the infrared spectrum of this material (sample 1) proved the presence of celluloid (bands at 3,437 cm^{-1} (O–H), 2,970 cm^{-1} (C–H), 1,732 cm^{-1} (C═O), 1,653 cm^{-1} (N–O), 1,454 cm^{-1} (C–H), 1,278 cm^{-1} (N–O), and 839 cm^{-1} (N–O) (Derrik et al., 1999) a resin used frequently as an adhesive, consolidant, and coating of artifacts in many applications for the first half of the 20th century. Due to its disadvantageous properties such as flammability and tendency to yellow, it is no longer used for the consolidation of artifacts (Unger, Schniewind, & Unger, 2001).

As shown in Figure 10b, UVL revealed the presence of a greenish emission from luminescent materials on the gilded surface (Object GEM 3467), and its presence is much more extensive than what was discernible with the naked eye (Figure 10a). This luminescence presumably relates to the use of rosin mixed with paraffin wax in the previous restoration interventions as mentioned in previous work (Abdrabou et al., 2018) The infrared spectrum of this material (sample 2 and 3) proved the presence of rosin (bands at 3,427, 1,695, 1,462, 1,379, 1,273, 1,173, and 1,051 cm^{-1}, Figure 10c) mixed with paraffin wax (bands at 2,926, 1,462, and 729 cm^{-1}, Figure 10c), (Abdrabou et al., 2019; Derrik et al., 1999). Rosin, a natural organic resin, has been used frequently as a surface treatment agent and in mixtures with waxes for filling cracks and voids in wood artifacts in recent decades (Abdallah, Kamal, & Abdrabou, 2016; Abdrabou et al., 2018; Unger et al., 2001). As for the previous yellow plaster fill material used in the object (GEM 3651), the UVL image makes these areas clearly visible as they do not emit any fluorescence under UV light and appear as characteristic black spots. The XRF spectrum revealed the presence of Fe, which may indicate the presence of modern iron-based pigments. Small amounts of S, Zn, and Ba are also observed. These elements suggest the use of lithopone ($ZnS + BaSO_4$), which was confirmed by XRD analysis. In the infrared spectrum of sample 4, yellow-based pigments (bands at 1,080, 904, and 794 cm^{-1}) were detected, bound by paraffin wax (bands at 2,916, 1,463, and 728 cm^{-1}). In the same spectrum, the presence of sulfate groups (SO_4) at 1,124, 669, and 609 cm^{-1} were also observed. These results indicate the use of different materials for these shawabtis during several restoration interventions from the discovery of the tomb in 1922 until their transportation to the GEM CC in 2016.

4 Conclusion

The use of an imaging-based protocol combined with data from single-spot techniques such as XRF and Vis-RS, as well as complementary results from XRD and FTIR, provided an excellent insight into the chemical compositions of the materials used for the pictorial polychrome layers of the studied shawabtis and presented a panoramic view of the previous restoration materials and their distribution on the shawabtis. In general, the findings can be used to explain the ancient technology of King Tutankhamun's polychrome wooden shawabtis.

The results revealed that the materials used for the original polychrome layers of the studied shawabtis were nearly identical to those used in ancient Egyptian polychrome artifacts with respect to a chromatic palette and the painting technique. The chromatic palette used in these shawabtis was identified as red ochre for the red painted layers, cuprorivaite mixed with gypsum for the blue painted layers, cuprorivaite mixed with calcite and quartz for greenish hue areas, orpiment for the yellow painted layer, and gypsum for the white painted layer. Carbon obtained from charred bone black and lamp black or soot was used for the black painted layers. The variation in the composition of gilded layers is in agreement with the composition of the other ancient Egyptian gold leaf previously determined. The application of imaging techniques provided useful information about the spatial distribution of pigments, in particular VIL, which allowed the spatial distribution of the surviving Egyptian blue pigment to be mapped. Moreover, UVL presented a panoramic view of the spatial distribution of the materials used in the previous restoration interventions, providing necessary data for future conservation work. This study provided preliminary information

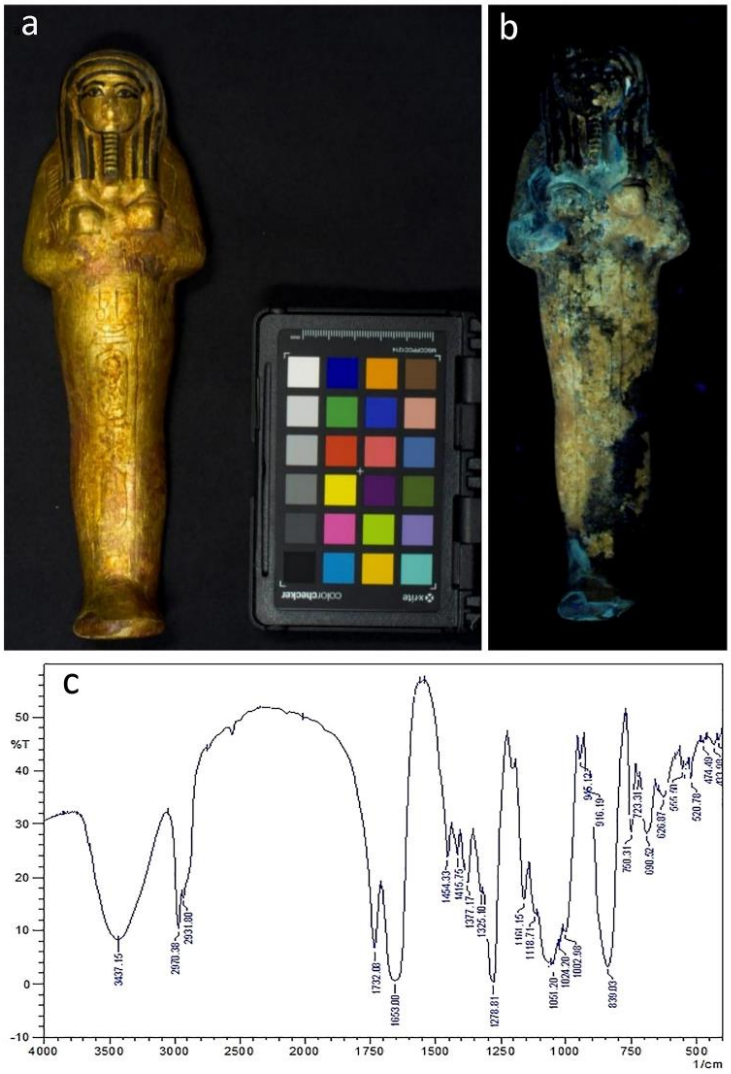

Figure 9: Analytical characterization of the previous consolidation material on the outer surface of Object GEM 3452: (a) visible image; (b) UV luminescence image; and (c) FTIR spectrum of sample 1.

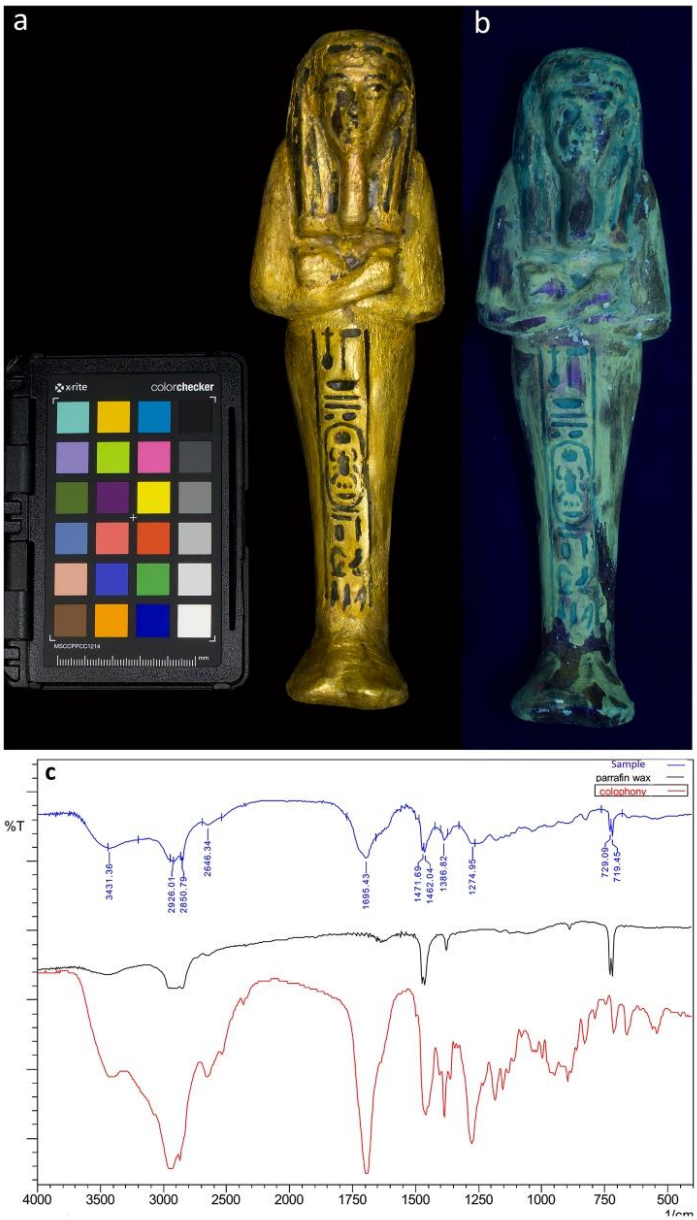

Figure 10: Analytical characterization of the previous resinous fills on the outer surface of Object GEM 3467: (a) visible image; (b) UV luminescence image; and (c) FTIR spectrum of sample 2.

concerning the original materials used in some polychrome wooden shawabtis of King Tutankhamun and the materials added during the previous treatment interventions, which provides not only important information for the study of polychrome wooden shawabtis from the same collection of Tutanhkamun and other periods in ancient Egypt but also essential data for the follow-up treatment and conservation works of these shawabtis.

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References

Abadir, M. I. (2014). *Experimental study of the Egyptian blue degradation by the copper chloride cancer, Bachelor Degree in chemical engineering*. Barcelona: Escola Tècnica Superior d'Enginyeria Industrial de Barcelona, Universitat politecnica de Catalunya (UPC).

Abdallah, M., & Abdrabou, A. (2018). Tutankhamen's small shrines (naoses): Technology of woodworKing and identification of wood species. *International Journal of Conservation Science*, *9*(1), 91–104.

Abdallah, M., Abdrabou, A., & Kamal, H. M. (2018). Analytical study and conservation processes of Tutankhamen decorated stick: A case study. *Scientific culture*, *4*(1), 93–100.

Abdallah, M., Abdrabou, A., & Kamal, H. M. (2020). Multiscientific analytical approach of polychrome greco-roman palette applied on a wooden model naos: Case study. *Mediterranean Archaeology and Archaeometry*, *20*(2), 45–65.

Abdallah, M., Kamal, H., & Abdrabou, A. (2016). Investigation, preservation and restoration processes of an ancient Egyptian wooden offering table. *International Journal of Conservation Science*, *7*(4), 1047–1064.

Abdrabou, A., Abdallah, M., & Kamal, H. M. (2017). Scientific investigation by technical photography, OM, ESEM, XRF, XRD and FTIR of an ancient Egyptian polychrome wooden coffin. *Conservar Património*, *26*, 51–63.

Abdrabou, A., Abdallah, M., Nabil, E., Matsuda, Y., & Kamal, H. M. (2019). Preliminary investigation of the materials and techniques used in a decorated wooden stick of King Tutankhamun. *Conservar Património*, *30*, 9–19.

Abdrabou, A., Abdallah, M., Shaheen, I. A., & Kamal, H. M. (2018). Investigation of an ancient Egyptian polychrome wooden statuette by imaging and spectroscopy. *International Journal of Conservation Science*, *9*(1), 39–54.

Abdrabou, A., El Hadidi, N. M., Hamed, S., & Abdallah, M. (2018). Multidisciplinary approach for the investigation and analysis of a gilded wooden bed of King Tutankhamun. *Journal of Archaeological Science: Reports*, *21*, 553–564.

Abdrabou, A., Hussein, A., Sultan, G. M., & Kamal, H. M. (2022). New insights into a polychrome Middle Kingdom palette applied to a wooden coffin: A multidisciplinary analytical approach. *Journal of Cultural Heritage*, *54*, 118–129.

Accorsi, G., Verri, G., Bolognes, M., Armaroli, N., Clementi, C., Miliani, C., & Romani, A. (2009). The exceptional near-infrared luminescence properties of cuprorivaite (Egyptian blue). *Chemical Communications*, *23*, 3392–3394.

Aceto, M., Agostino, A., Fenoglio, G., Idone, A., Gulmini, M., Picollo, M., … Delaney, J. K. (2014). Characterisation of colorants on illuminated manuscripts by portable fibre optic UV-visible-NIR reflectance spectrophotometry. *Analytical methods*.

Ambers, J. (2004). Raman analysis of pigments from the Egyptian Old Kingdom. *Journal of Raman Spectroscopy*, *35*, 768–773.

Amenta, A. (2014). The Vatican Coffin project. In E. Pischikova, J. Budka, & K. Griffen (Eds.), *Thebes in the first millennium* (pp. 483–499). Cambridge: Cambridge Scholars Publishing.

Bonizzoni, L., Bruni, S., Gargano, M., Guglielmi, V., Zaffino, C., Pezzotta, A., … Ludwig, N. (2018). Use of integrated non-invasive analyses for pigment characterization and indirect dating of old restorations on one Egyptian coffin of the XXI dynasty. *Microchemical Journal*, *138*, 122–131.

Bonizzoni, L., Bruni, S., Guglielmi, V., Milazzo, M., ... Neri, O. (2011). Field and laboratory multi-technique analysis of pigments and organic painting media from an Egyptian coffin (26th dynasty). *Archaeometry*, *53*(6), 1212–1230.

Bracci, S., Caruso, O., Galeotti, M., Iannaccone, R., Magrini, D., Picchi, D., ... Porcinai, S. (2015). Multidisciplinary approach for the study of an Egyptian coffin (late 22nd/early 25th dynasty): Combining imaging and spectroscopic techniques. *SpectrochimicActa Part A: Molecular and Biomolecular Spectroscopy*, *145*, 11–522.

Brunel-Duverger, L., Laval, E., Lemasson, Q., Brodie-Linder, N., & Pagès-Camagna, S. (2019). The contribution of non-invasive and non-destructive techniques to the understanding of the 21st Dynasty Egyptian Yellow Coffins complex stratigraphy: Case of study of E 20043 from the Louvre Museum. *The European Physical journal Plus*, *134*, 1–11.

Carter, H. (1933). *The tomb of Tut ankh amen* (Vol. III ed.). London: Cassell and Company, Ltd.

Cavaleri, T., Giovagnoli, A., & Nervo, M. (2013). Pigments and mixtures identification by visible reflectance spectroscopy, youth in conservation of cultural heritage, YOCOCU 2012. *Procedia Chemistry*, *8*, 45–54.

Colinart, S. (2001). Analysis of inorganic yellow colour in ancient Egyptian painting. In W. V. Davies (Ed.), *Colour and painting in ancient Egypt* (pp. 1–4). London: The British Museum Press.

Corcoran, L. H. (2016). The color blue as an "Animator" in ancient Egyptian art. In R. B. Goldman (Ed.), *Essays in global color history*, Interpreting the Ancient Spectrum (pp. 41–67). Piscataway, New Jersey: Gorgias Press.

Cosentino, A. (2015). Practical notes on ultraviolet technical photography for art examination. *Conservar Património*, *21*, 53–62.

Daniels, V., Stacey, R., & Middleton, A. (2004). The blackening of paint containing Egyptian blue. *Studies in Conservation*, *49*, 217–230.

Davies, W. (1995). Ancient Egyptian timber imports: An analysis of wooden coffins in the BritishMuseum. In W. Davies & L. Schofield (Eds.), *Egypt, the Aegean and the Levant: Interconnections in the second millennium BC* (pp. 146–156). London: British Museum Press.

Derrik, R. M., Stulik, D., & Landy, M. J. (1999). *Infrared spectroscopy in conservation science*. Los Angeles: The Getty Conservation Institute.

Dyer, J., & Sotiropoulou, S. (2017). A technical step forward in the integration of visible-induced luminescence imaging methods for the study of ancient polychromy. *Heritage Science*, *5*, 24.

Dyer, J., Verri, G., & Cupitt, J. (2013). *Multispectral imaging in reflectance and photo-induced luminescence modes: A user manual* (1st ed.). Available from: http://www.britishmuseum.org/pdf/charismamultispectral-imaging-manual-2013.pdf.

Edreira, C. M., Feliu, J. M., Fernández-Lorenzo, F. C., & Martín, J. (2003). Spectroscopic study of Egyptian blue mixed with other pigments. *Helvetica Chimica Acta*, *86*, 29–49.

Edreira, M., Feliu, M., Fernández-Lorenzo, C., & Martín, J. (2001). Roman wall paintings characterization from Cripta del Museo and Alcazaba in Mérida (Spain): Chromatic, energy dispersive X-ray flurescence spectroscopic, X-ray diffraction and Fourier transform infrared spectroscopic analysis. *Analytica Chimica Acta*, *434*, 331–345.

Frantz, H. J., & Schorsch, D. (1990). Egyptian red gold. *Archeomaterials*, *4*, 133–152.

Ganio, M., Salvant, J., Williams, J., Lee, L., Cossairt, O., & Walton, M. (2015). Investigating the use of Egyptian blue in Roman Egyptian portraits and panels from Tebtunis, Egypt'. *Applied Physics A*, *121*(3), 813–821.

Gard, F. S., Bozzano, P. B., Santos, D. M., Daizo, M. B., Halac, E. B., & Reinoso, M. (2020). A multi-analytical approach for the study of the pigments used to decorate an Egyptian cartonnage from ptolemaic period. *Microscopy and Microanalsis*, *26*, 1–2.

Giménez, J. (2015). Egyptian blue and/or atacamite in an ancient Egyptian coffin. *International Journal of Conservation Science*, *6*(4), 573–586.

Grant, M. S. (2000). 'The use of ultraviolet induced visible-fluorescence in the examination of museum objects, Part II'. *Conserve O Gram*, *1*(9), 1–3.

Green, L. (2001). Colour transformations in ancient Egyptian pigments. In W. Davies (Ed.), *In colour and painting in ancient Egypt* (pp. 43–48). London: British Museum Press.

Guglielmi, V., Andreoli, M., Comite, V., Baroni, A., & Fermo, P. (2021). The combined use of SEM-EDX, Raman, ATR-FTIR and visible reflectance techniques for the characterisation of Roman wall painting pigments from Monte d'Oro area (Rome): An insight into red, yellow and pink shades. Environmental Science and Pollution Research, 1–19.

Hatchfield, P., & Newman, R. (1991). Ancient Egyptian gilding methods. In D. Bigelow, E. Cornu, G. Landre, & C. V. Home (Eds.), *Gilded wood conservation and history* (pp. 291–299). Madison CT: Sound View Press.

Hatton, G., Shortland, A., & Tite, M. (2008). The production technology of egyptian blue and green frits from the second millennium BCE Egypt and Mesopotamia. *Journal of Archaeological Science, 35*, 1591–604.

Heywood, A. (2001). The use of huntite as a white pigment in ancient Egypt. In W. Davies (Ed.), *Colour and painting in ancient Egypt* (pp. 5–9). London: The British Museum Press.

Hoving, T. (1978). *Tutankhamun: The untold story*. New York: Simon and Schuster.

Ismail, Y., Abdrabou, A., & Abdallah, M. (2016). A non-destructive analytical study and the conservation processes of Pharaoh Tutankhamun´s painted boat model. *International Journal of Conservation Science*, *7*(1), 15–28.

Jaksch, H., Seipel, W., Weiner, K., & El Goresy, A. (1983). Egyptian blue – cuprorivaite a windowto ancient Egyptian technology. *Naturwissenschaften*, *70*(11), 525–535.

James, T. G. H. (1972). Gold technology in ancient Egypt, mastery of metal working methods. *Gold Bulletin*, *5*, 38–42

Lee, L. (1997). *Investigation into degraded pigments on the Coffin of Amenemope, EA 22941*. British Museum Conservation Research Group CA1997/20.

Lee, L., & Quirke, S. (2000). Painting materials. In P. Nicholson & I. Shaw (Eds.), *Ancient Egyptian materials and technologies* (pp. 104–121). Cambridge: Cambridge University Press.

Mahmoud, H. (2014). Investigations by Raman microscopy, ESEM and FTIR-ATR of wallpaintings from Qasr el-Ghuieta temple, Kharga Oasis, Egypt. *Heritage Science*, *2*, 18–29.

Martinez, K., & Cupitt, J. (2005). VIPS – a highly tuned image processing software architecture. *IEEE International Conference on Image Processing* (pp. 574–577).

McCarthy, B. (2001). Technical analysis of reds and yellows in the tomb of Suemniwet, Theban tomb 92. In W. V. Davies (Ed.), *Colour and painting in ancient Egypt* (pp. 17–21). London: The British Museum Press.

Miriello, D., Bloise, A., Crisci, G. M., De Luca, R., De Nigris, B., Martellone, A., … Ruggieri, N. (2018). Non-destructive multi-analytical approach to study the pigments of wall painting fragments reused in mortars from the archaeological site of Pompeii (Italy). *Minerals*, *8*(134), 1–15.

Mirti, P., Appolonia, L., Casoli, A., Ferarri, R., Laurenti, E., Amizano, A., & Chiari, G. (1995). Spectrochemical and structural studies on a roman sample of Egyptian Blue. *Spectrochimica Acta, Part A: Molecular and Biomolecular pectroscopy*, *51*(3), 437–46.

Pagès-Camagna, S., & Guichard, H. (2010). Egyptian colours and pigments in French collections: 30 years of physicochemical analyses on 30 objects. In J. Dawson, C. Rozeik, & M. Wright (Eds.), *Decorated surfaces on ancient Egyptian objects technology, deteriorationand conservation* (pp. 25–31). London: Archetype Publications.

Pradell, T., Salvado, N., Hatton, G., & Tite, M. (2006). Physical processes involved in production of the ancient pigment, Egyptian blue. *Journal of the American Ceramic Society*, *89*(4), 1426–1431.

Reeves, N. (1990). *The complete Tutankhamen* (*the King-the Tomb-the Treasury*). Cairo: The American University in Cairo Press.

Riederer, J. (1977). Egyptian blue in artists. In E. FitzHugh (Ed.), *Pigments* (pp. 23–45). Oxford: Oxford University Press.

Rifai, M., & El Hadidi, N. M. (2010). Investigation and analysis of three gilded wood samples from the tomb of Tutankhamun. In J. Dawson, C. Rozeik, & M. M. Wright (Eds.), *Decorated surfaces on ancient Egyptian objects technology, deterioration and conservation* (pp. 16–24). London: Archetype Publications.

Sakr, A. A., Ghaly, M. F., Abdulla, M., Edwards, H., & Elbashar, Y. H. (2020). A new light on the grounds, pigments and bindings used in ancient Egyptian cartonnages from Tell Al Sawa, Eastern Delta, Egypt. *Jornal of Optics*, *49*(2), 230–247.

Schneider, H. (1977). *Shabtis: An Introduction to the history of ancient Egyptian funerary statuettes, with a catalogue of the collection of Shabtis in the National Museum of Antiquities at Leiden*. Leiden: Rijksmuseum van Oudheden.

Scott, D. A., Dodd, L. S., Furihata, J., Tanimoto, S., Keeney, J., Schilling, M. R., … Cowan, E. (2004). An ancient Egyptian Cartonnage Broad Collar: Technical examination of pigments and binding. *Studies in Conservation, 49*(3), 177–192.

Scott, D., Warmlander, S., Mazurek, J., & Quirke, S. (2009). Examination of some pigments, grounds and media from Egyptian cartonnage fragments in the Petrie Museum, University College London. *Journal of Archaeological Science, 36*(3), 923–932.

Stuart, B. H. (2007). *Analytical techniques in materials conservation*. England: Wiley.

The Griffith institute (2000–2004). *Tutankhamun: Anatomy of an excavation, the Howard carter archives, handwritten object cards by H. Carter*. [Online] Available at: http://www.griffith.ox.ac.uk/gri/carter/.

Tissot, I., Troalen, L., Manso, M., Ponting, M., Radtke, M., Reinholz, U., … Guerra, M. F. (2015). A multi-analytical approach to gold in Ancient Egypt: Studies on provenance and corrosion. *Spectrochimica Acta Part B, 108*, 75–82.

Unger, A., Schniewind, A., & Unger, W. (2001). *Conservation of wood artefacts, hand book*. Berlin Heidelberg: Springer-Verlag.

CHAPTER 7

Applying Gel-Supported Liquid Extraction to Tutankhamun's Textiles for the Identification of Ancient Colorants: A Case Study

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Greta Peruzzi [1,2,*], Alessandro Ciccola [3,*], Adele Bosi [3,4], Ilaria Serafini [3], Martina Negozio [5], Nagmeldeen Morshed Hamza [4,5,6], Claudia Moricca [5], Laura Sadori [5], Gabriele Favero [5], Valentina Nigro [7], Paolo Postorino [2] and Roberta Curini [3]

1 Institute for Complex System, National Research Council, Sapienza University, Piazzale Aldo Moro 5, 00185 Rome, Italy
2 Department of Physics, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy; paolo.postorino@roma1.infn.it
3 Department of Chemistry, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy; adele.bosi@uniroma1.it (A.B.); ilaria.serafini@uniroma1.it (I.S.); roberta.curini@uniroma1.it (R.C.)
4 Department of Earth Sciences, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy; nagmeldeenmorshed.hamza@uniroma1.it
5 Department of Environmental Biology, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy; negozio.1920109@studenti.uniroma1.it (M.N.); claudia.moricca@uniroma1.it (C.M.); laura.sadori@uniroma1.it (L.S.); gabriele.favero@uniroma1.it (G.F.)
6 Grand Egyptian Museum, Conservation Center, Al Remaya Square, Giza 3513204, Egypt
7 ENEA C.R. Frascati, Fusion and Technologies for Nuclear Safety and Security Department, Via E. Fermi 45, 00044 Frascati, Italy; valentina.nigro@enea.it
* Correspondence: gretaperuzzi@icloud.com (G.P.); alessandro.ciccola@uniroma1.it (A.C.)

Abstract: The identification of the dyes present on a linen fragment from the tomb of Pharaoh Tutankhamun is the objective of the present study. Fiber optic reflectance spectroscopy (FORS) was applied to the archaeological sample for preliminary identification of the dyes and to better choose the extraction methodology for different areas of the sample. The innovative gel-supported microextraction with agar gel and the Nanorestore Gel® High Water Retention (HWR) gel were applied to the archaeological sample after testing of the best concentration for the extraction of the agar gels substrates, performed on laboratory mock-ups by means of UV–Vis transmittance spectroscopy. Immediately after extraction, Ag colloidal pastes were applied on the gel surface and Surface Enhanced Raman Scattering (SERS) analysis was performed directly on them. The combination of information deriving from FORS and SERS spectra resulted in the successful identification of both indigo and madder and, in hypothesis, of their degradation products.

Keywords: SERS; dyes; gel; micro-extraction; non-invasive; archaeological textile

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1. Introduction

The use of dyed threads or dyed clothes in ancient Egypt could tentatively be traced back to the First Dynasty (3150–2925 BC), but it is only from the New Kingdom onwards (18th Dynasty, 1543–1292 BC) that cloth woven with colored thread was increasingly employed [1]. The most used fiber was linen and since not everyone could afford high quality linen, thread was often dyed using ochres rather than plant dyes [2]. The first was indeed utilized for dyeing in brown or dark red shades by hydrating iron oxide and mixing it with clay. Vibrant red textiles were obtained through the use of Mediterranean plants such as madder (*Rubia tinctorum*) or alkanet (*Alkanna tinctoria*) [3,4]. Yellow tones, instead, were achieved from safflower (*Carthamus tinctorius*) or turmeric (*Curcuma longa*), while indigo (*Indigofera tinctoria*) was the main source of blue colors. Other shades, such as green colors, were obtained by mixing blue and yellow dyestuffs [2,4].

In recent decades, the interest in studying organic dyestuff historically used to dye textiles has grown. These objects, in fact, possess great cultural importance and, in particular, the study of organic colorants allows for achieving historical and technological information about manufacturing, trades, exchanges, and civilization evolutions. However, the analysis of historical textile samples represents a complex challenge from the analytical point of view due to the usually limited amount of samples available, the low concentration of chromophores in the original material, and the presence of possible degradation products [5]. Textile fibers are generally susceptible to degradation mechanisms involving physicochemical and, consequently, mechanical processes that are eventually caused by microbiological attacks. These can result in the integrity loss of the fiber itself. The dye, in turn, can be subjected to photo-oxidative reactions, leading to fading and alteration of the original chromatic features [6]. Consequently, the place and methods of preservation of textile are fundamental for defining its current state of conservation. From this point of view, the archaeological contexts could represent "extreme" situations in which the microbiological attack is disadvantaged. In several cases (e.g., deserts, acidic peat bogs, alkaline lake muds, and perennial ice [7]), it is not uncommon to find remains of dyed fabric preserved up to the present day. Textile artifacts that survive are therefore extremely valuable; the definition of the dyeing matrices and the technologies employed to make the object not only help in reconstructing ancient cultures but also play a crucial role in developing and fine-tuning specific conservation methods.

Consequently, in the field of chemistry applied to cultural heritage, the analysis of the dye composition represents a powerful instrument, but it is also one of the most interesting analytical challenges. High pressure liquid chromatography coupled with an appropriate detector (diode array, HPLC-DAD; mass spectrometer, HPLC-MS) remains the most reliable and versatile identification method for organic colorants today [5,8–11], while the identification of dyes directly on fabrics without separative methods is often complex because of the organic matrix, which interferes in most analytical techniques. Nonetheless, when dealing with objects of art, the application of techniques which require sampling is discouraged and a multi-technical approach which includes both non-invasive and micro-invasive techniques is always preferred. During the last decades, great effort has been undertaken to develop minimally invasive techniques with increased sensitivity. In this sense, fiber optic reflectance spectroscopy (FORS) and hyperspectral imaging in the UV–Vis–NIR range have been demonstrated to be efficient tools for the rapid, non-invasive, in situ preliminary characterization of many artistic materials [9,12–18]. Additionally, Raman and Surface Enhanced Raman Scattering (SERS) spectroscopies have attracted the interest of many research groups for their ultrasensitive and high detection capability. In particular, in the latter case, SERS spectroscopy has proved to be a valid technique for the characterization and study of organic dyes [19–24]. Indeed, by exploiting metallic substrates, such as, for example, silver nanoparticles, it is possible to amplify the Raman signal significantly, and this enhancement allows for overcoming the problem of strong fluorescence emission—typical of organic compounds—due to a localized surface plasmon resonance (LSPR) phenomenon, which comes into play when the incident light has the same vibrational frequencies of the valence electrons in the metal nanoparticle [25,26]. In this perspective, the use of gel substrates for the micro-invasive extraction of dyes and their consequent analysis by the SERS technique has recently been applied to the study of cultural heritage [27–31]. The most used in this sense is the agar gel because it favors the interaction of silver nanoparticles with consequent enhancement of the SERS signals due to the shrinkage of its structure after drying [28]. For example, in 2015, Platania and colleagues [32] presented a methodology for the extraction and detection of indigo dyes in painting and textiles involving Ag-agar gel soaked into a reducing solution, resulting in a safe procedure for both laboratory samples and works of art. Despite this, other types of gels have been used, such as, for example, the Nanorestore Gel® High Water Retention (HWR) gel—patented for cleaning surfaces—which was tested for the first time by Germinario in 2020 for the extraction of dyes from textiles [33]. Both agar and Nanore-

store Gel® were employed in the aforementioned work for the extraction of madder and cochineal from wool mockups using the state-of-the-art ammonia-based solution devised in 2016 by Lombardi and colleagues [34]. This methodology, stated as 'mild', tested, for the first time, a basic environment using ammonia, Na_2EDTA, and NaCl for the extraction of anthraquinones in order to preserve glycosylated moieties, which are sensitive and may be lost in traditional acidic methodologies. The work by Germinario, through a multi-technical approach, proved highly successful for SERS analysis, allowing discrimination between madder and cochineal on both gels. The research was pushed forward and the methodology was revised and implemented for hydrophilic paint layers in the study by Bosi [30], where agar at different concentrations (ranging from 1% to 12%) and Nanorestore Gel® were tested for the extraction of madder lake pigments showing positive outcomes in terms of non-invasiveness. Agar gel concentrations below 4% permitted the extraction without ripping the paint and did not show any color change on the mockups. From the operative point of view, moreover, the methodology (which is defined as "gel-supported liquid extraction") is even very simple. Briefly, the gel is cut into cylinders and soaked in the extraction solution for a certain amount of time. Then, these substrates are removed with tweezers and put in contact with the sample surface in order to extract the analytes present on the surface [30]. This makes this approach suitable for different typologies of laboratories.

For all these reasons, in the present work, we decided to investigate more deeply the behavior of extraction of agar gel in the range of concentrations between 2% and 4%. In this way, in comparison to the previous literature, a deeper insight was accomplished in order to individuate the agar gel concentration, coupling the best extraction performances to handing features for the analytical methodology [30]. Agar gel was tested along with Nanorestore Gel® on both paint and textile laboratory mock-ups tinted with madder and indigo; spectroscopic analysis including UV–Vis and Raman SERS spectroscopy were performed on these samples and also served as a reference spectral database. In this way, the methodology was hence applied to a wider set of artist matrices (for instance, the tempera mock-ups) and with a systematic approach in comparison to our previous works [30,33]. It was also employed for the diagnostics of a precious sample, an archaeological textile fragment from Tutankhamun's tomb. Preliminary spectroscopic analysis was conducted on the archaeological sample using FORS to understand which extraction procedure should be followed. Gels were then soaked in two different solutions according to the area of extraction (blue or red), Ag-colloidal pastes were applied immediately after extraction, and SERS analysis was performed to characterize the dyes. The combination of gel-supported liquid extraction with the use of colloidal paste represents a different approach in comparison to the previous studies [30,33,35], which takes advantage from the formation of extended nanoclusters for the enhancement of the Raman signal. The application of this sample allowed for achieving an actual valorization in a real case study, and it integrated the gel-supported liquid extraction in an analytical protocol that also involved non-invasive techniques: this promotes the transferability of the new technology in routine cultural heritage diagnostics.

2. Results and Discussion

2.1. UV–Vis Spectroscopy

The UV–Vis spectra of agar gel in the concentration range between 2% and 4% have been compared to determine the concentration of agar that is the most appropriate to employ for the extraction of the real case study. The spectrometric measurements of agar are taken before the extraction and after the extraction both on the paint and textile mock-ups. In Figure 1, the transmittance spectra at three different concentrations (2%, 3%, 4% in water w/w) are reported. For all tested concentrations, the transmittance is higher in the samples pre-extraction than in the samples post-extraction. Furthermore, the spectra of agar gel after the extraction on the textile sample show lower transmittance values, probably due to the higher absorption of incident radiation of the extracted dye.

The UV–Vis spectra of agar gel exhibit the same pattern at all concentrations after being soaked in the ammonia solution, along with Na_2EDTA and NaCl. Up until 300 nm, transmittance values are constant, then they start to quickly rise. The spectra of agar gel after extraction on the paint mock-up show a wide absorption band between 480 nm and 550 nm, which is due to the electron transitions $n \rightarrow \pi^*$ of carbonyl groups present in the chromophore alizarin [36]. Samples with 2% and 3% of agar gel post-extraction are more susceptible to the UV–Vis radiation than samples at C_w = 4%, with a more evident decrease in transmittance around 400 and 500 nm, which is due to the typical absorbance bands of madder, as shown in Figure 1. Spectra of agar gel after the extraction on the textile mock-up show slight absorption characteristic bands that confirm the major capability of extracting the dye from paint rather than textile. Nevertheless, when comparing all the concentrations, 2% agar gel and 3% agar gel clearly show the absorption band opposed to all other tested concentrations.

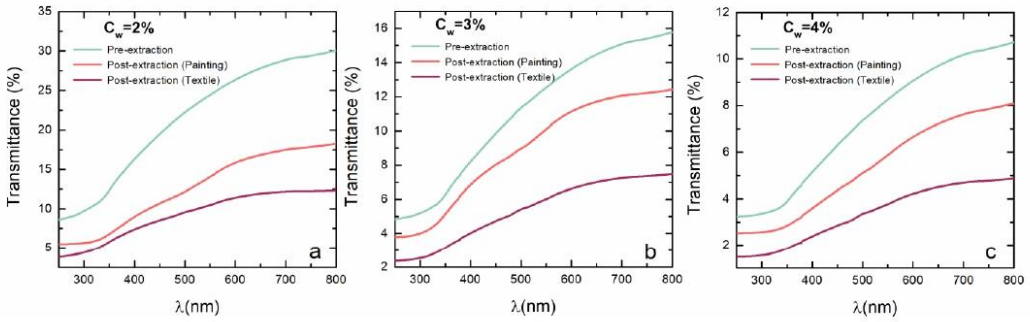

Figure 1. UV–Vis transmittance spectra of agar gel at (**a**) C_w = 2%, (**b**) C_w = 3%, and (**c**) C_w = 4% of the gel before extraction (green), after extraction from the madder paint mockup (orange), and after extraction from the madder textile mockup (red).

An evaluation of the invasiveness of the procedure for the mock-ups was performed by means of optical analysis and FORS-colorimetry. The observation at the microscope did not evidence gel residues on the surface of the mock-ups and no observation about the morphology of the mock-up was observed. From the point of view of color changes, the calculation of color variation ΔE_{00} using the CIEDE2000 formula resulted in values lower than three, which were considered as the upper limit of rigorous color tolerance. With the reference to these results, the methodology can be considered remarkably micro-invasive because it extracts the analytes without causing damage or visible color variations on the artist matrices. However, with reference to more sensitive materials, further details about these aspects can be found in previous works [30].

2.2. Case Study: Archaeological Textile Fragment from Tutankhamun's Tomb

2.2.1. Gel Micro-Extraction In Situ

Based on tests conducted on laboratory mock-ups and results obtained with UV–Vis spectroscopy, it was decided to use 3% agar gel for the extraction in the real case study as it showed the best results in terms of extraction capacity without leaving any residue.

Both agar gel and Nanorestore Gel® worked well with the ammonia-based solution for the extraction of the red dyes. On the contrary, in the blue area, it was possible to use only agar gel since Nanorestore Gel® appeared to be incompatible with the indigo reducing solution. Further evaluations must be performed in this aspect since it could be interesting to understand if the solution causes polymer degradation rather than sodium dithionite concrete forming inside the gel (Figure 2).

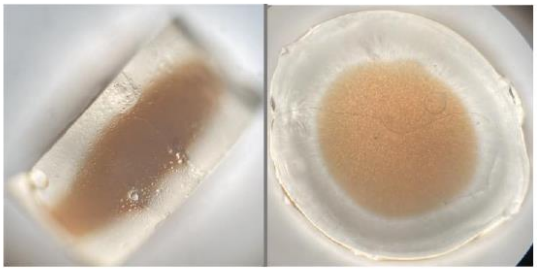

Figure 2. Optical microscope (4× magnification) images of Nanorestore Gel® soaked with the reducing extraction solution used for indigo extraction.

2.2.2. Fiber Optic Reflectance Spectroscopy (FORS)

FORS analyses on the archaeological samples were useful to hypothesize a first characterization of the dyes present. Indeed, by comparing spectra acquired on the bluish area and that of the indigo mockup (Figure 3a), it is possible to underline spectral similarities in the 650–700 nm region where a broad absorption band typically attributed to the $\pi \rightarrow \pi^*$ of the C=C double bond is present [37]. Moreover, through applying the first derivative, it is possible to observe a flex around 700 nm, which is perfectly in line with what is reported in the literature for indigo and woad [37]. However, it is important to highlight that the weak maximum in the violet region, observable in the mock-up and cited in the literature, is not visible in the spectrum of the archaeological sample [37].

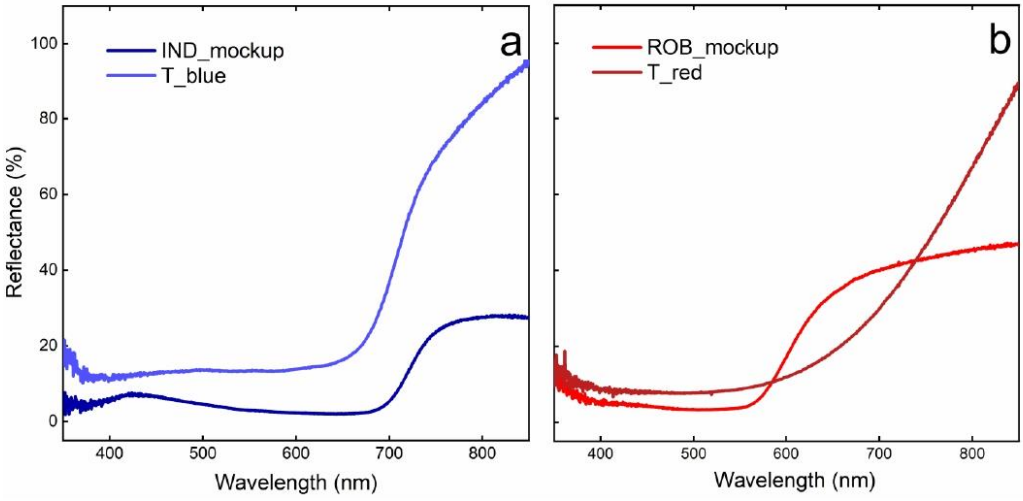

Figure 3. (**a**) Comparison between FORS spectra of indigo mockup (dark blue) and of the blue area of the archeological sample; (**b**) comparison between FORS spectra of madder mockup (red) and of the red area of the archaeological fragment (Bordeaux).

Quite the opposite is evident when comparing the results obtained from the reddish area with the reference spectra of red dyes and laboratory mockups (Figure 3b, showing the comparison with a reference mock-up of madder mockup). Indeed, the presence of a wide absorption band between 400 and 700 nm disallows any attribution to typical spectral features of known colorants of reddish shade.

2.2.3. Surface Enhanced Raman Scattering (SERS)

Results obtained from SERS spectra acquired on agar gel after extraction (Figure 4a) confirm the presence of an indigo dye on the blue area of the Tutankhamun's fragment. Nonetheless, by comparing results obtained from the extraction of the archaeological sample and those from the indigo mockup (Figure 4b), the absence of the most intense signals of indigo is evident for the former, although characteristic peaks of both indigotin (1073 w, 1176 vw, 1369 w, 1470 vw) and indirubin (645 m, 971 m, 1404 w, 1585 vw) are present. Indeed, signals of indirubin are generally more intense than those of indigotin, probably due to thermal degradation of the dye [38]. In particular, peaks at 645, 971 and 1404 cm^{-1} can be attributed to bending modes of the C-C, C-H, and N-H bonds of indirubin [39], while the signal at 1073 cm^{-1} is attributed to a ring stretching and C-O rocking of indigotin [32]. The broad band centered at 1176 cm^{-1} also refers to indigotin and corresponds to C-C stretching and C-H bending [32]. The band at 1585 cm^{-1}, instead, can be attributed to stretching modes of the C-C, C=O, and C=C bonds of indirubin [39]. Finally, the peak at 673 cm^{-1} is probably due to agar gel, as proven by the comparison in Figure 4a, while the very intense signal at 1037 cm^{-1} has been hypothesized to be a degradation product, probably anthranilic acid, which is a known degradation product of indigotin. Indeed, according to Poulin [40], one of the main degradation products of indigo is isatin, which is formed when indigotin oxidizes. If the degradation goes further and a secondary reaction takes place, anthranilic acid is formed. In 2014, Chadha and colleagues reported a very intense SERS band at 1036 cm^{-1} attributed to the phenolic ring bending and to the NH_2 rocking of anthranilic acid [41]. The results about the strong affinity between anthranilic acid and the Ag-NPs reported in the aforementioned work support both the hypothesis of the presence of anthranilic acid as a degradation compound on the blue area of the Tutankhamun's textile fragment and also justify the relatively strong intensity of the SERS signal we observed. However, further analysis through HPLC/MS could help to clarify the nature of this compound.

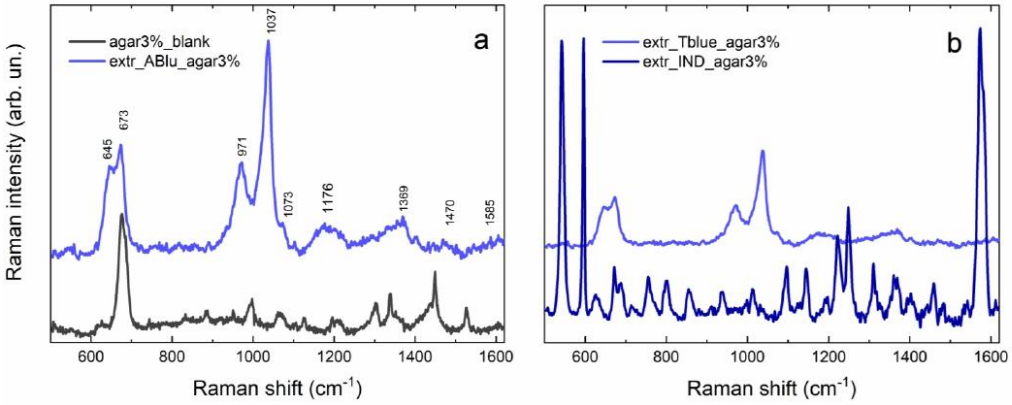

Figure 4. (**a**) Comparison between SERS spectra of 3% agar blank (gray) and 3% agar after extraction of the blue area (light blue); (**b**) comparison between SERS spectra of 3% agar after extraction of the indigo mockup (dark blue) and 3% agar after extraction of the blue area (light blue).

SERS analysis performed on gels after extraction of the red area of Tutankhamun's fragment gave good quality spectra for both agar (Figure 5a) and Nanorestore Gel® (Figure 5b). In both cases, it is possible to recognize the characteristic peaks of madder; most notably, by comparing the spectrum acquired on agar gel after extraction from the archaeological sample and from the madder mockup, a clear correspondence between the peaks is observed (Figure 5a). In particular, the peak at 1448 cm^{-1} is present in the spectrum acquired

on agar and is attributable to C-O stretching, C-O-H bending, and C-H bending [20,28,42]; whereas, the peak around 1400 cm^{-1}, which is a characteristic signal of purpurin, is very clearly seen in the spectrum acquired on the Nanorestore Gel®. Furthermore, the band around 1330 cm^{-1} present in both spectra is typical of madder and refers to the content of alizarin and purpurin, while the shoulder at 1290 cm^{-1} and the band around 1590 cm^{-1} are typical signals of anthraquinone molecules and correspond to the C-C and C=O stretching of the ring, respectively [28]. Results for madder were eventually confirmed and supported by LC/MS data obtained after re-extraction from the gels, whose results are the subject of another publication by the same authors.

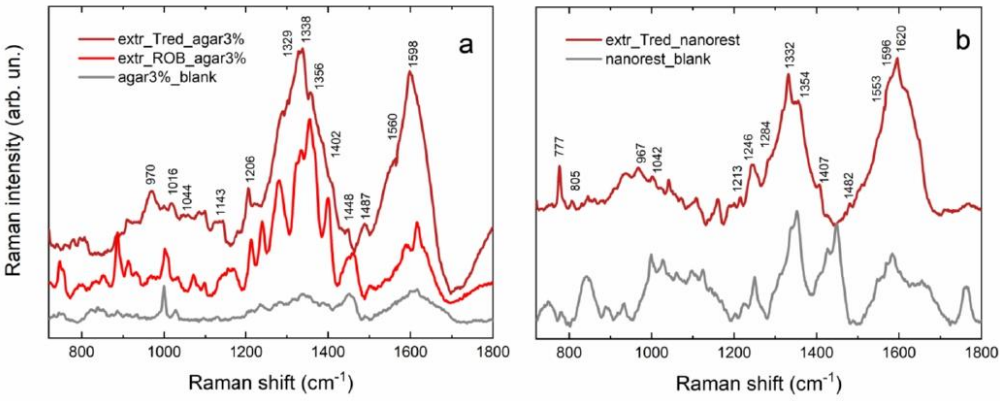

Figure 5. (**a**) Comparison between SERS spectra of 3% agar gel blank (gray), 3% agar gel after extraction from madder mockup (light red), and 3% agar gel after extraction from red area of archaeological sample (Bordeaux); (**b**) comparison between SERS spectra of Nanorestore Gel® blank (gray) and Nanorestore Gel® after extraction from red area of archaeological sample (Bordeaux).

3. Conclusions

The multi-technical approach pursued in this research study, composed of gel microextraction and the subsequent spectroscopic characterization, enabled the detection of the dyes present on Tutankhamun's textile fragment without posing any threat to the artifact.

First, tests conducted on laboratory mock-ups permitted to investigate how different concentrations of agar gel and UV–Vis transmittance spectroscopy have contributed to the choosing of 3% agar gel as the suitable concentration.

The application of the FORS technique on the archaeological sample was useful to obtain preliminary data in a totally non-invasive way and to consequently hypothesize the composition of the dyes present. This was true for the bluish area where the presence of an indigoid compound was even supported by the comparison with a reference spectrum of indigo. On the contrary, for the reddish area, the reflectance spectrum did not allow us to retrieve any information because of the wide absorption in the visible light range.

The application of the gel-supported liquid extraction protocol allowed a microsampling of the dyes both on the blue and red areas. The consequent detection was performed by SERS spectroscopy directly on the gel by contact of its surface with a silver colloidal paste. The SERS spectra confirmed the presence of indigo dye, from the characteristic signals of indirubin and (with lower intensity) indigotin, while, for the red area, it was possible to observe the characteristic signals of madder dye by comparison with reference spectra of the madder mockup. It is interesting to evaluate the potential and the complementarity of SERS applied to the gel extraction in comparison to FORS. For the indigo dye, the combination of FORS and SERS was useful in effectively detecting the presence of the blue colorants and in providing information about degradation processes.

While FORS suggested the presence of indigotin, the SERS data results were indicative of decomposition products. In the case of madder, FORS could not provide information for the dye identification, and only the on-gel approach allowed a clear identification of the chromophores. These aspects highlight the effectiveness and the information potential resulting from the gel-supported liquid extraction methodology.

It is fundamental to mention some aspects of the study, which require further research. At first, in this work, we limited the testing only to one typology of laboratory-made gel and to one parameter, the polymer/gel concentrations, but deeper studies would be necessary for better optimization of the gel-supported liquid extraction methodologies. Further gels could be studied, and the influence of other aspects (for instance, the thickness of the gel) should also be evaluated. The final protocol adopted for the historical sample requires integrating further analytical techniques, such as HPLC/MS, in order to provide the complete characterization of the dye chromophores and their final characterization. These aspects are the object of a future publication. Finally, regarding the hypotheses about degradation products observed by means of SERS, further analyses must be performed in order to confirm the presence of anthranilic acid.

4. Materials and Methods

4.1. Commercial Products

For the preparation of mock-ups, madder roots (*Rubia tinctorum* L.) and alum were purchased from Chroma Srl (Milano, Italy), while indigo in powder (*Indigofera tinctoria* L.) was purchased from Kremer Pigmente (Berlin, Germany). Cream of tartar (99.9%) and sodium carbonate (99.9%) were purchased at a local grocery shop.

Agar in powder (ash 2.0–2.4%), solvents, and salts such as K_2CO_3 (with impurities $\leq$55.0 ppm), ammonia (30–33%), NaOH ($\geq$95%), hydroxylamine hydrochloride (99.9%), NaCl (with impurities $\leq$0.005% as insoluble matter), Na_2EDTA (with impurities $\leq$0.005% as insoluble matter), and silver nitrate ($\geq$99.0%) were purchased from Sigma-Aldrich (Burlington, MA, USA). The Nanorestore Gel® High Water Retention Gel is produced by the Italian Center for Colloids and Surface Science (CSGI); information about this kind of gel is available on the Nanorestore Gel® High Water Retention Gel technical data sheet and in the previous literature [43–48].

4.2. Mock-Ups Preparation

A paint mock-up was prepared following traditional procedures reported in the literature [49]. First, preparation was performed by soaking 17 g of animal glue in 250 mL of water and leaving it overnight. The glue was then heated up at 45 °C until completely melted. Later, approximately 100–150 g of gypsum was added to the solution, which was applied on a brick in eight perpendicular coats. The whole was left drying for one week and then polished with sandpaper to make the surface smooth and homogeneous. The madder lake pigment was prepared following a recipe from Daniels et al. [49]. In brief, 5 g of madder roots were soaked in 150 mL distilled water and left overnight. The roots in water were heated up to 70 °C for 30 min. After filtration, 2.5 g potassium alum was added to the solution and the temperature was brought to 80 °C. Meanwhile, 0.94 g K_2CO_3 was dissolved in 25 mL water and gently poured into the dye bath under continuous stirring. The lake pigment was left precipitating overnight, filtered, and ground. Lake pigment was hence mixed with egg yolk and applied in layers on the previously prepared brick.

Laboratory textile mock-ups were prepared by wrapping and compacting dyed wool yarn around a microscope slide to simulate the surface of a fabric. For the dyeing process, ancient recipes already described in the literature and historically employed for dyeing with natural dyestuffs were followed [4,33,34]. Concerning madder, the procedure was divided into two steps: mordanting and dyeing. The mordanting bath was prepared by mixing 310 mg of alum and 60 mg of cream of tartar in 250 mL of distilled water and the solution was heated up to 40 °C for ten minutes. Then, it was left to cool to 25 °C before adding 1 g of raw purged wool into the bath. Following this, the temperature was

slightly raised to 80 °C over a period of 40 min, and the wool was maintained in the bath at this temperature for 1 h under gentle magnetic stirring. After that time, the bath was cooled at room temperature for over 20 min and the yarn was squeezed out and left to dry. A dyeing bath was prepared by soaking 1 g of crumbled madder roots in 400 mL of distilled water. The mordanted wool was added to a lukewarm bath while the temperature was increased to 80 °C over a period of 40 min and kept for 1 h under gentle magnetic stirring. Subsequently, the wool was left cooling in the bath for 30 min, then squeezed and washed repeatedly until the water was completely clear. Finally, it was left to dry.

The mechanism for dyeing with indigo involves a redox reaction (vat dyeing) in which indigo—which is usually insoluble in water—is reduced to its soluble leuco-form (leuco indigo) in alkaline conditions [4,50]. This allows the dye to penetrate into the fibers; the final color is reached and maintained through oxidation during the drying process, which makes indigo insoluble in water and impossible to be washed out [50]. A dyeing bath was hence prepared by mixing 0.6 g of minced indigo powder in 10 mL of distilled water previously warmed at 45 °C. Then, a solution of 0.6 g of sodium carbonate dissolved in 6 mL of water and a solution of 1.5 g of sodium dithionite dissolved in 50 mL of lukewarm water (40–50 °C) were added. The whole solution was thus heated up to 55 °C and left at this temperature for 20 min. After that time, 3 g of raw purged wool was soaked into the bath and left for 10 min. The yarn was then extracted from the bath, squeezed, and left to air dry in order to allow the indigo to oxidize again and reach the final color. Finally, the wool was rinsed with distilled water until it was clear and then left to dry.

4.3. Archaeological Sample: Textile Fragment from Tutankhamun's Tomb

After collecting and cataloging the most valuable remains from the tomb of Pharaoh Tutankhamun, the archaeologist Howard Carter swept the remaining materials from the surfaces of the tomb and deposited them in a wooden box. The box was closed in 1933 and stored in the Egyptian Museum in Cairo until 2017. One year later, it was moved to the Grand Egyptian Museum in Giza (Egypt), where its materials started to be subjected to scientific analyses.

Fragments presented here are linen-dyed textile pieces dating back to 1325 BC, the year of Pharaoh Tutankhamun's death. They are part of a wider collection of textile objects (more than 750) discovered in the tomb and constitute the sole surviving royal wardrobe from the pharaonic period. This large number of textiles offers a significant glimpse into the use of fabrics in ancient Egypt, particularly during the 18th Dynasty. The textile under examination is woven with the tapestry technique, a distinctive method of weaving that incorporates decorative designs using colored threads on a loom. In particular, the fragments presented here have some tinted blue and red areas with some striped sections with both colors in them. More information about the sample can be found in the Figures S1–S3 and in previous publications [51].

4.4. Gel Preparation and Micro-Extraction In Situ

The micro-extraction in situ was carried out with two different kinds of hydrogels: agar gel and Nanorestore Gel® High Water Retention (HRW) [33]. In previous studies, different concentrations between 1% and 12% have been tested with the best results for dye extraction observed at concentrations below 4% [9]. Therefore, here, we tested different concentration between 2% and 4% (2%, 2.5%, 3%, 3.5%, 4% in water *w*/*w*) of agar gel on naturally dyed wool and paint layer mockups before leading extraction on real case study samples. In brief, 0.16, 0.20, 0.24, 0.28 and 0.32 g of agar powder were dissolved in 8 mL of water, respectively, in three different beakers properly chosen to obtain a suitable thickness (~2 mm). The solution of agar was then heated up in a bain marie at 100 °C for 10 min and then cooled down for half an hour; afterwards, gels were stored in the fridge overnight before use. The Nanorestore Gel® was utilized as a factory product. For UV–Vis analysis, agar gels at different concentrations were cut in squares with 2 cm sides, while, for SERS measurements, the extraction was performed using both Nanorestore Gel® and agar gel

cut in small cylinders (cylinders were cut in half for the extraction of the archaeological sample because of the fragments' dimensions for a final size of about 3.5–4 mm of diameter) obtained with the back of a Pasteur pipette and loaded in the respective extracting solutions for 90 min [33].

For the extraction of the red area, we prepared a solution of 1 mM NH_3/Na_2EDTA (1:1) with 4.7 mM NaCl following the procedure pointed out in [34]. Both agar and Nanorestore Gel® were thus applied on the surface of the red area after the gel had lost 5% of its weight and it was left in extraction for 3 h. On the blue area, instead, the dye was extracted using a reducing solution containing NaOH/$Na_2S_2O_4$ (1:2) dissolved in water [32]. Gels were subsequently applied directly on the area of extraction at 100% of their weight but the exceeding solution was removed using adsorbent paper and letting it absorb the solution for 5 min [30]. The limited amount of time with respect to the ammonia-based solution extraction was decided, in this case, after several tests on the indigo mock-up in order to prevent the formation of a salty halo on the archaeological sample, which is due to, probably, the presence of sodium dithionite.

4.5. Preparation of Ag-Colloidal Pastes

Ag-colloidal pastes were prepared by adapting the procedure already described in [52,53]. In brief, Ag-colloids were prepared following Leopold and Lendl's methodology [54]: two solutions, one containing 0.021 g of NH_2OH HCl in 5 mL of MilliQ water and the other one with 0.02 g of NaOH in 5 mL of MilliQ water, were added to a solution of 0.017 g of $AgNO_3$ in 90 mL of MilliQ water under gentle magnetic stirring to induce the formation of colloids. Then, 10 mL of Ag-colloids were centrifuged for 20 min at 4500 rpm and the supernatant was removed. Afterwards, colloidal pastes were applied onto the gel surface immediately after dye extraction using a Pasteur pipette, and the gels were left drying for 12 h [30].

4.6. Spectroscopic Analysis

UV–Vis transmittance spectra were collected in the wavelength range between 190 and 800 nm using a Perkin Elmer Lambda 1050+ spectrophotometer in the ENEA C. R. Frascati laboratories. Measurements were led by housing the sample in a homemade support specifically designed for measurements on solid samples and by exposing both sides of the gel (i.e., the one in direct contact with the mockup where the extraction was performed and the opposite side) to the radiation. For each side, three measurements were acquired in three different positions. Spectra were then averaged and processed using Origin9 (©OriginLab, Northampton, MA, USA).

Raman-SERS data were collected using a Horiba Jobin-Yvon HR-Evolution Raman spectrometer (Kyoto, Japan) coupled with a microscope equipped with a series of interchangeable objectives. In this case, 20x magnification was chosen to select the area of analysis, while 100× objective was used to focus the laser beam on the Ag-colloid spots observed on the gels to obtain good quality SERS spectra. Samples were excited using a He–Ne laser (λ = 633 nm), whose intensity varied between 0.15 and 0.75 mW. The acquisition time and number of acquisitions were varied for each sample to optimize the signal-to-noise ratio; up to five spectra were acquired in different points of the gels and, to ensure reproducibility, data were averaged and processed using Origin9 (©OriginLab). Fifth-grade polynomial baseline was subtracted for the background and the adjacent–averaging smoothing method was applied to reduce noise.

Preliminary analysis was led on the archaeological fragment using a BELPhotonic optical microscope (Bengaluru, India) equipped with interchangeable objectives. After a general visual evaluation, fiber optic reflectance spectroscopy (FORS) measurements were performed to have a first non-invasive hypothesis of the chemical class to which natural dyes belong to better understand the procedure of extraction to follow. Spectra were acquired using the EXEMPLAR LS BW TECH spectrometer (Plainsboro Township, NJ, USA), operating in the range of 180–1100 nm with a variable resolution from

0.6 to 6.0 nm. Samples were illuminated with a 5W BW TECH BPS101 halogen lamp with an emission spectrum between 350 and 2600 nm and a color temperature of 2800 K. Radiation was sent to (and collected from) the samples using THORLABS RP22 optical fiber bundles provided with a measuring head of 45° inclination, which was suited to avoid the collection of specular reflectance radiation. Five measurements were acquired for each area (red and blue), and then spectra were averaged and processed using Origin9 (©OriginLab). The same instruments and methodology were used to evaluate chromatic variations in the mock-ups of yarns dyed with madder and the painting layer constituted by madder lake in egg tempera by exploiting the color analysis tool of the software BWSpec version 4.10. For the evaluation of the color invasiveness, further details about the experimental procedure are provided in [30].

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/gels9070514/s1, Figure S1: The historical samples from Tutankhamun's tomb analysed in the paper, constituted by archaeological textile fragments; Figure S2: A 10x magnification image of a red area on the analysed textile fragment; Figure S3: A 10x magnification image of a bluish area on the analysed textile fragment.

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References

1. *The Oxford Encyclopedia of Ancient Egypt*, 1st ed.; Oxford University Press: Oxford, UK, 2001; ISBN 978-0-19-510234-5.
2. Ahmed, H.E. History of Natural Dyes in North Africa 'Egypt'. In *Handbook of Natural Colorants*; Bechtold, T., Mussak, R., Eds.; John Wiley & Sons, Ltd.: Chichester, UK, 2009; pp. 27–36, ISBN 978-0-470-74497-0.
3. Angelini, L.G.; Pistelli, L.; Belloni, P.; Bertoli, A.; Panconesi, S. Rubia Tinctorum a Source of Natural Dyes: Agronomic Evaluation, Quantitative Analysis of Alizarin and Industrial Assays. *Ind. Crops Prod.* **1997**, *6*, 303–311. [CrossRef]
4. Cardon, D. *Natural Dyes: Sources, Tradition, Technology and Science*; Archetype: London, UK, 2007; ISBN 978-1-904982-00-5.
5. Degano, I.; Ribechini, E.; Modugno, F.; Colombini, M.P. Analytical Methods for the Characterization of Organic Dyes in Artworks and in Historical Textiles. *Appl. Spectrosc. Rev.* **2009**, *44*, 363–410. [CrossRef]
6. Sousa, M.M.; Miguel, C.; Rodrigues, I.; Parola, A.J.; Pina, F.; Seixas de Melo, J.S.; Melo, M.J. A Photochemical Study on the Blue Dye Indigo: From Solution to Ancient Andean Textiles. *Photochem. Photobiol. Sci.* **2008**, *7*, 1353. [CrossRef]
7. Cybulska, M. Archaeological Textiles—A Need for New Methods of Analysis and Reconstruction. *Fibres Text. East. Eur.* **2007**, *15*, 64–65.
8. Shahid, M.; Wertz, J.; Degano, I.; Aceto, M.; Khan, M.I.; Quye, A. Analytical Methods for Determination of Anthraquinone Dyes in Historical Textiles: A Review. *Anal. Chim. Acta* **2019**, *1083*, 58–87. [CrossRef] [PubMed]

9. Calà, E.; Gosetti, F.; Gulmini, M.; Serafini, I.; Ciccola, A.; Curini, R.; Salis, A.; Damonte, G.; Kininger, K.; Just, T.; et al. It's Only a Part of the Story: Analytical Investigation of the Inks and Dyes Used in the Privilegium Maius. *Molecules* **2019**, *24*, 2197. [CrossRef] [PubMed]
10. Calà, E.; Benzi, M.; Gosetti, F.; Zanin, A.; Gulmini, M.; Idone, A.; Serafini, I.; Ciccola, A.; Curini, R.; Whitworth, I.; et al. Towards the Identification of the Lichen Species in Historical Orchil Dyes by HPLC-MS/MS. *Microchem. J.* **2019**, *150*, 104140. [CrossRef]
11. Han, J.; Wanrooij, J.; Van Bommel, M.; Quye, A. Characterisation of Chemical Components for Identifying Historical Chinese Textile Dyes by Ultra High Performance Liquid Chromatography—Photodiode Array—Electrospray Ionisation Mass Spectrometer. *J. Chromatogr. A* **2017**, *1479*, 87–96. [CrossRef]
12. Aceto, M.; Agostino, A.; Fenoglio, G.; Idone, A.; Gulmini, M.; Picollo, M.; Ricciardi, P.; Delaney, J.K. Characterisation of Colourants on Illuminated Manuscripts by Portable Fibre Optic UV-Visible-NIR Reflectance Spectrophotometry. *Anal. Methods* **2014**, *6*, 1488. [CrossRef]
13. Picollo, M.; Cucci, C.; Casini, A.; Stefani, L. Hyper-Spectral Imaging Technique in the Cultural Heritage Field: New Possible Scenarios. *Sensors* **2020**, *20*, 2843. [CrossRef] [PubMed]
14. Cazenobe, I.; Bacci, M.; Picollo, M.; Radicati, B.; Bacci, G.; Conti, S.; Lanterna, G.; Porcinai, S. Non-Destructive Spectroscopic Investigation of Dyed Textiles: An Application to Yellow Dyed Wool Samples. In Proceedings of the Preprints of the 13th Triennial ICOM Meeting, Rio de Janeiro, Brazil, 22–27 September 2002; Volume 1, pp. 238–244.
15. Peruzzi, G.; Cucci, C.; Picollo, M.; Quercioli, F.; Stefani, L. Non-Invasive Identification of Dyed Textiles by Using Vis-NIR FORS and Hyperspectral Imaging Techniques. *Cult. Sci.Color.-Color Cult. Sci. J.* **2021**, *13*, 61. [CrossRef]
16. Ciccola, A.; Tozzi, L.; Romani, M.; Serafini, I.; Ripanti, F.; Curini, R.; Vitucci, F.; Cestelli Guidi, M.; Postorino, P. Lucio Fontana and the Light: Spectroscopic Analysis of the Artist's Collection at the National Gallery of Modern and Contemporary Art. *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* **2020**, *236*, 118319. [CrossRef]
17. Ciccola, A.; Serafini, I.; D'Agostino, G.; Giambra, B.; Bosi, A.; Ripanti, F.; Nucara, A.; Postorino, P.; Curini, R.; Bruno, M. Dyes of a Shadow Theatre: Investigating Tholu Bommalu Indian Puppets through a Highly Sensitive Multi-Spectroscopic Approach. *Heritage* **2021**, *4*, 1807–1820. [CrossRef]
18. Alp, Z.; Ciccola, A.; Serafini, I.; Nucara, A.; Postorino, P.; Gentili, A.; Curini, R.; Favero, G. Photons for Photography: A First Diagnostic Approach to Polaroid Emulsion Transfer on Paper in Paolo Gioli's Artworks. *Molecules* **2022**, *27*, 7023. [CrossRef] [PubMed]
19. Pozzi, F.; Leona, M. Surface-Enhanced Raman Spectroscopy in Art and Archaeology: SERS in Art and Archaeology. *J. Raman Spectrosc.* **2016**, *47*, 67–77. [CrossRef]
20. Whitney, A.V.; Van Duyne, R.P.; Casadio, F. An Innovative Surface-Enhanced Raman Spectroscopy (SERS) Method for the Identification of Six Historical Red Lakes and Dyestuffs. *J. Raman Spectrosc.* **2006**, *37*, 993–1002. [CrossRef]
21. Leona, M. Sub-Nanogram Level Identification of Alizarin by Surface Enhanced Raman Scattering. In Proceedings of the IRUG6, Florence, Italy, 29 March–1 April 2004; pp. 105–112.
22. Chang, J.; Cañamares, M.V.; Aydin, M.; Vetter, W.; Schreiner, M.; Xu, W.; Lombardi, J.R. Surface-Enhanced Raman Spectroscopy of Indanthrone and Flavanthrone: SERS from Indanthrone and Flavanthrone. *J. Raman Spectrosc.* **2009**, *40*, 1557–1563. [CrossRef]
23. Colantonio, C.; Lanteri, L.; Ciccola, A.; Serafini, I.; Postorino, P.; Censorii, E.; Rotari, D.; Pelosi, C. Imaging Diagnostics Coupled with Non-Invasive and Micro-Invasive Analyses for the Restoration of Ethnographic Artifacts from French Polynesia. *Heritage* **2022**, *5*, 215–232. [CrossRef]
24. Serafini, I.; Ciccola, A. Nanotechnologies and Nanomaterials. In *Nanotechnologies and Nanomaterials for Diagnostic, Conservation and Restoration of Cultural Heritage*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 325–380, ISBN 978-0-12-813910-3.
25. Stiles, P.L.; Dieringer, J.A.; Shah, N.C.; Van Duyne, R.P. Surface-Enhanced Raman Spectroscopy. *Annu. Rev. Anal. Chem.* **2008**, *1*, 601–626. [CrossRef]
26. Kneipp, K.; Moskovits, M.; Kneipp, H. (Eds.) *Surface-Enhanced Raman Scattering: Physics and Applications*; Topics in Applied Physics; Springer: Berlin/Heidelberg, Germany; New York, NY, USA, 2006; ISBN 978-3-540-33566-5.
27. Leona, M.; Decuzzi, P.; Kubic, T.A.; Gates, G.; Lombardi, J.R. Nondestructive Identification of Natural and Synthetic Organic Colorants in Works of Art by Surface Enhanced Raman Scattering. *Anal. Chem.* **2011**, *83*, 3990–3993. [CrossRef]
28. Lofrumento, C.; Ricci, M.; Platania, E.; Becucci, M.; Castellucci, E. SERS Detection of Red Organic Dyes in Ag-Agar Gel: Detection of Red Organic Dyes in Ag-Agar Gel. *J. Raman Spectrosc.* **2013**, *44*, 47–54. [CrossRef]
29. Becucci, M.; Ricci, M.; Lofrumento, C.; Castellucci, E. Identification of Organic Dyes by Surface-Enhanced Raman Scattering in Nano-Composite Agar-Gel Matrices: Evaluation of the Enhancement Factor. *Opt. Quant. Electron.* **2016**, *48*, 449. [CrossRef]
30. Bosi, A.; Ciccola, A.; Serafini, I.; Peruzzi, G.; Nigro, V.; Postorino, P.; Curini, R.; Favero, G. Gel Microextraction from Hydrophilic Paint Layers: A Comparison between Agar-Gel and Nanorestore Gel® HWR for Spectroscopic Identification of Madder. *Microchem. J.* **2023**, *187*, 108447. [CrossRef]
31. Gagliano Candela, R.; Lombardi, L.; Ciccola, A.; Serafini, I.; Bianco, A.; Postorino, P.; Pellegrino, L.; Bruno, M. Deepening Inside the Pictorial Layers of Etruscan Sarcophagus of Hasti Afunei: An Innovative Micro-Sampling Technique for Raman/SERS Analyses. *Molecules* **2019**, *24*, 3403. [CrossRef]
32. Platania, E.; Lofrumento, C.; Lottini, E.; Azzaro, E.; Ricci, M.; Becucci, M. Tailored Micro-Extraction Method for Raman/SERS Detection of Indigoids in Ancient Textiles. *Anal. Bioanal. Chem.* **2015**, *407*, 6505–6514. [CrossRef]

33. Germinario, G.; Ciccola, A.; Serafini, I.; Ruggiero, L.; Sbroscia, M.; Vincenti, F.; Fasolato, C.; Curini, R.; Ioele, M.; Postorino, P.; et al. Gel Substrates and Ammonia-EDTA Extraction Solution: A New Non-Destructive Combined Approach for the Identification of Anthraquinone Dyes from Wool Textiles. *Microchem. J.* **2020**, *155*, 104780. [CrossRef]
34. Lombardi, L.; Serafini, I.; Guiso, M.; Sciubba, F.; Bianco, A. A New Approach to the Mild Extraction of Madder Dyes from Lake and Textile. *Microchem. J.* **2016**, *126*, 373–380. [CrossRef]
35. Platania, E.; Lombardi, J.R.; Leona, M.; Shibayama, N.; Lofrumento, C.; Ricci, M.; Becucci, M.; Castellucci, E. Suitability of Ag-Agar Gel for the Micro-Extraction of Organic Dyes on Different Substrates: The Case Study of Wool, Silk, Printed Cotton and a Panel Painting Mock-up: Suitability of Ag-Agar Gel. *J. Raman Spectrosc.* **2014**, *45*, 1133–1139. [CrossRef]
36. Fonseca, B.; Schmidt Patterson, C.; Ganio, M.; MacLennan, D.; Trentelman, K. Seeing Red: Towards an Improved Protocol for the Identification of Madder- and Cochineal-Based Pigments by Fiber Optics Reflectance Spectroscopy (FORS). *Herit. Sci.* **2019**, *7*, 92. [CrossRef]
37. Gulmini, M.; Idone, A.; Diana, E.; Gastaldi, D.; Vaudan, D.; Aceto, M. Identification of Dyestuffs in Historical Textiles: Strong and Weak Points of a Non-Invasive Approach. *Dye. Pigment.* **2013**, *98*, 136–145. [CrossRef]
38. Bloxam, W.P.; Perkin, A.G. CXXXV—Indirubin. Part I. *J. Chem. Soc., Trans.* **1910**, *97*, 1460–1475. [CrossRef]
39. Ju, Z.; Sun, J.; Liu, Y. Molecular Structures and Spectral Properties of Natural Indigo and Indirubin: Experimental and DFT Studies. *Molecules* **2019**, *24*, 3831. [CrossRef] [PubMed]
40. Poulin, J. Identification of Indigo and Its Degradation Products on a Silk Textile Fragment Using Gas Chromatography-Mass Spectrometry. *J. Can. Assoc. Conserv.* **2007**, *32*, 48–56.
41. Chadha, R.; Maiti, N.; Kapoor, S. Interaction of Anthranilic Acid with Silver Nanoparticles: A Raman, Surface-Enhanced Raman Scattering and Density Functional Theoretical Study. *J. Mol. Struct.* **2014**, *1076*, 35–41. [CrossRef]
42. Bruni, S.; Guglielmi, V.; Pozzi, F. Historical Organic Dyes: A Surface-Enhanced Raman Scattering (SERS) Spectral Database on Ag Lee-Meisel Colloids Aggregated by $NaClO_4$: SERS Database on Historical Organic Dyes. *J. Raman Spectrosc.* **2011**, *42*, 1267–1281. [CrossRef]
43. *Nanotechnologies in the Conservation of Cultural Heritage*; Springer: New York, NY, USA, 2014; ISBN 978-94-017-9302-5.
44. Royal Society of Chemistry. *Nanoscience for the Conservation of Works of Art*; Baglioni, P., Chelazzi, D., Eds.; The Royal Society of Chemistry: London, UK, 2013; ISBN 978-1-84973-566-7.
45. Mastrangelo, R.; Chelazzi, D.; Poggi, G.; Fratini, E.; Pensabene Buemi, L.; Petruzzellis, M.L.; Baglioni, P. Twin-Chain Polymer Hydrogels Based on Poly(Vinyl Alcohol) as New Advanced Tool for the Cleaning of Modern and Contemporary Art. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 7011–7020. [CrossRef]
46. Domingues, J.A.L.; Bonelli, N.; Giorgi, R.; Fratini, E.; Gorel, F.; Baglioni, P. Innovative Hydrogels Based on Semi-Interpenetrating p(HEMA)/PVP Networks for the Cleaning of Water-Sensitive Cultural Heritage Artifacts. *Langmuir* **2013**, *29*, 2746–2755. [CrossRef] [PubMed]
47. Baglioni, M.; Poggi, G.; Chelazzi, D.; Baglioni, P. Advanced Materials in Cultural Heritage Conservation. *Molecules* **2021**, *26*, 3967. [CrossRef]
48. Baglioni, M.; Guaragnone, T.; Mastrangelo, R.; Sekine, F.H.; Ogura, T.; Baglioni, P. Nonionic Surfactants for the Cleaning of Works of Art: Insights on Acrylic Polymer Films Dewetting and Artificial Soil Removal. *ACS Appl. Mater. Interfaces* **2020**, *12*, 26704–26716. [CrossRef]
49. Daniels, V.; Devièse, T.; Hacke, M.; Higgitt, C. *Technological Insights into Madder Pigment Production in Antiquity*; Archetype Books: London, UK, 2014.
50. Hofenk de Graaff, J.H.; Roelofs, W.G.T.; van Bommel, M.R. *The Colourful Past: Origins, Chemistry and Identification of Natural Dyestuffs*; Archetype Publ: London, UK, 2004; ISBN 978-1-873132-13-5.
51. Deutsches Archäologisches Institute (Ed.) *Mitteilungen des Deutschen Archäologischen Instituts*; Harrassowitz: Berlin, Germany, 2019; ISBN 978-3-447-11194-2.
52. Idone, A.; Gulmini, M.; Henry, A.-I.; Casadio, F.; Chang, L.; Appolonia, L.; Van Duyne, R.P.; Shah, N.C. Silver Colloidal Pastes for Dye Analysis of Reference and Historical Textile Fibers Using Direct, Extractionless, Non-Hydrolysis Surface-Enhanced Raman Spectroscopy. *Analyst* **2013**, *138*, 5895. [CrossRef]
53. Saviello, D.; Alyami, A.; Trabace, M.; Giorgi, R.; Baglioni, P.; Mirabile, A.; Iacopino, D. Plasmonic Colloidal Pastes for Surface-Enhanced Raman Spectroscopy (SERS) of Historical Felt-Tip Pens. *RSC Adv.* **2018**, *8*, 8365–8371. [CrossRef] [PubMed]
54. Leopold, N.; Lendl, B. A New Method for Fast Preparation of Highly Surface-Enhanced Raman Scattering (SERS) Active Silver Colloids at Room Temperature by Reduction of Silver Nitrate with Hydroxylamine Hydrochloride. *J. Phys. Chem. B* **2003**, *107*, 5723–5727. [CrossRef]

CHAPTER 8

Overcoming the limit of in situ gel supported liquid microextraction: development of the new InGel-LC-MC analyses, a smart methodology for the identification of natural dyes from Tutankhamun tomb relics

Serafini I., Bosi A., Vincenti F., Peruzzi G., McClure K. R., Ciccola A., Hamza N. M., Moricca C., Sadori L., Montesano C., Sergi M., Favero G., Curini R.

Overcoming the limit of *in situ* gel supported liquid microextraction: development of the new InGeL-LC-MS analyses, a smart methodology for the identification of natural dyes from Tutankhamun tomb relics

Ilaria Serafini*[1], Adele Bosi[1,2], Flaminia Vincenti[1], Greta Peruzzi[1], Kathryn Raeburn McClure[1], Alessandro Ciccola[1], Nagmeldeen Morshed Hamza[2,3,4], Claudia Moricca[4], Laura Sadori[4], Camilla Montesano[1], Manuel Sergi[1], Gabriele Favero[4], Roberta Curini[1]

1- Department of Chemistry, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome Italy
2- Department of Earth Sciences, Sapienza University of Rome, P. le Aldo Moro 5, 00185, Rome
3- Grand Egyptian Museum, Conservation Center, Al Remaya Square, Al Haram, Giza Governorate, 3513204, Egypt
4- Department of Environmental Biology, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome Italy

Dyes, dLLME, dispersive liquid-liquid micro-extraction, HPLC-MS, archaeological remains

corresponding author: ilaria.serafini@uniroma1.it

ABSTRACT: In this paper, an innovative approach for the extraction and clean-up of natural dyes is presented.
Background. Building upon recent research highlighting the benefits of an ammonia-based protocol for extraction and preservation of glycosidic moieties present in natural dyes, this work overcomes the limits of working on minimal quantities of materials.
Results. In this work, a *IN situ* Gel supported Liquid micro-extraction (InGEL) methodology based on this ammonia extraction protocol is combined with a novel clean-up strategy to improve re-extraction from the gel and increase the quantities and purities of dye available for chromatographic analysis. For this reason, a dispersive liquid-Liquid MicroExtraction (dLLME) clean-up methodology, novel to heritage science, is developed and applied to purify and preconcentrate the dye molecules from both in-solution and gel extraction systems. The extraction recoveries of a variety of disperser and extraction solvents were assessed using HPLC coupled with tandem mass spectrometry. When the best conditions were defined, the InGEL protocol was applied successfully to archaeological dyed textiles from the Tutankhamun tomb relics. For these valuable archaeological samples, two extractions were performed: one in-solution from a sampled thread and one with the new gel system in situ.
Significance and novelty. The results showed significant improvements in the recovery of natural dye analytes compared to current methods, as well as increased precision and efficiency. The success of both methods for the identification of dyes confirmed the success of the protocol for small-scale, minimally invasive dye analysis, not only in cultural heritage but it also offers a successful model for other fields, such as textiles industries or cosmetics, where the analysis on these analytes is required.

1. Introduction

Natural dyes have long histories in human culture, with material evidence of dyeing tracing back at least 6000 years [1]. Through museology and archaeology, we are left today with the precious remains of dyed household objects and artistic works from a wide range of historical periods. The scientific study of these remains can provide useful information about past societies, technologies, artistic practices, and the objects themselves (including their conservation and preservation requirements). To fully understand and appreciate this information, raw scientific data acquired from the analysis of

dyed fibres must be combined with in-depth knowledge of the historical context from which the object under study originates.

In the field of chemistry applied to heritage (which encompasses the study of archaeological materials, historical artefacts, and works of art), textile dye analysis poses particular challenges. Natural dyes are not a single molecular structure – but a mix of chromophoric compounds. For this reason, much research has sought to investigate the mixtures of compounds which are fixed on yarns in the different natural dye baths. Studies of madder, weld, and other natural dyes have yielded information about the geographical provenances of the dye plants – with the aim of differentiating even within a single plant species [2-8], trying to define criteria for their distinction. The intrinsic chemical variability of natural dyes means that for analysis, dyes must generally be extracted from their fibres and analysed by chromatographic methods to obtain detailed information about their constitutions. This necessitates removal of a sample from the object, which raises ethical questions regarding acceptable sample size for preserving the overall object. There has been significant recent progress in the use of spectroscopic techniques for dye analysis, namely Fibre Optics Reflectance Spectroscopy (FORS), Fluorescence Spectroscopy and Surface Enhanced Raman Spectroscopy (SERS) [9-14]. However, whilst these techniques may allow identification of molecular class or significant marker compounds – they are unable to provide detailed characterisation of the molecular mixture which composes a single natural dyestuff. Micro-invasive analyses are therefore still of primary importance in the complete characterisation of natural dyes. Liquid chromatography–mass spectrometry (LC–MS) analyses (especially MS/MS fragmentation patterns) are essential tools for the identification of all components of natural dye mixtures. In particular, the combination of chromatographic techniques with high-resolution MS (HRMS) may allow detailed analysis of dye components which are isomers or very close in ion mass [15].

Optimising the extraction process is a complex task which requires effective removal of the dye from the fibre whilst preserving the molecular structure of the dye molecules for analysis. For this reason, the field has moved away from the use of hard procedures, such as strong acids at high temperatures in extractions [3-4, 6,16-19], which were suitable for the identification of one or a few markers but were found to cleave sensitive bonds within certain dye molecules [5]. There is, therefore, a great interest towards the principle of preserving as much of overall molecular pattern that has been fixed on fibre as possible, in order to maximise information obtained. To achieve this goal, 'soft' extraction processes to preserve the molecular structures of the sensitive dye components have been developed [19-23]. The importance of preserving sensitive dye components through the correct extraction protocol was illustrated in a 2017 study of cochineal, when a novel 'soft' extraction protocol employing ammonia, which is the first method that takes advantage of a basic environment as the extraction agent, allowed the first ever detection of the molecule pp6 (an O-glycoside of flavokermesic acid) in an American cochineal extract [24]. This molecule had previously been considered a characteristic species marker of Polish cochineal [7,8, 25-27]. These findings demonstrated the importance of employing suitable extraction methodologies, illustrating that even mild acid conditions can result cleavage of sensitive glycosyl bonds and loss of information.

Recent research has also focussed on the development of gel-based systems to extract the dye *in situ*. This is a low invasive approach which helps to maintain the overall integrity of the object under analysis by minimising the material removed. This technique has been primarily used in preparation of samples for SERS analysis [28-33]. These gel-based approaches primarily employed Agar-gel, as it is a well-known safe material used widely for the cleaning of artworks and is also well-suited to SERS analyses [32, 34-35]. These successful applications of gels illustrate their potential for minimally invasive dye extractions for analysis using highly informative laboratory techniques. However, the crucial challenge facing gel use is the need to develop a procedure which optimises the quantity of dye extracted from the object by the gel.

In 2020, Germinario et al. [11] proposed the first double workflow micro-sampling gel system (combining both spectroscopic and mass spectrometric analysis). This approach employed two different gel systems, Agar and Nanorestore® gels, to extract natural anthraquinone dyes (madder

and cochineal) from wool dyed mock-ups. This work combined the use of gels with the aforementioned ammonia extraction method [18, 24] by soaking the gel-systems in ammonia solutions of different concentrations and compositions and applying these to the dyed wool. SERS analysis was then performed directly on the gel substrate before re-extracting the dye samples from the gel for HPLC-HRMS investigation. In this context, the ammonia extraction method has significant benefits: it does not require the use of high temperatures so it can be performed in situ; and it is effective in extracting aglycones and glycosyl moieties without degrading sensitive bonds within the molecules. This approach proved highly successful for SERS analysis, allowing discrimination between madder and cochineal on both gels. HPLC-HRMS analyses showed the presence of alizarin and few markers from madder, testifying the extraction power of the gel-ammonia system. However, the re-extraction employed a liquid-liquid extraction protocol (LLE) [24] clean-up step, initially developed for 1 mg samples of dyed yarns. This step resulted in a loss of material during clean-up and was therefore not suitable for the quantities of dyes extracted in the gel, which were significantly lower than those [11]. These results showed that further development of the clean-up procedure was essential to avoid the loss of precious, low-concentration analyte during sample preparation – allowing detection of smaller quantities of dyestuff and minimising invasiveness.

Building upon this previous work and following recent trends towards developing small-scale, controlled, high-precision precision strategies for sample preparation in this field [36-37], this research aims to overcome the issue of sample loss whilst still employing the glycoside preserving ammonia-based extraction methodology, which requires the additional clean-up step (not used in acid extractions) [11]. To address this, an ultrasound assisted dispersive liquid-liquid micro-extraction (dLLME) is employed for clean-up in place of the traditional liquid-liquid extraction (LLE) for the first time in the field of natural dyes research.

dLLME was first developed in 2006 [38] for the detection of polycyclic aromatic hydrocarbons (PAHs) in water samples and is now widely used in analytical chemistry fields including forensics, food science, and environmental biology [39-40]. Based on a ternary solvent system, dLLME involves the rapid injection of an organic extracting solvent and a disperser solvent into an aqueous solution containing the analytes of interest. The disperser solvent is miscible in both phases and encourages a fine dispersion of organic micro-droplets in the aqueous solution, which visually appears as a ‘cloudy solution’. This fine dispersion maximises the contact area between the two phases, increasing the opportunities for analytes to migrate into the organic phase. The dispersion is further enhanced by sonication in an ultrasonic bath. Compared to traditional LLE, dLLME requires a far smaller quantity of organic solvent, allowing for sample enrichment during clean-up [38-41]. A further benefit of this is a reduction in the environmental implications related to organic solvent usage [42]. For historic dye analysis, the development and application of the ultrasound assisted dLLME protocol for the ammonia extraction method, together with the IN situ GEl supported Liquid micro-extraction- InGEL system has a significant benefit: increasing the recovery of natural dyes from micro-samples which allows the use the minimally invasive gel system. This makes the ammonia extraction method, which preserves very sensitive dye component molecules, suitable for the detection of extremely low-concentration dye components. Furthermore, dLLME is significantly more efficient than traditional LLE, meaning that several samples can be lined up and extracted rapidly, washing only the syringe between injections. The whole sample set can then undergo the sonication and centrifugation steps simultaneously. This paper sets out the fine tuning of the extraction and dLLME protocol for natural dyes and its use in a case study of dyed textiles from the Tutankhamun tomb. Furthemore, considering the increasing interest on natural dyes in different fields, such as textile industries and cosmetics [43], particularly with regard to a green chemistry approach, this study proposes a successful model for every field where the analysis on these analytes is required.

2. Materials and methods

2.1. Commercial Materials

High purity analytical standards of Alizarin (1,2-dihydroxyanthraquinone), Purpurin (1,2,3-dihydroxyanthraquinone), Carminic Acid and Luteolin 7-O-β-D- glucoside were purchased from Sigma Aldrich. Solvents, acids, and bases were purchased from Sigma Aldrich and used without further purification. Disodium EDTA was purchased from Carlo Erba while other salts were purchased from Sigma Aldrich. For the microextraction in situ, Nanorestore® gel HWR (High Water Retention) was purchased by CSGI, while Agar powder was purchased by Sigma Aldrich.

2.2. Sample preparation

2.2.1. Sample extraction

a) Thread sample extraction

1 mg of thread was sampled from the red area and put in the extracting solution (1.6 mL of NH_3/Na_2EDTA 1mM (1:1) and 4.4 mg NaCl) for 24h [24]. Ultrasound assisted dLLME was then performed as described in the following section.

b) InGEL micro-extraction

Microextraction on the archaeological textile sample was carried out by cutting the Nanorestore® and Agar gels into small cylinders using the back of a Pasteur pipette. Gel pieces were then soaked for 90 minutes into an extracting solution containing ammonia (30%)/Na_2EDTA 1mM (1:1) and NaCl 4.7 mM. The solution was allowed to evaporate from the gels so that they would lose 5% of weight before the 3h extraction.

The soaked gels were applied to a red area of the archaeological textiles for 3 hours. After extraction, each gel piece was put in a solution with 0.8 mL of NH_3 , 0.8 mL of Na_2EDTA 1mM and 4.4 mg of NaCl and left for 24h in the re-extraction solution. The gels were then removed from the solution and dLLME was performed.

2.2.2. Clean up -Ultrasound assisted dLLME

The clean-up has followed these steps: 495.6 mg NaCl, 1 mL HCl and 0.8 mL formic acid were added to the extraction solution, alongside 250 uL 1-pentanol. 200 uL of 1-pentanol and 100 uL 2-propanol were then rapidly injected to perform dLLME. Samples were centrifuged and washed with a solution of NaCl (66 mg mL^{-1}) and water until a pH of 4.5-5. The aqueous fraction was discharged, while pentanol was dried out at 65°C under N_2 flux and then reconstituted with 200 ul of H_20/MeOH 1:1 before being injected into the chromatographic system. Recoveries were calculated by performing peak area integrations with the Sciex Analyst software, and these areas were compared to results from the pre-extraction 0.5 mg L^{-1} analytical standards in Microsoft Excel. HPLC-MS analysis was carried out as described in the section 2.5.

2.3. Sample collection

2.3.1. Archaeological textile from the tomb of Tutankhamun

The proposed method was used for the analysis of dyes on linen textile fragments from the tomb of Pharaoh Tutankhamun dating back to 1325 BC, the year of his death. After collecting and cataloguing the most valuable remains, the archaeologist Howard Carter swept the remaining materials from the surfaces of the tomb and deposited them in a wooden box. The box was closed in 1933 and was stored in the Egyptian Museum in Cairo until 2017. In 2018 it was moved to the Grand Egyptian Museum, where its materials started to be subjected to scientific analyses. The analysis was performed using two different techniques which both employed the validated dLLME clean-up protocol. First, a gel microextraction *in situ* was performed and the extract analysed. The second analysis was performed on the same fragment and employed a direct extraction from the thread.

2.3.2. Sample thread. For the analyses of dyes following the dyed yarn traditional extraction procedure, a detached fragment of 1 mg from the archaeological textile was considered.

2.3.4. INGEL analyses

Based on previous research by Germinario [11], two types of gels were tested for the extraction of dyes from the textile matrix: Nanorestore® gel HWR (High Water Retention) and Agar gel. The Nanorestore® gel is a commercial product produced by CSGI and therefore it didn't require any preparation. The Agar gel was prepared fresh in the laboratory the day before extraction in order to prevent it from drying out. The preparation of the gel involved dissolving 0.24 g of agar powder in 8 mL distilled water and heating in a bain marie at 100°C for 10 minutes. The gel was then allowed to cool down at room temperature and stored in the fridge overnight before performing the extraction the following day.

2.4. Method development

The development of the dLLME clean-up step was performed on a mixed reference sample solution containing 0.5 mg L^{-1} of alizarin, purpurin, carminic acid, and luteolin O-7-β-D-glucoside. The analytes were dissolved to 500 mg L^{-1} and diluted to 100 mg L^{-1} in ethanol and the final dilution to 0.5 mg L^{-1} was then performed in methanol and isopropanol, 1:1. All experiments were repeated three times and each replicate was analysed twice by mass spectrometry to obtain an average.

The method was developed in three steps. First, the best conditions for analytes recovery were assessed in water by trialing different solvents, aqueous solution volumes and NaCl concentrations. The best performing combination of disperser and extracting solvent, aqueous solution volume and NaCl concentration was then selected for trials which included the from the ammonia-EDTA extraction solution. At this stage, different procedures to neutralise ammonia and acidify the solution before dLLME were tested.

2.4.1. Ultrasound assisted dLLME Method Development

Three extraction solvents were trialled: 1-pentanol, chloroform and dichloromethane, with four different dispersers: methanol, isopropanol, acetone, and acetonitrile.

a) dLLME Solvent Trials

- 1-pentanol.

Trials with 1-pentanol as the extracting solvent were based on the traditional LLE methodology for solvent quantities and ratios[24] and performed as follows. 100 µl of the 0.5 mg L^{-1} reference mixture in methanol was placed in an Eppendorf tube alongside 900 ml Millipore water. 100 µl 1 M HCl was added to the solution (until it reached pH=2) followed by 200 µl of disperser solvent. 200 µl 1-pentanol and 100 µl of disperser were then rapidly injected into the aqueous phase, forming the cloudy solution. The solution was then vortexed before being placed in an ultrasonic bath for 10 min. After sonication, the mixture was centrifuged at 12500 rpm for 10 min to separate the layers. The lower aqueous layer was removed with a syringe leaving the organic layer in the Eppendorf.

Tests with litmus paper found that the organic phase possessed acidic character, so the solution was washed. For washing, 500 µl Millipore water was added to the organic layer, shaken, centrifuged, and the aqueous phase was then removed. This process was repeated with 250 µL MilliQ water aliquots until a it reached a final pH value of 4/5. After extraction, the organic layer was transferred into a vial and the extract dried under N_2 flow and 65°C heat.

- Chloroform and Dichloromethane

Trials with dichloromethane and chloroform were adapted from an approach based on anthraquinone derivative detection in urine analysis from Shi et al. [45]. In this approach, 100 µl of 0.5 mg L^{-1} mixed

standard in methanol was added to a Falcon tube. 1.7 ml Millipore water was added to the tube alongside 200 µl 1 M HCl. 800 µl disperser and 80 µl extracting solvent were rapidly injected into the aqueous phase, forming the cloudy solution. The mixture was then vortexed, sonicated for 10 minutes, and then centrifugation at 4200 rpm for 5 minutes. The bottom organic layer was then removed with a syringe and placed in a vial where it was dried under N_2 flow.

- Traditional LLE for comparison

Traditional LLE was carried out on samples identical to those above for comparison with the novel dLLME methods. The method was performed as described by Serafini et al. [24]. 900 µL Millipore water, 100 µL 1M HCl and 100 µL 0.5 mg L^{-1} mixed standard were combined. The solution was shaken in a small separating funnel with 250 µL 1-pentanol before separating. The process was repeated with the aqueous layer, and the resulting organic layers were combined and washed once with 500 µL Millipore water, then thrice with 250 µL Millipore water. The organic phase was then extracted and dried at 65ºC under N_2.

b) dLLME aqueous solution conditions

- Aqueous solution volume and NaCl concentration

Once the nature and ratio of extraction and disperser solvents had been chosen, they were tested on different volumes of aqueous phase and NaCl concentration. Aqueous phases of 1 mL and 3 mL volumes were trialled, and two different NaCl concentrations were tested for each: 1.5 mg/mL and 66 mg. The trials were performed as follows: 100 µl of 0.5 mg L^{-1} reference mixture in isopropanol was placed in an Eppendorf test tube and made up to the overall volume with Milli-Q water. The required quantity of NaCl and 100 µl HCl 1 M were then added to the solution (pH 2). To form the cloudy solution, 150 µl of isopropanol was added, and 200 µl 1-pentanol plus 100 µl isopropanol were rapidly injected into the aqueous phase. The samples were vortexed before being placed in an ultrasonic bath for 10 min. After sonication, the mixture was centrifuged at 12500 rpm for 10 min to separate the layers. The lower aqueous phase was removed and the 1-pentanol was washed twice using 500 µl and 250 µl Milli-Q water and NaCl (the same NaCl concentration used for the initial aqueous phase). After extraction, the organic layer was transferred into a vial and the extract dried under N_2 flow and 65°C heat.

c) Ammonia-based extraction solution trials

The best performing combination of disperser and extracting solvent, aqueous phase volume and NaCl concentrations were selected for ammonia-EDTA based trials. The trials were carried out starting from 800 µl NH_3 (30-33%), 800 µl Na_2EDTA (1 mM), 4.4 mg NaCl and 100 µl of the 0.5 mg L^{-1} reference mixture in isopropanol [24]. Three main methods of neutralising the ammonia were compared to recreate the pH conditions obtained during the trials in water. In detail:

i. N_2 neutralisation and HCl acidification

The ammonia solution containing the reference mixture in isopropanol was placed under N_2 flow to allow the ammonia to evaporate. Once the solution had reached a volume of 0.5 ml, it was made up to 3 ml with Millipore water and 100 µl HCl 1 M. After dLLME, the organic phase was washed twice using 500 µl and 250 µl NaCl in Millipore water (66 mg/ml).

ii. HCl neutralisation and acidification

The ammonia solution with reference mixture in isopropanol was acidified using 2.2 ml HCl 6 M until the pH reached 2/3. After dLLME, the organic phase was washed using 1.2 ml NaCl in Millipore water (66 mg/ml).

iii. HCl neutralisation and acidification with buffers

1 ml HCl 6 M was added to the ammonia solution with reference mixture in isopropanol until the pH reached 9. At this point, the ammonium formed in the solution was used to create a buffer with citric acid or with formic acid. In the first case, 2.7 g and 2.5 g citric acid were added until the pH reached 2 and 3, respectively. In the second case, 400 µl and 800 µl formic acid were added until the pH reached 4 and 3, respectively. After dLLME, the organic phase was washed using 1.5 ml NaCl solution for samples with citric buffer and 3.1 ml NaCl solution for samples with formic buffer.

After acidification, all the samples were processed as follows: the solution was made up to 3 ml and 195.6 mg NaCl was added to reach a final concentration of 66 mg/ml. 150 µl of isopropanol was added to the solution, 200 µl 1-pentanol and 100 µl isopropanol were rapidly injected into the aqueous phase to obtain the cloudy solution. The samples were vortexed before being placed in an ultrasonic bath for 10 min. After sonication, the mixture was centrifuged at 12500 rpm for 10 min to separate the layers. The aqueous layer was removed and 1-pentanol was washed using NaCl in Millipore water (66 mg/ml) until a pH value of 4/5 was obtained. The organic phase was then transferred into a vial and dried under N_2 flow and 65°C heat. The extract was reconstituted with 100 µl methanol for analysis by HPLC-MS.

2.5. UHPLC-MS/MS analyses

Analyses on selected samples and for the validation of the method were carried out through targeted UHPLC-MS/MS. The instrumental apparatus system consisted of a ExionLC AD System from Sciex (Framingham, MA, USA) coupled with a QTrap 6500+, hybrid quadrupole-linear ion trap mass spectrometer, from Sciex equipped with an ESI source, operating in negative ionisation mode. An Acquity UPLC BEH C18 (100 x 2.1 mm i.d., packed with 1.7µm particles) from Waters was used. Column was maintained at 40°C.

A binary solvent system was used, employing the following mobile phases: Phase A - 0.1% formic acid in acetonitrile (ACN) and Phase B - 0.1% formic acid in Millipore water. The following gradient elution programme was used: minute 0 Phase A – 5%; minute 2 Phase A – 5%; minute 8 Phase A – 100%; minute 11 Phase A – 100%, minute 11.5 Phase A – 5%; minute 14.5 Phase A – 5%.

The multiple reaction monitoring (MRM) targets are listed in Table 1. MRM transitions of other madder chromophores were derived from literature in the absence of analytical standards, these are listed in the same table in light blue.

Dye	Parent Ion (m/z)	Fragments (m/z)	DP (V)	EP (V)	CE (V)	CXP (V)
Alizarin	239	210; 167	-120	-8.1	-49; -40	-15; -10
Purpurin	255	227; 171	-109	-8.3	-38; -42	-21; -13
Carminic acid	491	447; 357	-99	-3	-34; -48	-42; -35
Luteolin O-7- β-D-glucoside	447	285; 283	-61	-8.5	-46; -54	-27; -23
Xanthopurpurin	239	211, 195	-130	-8.1	-38; -42	-21; -13
Munjistin	283	239	-130	-8.1	-38; -42	-21; -13
Ruberithyc acid	533	239	-130	-8.4	-34; -48	-42; -35
Rubiadin	547	253	-130	-8.4	-34; -48	-42; -35

Table 1: Natural dye standard mass spectrometry targets, together with MRM transitions derived from literature [15,44] – in light blue.

2.6. Method Validation

The presented method was validated as a semi-quantitative method. The following parameters were investigated: limit of detection (LOD) and limit of quantification (LOQ), linearity, specificity, precision, recovery. For LOD and LOQ determination, extracting solutions with an increasingly diluted mix of analytes (0.025, 0.05, 0.075, 0.1, 0.25, 0.5 and 0.75 mg L^{-1}) were injected after dLLME. LOD was evaluated as the minimum concentration visible with intensity three times higher than background noise. LOQ was evaluated similarly, verifying the minimum concentration which produced a signal-to-noise ratio of at least ten.

To evaluate linearity, calibrator solutions containing a mixture of all analytes were prepared in methanol at six selected concentrations: 0.025, 0.05, 0.075, 0.1, 0.25, 0.5 and 0.75 mg L^{-1}. Every sample mix was analysed twice and averaged. The calibration curves were derived by plotting the peak area of analytes versus their respective concentrations. The squared correlation coefficient (R^2) was used to estimate linearity.

Precision was calculated at two concentrations: 0.75 and 0.25 mg L^{-1}. For this purpose, quality control samples (QCs) were prepared freshly every day spiking the extracting solution with a mix of analytes at chosen concentration before dLLME. QCs were analyzed on different days and each day three identical samples were prepared and injected twice in the instrument. Precision was evaluated by calculating %RSD (relative standard deviation) both within the same day (inter-day precision) and over several days (intra-day precision).

To evaluate recoveries, three sets of samples (three replicates for each) were prepared at 0.75 and 0.25 mg L^{-1}. The first set was obtained by spiking samples with a mix of analytes at the chosen concentration before the extraction (M); the second group of samples was obtained in the same way, fortifying after dLLME (V); the third set consisted of reference mix solutions of analytes in methanol (Ref). Recoveries were evaluated by comparing the peak areas obtained from the analysis of the first set with the ones of the second set as follows: R%=(M/V)*100.

3. Results and discussion

The choice of dye analytes (two aglycones and two glycosylated compounds) for assessing dye recovery was based on a few main factors. Firstly, it is essential that an effective analytical method allows identification of both classes, as this allows a fuller picture of the complete molecular pattern to be identified. The aglycones, alizarin and purpurin, were chosen as they are two major molecular components of red madder dyes. Furthermore, the choice of carminic acid (a C-glucoside) and luteolin-o-glucoside (an O-glucoside) allowed for monitoring of the recovery of these two different glycosidic sub-classes.

In progressively building the methodology, the best performing combination of disperser, extracting solvent, aqueous solution volume, and NaCl concentration were first assessed. The ammonia-EDTA solution was then added to the best performing combination of disperser and extracting solvent and trialed to verify coherence of the overall method, considering the ammonia-based extraction solution as the matrix. At this point, several methods for neutralising ammonia and acidifying the solution before dLLME were trialled – this step was critical in promoting the migration of analytes from the aqueous to the organic phase. The final conditions were chosen based on the results of chromatographic separation and ion signal, and in terms of extraction/clean-up [40].

3.1 Chromatographic conditions

To set-up the mobile phases for natural dyes, three trials were performed:

i. Phase A: Methanol
   Phase B: 5mM ammonium acetate in water

ii. Phase A: Acetonitrile:Methanol (1:1)
Phase B: 2.5mM ammonium acetate and 2.5mM formic acid in water
iii. Phase A: 0.1% formic acid in acetonitrile Phase B: 0.1% formic acid in water

Only the solvent system employing 0.1% formic acid in acetonitrile and 0.1% formic acid in water provided adequate separation of the analytes, and they were therefore used as the mobile phases. The gradient elution program was optimised by trial and error based on the measured retention times of the analytes to ensure adequate separation. A Luna-C18 and a Kinetex XB-C18 chromatographic column were tested. Whilst both columns achieved adequate separation of the analytes, the Luna-C18 column allowed a shorter run time, and it was hence chosen as the column for analyses.

3.2 Ultrasound assisted dLLME development trial

As previously discussed, the set-up of the dLLME conditions for the chosen analytes (Fig. 1) was initially performed from the standard mixed solution of the four analytes. The results of these disperser and extracting solvent trials are displayed in Fig. 2.

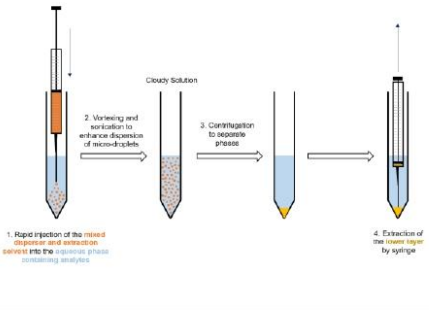

Fig. 1- Schematic workflow of dLLME. Picture realized by Kathryn Raeburn McClure

a) dLLME Solvent Trials

As discussed in the experimental section, the protocol for dichloromethane and chloroform extractions was adapted from an approach by Shi et al., who were performing extraction of anthraquinone derivatives from urine [45]. It must be noted that this paper also trialled carbon tetrachloride and found this very successful, but this research chose to avoid CCl_4 due to its toxicity. It must also be noted that the dLLME protocols using dichloromethane and chloroform trials were performed using a slightly different methodology than was used for trials with 1-pentanol, in order to obtain the best conditions for each solvent. A trial combining the methodology for chloroform and dichloromethane (derived from the methodology by Shi et al.[45]) and the use of 1- pentanol as the extracting solvent was performed. In this case, 1-pentanol was not separated from the aqueous phase and this method was therefore not applicable for this extracting solvent.
In trials with dichloromethane as the extracting solvent, three of the four trials resulted in no separation of the organic phase. Where separation was observed – when acetone was used as the disperser – recoveries were low for all analytes. Dichloromethane was therefore disregarded as a candidate for the extracting solvent.

With chloroform as the extracting solvent, separation of the organic layer was achieved in all trials. Where chloroform was combined with the use of acetonitrile or acetone as the disperser, excellent recoveries were obtained for alizarin and purpurin. In all cases however, very poor recovery was observed for carminic acid and luteolin, with less than 5% recovery of luteolin with all dispersers. The low recovery of these analytes is attributed to their high relative polarity compared to alizarin and purpurin. This high polarity gives carminic acid and luteolin an affinity for water, and the non-polar nature of chloroform means they are unlikely to migrate into the organic phase; despite the ample opportunity offered by the high surface area provided by DLLME. The recovery of these analytes was considered insufficient for the protocol and hence chloroform was disregarded as a candidate for the extracting solvent.

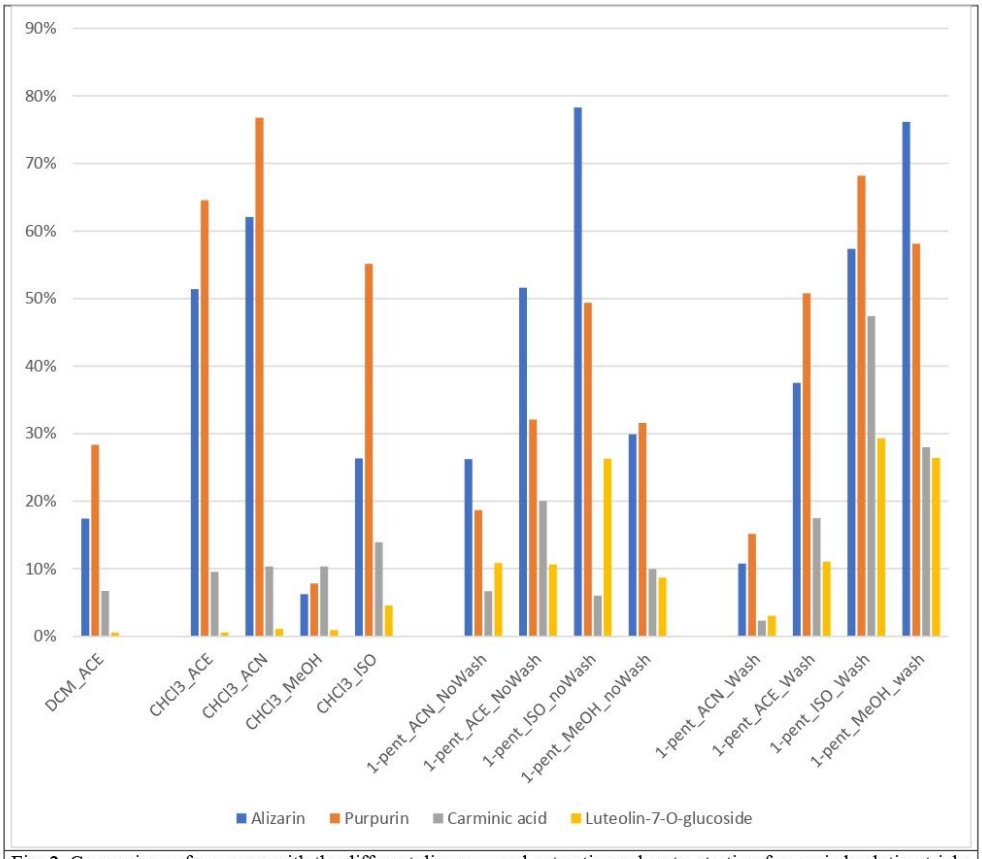

Fig. 2. Comparison of recovery with the different disperser and extracting solvents, starting from mied solution trials. In detail:
1st group: Disperser- Acetone (ACE)/Extracting solvent- Dichloromethane (DCM)
2nd group: Disperser -Acetone (ACE), Acetonitrile (ACN), methanol (MeOH), Isopropanol (ISO)/Extracting solvent- Chloroform

3rd group: Disperser - Acetonitrile (ACN), Acetone (ACE), Methanol (MeOH), Isopropanol (ISO) without final washing of organic phase/Extracting solvent- 1-pentanol
4th group: Acetonitrile (ACN), Acetone (ACE), Methanol (MeOH), Isopropanol (ISO) with final washing of organic phase/Extracting solvent- 1-pentanol

When 1-pentanol was used as the extracting solvent – the method was tried both with and without washing. The washing step was considered as in the traditional LLE extractions; washings were required to deacidify the organic phase. Acidifying the aqueous phase was necessary to promote the migration of dye analytes into the organic phase, but neutralisation of the organic phase is required before drying to avoid cleavage of the glucoside bonds in carminic acid and luteolin and in general glycosides moieties as observed in previous acid extraction methods[20,22,24]. However, it is important to note that this additional washing step did incur some loss of organic phase, which resulted in a reduced recovery of the aglycones.
In general, trials using 1-pentanol as the extracting solvent achieved higher recovery of carminic acid and luteolin than was observed with chloroform, strongly improving the recovery of dyes when compared with traditional LLE as previously published [18], fig 3. A likely hypothesis for this is that these polar analytes have more affinity for 1-pentanol than chloroform due to its higher relative polarity. Considering the results of the disperser tests, acetonitrile and acetone were disregarded as dispersers as their recoveries were exceeded by methanol and isopropanol. The two best sets of recoveries for all four analytes were obtained in the following conditions: isopropanol and methanol as a disperser with 1-pentanol as the extracting solvent and the use of a final wash of the organic phase.

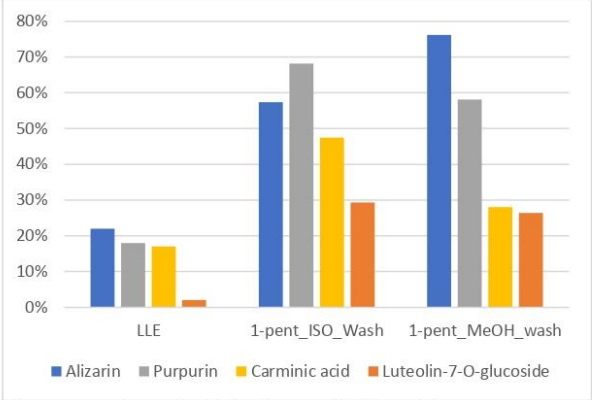

Fig. 3. Comparison of traditional LLE with dllme trials.

From these considerations, the use of isopropanol with the addition of a final wash was chosen as the most effective for the recovery of the analytes. Based on these results, further tests used the isopropanol and 1-pentanol dLLME system and trialled different volumes and salt concentrations in the aqueous starting solution.

b) dLLME aqueous solution conditions

After selection of the best solvent system (isopropanol as the disperser and 1-pentanol as the extraction solvent), this procedure was used to assess the optimum aqueous phase volume and NaCl concentration. NaCl plays a double function as it prevents hydroxyl groups of anthraquinone colourants from modification induced by ammonia [18, 24], and it promotes migration of analytes into the organic phase. Analyte recovery in Fig. 4 shows that a higher NaCl concentration promotes

dye extraction to the organic phase. At the best NaCl concentration, an aqueous phase volume of 3 ml provided better results than 1 ml.

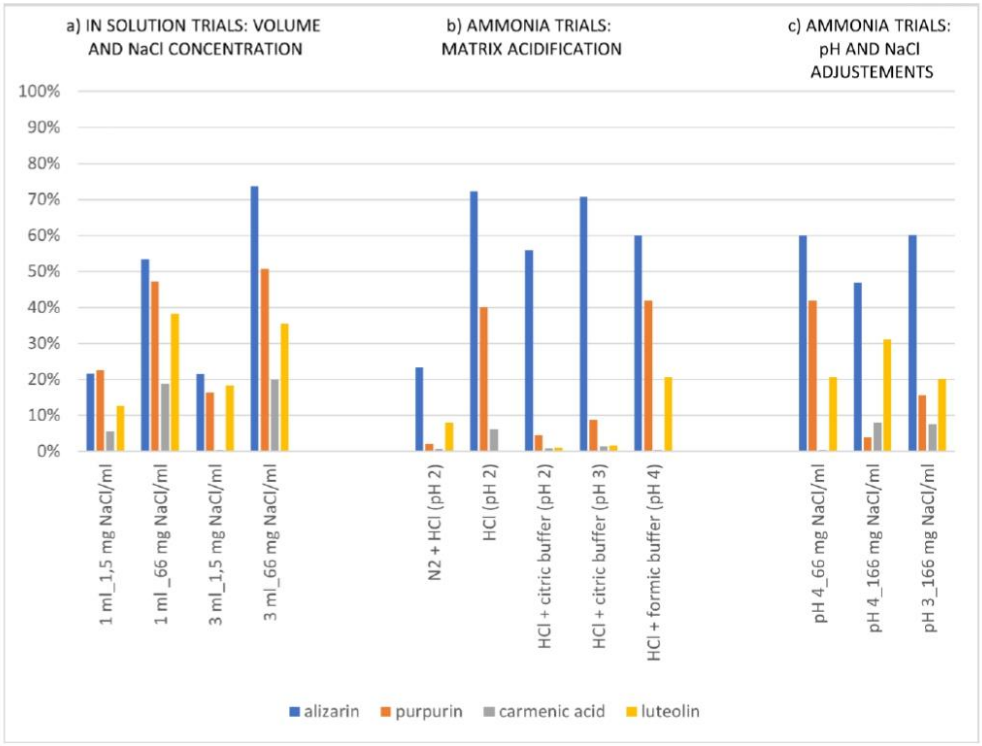

Fig. 4. Analyte recovery, basing on different trials. a) Analyte recovery varying the volume/NaCl ratio; b) Analyte recovery using different approaches to reach the neutralization of ammonia c) Analyte recovery working on different pH and NaCl concentration.

a) Ammonia-based extraction solution trials

Three main workflows were compared to recreate the pH conditions obtained during the trials in water. In the a) test, ammonia was neutralised using N_2 flow and HCl was used to bring the pH of the solution to 2. In the b) test, direct acidification with HCl until the solution reached a pH of 2 was used. In the c) test, the ammonia was mostly neutralised using HCl and two different buffers were trialled to achieve a better control of pH conditions. Citric acid and formic acid were compared to stabilise the solution pH at 2, 3 and 4.

Trial a) resulted in poor recovery and poor reproducibility. In trial b), aglycone recovery significantly improved. However, the glycosidic moieties were not recovered in this process. Furthermore, the addition of a strong acid to the ammonia solution resulted in poor control of the final pH value. Trial c), in which a buffer was used, provided much better reproducibility. The use of citric acid to form a buffer with ammonium citrate resulted in a poor recovery of the glycosides and purpurin at both at pH 2 and pH 3. The use of formic acid for buffering at pH 4 improved the recoveries of luteolin and purpurin significantly. However, at pH 4, the most critical analyte in recovery, carminic acid, is mainly undissociated and thus remains in the aqueous solution and does not migrate to the organic phase. To overcome this, the procedure was adjusted to promote carminic acid extraction in 1-

pentanol: by increasing NaCl concentration from 66 mg/ml to 166 mg/ml (Fig. 2c); and buffering at a lower pH by increasing the volume of formic acid added from 400 µl to 800 µl.

3.4 Validation results

The acquisition of all the analytes was performed in MRM mode. LODs of the method were observed at 0.020 and 0.025 mg L^{-1} for aglycone and glycosyl compounds respectively, while LOQs were observed at 0.6 and 0.75 mg L^{-1}, respectively (Table 2). For linearity, the correlation coefficient (R^2) was higher than 0.98 for the four analytes. The method showed good precision as reported in Table 2, and good specificity: blank samples, repeatedly analysed, did not show any signals related to the analytes.

The recoveries were reproducible and varied from 60% to 5% (Table 2).

	Alizarin		Purpurin		Carminic Acid		Luteolin	
LOD	0.020 mg L^{-1}		0.020 mg L^{-1}		0.025 mg L^{-1}		0.025 mg L^{-1}	
LOQ	0.06 mg L^{-1}		0.06 mg L^{-1}		0.075 mg L^{-1}		0.075 mg L^{-1}	
R^2	0.9955		0.9820		0.9965		0.9971	
mg L^{-1}	0.075	0.25	0.075	0.25	0.075	0.25	0.075	0.25
R(%)	60	58	12	16	8	5	18	20
Prec(%)	16.35	22.37	2.75	9.06	16.02	6.03	35.99	2.29

Table 2. Values of LOD, LOQ and precision obtained from validation tests.

3.5 Case Study: Archaeological textile from the tomb of Tutankhamun

When the best conditions for the recovery of natural anthraquinone dyes had been defined, the protocol was applied to dyed relics from the Tutankhamun tomb (Fig. 5).

Fig. 5. Detail of Tutankhamun fabrics. Picture by Dr. Nagmeldeen Morshed Hamza and Dr. Claudia Moricca

First, the methodology was applied directly to a detached yarn sample of about 1 mg. Secondly, the new protocol was applied onto gels which had been used for *in situ* micro-extraction to assess the protocol alongside a minimally invasive gel-based ammonia extraction. For this step, Nanorestore® and Agar gel were employed. Nanorestore® is normally used for removing varnishes or dirt on painting surfaces in conservation/restoration protocols. It is a chemical hydrogel based on a pHEMA / PVP network [46], and was evaluated as having optimal qualities for performing controlled extraction on textiles due to its high retention capacity for liquids [11]. Agar gel is a biopolymer with multiple scientific applications [47], amply employed in different fields, which can be easily synthetized.

The chromatograms of the extract from 1 mg of thread show excellent results with good intensity of the peaks (Fig. S1-S2). It is possible to observe both transitions of alizarin at t_R = 4.18 min, and those of purpurin, although the peak at t_R = 4.53 min has a very low intensity while there is another intense peak at t_R = 3.62 which could not be attributed. Furthermore, based on reference MRM transitions derived from literature [15,44] it was possible to hypothesise the presence of other molecules present in madder. In particular, there are peaks related to munjistin at t_R = 4.07 min, ruberythric acid at t_R = 3.18 min; rubiadin at t_R = 3.54 min (fig S3).

To identify the analytes extracted through the gel micro-extraction system, the gels were soaked into the extracting solution and thereafter dLLME was performed applying the protocol validated above. Similarly, the samples re-extracted from gels gave excellent results both for the Agar and Nanorestore® gels (Fig.S1-S2). The highest intensities were obtained for samples re-extracted from Agar, this is likely due to the high fluid retention properties of Nanorestore® retaining the analytes during re-extraction. An intense peak is observed in all chromatograms for the first transitions of alizarin at t_R = 4.19 min and for purpurin at t_R = 4.53 min. As with the thread sample, it was possible to hypothesise the presence of ruberythric acid and rubiadin (Fig. S4-S5) It was also possible to hypothesise the presence of munjistin, but only in the case of the sample re-extracted from Agar at t_R = 3.78 min (Fig. S4).

Comparing results of the two extractions, as expected, the most intense peaks are obtained for the sample extracted from the thread for all analytes. Only in the case of purpurin, results obtained on gels are better in accordance with the analysis of standards. Nonetheless, the application of this novel dLLME protocol to small quantities of analytes re-extracted from gels allowed very good results to be obtained and confirmed the presence of madder dye on the red area of the archaeological textile fragment.

Conclusions

Historical dyed textile artefacts are precious objects which hold the potential to provide information about the materials used in everyday human life, and about the art and colour of human history. These materials can provide us with extraordinary insights about ancient communities, their technologies, values, and stylistic preferences. Analytical methodologies, which preserve a complete picture of the dye mixtures present in a sample, are key resources for informing our understanding of the objects and their creation. For this reason, the ammonia extraction, which preserves minor, sensitive dye components is a key tool in the dye analyst toolbox. Furthermore, combining this with the use of gels for *in situ* micro-extraction reduces the invasiveness of analysis – preserving these prized objects for future generations. This paper presents the development and use of a clean-up protocol based upon ultrasound assisted dLLME with isopropanol and 1-pentanol as disperser and extraction solvents. This new dLLME protocol preserves the extracted dye sample, making the ammonia protocol applicable to gel-based extraction systems – and allowing detailed results from archaeological samples, such as those from Tutankhamun tomb. This approach also allowed the recovery of glycosyl moieties on this miniaturised scale, as would be expected for samples of around 1 mg. Further work is underway in evaluating the potential of this technique for the identification of natural dyes in paint

layers – developing upon previous work which has used this type of gel system for spectroscopic identification [48].

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References.

[1] J. C. Splitstoser, T.D., Dillehay, J. Wouters, A. Claro, Early pre-Hispanic use of indigo blue in Peru, Sci. Adv. (2016);2:e1501623.
[2] I. Degano, E. Ribechini, F. Modugno, M. P. Colombini, Analytical methods for the characterization of organic dyes in artworks and in historical textiles, Appl Spectrosc Rev. 44(5) (2009) 363-410.
[3] J. Wouters, High performance liquid chromatography of anthraquinones: analysis of plant and insect extracts and dyed textiles, Stud. Conserv. 30(3) (1985) 119–128.
[4] J. Wouters, A. Verhecken, The coccid insect dyes: hplc and computerized diode array analysis of dyed yarns, Stud. Conserv., 34 (4) (1989) 189–200.
[5] L. Ford, R. L. Henderson, C. M. Rayner, R. S. Blackburn, Mild extraction methods using aqueous glucose solution for the analysis of natural dyes in textile artefacts dyed with Dyer's madder (Rubia tinctorum L.), J. Chromatogr. A, 1487 (3) (2017) 36-46.
[6] L. Ford, M. C. Rayner, R. S. Blackburn, Degradation of lucidin: New insights into the fate of this natural pigment present in Dyer's madder (Rubia tinctorum L.) during the extraction of textile artefacts, Dyes Pigments, 154 (2018) 290–295.
[7] D. Mantzouris, I. Karapanagiotis, Armenian cochineal (Porphyrophora hamelii) and purpurin-rich madder in ancient polychromy, Coloration Technology 131 (5) (2015) 370 - 3731.
[8] K. Stathopoulou, L. Valianou, A. L. Skaltsounis, I. Karapanagiotis, P. Magiatis, Structure elucidation and chromatographic identification of anthraquinone components of cochineal (Dactylopius coccus) detected in historical objects, Anal Chim Acta, 804 (2013) 264–272.
[9] M. Aceto, A. Agostino, G. Fenoglio, A. Idone, M. Gulmini, M. Picollo, P. Ricciardi, J. K. Delaney, Characterisation of Colourants on Illuminated Manuscripts by Portable Fibre Optic UV-Visible-NIR Reflectance Spectrophotometry. Anal Methods, 6(5) (2014) 1488–1500.
[10] F. Pozzi, M. Leona, Surface-Enhanced Raman Spectroscopy in Art and Archaeology. J Raman Spectrosc, 47 (1) (2016) 67–77.
[11] G. Germinario, A. Ciccola, I. Serafini, L. Ruggiero, M. Sbroscia, F. Vincenti, C. Fasolato, R. Curini, M. Ioele, P. Postorino, A. Sodo, Gel substrates and ammonia-EDTA extraction solution: a new non- destructive combined approach for the identification of anthraquinone dyes from wool textiles, Microchem J, 155 (2020) 104780.
[12] A. Ciccola, I. Serafini, F. Ripanti, F. Vincenti, F. Coletti, A. Bianco, C. Fasolato, C. Montesano, M. Galli, R. Curini, P. Postorino, Dyes from the Ashes: Discovering and Characterizing Natural Dyes from Mineralized Textiles. Molecules, 25(6) (2020).
[13] A. Ciccola, I. Serafini, G. D'Agostino, B. Giambra, A. Bosi, F. Ripanti, A. Nucara, P. Postorino, R. Curini, M. Bruno, Dyes of a Shadow Theatre: Investigating Tholu Bommalu Indian Puppets through a Highly Sensitive Multi-Spectroscopic Approach. Heritage, 4(3) (2021) 1807–1820.

[14] C. Colantonio, L. Lanteri, A. Ciccola, I. Serafini, P. Postorino, E. Censorii, D. Rotari, C. Pelosi, Imaging Diagnostics Coupled with Non-Invasive and Micro-Invasive Analyses for the Restoration of Ethnographic Artifacts from French Polynesia. Heritage, 5(1) (2022) 215–232.
[15] K. Lech, E. Fornal, A Mass Spectrometry-Based Approach for Characterization of Red, Blue, and Purple Natural Dyes, Molecules, 25 (2020) 25, 322.
[16] L. Valianou, I. Karapanagiotis, Y. Chryssoulakis, Comparison of Extraction Methods for the Analysis of Natural Dyes in Historical Textiles by High-Performance Liquid Chromatography. Anal Bioanal Chem, 395 (2009) 2175–2189.
[17] M. Shahid, J. Wertz, I. Degano, M. Aceto, M. I. Khan, A. Quye, Analytical methods for determination of anthraquinone dyes in historical textiles: A review, Anal. Chim. Acta , 1083 (2019) 58-87.
[18] L. Lombardi, I. Serafini, M. Guiso, F. Sciubba, A. Bianco, A new approach to the mild extraction of madder dyes from lake and textile, Microchem J, 126 (2016) 373–380.
[19] I. Karapanagiotis, O. Abdel-Kareem, P. Kamaterou, D. Mantzouris, Identification of Dyes in Coptic Textiles from the Museum of Faculty of Archaeology, Cairo University, Heritage, 4 (2021) 3147–3156.
[20] X. Zhang, R. A., Laursen, Development of Mild Extraction Methods for the Analysis of Natural Dyes in Textiles of Historical Interest Using LC-Diode Array Detector-MS, Anal Chem, 77 (2005) 2022-2025.
[21] A. Serrano, M. van Bommel, J. Hallet, Evaluation between ultrahigh pressure liquid chromatography and high-performance liquid chromatography analytical methods for characterizing natural dyestuffs, J. Chromatogr. A., 1318 (2013) 102–11.
[22] A. Manhita, T. Ferreira, A. Candeias, C. B. Dias, Extracting natural dyes from wool—an evaluation of extraction methods. Anal. Bioanal. Chem., 400(5) (2011) 1501.
[23] E. Calà, M. Benzi, F. Gosetti, A. Zanin, M. Gulmini, A. Idone, I. Serafini, A. Ciccola, R. Curini, I. Whitworth, M. Aceto, Towards the identification of the lichen species in historical orchil dyes by hplc-ms/ms, Microchem. J., 150 (2019) 104140.
[24] I. Serafini, L. Lombardi, G. Vannutelli, C. Montesano, F. Sciubba, M. Guiso, R. Curini, A. Bianco, How the extraction method could be crucial in the characterization of natural dyes from dyed yarns and lake pigments: The case of American and Armenian cochineal dyes, extracted through the new ammonia-EDTA method, Microchem. J., 134 (2017) 237–245.
[25] A. Serrano, A. van den Doel, M. van Bommel, J. Hallett, I. Joosten, K. J. van den Berg, Investigation of crimson-dyed fibres for a new approach on the characterization of cochineal and kermes dyes in historical textiles, Anal. Chim. Acta, 897 (2015) 116-127.
[26] K. Lech, M. Jarosz, Identification of Polish cochineal (Porphyrophora polonica L.) in historical textiles by high-performance liquid chromatography coupled with spectrophotometric and tandem mass spectrometric detection, Anal. Bioanal. Chem., 408 (2016) 3349–3358.
[27] K. Lech, K. Witkoś, B. Wileńska, M. Jarosz, Identification of unknown colorants in pre-Columbian textiles dyed with American cochineal (Dactylopius coccus Costa) using high-performance liquid chromatography and tandem mass spectrometry, Anal. Bioanal. Chem. 407 (2015) 855–867.
[28] M. Leona, P. Decuzzi, T. A. Kubic, G. Gates, J. R. Lombardi, Nondestructive identification of natural and synthetic organic colorants in works of art by surface enhanced Raman scattering. Anal. chem., 83(11) (2011) 3990-3993.
[29] C. Lofrumento, M. Ricci, E. Platania, M. Becucci, E. Castellucci, SERS detection of red organic dyes in ag-agar gel, J. Raman Spectrosc., 44 (2013) 47–54.
[30] E. Platania, C. Lofrumento, E. Lottini, E. Azzaro, M. Ricci, M. Becucci, Tailored micro-extraction method for raman/sers detection of indigoids in ancient textiles, Anal. Bioanal. Chem., 407 (2015) 6505–6514.

[31] E. Platania, J. R. Lombardi, M. Leona, N. Shibayama, C. Lofrumento, M. Ricci, M. Becucci, E. Castellucci, Suitability of Ag-agar gel for the micro-extraction of organic dyes on different substrates: the case study of wool, silk, printed cotton and a panel painting mock-up, J. Raman Spectrosc., 45 (2014) 1133-1139.
[32] M. Ricci, C. Lofrumento, E. Castellucci, M. Becucci, Microanalysis of Organic Pigments in Ancient Textiles by Surface-Enhanced Raman Scattering on Agar Gel Matrices. J. Spectrosc., 3 (2016) 1-10.
[33] M. Becucci, M. Ricci, C. Lofrumento, E. Castellucci, Identification of organic dyes by surface-enhanced Raman scattering in nano-composite agar-gel matrices: evaluation of the enhancement factor, Opt. Quant. Electron., 48(9) (2016).
[34] D. Stulik, D. Miller, H. Khanjian, N. Khandekar, R. Wolbers, J. Carlson, W. C. Petersen, Solvent Gels for the Cleaning of Works of Art - The Residue Question. Edited by Valerie Dorge, the Getty Conservation Institute (2004)
[35] A. Sansonetti, M. Bertasa, C. Canevali, A. Rabbolini, M. Anzani, D. Scalarone, A review in using agar gels for cleaning art surfaces, J. Cult. Herit. 44(2020) 285-296.
[36] E. Sandstrom, H. Wyld, C. L. Mackay, L. G. Troalen, An optimised small-scale sample preparation workflow for historical dye analysis using UHPLC- PDA applied to Scottish and English Renaissance embroidery, Anal. Methods., 13 (2021) 4220-4227.
[37] E. van der Klift, A. Villela, G. C. H. Derksen, P. P. Lankhorst, T. A. van Beek, Microextraction of Reseda luteola-Dyed Wool and Qualitative Analysis of Its Flavones by UHPLC-UV, NMR and MS, Molecules, 26 (2021) 3787.
[38] M. Rezaee, Y. Assadi, M. R. Milani Hosseini, E. Aghaee, F. Ahmadi, S. Berijani, Determination of organic compounds in water using dispersive liquid–liquid microextraction, J. Chromatog. A., 1116 (2006) 1–9.
[39] H. Yan, H. Wang, Recent development and applications of dispersive liquid–liquid microextraction, J. Chromatogr. A., 1295 (2013) 1–15.
[40] F. Vincenti, C. Montesano, L. Cellucci, A. Gregori, F. Fanti, D. Compagnone, R. Curini, M. Sergi, Combination of pressurized liquid extraction with dispersive liquid liquid micro extraction for the determination of sixty drugs of abuse in hair, J. Chromatogr. A., 1605 (2019) 360348.
[41] A. Quigley, W. Cummins, D. Connolly, Dispersive liquid-liquid microextraction in the analysis of milk and dairy products: a review. J. Chem-NY. (2016).
[42] A. Spietelun, Ł. Marcinkowski, M. de la Guardia, J. Namieśnik, Recent developments and future trends in solid phase microextraction techniques towards green analytical chemistry. J. of Chromatogr. A., 1321 (2013) 1-3.
[43] N. P. Patil, A. G. Datar, Applications of natural dye from Ixora coccinea L. in the field of textiles and cosmetics, Colora. Technol., 132 (2016) 98–103
[44] L. Rafaelly, S. Heron, W. Nowik, A. Tchapla, Optimisation of ESI-MS detection for the HPLC of anthraquinone dyes, Dyes Pigments, 77(1) (2008), 191–203.
[45] Z. Shi, H. Jiang, J. Hu, Z. Li, H. Zhang, Dispersive liquid-liquid microextraction and high-performance liquid chromatographic determination of anthraquinone derivatives in human urine after oral administration of San-Huang tablets. J. Liq. Chromatogr. R. T., 37(14) (2014) 2062-2071.
[46] CSGI, «NANORESTORE GEL® DRY Scheda Tecnica», https://www.csgi.unifi.it/products/dry_ita.html
[47] O. Ayyard, D. Muñoz-Rojas, N. Agulló, S. Borrós, P. Gómez-Romero, High-concentration compact agar gels from hydrothermal synthesis, Soft Matter, 6 (2010) 2389–2391.
[48] A. Bosi, A. Ciccola, I. Serafini, G. Peruzzi, V. Nigro, P. Postorino, R. Curini, G., Favero, Gel microextraction from hydrophilic paint layers: a comparison between Agar-gel and Nanorestore Gel® HWR for spectroscopic identification of madder, Microchemical Journal, 187 (2023) 108447.

Overcoming the limit of *in situ* gel supported liquid microextraction: development of the new InGeL-LC-MS analyses, a smart methodology for the identification of natural dyes from Tutankhamun tomb relics

Ilaria Serafini*[1], Adele Bosi[1,2], Flaminia Vincenti[1], Greta Peruzzi[1], Kathryn Raeburn McClure[1], Alessandro Ciccola[1], Nagmeldeen Morshed Hamza[2,3,4], Claudia Moricca[4], Laura Sadori[4], Camilla Montesano[1], Manuel Sergi[1], Gabriele Favero[4], Roberta Curini[1]

The manuscript was written through contributions of all authors.
Ilaria Serafini: all aspects of the work. **Adele Bosi**: investigation, validation and writing- original draft. **Greta Peruzzi**: investigation, validation and writing- original draft. **Flaminia Vincenti**: conceptualization, data curation, validation. **Kathryn Raeburn McClure:** investigation, writing - Review & Editing. **Alessandro Ciccola**: conceptualization. **Nagmeldeen Morshed Hamza, Claudia Moricca, Laura Sadori:** case study and all preliminary investigation on Tutankhamun relics. **Camilla Montesano**: data curation, writing- Review &Editing, visualization, and supervision. **Manuel Sergi, Gabriele Favero, Roberta Curini:** supervision and project administration. All authors have given approval to the final version of the manuscript.

CONCLUSIONS

Despite of the tomb of Tutankhamun having been investigated thoroughly over the years, there is still a lot to be discovered. For this reason, the present PhD thesis represents an attempt to fill the gaps in the state of art and solve some of the mysteries surrounding the tomb of the pharaoh.

The study illustrated in this PhD thesis represents a multidisciplinary study of organic remains, long considered as garbage, found in the tomb of Tutankhamun.

The papers reported here are the result of a classical archaeobotanical approach combined with novel diagnostic techniques, allowing to obtain a 360° perspective on the precious contents of the tomb of Tutankhamun.

This dissertation highlights the need of evaluating the conservation state and past interventions to propose a suitable strategy to guarantee the accessibility of an archaeological collection to future generations. Scientific analyses are not only crucial to acquire knowledge on the studied archaeological context but can be a precious tool for the musealization of the Tutankhamun collection.

The conclusions here presented are based on the combination of data obtained in SECTION 1, Chapter 1 (Hamza et al., 2021. *ICOM-CC*), Chapter 2 (Hamza & Shaheen, 2021. *Colloque APROA-BRK*), Chapter 3 (Hamza, 2021. *Young Professionals Forum Proceedings*), SECTION 2, Chapter 4 (Hamza et al., in preparation. *Plants*), Chapter 5 (Moricca et al., in review. *Heritage*), and SECTION 3, Chapter 6 (Abdrabou et al., 2022. *Open Archaeology*), Chapter 7 (Peruzzi et al., 2023. *Gels*) and Chapter 8 (Serafini et al., in preparation. *Analytica Chimica Acta*).

Evaluating past conservation and restoration strategies is crucial to assessing the state of preservation of an artifact. An approach of "less is more" needs to be favored to ensure that the Tutankhamun collection is accessible to future generation. The results of such an approach can be seen on objects selected as case studies, which provide key information about the material culture during New Kingdom Egypt. In terms of musealization strategies, it is also important to keep in mind the different "stages of live" of an archaeological context and its contents. For this reason, a choice was made to highlight the role of the excavators of Tutankhamun's tomb and their daily life. A display was prepared for the common objects and materials that they used to wrap and secure the precious archaeological findings.

In terms of material evidence, archaeobotanical remains represent a precious source of information about funerary offerings. The perfect state of preservation, which occurred by mummification, allowed to preserve even the most fragile parts of plants. This made it possible to retrieve and identify compound fruits of *Beta vulgaris* (beet), which are typically not preserved. The carpological assemblage includes food, ornamental and medicinal plants, providing the king with anything he might need in his afterlife.

Coupling classical archaeobotanical analyses with micro-magnetic resonance imaging (μ-MRI), allowed to obtain additional information about the plants selected for the funerary assemblage. This method made it possible to identify an archaeological reed fragment as *Phragmites australis* when optical microscopy resulted inconclusive. Another advantage of this study was that of suggesting the use of a new technique, μ-MRI, for the study of archaeological findings. Hopefully, technological development will lead to an increase of image resolution and a decrease in costs.

Organic materials, namely wooden figurines and linen textile fragments were subjected to a wide array of other diagnostic techniques. These allowed the identification of pigments and dyes used to colour them. In case of the shawabtis, it was also possible to identify the materials used during previous treatment interventions, confirming the idea that it is necessary to keep track of the entire history of the objects to be displayed to ensure their conservation in time.

As far as the textile fragments are concerned, not only were indigo and madder identified as dyes used to colour the fabrics, but the proposed study also allowed to propose new protocols for a non-invasive sampling of dyes.

Despite the wide range of materials studied in the present PhD thesis, and the numerous techniques used, a lot can still be done to investigate the role and choice of plants in the tomb of Tutankhamun.

Future perspectives involve morphometric analyses of grape pips found in the box containing the sweepings of the tomb, and a comparison with modern varieties currently present in Egypt. Furthermore, considering the desiccated state of conservation, an attempt could be made to extract ancient DNA from the grape pips, providing more detailed information about their entity.

Paper contributions

1. Hamza N. M., Mie I., & Shaheen E. (2021). Conservation between scientific methodology and laboratory application: An integrated approach to past and present challenges. In *ICOM-CC 19th Triennial Conference 2021 Beijing* (pp. 1-7). ICOM-CC.

The manuscript was conceived and written by the candidate, who was responsible for data production, management and interpretation in collaboration with all the authors. He also elaborated all the figures.

2. Hamza N. M. & Shaheen E. (2021) Conservation of the Tutankhamun collection: strategy of conservation decision-making in the Grand Egyptian Museum (GEM) in Giza. In *Colloque APROA-BRK 2021 – Conservatie-restauratie in context* (pp. 116-123).

The manuscript was conceived and written by the candidate, who was responsible for data production, management and interpretation in collaboration with all the authors. He also elaborated all the figures.

3. Hamza N. M., (2021). Conservation of evidence: the significance of objects. Biography from Tutankhamun tomb. In: Young Professionals Forum Proceedings 2021 (pp. 121-125). Centro Conservazione "Restauro La Venaria Reale".

The manuscript was conceived and written by the candidate, who was responsible for data production, management, interpretation and elaboration of figures.

4. Hamza N. M., Moricca C., & Sadori L (in preparation). Trash or treasure? Plant remains from the tomb of Tutankhamun. *Plants.*

The candidate conceived the research and was responsible for data production and interpretation. He also collaborated in the writing of the manuscript.

5. Moricca C., Stagno V., Hamza N. M., Favero G., Sadori L., & Capuani S. (in review). The high potential of micro-Magnetic Resonance Imaging for the identification of archaeological reeds: the case study of Tutankhamun. *Heritage*.

The candidate took part in the conceptualization of the research presented in this manuscript, validation of results, writing of the original draft and review. He also played a key role in the acquisition of resources and funding.

6. Abdrabou A., Abdallah M., Sultan G. M., Mostafa M., Bayoumi H., Magdy R., Abd El Kaser M. A., Hamza N. M., Mamdouh D., Elsayed H. M., Abbas E., & Kamal H. M. (2022). Tutankhamun's polychrome wooden shawabtis: Preliminary investigation for pigments and gilding characterization and indirect dating of previous restorations by the combined use of imaging and spectroscopic techniques. *Open Archaeology*, 8(1), 30-54.

The candidate conducted the analysis of all the painted layers using a handheld XRF (HH_XRF) spectrophotometer. He was also involved in writing the manuscript.

7. Peruzzi, G., Ciccola, A., Bosi, A., Serafini, I., Negozio, M., Hamza, N. M., Moricca, C., Sadori, L., Favero, G., Nigro, V. & Curini, R. (2023). Applying Gel-Supported Liquid Extraction to Tutankhamun's Textiles for the Identification of Ancient Colorants: A Case Study. *Gels*, 9(7), 514.

The candidate was responsible for the separation of textile fragments from the tomb sweepings and for the selection of textile fragments analyzed in the manuscript.

8. Serafini, I., Bosi, A., Vincenti, F., Peruzzi, G., McClure, K.R., Ciccola, A., Hamza, N. M., Moricca, C., Sadori, L., Montesano, C., Sergi, M., Favero, G., Curini, R. (in preparation). Overcoming the limit of in situ gel supported liquid microextraction: development of the new InGeL-LC-MS analyses, a smart methodology for the identification of natural dyes from Tutankhamun tomb relics. *Analytica Chimica Acta*.

The candidate selected the case study and performed preliminary investigations on Tutankhamun relics. He was also responsible for the elaboration of figure 5.

9. Hamza, N. M. Limited technology and unlimited results from National Museum of Ras Al Khaimah collection and its sustainability for future generations accessibility. *2023 IMEKO TC-4 International Conference on Metrology for Archaeology and Cultural Heritage Rome, Italy, October 19-21, 2023*.

The candidate was the sole author of the manuscript, and was thus responsible for conceiving the paper, data production, management, interpretation, figure elaboration and writing.

Other products

Other than focusing of the Tutankhamun collection, during my PhD project I have had the opportunity to work on the National Museum of Ras Al Khaimah collection and assess its conservation state and accessibility for future generations.

The analysed cases are represented by artifacts from different time periods, including daggers, herbarium collections and pottery. The aim of the study was to present innovative approaches, using technology and the right methodology, to preserve and display artifacts in the Ras Al Khaimah Museum.

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Appendix A

Limited technology and unlimited results from National Museum of Ras Al Khaimah collection and its sustainability for future generations accessibility

Hamza N. M.

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Metrology for Archaeology and Cultural Heritage
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Limited technology and unlimited results from National Museum of Ras Al Khaimah collection and its sustainability for future generations accessibility

Nagmeldeen Morshed Hamza[1]

[1] *National Museum of Ras Al Khaimah*
Department of Antiquities and Museums,Ras Al Khaimah, United Arab Emirates,
nagm.h@museum.rak.ae
Sapienza University of Rome, nagmeldeenmorshed.hamza@uniroma1.it

***Abstract*— The National Museum of Ras Al Khaimah reflecting an eventful history, displaying a rich diversity of traditional architecture. Today the 'Late Fort'exhibits historical, ethnographical, and archaeological material relating to the emirate of Ras Al Khaimah and provides an interesting insight into the history and traditions of this area.**

The formulation of history through the study and preservation of the museum collection is a dynamic and collaborative process that apply a multidisciplinary approach, ethical considerations, and a commitment to sharing the richness of human history with current and future generations. The research focus and shade the light on the role of using technology in studying, investigating and Preservating collection can extracting information which formulate the history of collection. Different cases here representing different time periods, the Daggers, Herbarium collections, and pottery from National Museum of Ras Al Khaimah are valuable resources, a crucial process for formulating and understanding the history, culture and for research, education, and inspiration when studying and preserving it. The research offers innovative technology and solutions for collection that enhance the significant value, history, trading knowledge, protecting unique stories, knowledge, and environmental changes during the history. By adopting integrated study and preservation approaches, using technology and the right methodology of can extend the lifespan of collections and make it more sustainable.

Keywords* — *Collection, conservation, sustainable, preservation, accessibility, archaeobotanical, digital, herbarium.

I. INTRODUCTION

The National Museum of Ras Al Khaimah was an 'Early Fort' existed inside Ras Al Khaimah Old Town in close proximity to the Mohammed bin Salim Mosque. The Fort according to ancient documents and letters was destroyed twice, in 1621 by the Portuguese and in 1820 by the British. Serving as a residence for the ruling family it was eventually given up around 1920 for a bigger one, just 700m to the south. This 'Later Fort' had originally been built between the British attacks of 1809 and 1819 outside the town-wall and Ras Al Khaimah Old Town. Drawn on the ancient British maps as a squarish defence structure strengthened with three round towers and a single big tower, it was eventually developed into a larger fortified complex. It served as the residence of the ruling Quwasim family until 1964, when the late Ruler, H.H. Sheikh Saqr Bin Mohammed al-Qasimi, moved to a modern building in Mamoura. Later it became a police headquarters and a prison, before it was finally converted into the National Museum in 1987 to start a new adventure but this time with collection [*1*].

The National Museum of Ras Al Khaimah Reflecting an eventful history it has been continuously enlarged over time, displaying a rich diversity of traditional architecture. Today the 'Late Fort ' exhibits historical, ethnographical and archaeological material relating to the emirate of Ras Al Khaimah and provides an interesting insight into the history and traditions of this area.

The museum design like all other traditional houses in Ras Al Khaimah Old Town the 'Late Fort' was originally constructed from coral stone, a fossil building material originating from the sea. The massive rectangular tower represents the oldest part of the 'Late Fort'. It originally served as a single defence tower and, unlike today, stood outside the perimeter wall of Old Town Ras Al Khaimah. While its foundations and lower parts originate from 1809-1819, all further additions took place after the peace treaty

[1] National Museum of Ras Al Khaimah". Department of Antiquities and Museums. Retrieved 2021-11-19.

was signed with the British in 1820[2].
Today the 'Late Fort' is an interesting conglomerate of two-storey buildings surrounding a central courtyard. The big rectangular tower is still the most impressive feature and a smaller tower occupies the opposite corner. Another prominent building is the wind tower, representing the traditional 'air conditioning of the past. Its open sides are designed to catch the breeze from any direction and funnel it down into the room below keeping it cool and ventilated, especially during hot summer months. If desired the wind tower could be blocked with matting or specially cut pieces of wood during winter, when the weather was much cooler with occasional rainfall[3].
The museum gallery in the Late Fort is the rooms situated around the inner courtyard garden with antique wooden doors with traditional carved designs, are open to the public. The artifacts and collections were partly donated by members of the ruling Quwasim family and residents of Ras Al Khaimah. Archaeological excavations, surveys and various scientific research projects undertaken by the Department of Antiquities and Museums have provided further material and significant information about the culture and the traditions of the area [4].

II. METHODOLOGY OF CONSERVATION AND PRESERVATION

III. OBJECT CASE STUDIES

Three cases are discussed the two Daggers, Herbarium collection and pottery jars from recent excavation. The objects were kept in the museum storage and presented to receive the preservation, restoration, and conservation treatment in preparation for future display in the museum gallery for the first time.

A. DAGGER, JAMBIYA

TWO DAGGERS (RAK 430, RAK 11584)

Dagger in a form of Khanjar or Jambiya is a traditional worn by men for ceremonial occasions, it is a specific type of dagger with a short, curved blade with a medial ridge attached to a belt made of textile and/or leather usually worn around the lower abdomen, originated from the Middle East and the Arab world. Craftsmen have excelled in their manufacture and made it full of fine artistic inscriptions and decorations that made it an expensive masterpiece.

The two dagger consists of a curved, double-edged blade, usually made from a fine steel that does not seem to corrode or oxidize to form rust. The hilt/handle is the most important part that holds the most value. The best ones are made of rhino horn. The hilt/ handle is a flattened piece and ornamented, at the centre of the pommel and on the base, with two small circle disks of copper, silver, or gold looks like old coins. The hilt/handle of horn decorated from the front with many holes inlayed with silver.

The sheath where the steel blade is stored, made of ivory or wood covered with metal, cloth, or leather. Sheaths are of two types of Al-Hashidi, which is characterized by the small angle of curvature at the back of the sheath, and its shape resembles the Arabic letter L, and Second type is Al-Bakili, which is in the form of the Arabic letter R and is like the scabbard of a sword. the belt which the scabbard is generally permanently secured on it. The full jambiya outfit is not considered complete without such a belt. The belt is usually worn around the lower abdomen. the belt made of leather decorated with leather strips in different colour and other time made of textiles support decorated with embroidery decoration.

The main problem of the two dagger that there is no any data about them and the hilt of dagger RAK 430 was broken. The methodology started first with the aime of extracting all data with aid of technology to search about the significance value of the two objects.

Photographic documentation was applied to record the state of preservation of the objects and to document in details all the jambiya component (Fig. 1). Digital documentation also applied to obtain a digital facsimile from the original object (Fig. 2,3). Microscopic image using Dino-lit portable microscope was able to get deep magnification for the object component in different area.

Fig. 1 Two daggers RAK 430, and RAK11584.

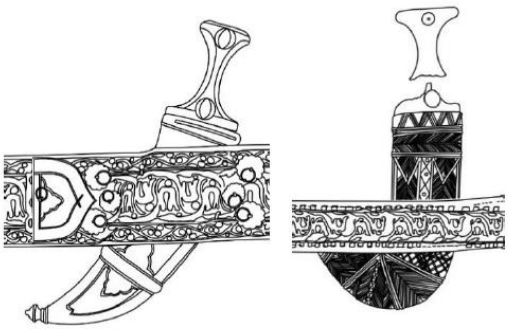


Fig. 2 Diagram of object RAK 430, RAK 11584.

[2] Exell, Karen (2016-03-10). Modernity and the Museum in the Arabian Peninsula. Routledge. ISBN 978-1-317-27901-3.

[3] Haza, Ruba (2020-10-15). "Ras Al Khaimah National Museum to reopen after six months". The National. Retrieved 2021-11-19.

[4] National Museum of Ras Al Khaimah Set to Reopen with Tamra (Date Palm) Exhibition - Ras Al Khaimah Media Office". Ras Al Khaimah Government Media Office. Retrieved 2021-11-19.

B. SIGNIFICANT OF THE OBJECTS

From the investigation of the decorated gold circle/flower, in the form of circular golden coins, this circle contains a significant sign which is the Christ standing with sixteen stars around and text in the circl fram: SIT T XPE DAT QTV REGIS ISTE DVCA

This decorated circle has a foreign feature may be affected or come from the medieval art (Fig.4,5). This metal circle is like gold coin minted by the Republic of Venice 13th centur. The face of the Christ is very simple it a circle with three dots inside and arc above it.

Fig. 4 Gold circle / flower diamete 1.8 cm, Christ standing with sixteen stars around.

Fig. 5 A digital facsimile copy from the visible traces of the golden circle.

As the hilt/handle the most significant part from the dagger, it was important the identify the type of material which the hilt made of. Microscopic image using Dino-lit portable microscope was able to identify the type of horn which proved that it made from rhino horn this was clear from the microscopic images for the Internal structure, Intertubular matrix and Horn tubules of rhino horn (Fig. 6,7) [5].

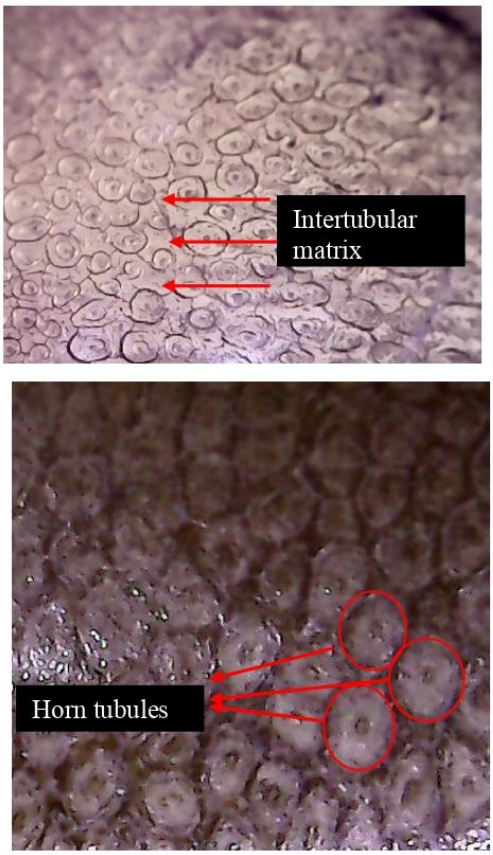


Fig. 6.7 Microscopic image for Internal structure of rhino horn.

Identification of the rhino horn in the hilt/handle of this jambiya can prove it is from the Al-Saifani type and the age of these jambiya bearing Saifani's head ranges between 400 and 1500 years and it is invaluable with the passage of time, the other hilt according microscopic inestigation not made from rhino horn.

These information can add as Object 'biography' which help us to understand what stage in an object's life we are studying, recording, representing and conserving Also help us to think about: how things are made (materials and technique) and how their significance varies.

SHEATH

The sheath shape in in the two dagger was different.The dagger RAK 11584 type of sheath known as Al-Bakili, which is in the form of the Arabic letter R, and is like the scabbard of a sword (Fig. 8). The dagger RAK 430 of sheath known as Al-Hashidi shape resembles the arabic letter L (Fig. 9). Belt decorative motif are similar from type know as Kepsi (Fig.10).The names given to sheath and motif

[5] Hieronymus, T.L., Witmer, L.M. and Ridgely, R.C., 2006. Structure of white rhinoceros (Ceratotherium simum) horn investigated by X - ray computed tomography and histology with implications for growth and external form. Journal of Morphology, 267(10), pp.1172-1176.

according to the name of family who created the design.

Fig.8 sheath from type of Al-Bakili, shape resembles the arabic letter R.

Fig.8 sheath from type Al-Hashidi, shape resembles the arabic letter L.

Fig.10 Belt decorative motif are similar from type know as Kepsi.

C. HERBARIUM COLLECTION

The Ras Al-Khaimah national museum Herbarium houses collections, acquired through collections of Mrs R. E. Ash and identified by Mr A. G. Miller.
were organized by the collector in this following: The collections comprise five files in which are contained the specimens in alphabetical order according to their family names (Fig.11). Each specimen, mounted on an individual card, shows a pressed example of the flower together with a photograph of the plant in its natural habitat. the details with each specimen include, name, family, Map co-ordinates of location and reference number.
The main problem of the collection was the missing of data which was the collectr's working notebooks of which there were three.
the missing of the collectr's working notebooks were include the identification sheets of the specimens made by Mr A. G. Miller, of the Royal Botanical Gardens, Edinburgh, Scotland. which give the following information: name, Arabic, family, locality, co-ords, habitat, description and uses, date and collection number which for example this is shown, as R. E. ASH 169. this is shown, as R. E. ASH 169.this number is shown on the specimen card as REA169 at the specimen and working notebooks.
duplicates of this collection are held at the royal botanical gardens, Edinburgh, Scotland, the herbarium at Oman national history museum, ministry of national heritage and culture, Muscat, sultanate of Oman and on the 18th of May 1992 for the national museum of Ras Al Khaimah.
This herbarium collection was kept at the national museum of Ras Al Khaimah storage from 1992 until 2023 without any sorting for data or preservation.

COLLECTION PRESERVATION STRATEGY

The main aim of the preservation of the herbarium collection was to apply a computerized cataloging of the collections to makes it available for scientific studies and research send information and material, upon request, to scholars from all over the world (Fig.12).
Organizes guided tours for individual users, as well as for schools of all levels to see the collection, design an exhibition and study days, both in the academic and popular fields; participates in projects aimed at the dissemination of scientific culture and finally promotes editorial activities aimed at disseminating knowledge of flora.

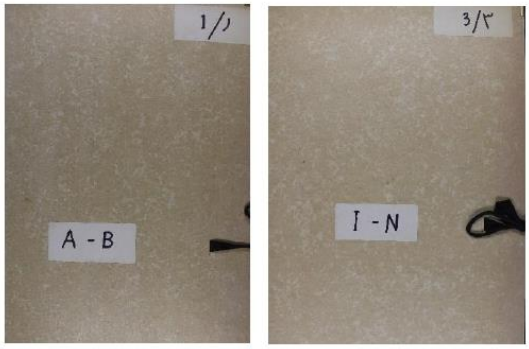


Fig.11 Files in which are contained the specimens

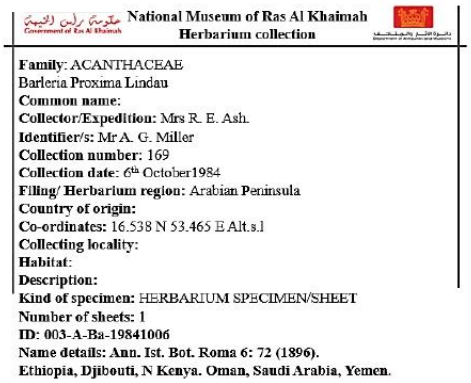
Fig.12 Example of The computerization and digitization of the collections

COMPUTERIZATION AND DIGITIZATION OF THE COLLECTIONS

The computerization and digitization of the collections was the first procedures to apply. The computerization of the collections was the first procedures to apply which was counted in 34 Family and 78 species, followed by designing a digital sheet for each file of the fifth files (Fig.13). Describing, identifying, and cataloging plants, a designed card for each specimens include all data which were missed by losing the collection notebooks.

Fig.13 Example of The computerization and digitization of the collections

In the designed card for each specimens an ID number for each specimen was generated consisted of a serial number, the first alphabetic of the family, the two-starting alphabetic of the specimen and the date of collection (Fig.14).



National Museum of Ras Al Khaimah
Herbarium collection

Family: ACANTHACEAE
Barleria Proxima Lindau
Common name:
Collector/Expedition: Mrs R. E. Ash.
Identifier/s: Mr A. G. Miller
Collection number: 169
Collection date: 6th October1984
Filing/ Herbarium region: Arabian Peninsula
Country of origin:
Co-ordinates: 16.538 N 53.465 E Alt.s.l
Collecting locality:
Habitat:
Description:
Kind of specimen: HERBARIUM SPECIMEN/SHEET
Number of sheets: 1
ID: 003-A-Ba-19841006
Name details: Ann. Ist. Bot. Roma 6: 72 (1896).
Ethiopia, Djibouti, N Kenya. Oman, Saudi Arabia, Yemen.

Fig.14 Example of specimen new card

Digitization and digital innovation will allow data sharing of images and data to countries of origin which is Oman, more accessible to botanists and others around the world. Finally, we are building an electronic Herbarium Catalogue containing images of the specimens and information taken from their collection labels and some new data which were added for the sustainable development and systematics of collection [6].

D. POTTERY FROM EXCAVATION

Two big pottery jar from recent excavation were discovered in archaeological site Al Hudaibah,RAK,UAE (Fig. 15). The two jars were moved from archaeological site to museum laboratory to recieve conservation. The two jars were filled of soils from the excavation site. The methodology applied started to look first on the soil. employed to recover and analyze any materials or remains might be found.

Fig.15 The two pottery jars at the excavation site.

The materials collected from flotation carefully transferred to trays or containers for further analysis. The collected botanical materials are dried and sorted under controlled conditions.The materials classified as follow: pottery shards, fragments of glazed pottery, glass fragments, archaeobotanical remains, seashells and large group of bones (Fig. 16).

Fig.16 The seprated remains after flotation.

The investigated and analyzed remains provide valuable insight about past societies and the site itself. The glazed fragments identified to have a relation with glazed dishs from RAK collection and the bone remains idenyified as fish bones might be for saw fish and Salmonidae most

[6] Kew, Royal botanical Garden.

probably for *Oncorhynchus keta*[7][8].Althought the excavation site was far from the sea these find can interoertate that might be the site was close to the sea in the past or might be the jars were used to store food .The methodology of conservation for the two jars was not only preserving the objects but more deep to an impotratnt idea about the historical context of them. This is linked to the idea that integrated conservation approaches promote interdisciplinary collaboration.

IV. DISCUSSION

Preservation and conservation of collections involves not only on practices to mitigate risks and maintain the integrity of collection but also to disseminating of knowledge and facilitate the exchange of ideas.Foster collaborations within the scientific community.make research findings accessible. serve as an archival record of scientific progress and research developments over time. provide a reference point for future researchers. This discussion explores the importance of preservation and extraction of data about collection. In the case of the two daggers explain how conservation is important when you identify the significant value of collections.

In herbarium collection the technological advancements offers new tools and methods for preserving collections, such as digitalization which allows for the creation of high-quality replicas, reducing the handling of fragile objects while providing access to a wider audience.

The preservation national museum of Ras Al-Khaimah collection is a huge challenge. Working in direct contact with the objects and studying the debates and practices of the past while reviewing our own practices revealed that present-day conservation decisions integrate decisions for the collection future sustainability. It was discovered that limited devices and materials, in combination with the condition or state of an object and past trends in scientific approach, affect an object's characteristics, even becoming part of it[*9*].

Our present-day conservation of our collection not only the remedial intervention that applied, but scientific methodologies and laboratory applications are both advancing and have different approaches. The challenge to discover a new information about collection and sorting its data can add more value to it.

V. CONCLUSION

The Preservation and conservation efforts help safeguarding collection, ensuring their physical existence, and protecting their unique stories and knowledge for future generations. Collections serve as valuable resources for research, education, and inspiration. By preserving and conserving collections, can ensure that future scholars, students, and enthusiasts have access to these materials, enabling them to deepen their understanding of history, culture, science, and various disciplines.

Digitization initiatives make collections accessible to a broader audience, transcending physical boundaries. Online platforms, virtual exhibitions, and digital archives provide easy and remote access, ensuring future generations can explore and learn from collections.

Preservation and conservation are crucial for ensuring the sustainability and accessibility of collections for future generations. conservation and preservation in museums have changed to adapt to changing technologies, scientific development, and philosophical approaches. By adopting integrated preservation approaches, using technology and the right methodology of preservation and conservation extend the lifespan of collections and make it more sustainable.

FUTURE RESEARCH

The author suggests further investigation and analysis techniques such as optical microscope for identification of fibers and leather . X-ray Fluorescence (XRF) as non-destructive technique for the measurements of the elemental composition of metal parts and pottery shards. Spectroscopic techniques with the aid of High-performance liquid chromatography for the identification of dyes on textile parts. carbon dating for the bone remains.

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REFERENCES

1. Beisaw, A. M. (2013). Identifying and interpreting animal bones: A manual (Vol. 18). Texas A&M University Press.
2. Exell, K. (2016). Modernity and the Museum in the Arabian Peninsula. Routledge.
3. Hamza, N. M. A. M., Ishii, M., & Eslam, S. (2021). Conservation between scientific methodology and laboratory application: An integrated approach to past and present challenges. In ICOM-CC 19th Triennial Conference 2021 Beijing (pp. 1-7). ICOM-CC.
4. Haza, R. (2020, October 15). Ras Al Khaimah National Museum to reopen after six months. The National. Retrieved from https://www.thenationalnews.com/arts-culture/art/ras-al-khaimah-national-museum-to-reopen-after-six-months-1.1095757
5. Hieronymus, T. L., Witmer, L. M., & Ridgely, R. C. (2006). Structure of white rhinoceros (Ceratotherium simum) horn investigated by X-ray computed tomography and histology with implications for growth and external form. Journal of Morphology, 267(10), 1172-1176.
6. Royal Botanic Gardens, Kew. (n.d.). Herbarium Catalogue. Retrieved from http://apps.kew.org/herbcat/navigator.do

[7] Yee, Debbi. "Marine Fish Osteology: A Manual for Archaeologists." (1987).

[8] Beisaw, April M. Identifying and interpreting animal bones: a manual. Vol. 18. Texas A&M University Press, 2013.

[9] NagmEldeen Morshed Hamza, Mie Ishii, Eslam Shaheen,Conservation between scientific methodology and laboratory application: An integrated approach to past and present challenges, ICOM-CC 19th Triennial Conference 2021 Beijing.

7. Ras Al Khaimah Government Media Office. (n.d.). National Museum of Ras Al Khaimah Set to Reopen with Tamra (Date Palm) Exhibition. Retrieved from https://www.mediaoffice.raa.gov.ae/en/news/2021/October/15-10/national-museum-of-ras-al-khaimah-set-to-reopen-with-tamra-date-palm-exhibition
8. Department of Antiquities and Museums. (n.d.). National Museum of Ras Al Khaimah. Retrieved from https://www.rakheritage.rak.ae/en/Pages/Museums/National-Museum-of-Ras-Al-Khaimah.aspx.

9. Yee, D. (1987). Marine Fish Osteology: A Manual for Archaeologists.