ON THE STUDY OF HISTORICAL ARRAIOLOS TAPESTRIES

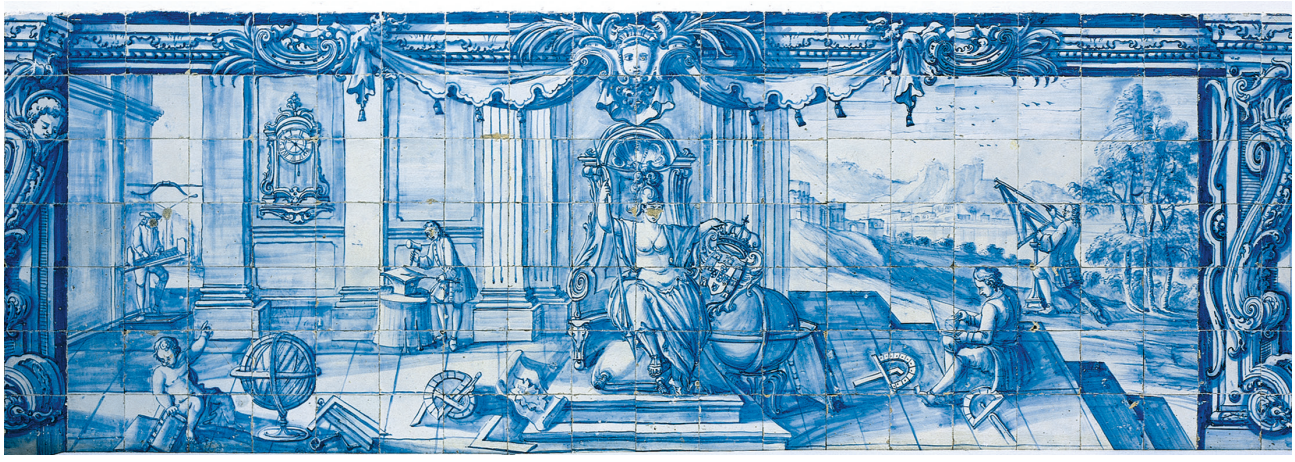
AN INTEGRATED APPROACH

Ana Cristina Cabaça Manhita

Tese apresentada à Universidade de Évora
para obtenção do Grau de Doutor em Química

ORIENTAÇÃO: *Professora Doutora Cristina Maria Barrocas Dias Teixeira da Costa*
CO-ORIENTAÇÃO: *Professor Doutor António José Estevão Grande Candeias*

ÉVORA, JULHO DE 2012



INSTITUTO DE INVESTIGAÇÃO E FORMAÇÃO AVANÇADA

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Este trabalho de Doutoramento recebeu financiamento da Fundação para a Ciência e Tecnologia (Bolsa de Doutoramento SFRH/BD/22411/2005) no âmbito do Programa Operacional Potencial Humano do Quadro de Referência Estratégico Nacional (POPH – QREN), comparticipado pelo Fundo Social Europeu e por Fundos Nacionais do Ministério da Educação e Ciência.

UNIÃO EUROPEIA
Fundo Social Europeu

GOVERNO DA
REPÚBLICA PORTUGUESA

Aos meus Pais,
Maria Cândida e José Francisco

ACKNOWLEDGEMENTS

First of all, I would like to thank my supervisors, Prof. Cristina Dias and Prof. António Candeias for all their guidance and support through this research. Finishing this thesis was not easy and I have to thank Cristina for her friendship and for believing in me when everything seemed to be falling apart...

Very special thanks go to Prof. Teresa Ferreira for being my 'supervisor', for all her help and advice and above all, for her friendship.

I also want to acknowledge architect Teresa Pacheco Pereira for the careful selection of the Arraiolos carpets and Mr. Luís Pedro for his help in collecting the historical samples.

At the José de Figueiredo Conservation and Restoration Laboratory, I would like to thank Eng. Isabel Ribeiro and Dr. Helena Vargas for all the help with the accelerated ageing and colourimetric studies.

To Prof. Pat Sandra and Dr. Lieve Balcaen, from the University of Ghent, I am thankful for the ICP-MS analyses. I also have to thank Prof. Sandra for inviting me for a short stay at his laboratory in Ghent University.

I am also thankful to all the colleagues and professors who have worked in the Natural Products Chemistry laboratory during the first years of this research project, especially Vanda Santos for dyeing the red wool samples and Prof. Dora Teixeira for helping with the madder LC-MS/MS analyses.

I also have to acknowledge all the members at the HERCULES Laboratory team who were always willing to share with me their time and knowledge. It was a huge privilege for me to be a part of this great group from its beginning!

Special thanks go to Conceição Borges and Carlos for their friendship and for being a second family to me during my frequent stays in Lisbon in the first years of this PhD work.

To José Pinto, I would like to thank for his friendship and support through the good and harder times of this research project.

Finally, I want to acknowledge my brother José António and especially my parents Maria Cândida and José Francisco, to whom this thesis is dedicated, for their unconditional love and support during these last five years. It was your help and guidance that made it possible for me to be here today. Thank you!

ABSTRACT

Wool samples collected from thirteen Arraiolos tapestries from the 17th-19th century belonging to the National Museum of Ancient Art (Lisboa, Portugal) collection were analysed to identify the natural dyes and mordants used in their making. Weld, indigo, spurge flax, brazilwood, madder and cochineal were identified in the fibres. Alum was the most commonly used mordant, but the presence of iron and zinc was also detected in some samples.

Contemporary dyed fibres were used to evaluate the available methodologies for dye extraction from wool fibres. A procedure with Na_2EDTA in water/DMF (1:1, *v/v*) proved to be the most efficient for recovering the different chromophores.

The influence of mordant metal ion (aluminium, iron and copper) and dyeing technique on the fibre hue and photodegradation was studied in contemporary wool samples dyed with madder or brazilwood. Colour hue was strongly influenced by dyeing methodology and alum mordanted fibres showed the poorest lightfastness.

TÍTULO: À redescoberta dos materiais dos Tapetes de Arraiolos

RESUMO

Amostras de lã recolhidas a partir de treze tapetes de Arraiolos dos séculos XVII a XIX, pertencentes à colecção do Museu Nacional de Arte Antiga (Lisboa, Portugal), foram analisadas para identificação dos corantes naturais e mordentes utilizados no seu fabrico. Lírio-dos-tintureiros, índigo, trovisco, pau-brasil, garança e cochinilha foram identificados nas fibras. O alúmen foi o mordente mais utilizado, embora ferro e zinco também tenham sido detectados em algumas amostras.

Para avaliação dos métodos de extracção de corantes a partir de fibras de lã, utilizaram-se fibras tingidas em laboratório. O procedimento mais eficaz para a remoção dos diferentes cromóforos utilizou Na_2EDTA aquoso/DMF (1:1, *v/v*) como solvente de extracção.

A influência do ião metálico mordente (alumínio, ferro e cobre) e da técnica de tingimento na cor das fibras e no processso de fotodegradação foi estudada em amostras de lã tingidas em laboratório com garança ou pau-brasil. A tonalidade das fibras foi fortemente influenciada pela técnica de tingimento e as fibras mordidas com alúmen foram as que apresentaram menor resistência à luz.

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LIST OF ABBREVIATIONS

ΔE	-	Colour variation
ANOVA	-	Analysis of variance
APCI	-	Atmospheric pressure chemical ionization
C^*	-	Chroma
CE	-	Capillary electrophoresis
CID	-	Collision induced dissociation
CIE	-	Commission internationale de l'éclairage
Da	-	Atomic mass unit dalton
DAD	-	Diode-array detector
DMF	-	Dimethylformamide
DMSO	-	Dimethyl sulfoxide
DMTA	-	Dynamic mechanical thermal analysis
DNA	-	Deoxyribonucleic acid
DSC	-	Differential scanning calorimetry
EDS	-	Energy dispersive X-ray spectroscopy
EDTA	-	Ethylenediaminetetraacetic acid
ESI	-	Electrospray ionization
FAAS	-	Flame atomic absorption spectroscopy
FORS	-	Fibre optics reflectance spectroscopy
FTIR	-	Fourier transform infrared spectroscopy
GC	-	Gas chromatography
h°	-	Hue
HPLC	-	High-performance liquid chromatography
ICP	-	Inductively coupled plasma
L^*	-	Lightness
LC	-	Liquid chromatography
LOD	-	Limit of detection
m/z	-	Mass-to-charge ratio
M+D	-	Simultaneous mordanting
MD	-	Pre-mordanting

MeOH - Methanol
MRM - Multiple reaction monitoring
MS - Mass spectrometry
MS/MS - Tandem mass spectrometry
NMAA - National Museum of Ancient Art
NMR - Nuclear Magnetic Resonance
OES - Optical emission spectroscopy
OM - Optical microscopy
p.a. - *pro analysi*
PIXE - Particle-induced X-ray emission
Py - Pyrolysis
RDA - Retro-Diels-Alder fragmentation
RF - Radiofrequency
RSD - Relative standard deviation
SEM - Scanning electron microscopy
SERS - Surface-enhanced Raman spectroscopy
SIM - Selected ion monitoring
SIMS - Secondary ion mass spectroscopy
TEM - Transmission electron microscopy
TFA - Trifluoroacetic acid
TLC - Thin-layer chromatography
ToF - Time-of-flight
UV - Ultraviolet
Vis - Visible
XPS - X-ray photoelectron spectroscopy
XRF - X-ray fluorescence

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1. Introduction

'*I made a provision of rugs for my journey, all with grotesque design and gaudy colours (...) I had them spread out around the bed, and looking at them, their diversity of colours offered me a flaming exotic effect*'[1].

These '*flaming exotic*' rugs, as described by William Beckford in 1787, refer to the well-known Arraiolos tapestries. The embroidered tapestries of Arraiolos constitute one of the most interesting popular artistic industries born in Portugal but, despite that, very little is known about their production since the historical sources are scarce and there is only one literature report concerning the analysis of two Arraiolos rugs from the Museum Machado de Castro[2].

The aim of this work was to investigate the nature of mordants and dyestuffs employed in the traditional Arraiolos tapestries dyeing process, in a way to complement and improve actual knowledge on these rugs. Wool samples were collected from thirteen historical tapestries, carefully selected from the National Museum of Ancient Art (Lisboa, Portugal) collection (17^{th}-19^{th} century period).

A study to assess the most suitable procedure to extract the natural dyes chromophores from the wool fibres was firstly conducted. The efficiency of eight different procedures was evaluated using contemporary wool samples dyed with cochineal, madder, woad, weld, brazilwood and logwood. Samples were analysed by high-performance liquid chromatography with diode-array detection (HPLC/DAD), and the comparison between methods was based on the HPLC/DAD peak areas of the natural dyes' main components, extracted from the wool samples.

Natural dyes were extracted from the Arraiolos historical wool fibres using the previously identified most efficient method followed by high-performance liquid chromatography with diode-diode array and mass spectrometry detection (HPLC/DAD/MS) for compound identification. Colourimetry was used to measure colour parameters in all historical samples.

The morphology of the wool fibres was evaluated using scanning electron microscopy (SEM). Microanalyses by scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDS) and micro-particle-induced X-ray spectrometry (μ-PIXE) were used for mordant identification. The quantification of mordants in the historical fibres was carried out by inductively coupled plasma mass spectrometry (ICP-MS).

The influence of mordant metal ion (aluminium, iron and copper) and dyeing technique on the fibre hue and chromophore photodegradation was studied in contemporary wool samples dyed with madder or brazilwood. Colourimetry was used to measure the fibre colour before and after the samples were subjected to accelerated light ageing. HPLC/MS was used for chromophore analysis on unaged and artificially aged wool fibres. Flame atomic absorption spectroscopy (FAAS) and inductively coupled plasma optical emission spectroscopy (ICP-OES) were used for mordant quantification.

2. Arraiolos tapestries

Origins

Arraiolos tapestries are probably one of the richest artistic Portuguese expressions in terms of textile heritage. Produced in the southern village of Arraiolos, in the Alentejo region, they consist in an embroidery of wool worked on a canvas, usually of linen or hemp. The long-armed cross stitch (a variant of the ordinary cross stitch) is the basis of the technique used for making these tapestries and has been in use in the Iberian Peninsula since the 12th century[3,4]. The design of the earlier Arraiolos tapestries exhibits the Western European fascination for oriental rugs, namely Indo-Persian rugs[5].

The origin of the first Arraiolos tapestries is quite controversial – the lack of documentation and references makes extremely difficult to pinpoint the beginning of Arraiolos' manufacturing and different theories arise among historians.

One of the theories proposes a Moorish origin for the first Arraiolos rugs[3,6]. On December 5th of 1496, King Manuel I declared the expulsion of Jews and Moors from the kingdom of Portugal, under death penalty and confiscation of assets in favour of those who denounced them. However, as the Portuguese monarch didn't want to lose this huge number of merchants and their resources, he forced their conversion to Catholicism[3,5]. This conversion led to distress within the Moorish and Jewish communities and it was in this context that they abandoned the large communities where they lived and looked for refuge in other parts of the kingdom, away from the pressures of religion persecution[3,5]. These communities, particularly the Moors, were commonly dedicated to the production of carpets, and in Arraiolos they found conditions to establish themselves and further develop their crafts[5]. With the installation of the Moors, the embroidery of Arraiolos was probably born, reflecting the influence of Moroccan rugs[3]. The local population would have seen in this new

community an opportunity to sell the wool from their flocks, in addition to the professional activities that would develop around the carpets: from carding and spinning wool to its dyeing and canvas weaving[3,5]. For some authors, this was the birth of Arraiolos rugs, although it does not gather full consensus of all historians.

A different vision is suggested by Reynaldo dos Santos: Arraiolos tapestries have oriental origin[7]. According to him, there is '*an influence of the Orient on our seventeenth-century decorative arts covering the origin of Arraiolos*'[7]. In the 17th century, Portugal was in the middle of a political, economic and even artistic crisis. The Oriental world emerged as a source of inspiration that influenced not only Arraiolos tapestries, but also furniture, fabrics and quilts, ceramics and ivory[5].

To Reynaldo dos Santos, 17th century rugs were most likely produced in convents, as they would have easier access to Oriental rugs than the common people[7]. Although the presence of Arraiolos tapestries in the Alentejo convents is remarkable (most of the tapestries belonging to the national museum's collections proceed from these institutions[4]), this is not enough to affirm that the manufacture of these carpets began in those convents.

In the 1710 inventory of Coimbra Cathedral, referring to Dom Frei Álvaro (Bishop of Coimbra between 1672 and 1683), a tapestry is described: '*There is a large rug made in the Alentejo for the choir and donated by Dom Frei Álvaro*'[4,8]. For a long time, this was regarded as the first description of an Arraiolos tapestry. In 1699, in a guideline of Lisboa customs, another reference is found: '*Arrayollos tapestries paying 40 thousand réis a yard*', being this the first known reference of a tapestry produced in the Arraiolos village until the 1990's. During the 1990's the Arraiolos Municipal Historical Archive underwent extensive organization and earlier references were found in some farmers inventories[9]. The first reference is dated 1598: '*a new carpet from the village evaluated in two thousand réis*'. Less than four years later, a new reference: '*a small carpet made in the village evaluated in six hundred réis*'[9]. Therefore, based on written documentation, the beginning of Arraiolos rugs production can go back to the late 16th century, which allows to suppose that Arraiolos rugs were already in production previously to that[5]. But this production is not described in the available documentation and further historical investigations are required.

Classification

'*Even in 1878, in Évora, Portuguese hand-embroidered rugs were confused with woven rugs of diverse origins both European and exotic. For those admirers of antique objects, with very few*

exceptions, everything was from Arraiolos. And even today the confusion is deplorable, since neither is their multiple colouring rigorously defined, nor the various patterns of their design are methodically defined at all... Fauna and flora are also unidentified'[10]. In response to this outburst by Joaquim de Vasconcelos, José Pessanha printed, in 1906, an unpublished study by Cunha Rivara on the dyeing process of wool[1]. Sebastião Pessanha, ten years later, issued the first chronological and typological classification for Arraiolos rugs[11]. In an attempt to systematize the known patterns of the rugs, he established the following division:

- 1st Period (second half of the 17th century): '*Products of individual curiosity or the work of convents in the Alentejo*'. Embroidered in linen, they are distinct by their meticulous copying of motifs from Persian carpets, mixed with an infinite number of animal designs. The stitching does not follow one particular direction and is smaller. The rich colouring and its good state of preservation show almost perfect dyeing techniques[11].
- 2nd Period (first two-thirds of the 18th century): '*Flourishing period of the cottage industry in Arraiolos*'. The Oriental motifs almost completely disappear at this time, giving place to those inspired in nature and with aesthetic compositions of the seamstresses[11].
- 3rd Period (last third of the 18th century and first half of the 19th century): '*Decline of the industry*'. In the designs, nothing is left of the Persian motifs, or even the picturesque compositions in which regional flora and domestic fauna were widely depicted. Simple bunches of large flowers, perhaps taken from rough sketches, seek to animate the monotonous brown backgrounds[11].

Considering that not all patterns were covered in Pessanha's classification, almost forty years later, a new approach was presented by Maria José de Mendonça[6]. A pattern-based classification was made for the 17th and 18th century rugs, as follows:

- 17th century rugs: different types of patterns can be found, which were grouped under the names '*geometric pattern*', '*floral pattern*', '*rosette pattern*' and '*oriental pattern*'. The '*geometric pattern*' and '*rosette pattern*' are likely influenced by the Spanish carpets from Alcatraz and Cuenca of the 15th and 16th centuries, with some of the examples of '*rosette patterns*' having insertions from Indo-Portuguese quilts - hunting scenes, human representations of the five senses, etc. With the '*floral patterns*', the influences are more difficult to determine. '*In the composition of*

the rugs grouped in this series, ornamentations inspired by foliage predominate, more or less stylised and revealing the influence of a number of models (...) Therefore, the 17th century would have been a period of Spanish influence, to be followed at the end of the century, by the oriental influence of Persian carpets of the 16th century and the first half of the 17th century'[6].

- 18th century rugs: patterns are grouped under the names of '*animal pattern*', '*foliage pattern*', '*geometric pattern*' and '*floral pattern*'. The '*animal pattern*' is representative of the Arraiolos industry during the first half of the 18th century. Animal figuration predominates of both erudite and popular inspiration, combined with floral elements, denoting, for the most part, the influence of the Orient. The centre of the field is generally occupied by a medallion, inspired by the medallions in Persian rugs or Indo-Portuguese quilts, or composed from the imagination of the artisan with floral elements which denote, in a more or less significant way, the influence of the Orient. As for the '*foliage pattern*', the field is entirely filled with foliage which begins at the top and sides and meets in the centre of the composition, which has no central ornament; foliage is done in shades of blue over a yellow background, originally red in colour. In the '*geometric pattern*', there are designs suggesting proximity to models of the geometric type from Asia Minor, others which recall the patterning on tiles from the second half of the 18th century; the ornamentation is made in shades of blue and yellow on an originally red background. Finally, in the '*floral pattern*', the colouring is transformed with the design; the red backgrounds of the fields are maintained, but others are in dark or pale blue, in dark or bright green, or in shades of brown[6].

Dyeing in Arraiolos

As already mentioned in subchapter '*Classification*', it was in 1906 that José Pessanha printed an unpublished study by Cunha Rivara on the dyeing process for wool[1]. Collected in 1834, these few dyeing recipes constitute, unfortunately, the only known reference for the traditional dyeing process in Arraiolos[12]. *Table 1* presents a list of the colours and materials used in the traditional Arraiolos dyeing process, according to Cunha Rivara[12].

Table 1. *Colours and materials used in Arraiolos dyeing process in the 19th century. (Adapted from [1])*

Colours	Dyes, mordants and auxiliaries
Black	Logwood
Blue	Indigo and urine
Brown	Natural black wool immersed in red wool dyeing bath
Green	Blue wool re-dyed with weld; addition of caparosa (sulfate of iron, zinc or copper)
Purple	Red wool and lye
Red	Brazilwood, spurge flax, urine and/or alum
Rose	Brazilwood and alum
Yellow	Weld, alum and urine

Arraiolos collection from the National Museum of Ancient Art (NMAA)

Thirteen Arraiolos historical tapestries were selected from the NMAA (Lisboa, Portugal) collection (17th-19th century period). The pieces were carefully chosen by the NMAA curator Teresa Pacheco Pereira to reflect the most representative periods of the Arraiolos' production. While some of the rugs are still well preserved (and in some cases, with brilliant and vivid colours), others are severely degraded. Different portions of embroidery wool yarns between 3 and 5 mm with the same hue were sampled in distinct parts of the carpets, in a total of 148 samples. Details of the individual tapestries that were sampled are listed below. Tapestries description is based on the works published by Teresa Pacheco Pereira[4,13]. Pictures from tapestries J, L, M and N were adapted from [13].

Tapestry A

NMAA 88

(9 samples taken)

17th century

Materials: Linen and wool

Dimensions: 294 × 168 cm

Provenance: Purchased

Description: Blue background field. Design based (?) on '*Holbein*' (?) type rugs.

Border: Yellow background. Corners forming a clearly defined square.

Tapestry B

NMAA 38

(12 samples taken)

18th century

Materials: Linen and wool

Dimensions: 280 × 218 cm

Provenance: Santa Clara Convent, Vila do Conde

Description: Green central medallion, decorated with yellow lace. Yellow background field with animal pattern symmetrically disposed towards the centre.

Border: Blue background with floral elements and birds structured by zigzag threads.

Tapestry C

NMAA 29

(11 samples taken)

18th century (end)

Materials: Linen and wool

Dimensions: 190 × 105 cm

Provenance: Semide Convent

Description: Salmon-pink background field, faded. Uncharacteristic design.

Border: No border.

Tapestry D

NMAA 36

(14 samples taken)

18th century

Materials: Linen and wool

Dimensions: 169 × 112 cm

Provenance: Bom Pastor Convent, Viana do Alentejo

Description: Yellow background field. Very typical '*animal pattern*'.

Border: Dark blue background with '*Oriental*' motifs.

Tapestry E

NMAA 74

(14 samples taken)

17th century (second half)

Materials: Linen and wool

Dimensions: 303 × 172 cm

Provenance: Chapel of Nª Sª da Conceição dos Matos, Arronches

Description: Blue background field. Three large flowers fill the field. Figures representing huntsmen on foot and on horseback; men and women playing musical instruments; horsemen, duelers, birds and other animals complete the composition.

Border: Green background. Wide, with inner frame, with flowers, birds, tigers and figures of men and women drinking and hunting.

Tapestry F

NMAA 25

(10 samples taken)

17th-18th century

Materials: Linen and wool

Dimensions: 221 × 134 cm

Provenance: São Bento de Avé Maria Convent, Porto

Description: Yellow background field. Design based on '*central medallion*' rugs made in Persia.

Border: Blue background.

Tapestry G

NMAA 55

(9 samples taken)

17th century

Materials: Linen and wool

Dimensions: 375 × 182 cm

Provenance: Madre de Deus Convent, Lisboa

Description: Dark red background field. Geometric design forming a quadrangular grid. In the corner of the square, knots in the form of a swastika. A rose fills the centre of the squares. Design possibly based on Spanish rugs from Alcatraz or Cuenca.

Border: Narrow, with geometric design forming Ss.

Tapestry H

NMAA 40

(12 samples taken)

19th century (first half)

Materials: Linen and wool

Dimensions: not available

Provenance: not available

Description: Dark brown background field. Floral design, with central flower.

Border: Floral motifs, with yellow background.

Tapestry I

NMAA 114

(13 samples taken)

17th century

Materials: Linen and wool

Dimensions: not available

Provenance: not available

Description: Yellow background field. Large dark blue '*central medallion*'. Some animal motifs.

Border: Yellow background. Corners forming a clearly defined green background square.

Tapestry J

NMAA 19

(8 samples taken)

17th century

Materials: Linen and wool

Dimensions: 230 × 128 cm

Provenance: Paraíso Convent, Évora

Description: Blue background field. Design probably based on '*Holbein*' type rugs made in Anatolia.

Border: Yellow background.

Tapestry L

NMAA 89

(19 samples taken)

17th century (second half)

Materials: Linen and wool

Dimensions: 229 × 138 cm

Provenance: Purchased

Description: Green background field. Design based on '*Indo-Persian*' rugs made in India in the 17th century.

Border: Dark brown background filled with '*palmettes*', here transformed into lozenges.

Tapestry M

NMAA 24

(8 samples taken)

18th century

Materials: Linen and wool

Dimensions: 240 × 136 cm

Provenance: São Bento da Avé Maria Convent, Porto

Description: Yellow background field. '*Animal pattern*'; birds and tulips surround the '*central medallion*' in a quadrangular form.

Border: Green background with design of Persian influence. Interior frame.

Tapestry N

NMAA 30

(9 samples taken)

18th century (end)

Materials: Linen and wool

Dimensions: 213 × 119 cm

Provenance: Semide Convent

Description: Yellow background field. Design with broad symmetrical foliage in relation to the two octagonal axes.

Border: Blue background. Regular design recalling Oriental originals.

3. Dyes and dyeing

Introduction

'*Dyeing, as the word is commonly used, is the art of communicating colour of some considerable degree of permanence to articles used in clothing*[14].' (The British Encyclopaedia, 1818)

The origins of dyeing are uncertain – the production of fabrics and their colouration precedes recorded history – but it is believed that several cultures had established dyeing technologies before 3000 BCE[15,16]. It is likely that the ancient art of dyeing originally spread westwards from India, and it may well have been accidental staining from berries and fruit juices that initially stimulated its development[16]. Natural dyes were extracted from plant and animal sources with water, sometimes under conditions involving fermentation. Fabric was dyed by soaking it in the aqueous extract and drying[15].

Although water-dispersed colourants were used in paints 30 000 years ago, they easily washed off from any material coloured with them. Dyes had only a limited range of dull colours and the dyeings invariably had poor fastness to washing and sunlight[15]. The fastness of a dye is a measure of its resistance to fading, or colour change, on exposure to a given agent or treatment. Wash-fastness can be improved if the fabric was first treated with a solution containing a salt of, for example, aluminium, copper or iron[15]. These metal salts are called mordants. When the pre-mordanted fabric is soaked in a bath of a suitable natural dye (or soaked in a bath containing the dye and the mordant), the dye penetrates into the fibres and reacts with the metal ions present. This reaction decreases the water solubility of the dye so the colour is less likely to bleed out on washing[15].

In this chapter, a review on the literature concerning wool fibres, mordants and natural dyes is presented. Wool fibre structure and composition is discussed and a general review on mordants is given. An overview on the most common natural dyestuffs, including the ones that were more relevant in the Arraiolos tapestries production, is also presented.

Wool fibres

Animal hair is built up of complex proteins. Sheep and goat hair are particularly relevant for textiles; wool is definitely the most commonly used animal fibre, although cashmere and mohair are significant for their market value[15,17].

Proteins (also known as complex polyamides) are linear polymers built of monomer units called amino acids. They are formed by attachment of an amino acid α-carboxyl group to the α-amino group of another amino acid with a peptide bond (also called an amide bond)[16-18]. The formation of the dipeptide is accompanied by the loss of a water molecule (*Figure 1*)[16,18]. The equilibrium of this reaction lies on the side of hydrolysis rather than synthesis. Hence, the biosynthesis of peptide bonds is an endoenergetic process. Nevertheless, peptide bonds are quite stable kinetically and can resist up to 1000 years in the absence of a catalyst[18].

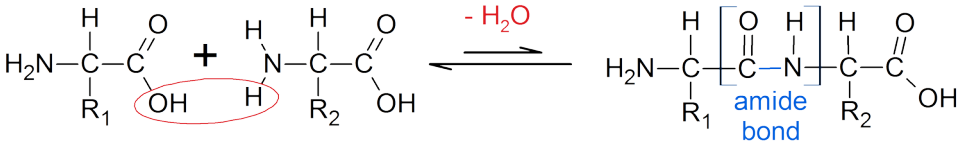


Figure 1. *Amide bond formation. The linking of two amino acids is accompanied by the loss of a water molecule. (Adapted from [16])*

The reversible interactions in biomolecules are due to three types of non-covalent bonds: electrostatic interactions, hydrogen bonds and van der Waals interactions, which vary in terms of geometry, strength and specificity. Moreover, they are strongly affected by the presence of water. Hydrogen bonds, although they are fundamentally weak electrostatic bonds, are fundamental to various biomolecules such as DNA and proteins[18].

In evaluating potential structures, Pauling *et al.*[19] considered which conformations of peptides were sterically allowed and which most fully exploited the hydrogen-bonding

capacity of the backbone NH and CO groups. The first of their proposed structures, the α-helix, is a rodlike structure. A tightly coiled backbone forms the inner part of the rod and the side chains extend outward in a helical array. The α-helix is stabilized by hydrogen bonds between the NH and CO groups of the main chain. In particular, the CO group of each amino acid forms a hydrogen bond with the NH group of the amino acid that is situated four residues ahead in the sequence[18,19].

Wool is a protein fibre and as such consists of the elements carbon, hydrogen, oxygen, nitrogen and sulfur[20]. It is essentially formed by the protein keratin (82%), which can present various forms, such as other animals hair, horns and nails[16,17,21]. Hard keratins, such as in wool, have a high sulfur content, mainly because of the incorporation of the double amino acid cystine. The remainder consists mainly of other proteins with a lower sulfur content[15]. Beside cystine, 20 other amino acid residues are found in wool[22]. Despite the fact that clean wool consists mainly of proteins, it also contains approximately 1% of non proteinaceous materials like fats, sterols and lipids, as well as a very small content in minerals and phosphorous[17,23]. Wool has therefore a different number of polypeptides with different molecular weights and different amino acid composition. Although total content of these amino acids in wool is known, their sequence in the protein is not. The relative amounts of different amino acids in wool depend on the part of the fibre analyzed, the species of sheep and their diet, and the influence of the effects produced by factors such as heat, humidity and light exposure. The structural components cuticle, cortex and medulla should also be considered. Despite these differences, wool keratin from different sources has been found to contain the same amino acids, with little quantitative variation (although most work has been carried out with Merino wool). Sulfur contents ranges from 2.7-4.1%[17,23].

In wool, individual polypeptide chains are joined together to form proteins by a variety of covalent (chemical bonds), called crosslinks, and non-covalent physical interactions[17,24] (*Figure* 2).

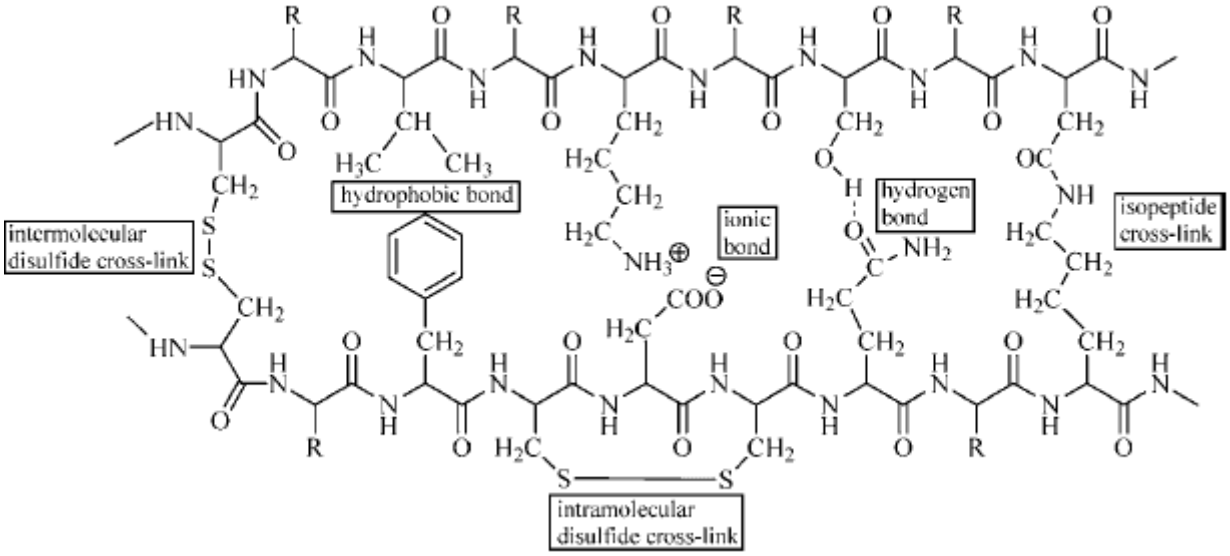


Figure 2. *Types of covalent and non-covalent bonds in wool*[17].

The most important of these interactions are the disulfide bonds[25]. They occur when pairs of cysteine residues form cystine (disulfide) linkages (—S—S—) between different polypeptide molecules, or between segments of the same molecules, as shown. They are important because they prevent movement of chains and chain segments and, thus, are responsible for the higher stability and lower solubility of wool fibres when compared with most proteins[16,17]. The carboxyl and amino groups in wool are also important because they give wool its amphoteric or pH buffering properties[16,24]. The ionic groups also control the dyeing behaviour of the fibre, as a result of their interactions with negatively charged dye molecules[24].

The ionic nature of the acidic and basic side-chains in wool leads to the formation of salt links between the protein chains[15,16,25]. Their formation is pH dependent, being at a maximum at the isoelectric point around pH 5.5 (*Figure 3*). This is the pH value at which the wool fibre has exactly the same number of cationic and anionic groups and is therefore electrically neutral. The work necessary to extend a wool fibre is at a maximum in the pH range from 5 to 9. In this pH range, the ionic salt links help to hold the protein chains together so that they resist elongation. The salt links cannot, however, exist under acidic conditions, when the anionic carboxylate groups are protonated (pH < 5), or under alkaline conditions, when the cationic ammonium ion groups are deprotonated (pH > 9). Wool contains about 820 mmol kg^{-1} of amino groups and a slightly lower number of carboxylic acid groups[15]. These are

responsible for its ability to absorb large amounts of alkalis and acids, and for dyeing processes involving ion exchange[15].

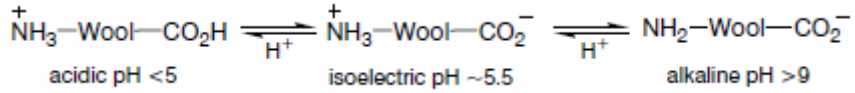


Figure 3. *Effect of pH on the structure of amino and carboxylic acid groups[15].*

Wool is a staple fibre from the fleece of various breeds of sheep. It is a multicellular, protein hair that tapers from the root to a point. Fibre lengths vary from 4 to 40 cm and diameters from 17 to 40 μm. Wool fibres have complicated structures. The main components are the scaly cuticle, the body of the fibre or cortex, and the cell membrane complex. The latter surrounds the cells of both the cuticle and cortex in a continuous phase. Coarse fibres may also have a medulla or inner core[15].

Microscopic examination shows the scales on the fibre surface that are characteristic of most animal fibres. The overlapping scales point towards the fibre tip[15,16]. The scales consist of several layers and are covered with a hydrophobic outer layer called the epicuticle. Below this hydrophobic epicuticle is the exocuticle, a cystine-rich component forming about two-thirds of the scale structure. The exocuticle just below the epicuticle is referred to as the A-layer, having a distinctly higher content of cystine (35%) than the rest of the exocuticle (B-layer, 15% of cystine residues)[25,26]. Below the exocuticle is the endocuticle. *Figure* 4 presents a diagram showing the typical structure of a wool fibre.

The cortex comprises the main bulk and determines many mechanical properties of the mammalian fibres. Cortical cells are long, polyhedral, and spindle-shaped and consist of intermediate filaments (microfibrils) embedded in a sulfur-rich matrix. The filament–matrix texture is organized into larger macrofibrillar units, and these are often observed in cortical cells[27]. Two different cellular textures are found in the cortex: ortho- and paracortex. They spiral around each other along the fibre, following the crimp, the orthocortex always being oriented towards the outside of the crimp wave. These two cortical regions differ in structure and reactivity. Basic dyes stain the more accessible orthocortex cells but acid dyes show no preference for either cortex[15].

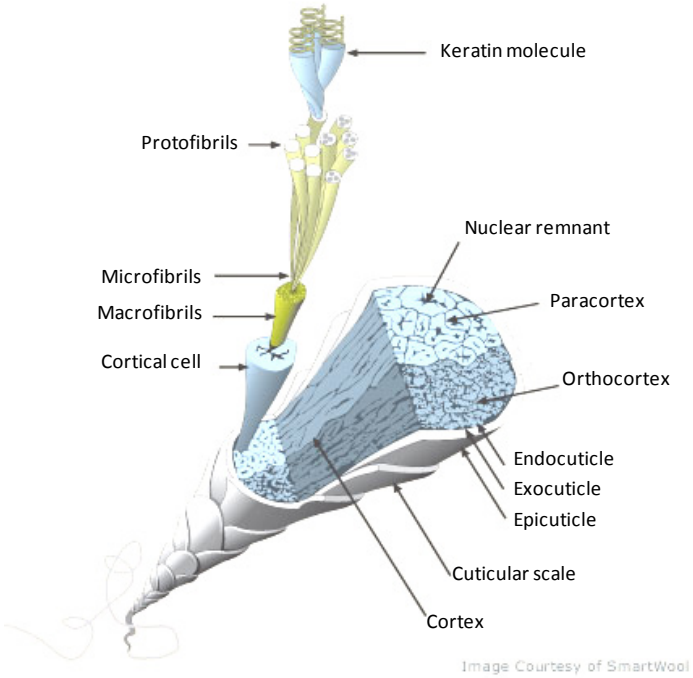


Figure 4. *Wool fibre structure*[28].

A continuous intercellular material is found between cuticle cells and cortical cells which, despite being a relatively minor fraction of the total fibre weight, is a material of increasing interest due to its presumed role in water and reagent penetration of fibres. The intercellular material and the apposing cellular membranes of cuticle and cortical cells encompass the cell membrane complex of fibres[17,27].

Wool is definitely a very complex fibre. Its morphology and chemical structure, as well as its physical properties, are of utmost importance for a thorough understanding of dyeing processes[29]. A model for dyeing based on transfer of the dye from the aqueous solution to the fibre surface, adsorption on the surface and diffusion into the fibre, seems simplistic on considering the complex morphology of the wool fibre. The wool cortex is far from being homogeneous. Different parts of a wool fibre have different degrees of dye absorption due to variations in permeability and chemical composition[15]. The three steps in dye transfer from an aqueous dyebath are[30]:

i) Diffusion of dye to fibre surfaces
ii) Dye transfer across the surfaces
iii) Diffusion of dye through wool fibre structures

Thorough liquor circulation during the dyeing process should eliminate the delays and uneven access to dyes implied in the first step. For the second step, the wool epicuticle acts as a surface barrier opposing dye penetration[15,30]. It is now accepted that dyes gain access to undamaged wool fibres mainly via junctions between wool cuticle scales[31]. They then diffuse throughout all the nonkeratinous regions, and also the endocuticle and intermacrofibrillar material regions of the cell membrane complex[32,33]. Hydrophobic lipids in the regions between the cuticle cells, where dye enters the fibre, are partly responsible for the observed dyeing barrier[15,30].

Dye then transfers from the cell membrane complex into the sulfur-rich proteins of the matrix that surrounds the cortical and cuticle cells, and finally into these cells themselves (*Figure 5*).

A further curious but informative observation from optical and electron microscope studies of dye location in wool fibres is that the initially favoured entry of dye through the cuticle scale junctions is also followed by dye transfer into the adjacent exocuticle and A-layer. The non-keratinous regions, which are so important in the early part of the dye cycle, are found to be almost totally devoid of dye at the completion of the dyeing process[30].

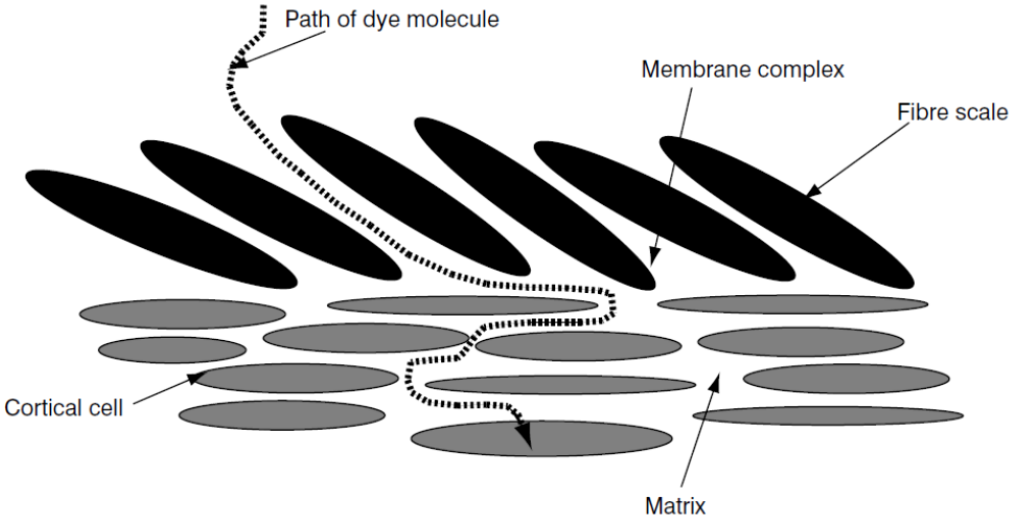


Figure 5. *Diffusion of dye molecules into the cortex of a wool fibre[15].*

Mordants

Dyes can bind to the surface of the fibre or be trapped within them. They are often bound to the textile with the aid of metallic ions known as mordants[34] (from the Latin verb

mordere, 'to bite')[35]. Mordants combine with the dye in such a way as to attach the colouring matter to the fibre by increasing affinity and/or strengthen interactions in some cases via a lasting chemical bond, sometimes making the colour stand fast against light and washing[36,37]. Mordants come from the salts of various metals, particularly aluminium, chromium, iron, copper, zinc or tin[37]. A mordant must have an affinity for the material being dyed and be chemically reactive; that is, the molecule must have free electrons that combine with the dyestuff[38].

Most of the natural dyes require some sort of mordant to set permanently in any fibre; these are called mordant dyes[34].

Mordants may be applied to the substrate either before, during, or after application of the dye. Pre-mordants, as the mordants applied before the dye are known, seem to have been the most commonly used in antiquity. Some mordants not only are involved in attaching the dye to the fibres but also alter the shade and even the hue of some dyes; a single dye often provides a range of hues when used with different mordants[39].

What is known for certain about ancient mordants is even less than what is known about dyes[36]. Alum, as a source of the aluminium ion, is an important historical mordant and was widely used in the past[34,40,41]. Potash alum (Aluminium potassium sulfate dodecahydrate, $KAl(SO_4)_2.12H_2O$) has been used since Greek and Roman times as a mordant to make vegetable dyes fast. In ancient Mesopotamia alum was used in tawing, dyeing, glass-making, washing, and in medicine[42].

Use of alum in the preparation of leather appears very early in the archaeological records. Analysis of the sheepskin lining of a Neolithic dagger sheath from Stade near Hamburg suggests that tawing may have been known in that area before the use of metals[43]. Tawed leather has been found from Predynastic times in Egypt. White and other coloured leather sandals are seen in tomb paintings from the New Kingdom which may have been fabricated by using alum[42]. Pliny and Dioscorides claimed Egyptian alum was superior to that found elsewhere, and both stated several types: white alum, for brighter colours and black alum, used for more sombre colours[43,44]. The former was presumably potash alum and the latter an alum with other metal ions[44]. Alum was used in preparing wool for dyeing with *hysginum* (a dark-red dye described by Pliny); other dyestuffs used with mordants included madder, kermes, saffron, orchil and weld[44].

The use of iron salts is reported for dyeing in black during the Middle Ages. To dye in black, the most common colourant materials were drawn from bark, roots, or fruits from

various trees: alder, walnut, chestnut, certain oak. By using a mordant of products rich in iron oxide (sometimes using mud or silt rich in iron salts), grey or brown tones were obtained that successive baths helped to make increasingly darker[45].

Natural dyestuffs

Natural dyes may be classified in several ways: method of application to textiles, chemical structure and colour.

Regarding the method of application to textiles, dyes can be classified as vat dyes, mordant dyes or direct dyes. Vat dyes (of which indigo and woad are the most important examples) are water-insoluble, but under reducing conditions, they can be converted into a 'leuco' form (soluble in alkaline aqueous solutions), which penetrates the fibres to be dyed. By exposure to air they are oxidised to their insoluble form, trapping the dye in the fibre[34,46,47]. Mordant dyes, as previously mentioned, require the treatment of the textile fibres with a mordant solution[46,47]. At last, direct dyes are applied directly to the fibre but frequently are less wash- and light-fast than vat or mordant dyes[46,47].

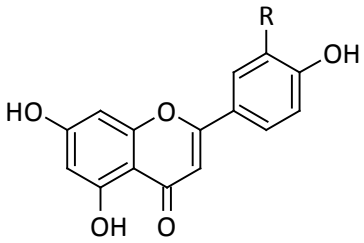
According to their chemical structure, natural dyes are commonly divided in the following categories: flavonoids, anthraquinones, homoisoflavonoids (or neoflavonoids), indigoids and tannins[47].

Yellow dyes

Yellow dyes, present in many herbs, are abundant all over the world in nearly all types of environments. In medieval to 19th century Europe, to supply the textile industry, some yellow dye plants were cultivated on a large scale, like weld (*Reseda luteola* L.). Others were massively collected in wild extensions of land, like dyer's greenweed (*Genista tinctoria* L.), sawwort (*Serratula tinctoria* L. Gaud.) or spurge flax (*Daphne gnidium* L.)[41,48]. The fruits of the different species of the *Rhamnus* spp. were also used for dyeing[47]. After the discovery of the New World, dyes were exported to Europe, among them the deep-yellow old fustic (*Chlorophora tinctoria* (L.) Gaud.) and quercitron bark (*Quercus velutina* L.)[49].

Weld, probably the oldest European-known yellow dye, is derived from the herbaceous plant *Reseda luteola* L., which is indigenous to central Europe[39,50]. The plant is inserted in the large group of flavonoid natural yellow dyes ('flavonoid' derives from the latin word *flavus*, meaning 'yellow')[46]. The main colouring components are the flavones luteolin and apigenin

(*Figure 6*), as well as some *O*-glycosides[34,51,52]. Dyeing with weld yields the purest and fastest shades of yellow. However, when used in conjunction with different mordants, it provides a variety of hues, that can range from green to brown[39,50]. Weld was also frequently used with the blue dyes woad or indigo to produce fast green hues[47].

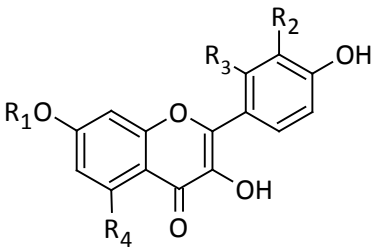


R=OH, luteolin
R=H, apigenin

Figure 6. *Flavones luteolin and apigenin, the main chromophores in weld.*

Flavonoid yellows are found in a variety of plants, including Persian berries (*Rhamnus* spp.), young fustic (*Cotinus coggygria* L.), and yellow wood (*Solidago virgaurea* L.). Other sources include onion skins (*Allium cepa* L.), marigold (*Chrysanthemum* spp.) or dyer's chamomile (*Anthemis tinctoria* L.)[34,41]. Flavonoid-based yellow chromophores can be divided in two large groups: flavones and flavonols. To the first group belong important chromophores such as the aforementioned luteolin and apigenin[34,53,54]. Some examples of flavonols are presented in *Table 2*: quercetin and kaempferol, major compounds in onion skins (*Allium cepa* L.) and quercitron bark (*Quercus velutina* L.); rhamnetin, from *Rhamnus cathartica* L.; morin, the yellow dye from old fustic (*Chlorophora tinctoria* (L.) Gaud.); fisetin, from young fustic (*Cotinus coggygria* L.) or sumac (*Rhus* spp.)[49].

Table 2. *Flavonols commonly found in flavonoid yellow dyes.*



R_1	R_2	R_3	R_4	Compound
H	OH	H	OH	Quercetin
H	H	H	OH	Kaempferol
CH_3	OH	H	OH	Rhamnetin
H	H	OH	OH	Morin
H	OH	H	H	Fisetin

Spurge flax (*Daphne gnidium* L.) is a common plant in the Mediterranean region. The plant is used in traditional hair and textile dyeing[55]. The composition of the main flavonoids and coumarins from this species seems to be quite well known: the flavonoids apigenin (*Figure 6*) and luteolin (*Figure 6*), together with some *O*-glycosides, and the coumarins daphnetin, daphnin (a glucoside of daphnetin) and daphnoretin (a di-coumarin) (*Figure 7*).

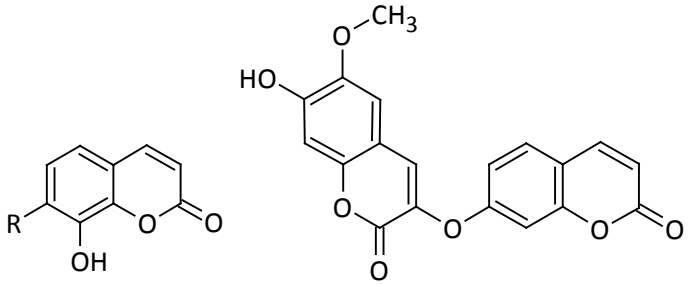


Figure 7. *Coumarins daphnetin, daphnin and daphoretin, from spurge flax.*

Saffron, a rare and costly dye in antiquity as well as in modern times, has been valued not only for dyeing textiles but also as a colouring and a fragrance in food. It is derived from the dried stigmas of the saffron plant, *Croccus sativus* L., a species of iris that grows naturally in central Asia as well as in the Spanish peninsula in Europe. When used as a direct dye, it gives a beautiful yellow orange colour and it can also be used with an alum mordant[40,47]. The colouring matter in saffron is the carotenoid crocin, a glucoside of crocetin (*Figure 8*). Because of its rarity and high cost, saffron was and still is often replaced or adulterated with the more common safflower and/or turmeric[39].

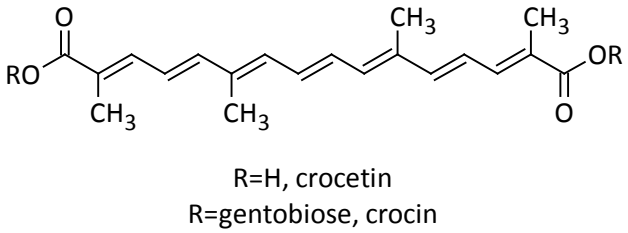
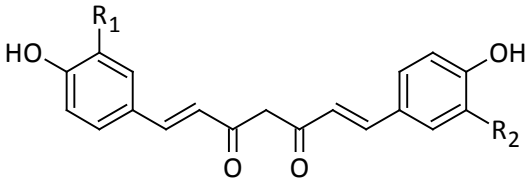


Figure 8. *Carotenoids crocetin and crocin, the main chromophores in saffron.*

Safflower, also known as 'bastard saffron', is a yellow dye that has been used for over three millennia, having been identified in fabrics from the Egyptian 12th Dynasty. It is derived

from the safflower plant, *Carthamus tinctoria* L., native to southern Asia and the Middle East. The colouring matter in the plants is a mixture of two components: one is yellow, known as safflower yellow B; the other, carthamin, is red. Safflower yellow B dissolves in water when fresh safflower flowers are washed with acidulated water[39].

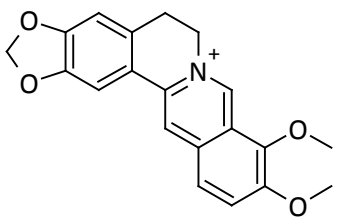
Turmeric, also known as curcuma, is an easily fading yellow direct dye that was used in Mesopotamia many centuries BCE and later became popular in ancient Rome. It is derived from the turmeric plant, *Curcuma longa* L., and other varieties of *Curcuma* indigenous to China and Southwest Asia. The main colouring matter in turmeric is a mixture of curcumin I, II and III[39,47] (*Figure 9*).



R_1=R_2=OCH_3, curcumin I
R_1=H, R_2=OCH_3, curcumin II
R_1=R_2=H, curcumin III

Figure 9. *Curcumins I, II and III, the main colouring compounds in turmeric.*

One of the first naturally occurring cationic dyes to be identified, berberine (*Figure 10*), occurs essentially in the stem, bark and roots of the barberry plant, *Berberis vulgaris* L.[56], a bush that grows indigenously in Europe as well as in North America. Silk and wool can be dyed directly with barberry root, yielding a yellow-green colour; however, for dyeing cotton, a mordant is required to attach the dye to the substrate fibres[39]. In the 16th century barberry plants were very popular in England as hedges, rather than as a source of vegetable dyes. At that time scarcely any other plant was used for dyeing purposes in England[56].



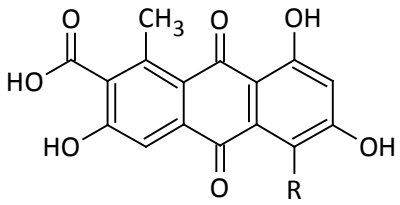
berberine

Figure 10. *Alkaloid berberine, the main chromophore in barberry plant.*

Red dyes

Quite a variety of red dyes of both vegetable and animal origin were used in antiquity, although only a few of them ever attained practical importance. Among these were madder, of vegetable origin, probably the most widely used and also the most important red dye, and kermes and cochineal, both derived from the bodies of insect red dyes[39].

Three red dyes of animal origin were known in the ancient world: kermes, cochineal, and lac; all three are derived from insects and were mainly used as mordant dyes. Kermes, which is scarlet red, is probably the earliest one on which there are records. Derived from the insect *Kermes vermilio* Planchon, which lives on oak trees of the species *Quercus coccifera* L.; it is widely distributed in Armenia, the Middle East, Northern Africa, and Spain. Kermes seems to have been the most important red dye known to the ancient Babylonians and was also used by the Egyptians, Greeks, Hebrews, and Phoenicians[39]. There are records of its usage in as early as 1727 BCE[49]. The major colouring matter in kermes are the anthraquinone compounds kermesic acid and flavokermesic acid[39,47] (*Figure 11*).

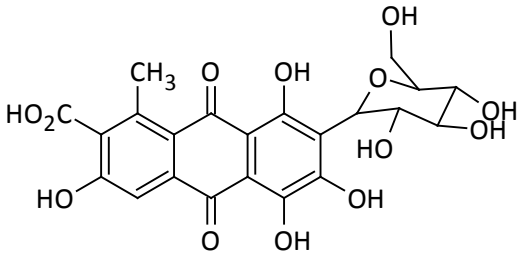


R=OH, kermesic acid
R=H, flavokermesic acid

Figure 11. *Anthraquinones kermesic and flavokermesic acid, the major colouring compounds in kermes.*

Cochineal, also derived from the bodies of insects, was known for many centuries to the pre-Columbian inhabitants of the Americas. Its native habitat seems to have been Mexico, although there is evidence that it was also used for dyeing in Peru during the Inca period. Cochineal was apparently unknown outside the American continent until the beginning of the 16th century, when, following the Spanish conquest of Mexico, it was brought to Europe. In ancient Mexico cochineal was extracted from the dried bodies of *Dactylopius coccus* Costa, a parasite of the cactus of the *Opuntia* family[39]. There also exists a Polish and an Armenian cochineal obtained from *Porphyrophora* spp., namely *Porphyrophora polonica* L. (Polish) and *Porphyrophora hamelii* L. (Armenian). Its use declined with the introduction of American

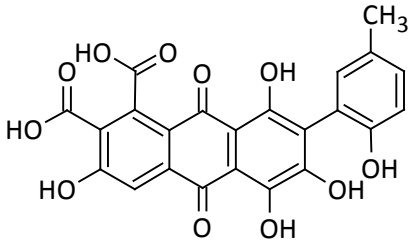
cochineal[47]. The colouring matter in cochineals is the anthraquinone carminic acid (*Figure 12*), but the various species have characteristic fingerprints of anthraquinone minor components, like some laccaic acids, kermesic acid and flavokermesic acid (*Figure 11*)[47].



carminic acid

Figure 12. *Anthraquinone carminic acid, the major constituent in cochineal.*

Lac is derived from lac resin, the hardened secretion of the lac insect (*Kerria lacca* Kerr), the only known resin of animal origin. The lac insect is a natural parasite of a variety of trees in large areas of southern Asia. The major constituents of lac are anthraquinones laccaic acid A and B[39,49,56] (*Figure 13*).



R=$CH_2NHCOCH_3$, laccaic acid A

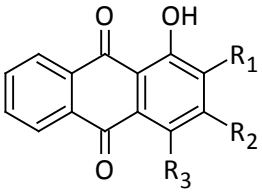
R=CH_2OH, laccaic acid B

Figure 13. *Anthraquinones laccaic acid A and B, from lac dye.*

Possibly, the most important dyestuff used since antiquity, was largely obtained from the roots of the madder plant[57]. Madder is a scarlet dye extracted from perennial herbaceous plants of the *Rubiacea* family, of which there are about 35 species[39]. A well-known plant from this family is *Rubia tinctorum* L., found naturally in Palestine and Egypt, abundant in Asia and Europe, and extensively cultivated in the ancient world[39,57]. The dyestuff is extracted from the dried roots of the plant[39,58]. Dyeing with madder yielded brilliant, permanent reds. When the dye was used in conjunction with different mordants, however, it provided a variety of hues[39], from pink to black, purple and red. The composition of the extracted anthraquinones

differs between the varieties of *Rubiaceae*. In *Rubia tinctorum* L., the major component forming the natural dye is alizarin (*Table 3*). Purpurin, xanthopurpurin, pseudopurpurin, and munjistin (*Table 3*) are also found in the roots of other species of the *Rubiaceae* family, such as Indian madder (*Rubia cordifolia* L.), wild madder (*Rubia pelegrina* L.) and the Himalayan species (*Rubia sikkimensis* Kurz)[47].

Table 3. *Anthraquinones commonly found in madder species.*

	R_1	R_2	R_3	Compound
	OH	H	H	Alizarin
	OH	H	OH	Purpurin
	H	OH	H	Xanthopurpurin
	OH	CO_2H	OH	Pseudopurpurin
	CO_2H	OH	H	Munjistin

Redwood dyes can be classified into two groups: soluble and insoluble redwoods. Soluble redwood dyes contain homoisoflavonoids as colouring principles and are readily soluble in water. They can be extracted from the bark of various species of the genus *Caesalpinia* (*C. sappan* L., *C. brasiliensis* L., *C. crista* L., *C. echinata* L.), which grows both in southern Asia and South America. Although there are several varieties of *Caesalpinia* species, they are frequently collectively known as brazilwood[47,59,60]. Brazilwood was known in Europe long before the discovery of South America because it was imported as a dyewood from the East Indies. Soon after the discovery of South America, large quantities of the valuable redwood were found in the forests along the Amazon[56], as recounted proudly by Camões in 1572, in his epic of the Portuguese people, '*Os Lusíadas*':

'Mas cá onde mais se alarga, ali tereis
Parte também, com o pau vermelho nota
De Santa Cruz o nome lhe poreis
Descobri-la-á a primeira vossa frota'
(Os Lusíadas, Canto X, 140)

'But where the land spreads broadest ye shall claim
The part that for its red wood is renowned
Of Santa Cruz ye shall bestow the name
Ye, by those fleet that land will first be found' [61]

This 'Santa Cruz' land, soon became called 'Brasil', due to the abundance of the redwood on its coast ('Brasil' derives from the Portuguese word 'brasa', suggesting 'red')[47,62]. The main

chromophores contained in these dyes are the homoisoflavonoids brazilin and brazilein (*Figure 14*). Logwood, native to South America, is extracted from the bark of *Haematoxylon campechianum* L. and is the only flavonoid dye to produce a dark colour (violet or brown or even black hues) if a suitable mordant is applied. It contains the homoisoflavonoids haematoxylin and haematein[63-65] (*Figure 14*). Homoisoflavonoids readily oxidize in air and are present in dyeing materials as redox couples, such as haematoxylin/haematein and brazilin/brazilein. They were used as mordant dyes[65,66] and for preparing lakes and inks. Brazilwood yields a red lake, whereas a dark ink is obtained from logwood[67-69].

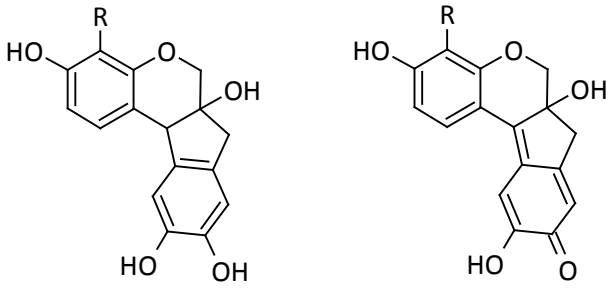


Figure 14. *Homoisoflavonoids from brazilwood and logwood.*

Insoluble redwood dyes are obtained from several hardwoods such as sandalwood (*Pterocarpus santalinus* L.f.) and narrawood (*Pterocarpus indicus* Willd.) from Asia, barwood (*Pterocarpus soyauxii* Taub. and *Pterocarpus erinaceus* Poir.) and camwood (*Baphia nitida* Lodd.), both from Africa, and were reported to be used in dyeing textiles as mordant dyes[41,47,70]. They yield much faster colours than the soluble class, but the colouring matter is scarcely soluble in water. They are mainly combined with other dyes to obtain dark red and brown colours[47]. The major colouring compounds are santalins and santarubins (*Figure 15*), condensed biflavonoids[71].

Blue and purple dyes

Blue is often found in the animal kingdom in butterflies wings, birds feathers and insect shells, but these colours are not extractable. Therefore, because of its scarcity, indigo was the most universally important blue dyestuff from prehistoric times. The earliest written occurrence of its use was probably in India around 2600 BCE and it is also mentioned in

Sanskrit writings from this time. It is certainly known that indigo was imported (probably from India) for the dyeing of mummy cloths in ancient Egypt, around 2300 BCE. Without it, there would have been practically no source of a permanent blue dyestuff until the invention of synthetic dyes in the late 19th century[57].

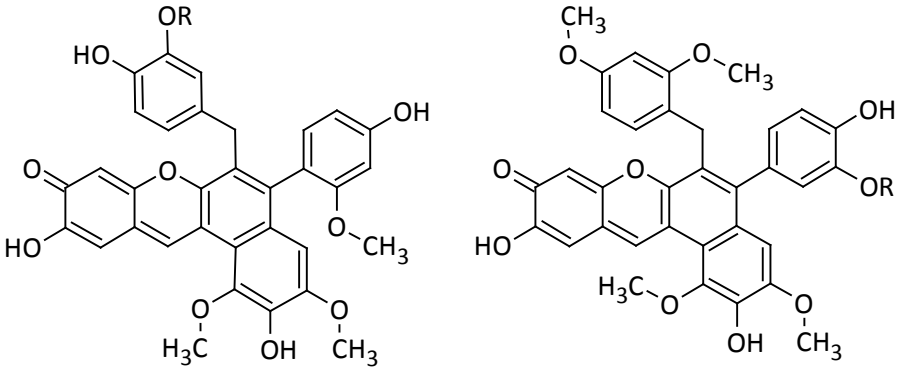


Figure 15. *Condensed biflavonoids santalin and santarubin, major colouring compounds in insoluble redwoods.*

Indigo is a vat dye that can originate from two different sources: indigo or woad. Indigo plants belong to the genus *Indigofera*, which grows naturally and was also cultivated in many areas of the world, e.g. Egypt, India, Brazil and Peru. The woad plant, *Isatis tinctoria* L., is native to Southeastern Europe, from where it spread quickly throughout Europe in prehistoric times. Although the dye in both types of plants is the same, namely, indigotin (*Figure 16*), in the *Indigofera* (indigo) plants the dye is about 30 times more concentrated than in *Isatis* (woad) plants. The dye is composed of a mixture of two different organic substances: indigotin, the major component, and small amounts of indirubin (*Figure 16*)[39]. When combined with red, yellow and brown dyes, produces purple, green and black colours[47].

Indigotin is not synthesised directly by the plant; it is a product derived from indole glucoside precursors, which are secondary metabolites. The indigo precursor, indoxyl (*Figure 16*), mainly in the form of the glucoside, indican (indoxyl-β-D-glucoside), is found in most indigo-producing plants. However, in *Isatis* spp., it was suggested that the precursor was the ester, isatan B (indoxyl-5-ketogluconate) rather than the glucoside, indican[57]. The hydrolysis of the glycosides leads to the indoxyl which either is oxidised to leuco-indigo (*Figure 16*), or

may be overoxidised to form isatin (*Figure 16*). Isatin may react with further indoxyl to form indirubin[60].

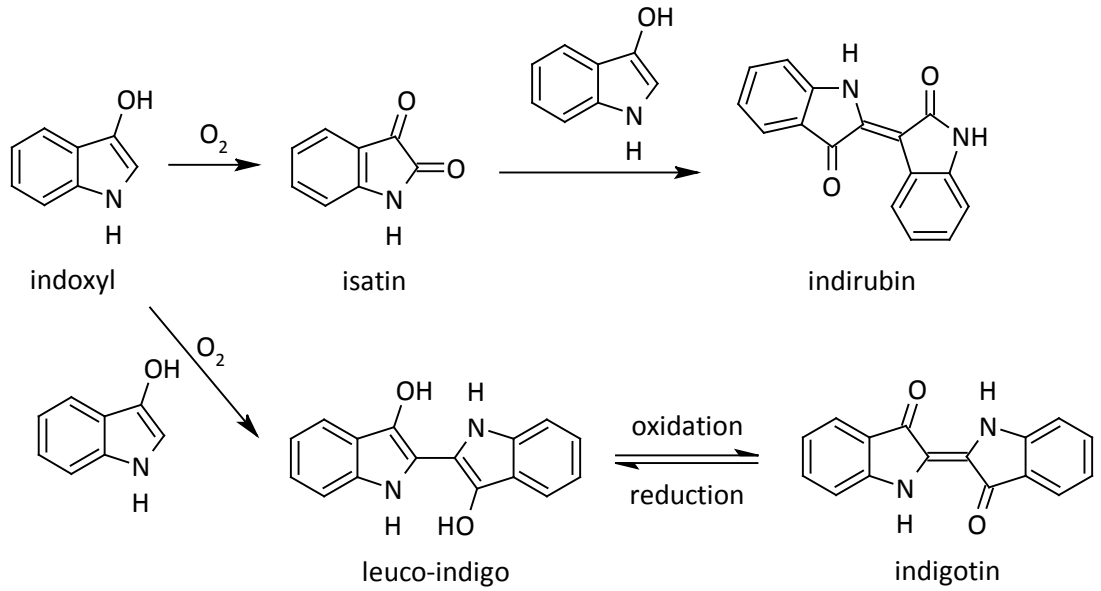


Figure 16. *Precursors and reactions leading to indigotin formation. (Adapted from [47])*

Two dyes were extensively used in antiquity for dyeing purple: Tyrian purple and orchil[39]. Tyrian purple, also known as royal purple or purple of the ancients, was undoubtedly, one of the most renowned and highly valued ancient dyes, which had a high standing as a symbol of wealth and distinction. Cloth dyed with Tyrian purple was very costly, and it seems that only royalty and priests could afford it[39]. The dye derives from the Mediterranean marine molluscs of the genus *Purpura* and *Murex*[49] (mainly *P. haemastoma* L., *M. brandaris* L. and *M. trunculus* L.). The dye occurs as the vat form of 6,6'-dibromoindigotin (*Figure 17*) in a colourless secretion contained in a small sac in the body of the molluscs[56]. When the secretion is exposed to oxygen in the air and to solar radiation, it acquires colour, turning first yellow, then green and finally purple[39].

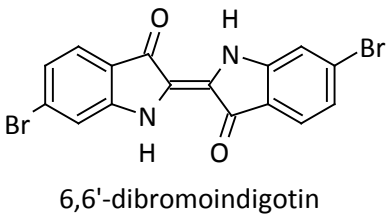


Figure 17. *6,6'-dibromoindigotin, the main chromophore in Tyrian purple.*

Orchil, also known as archil, lichen purple or 'poor person's purple', is derived, as the name suggests, from a variety of lichens[39]. Lichen dyes were occasionally used to produce 'false shelfish purples' and were obtained by fermentation of extracts from lichens of different species in the presence of ammonia and air[47]. The most important lichens were *Roccella tinctoria* D.C. and *Roccella fuciformis* D.C., which can be found on the French Atlantic coast, Holland, England and around the Mediterranean, as well as on the Azores, in South Africa (Cape Province), India and New Zealand[56]. The procedure for the ancient purple was to prepare a water extract with ammonia (urine) and to let the liquid stand in a warm place for several days[72]. During the resulting fermentation, depside or depsidone components were hydrolysed to orsellic acid, which, by further decarboxylation yields orsinol. Orsinol reacts by a sequence of condensation reactions incorporating nitrogen from ammonia, yielding several orcein derivatives. Depending on the pH and the presence of mordants, these can give bright red, purple or orange dyes[41,47].

Brown and black dyes

Brown and black dyes absorb light over a wide wavelength range and therefore their chemical structure is not easy to define[47]. Oak galls and sumac are well known mordant dyes for brown and black[47]. The chromophores derive from hydrolysable tannins which yield gallic acid (*Figure 18*) and sugars[60]. The galls are formed as a reaction of *Quercus infectoria* L. buds to the infection by the eggs of a *Cynips* spp. insect[47]. The buds are collected before the insect emerges, then dried and ground to produce a light-fast black dye when used with an iron mordant[47,60]. *Quercus infectoria* L. can be found in the Eastern Mediterranean and Asia Minor[47].

High levels of tannins are also found in the leaves of various sumac species (*Rhus* spp.)[60]. The species are indigenous to Europe, India, China, Japan, North Africa and America[47]. Sumac was known and used in Egyptian, Greek and Roman times[47]. Other hydrolysable tannins such as those derived from ellagic acid (*Figure 18*) are also found in the bark of oak species and chestnut tree[47]. Another important group are the condensed tannins which are polymers of catechin (*Figure 18*) and epicatechin (*Figure 18*) molecules[47] (from the bark of the oak species *Quercus robur* L.).

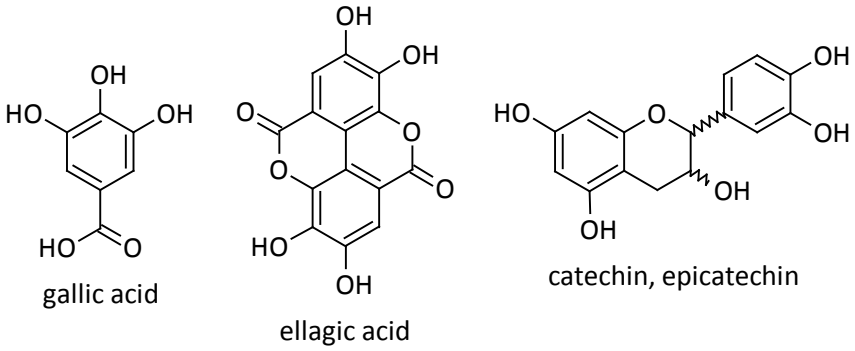


Figure 18. *Major chromophores in tannins.*

4. Analytical methods

High-Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) is nowadays the leading technique for chemical analysis and related applications, with an ability to separate, analyze, and/or purify virtually any sample[73]. Solvent is continually pumped throughout a column, and the separated compounds are continuously sensed by a detector as they leave the column. Optical detectors based on ultraviolet-visible (UV-Vis) absorption constitute over 70% of HPLC detection systems[73-75]. These detectors have a high sensitivity for many solutes, but samples must absorb in the UV (or visible) region (e.g., 190–700 nm)[73]. UV detectors come in three common configurations: fixed-wavelength detectors rely on distinct wavelengths of light generated from the lamp, whereas variable-wavelength and diode-array detectors (also known as photodiode-array detectors) select one or more wavelengths generated from a broad-spectrum lamp[73,75].

The mass spectrometry (MS) detector is the most popular hyphenated HPLC detector in use today. The detector interface is perhaps the most important factor in the successful application of mass spectrometry as an HPLC detection technique: MS detectors manipulate and detect ions in the gaseous phase, so for the MS to be useful as an HPLC detector, the mobile phase must be evaporated and sample ions must be generated[73]. The two most popular interfaces are electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI)[73].

The most widely used ionization technique in mass spectrometry today is ESI in which ions are created by electrically charging a flowing stream of liquid at atmospheric pressure, resulting in the emission of ions from the droplets in the subsequent spray[76] (*Figure 19*). Since the works attempting to adapt electrostatic spray techniques to mass spectrometry in the

early 1980s, the field has diverged in two general directions, high flow and low flow rate liquid introduction systems[76]. ESI is the most commonly used interface for bioanalytical applications because it is a 'softer' ionization technique and is less likely to cause undesirable analyte degradation[73].

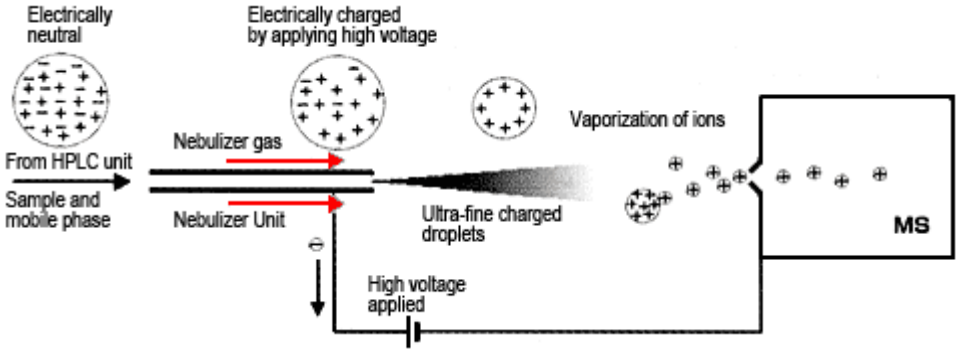


Figure 19. *ESI ionization. (Adapted from[77])*

APCI is a gas phase ionization process as opposed to the liquid phase ionization process of ESI. Ionization of the volatilized neutral analyte will only occur if it has sufficient gas phase basicity or acidity to extract or donate a proton from the reagent ion population present in great excess[76]. The APCI interface vaporizes the mobile phase first, and then uses a corona discharge to add a charge to the analyte in the gas phase[73]. The APCI technique is used for compounds that do not ionize well with ESI (often more stable, smaller molecular-weight compounds and some nonpolar compounds), but under harsher conditions, so it is more likely than ESI to cause sample degradation, especially with heat-labile compounds[73].

APCI and ESI have different ionization mechanisms, so the response and selectivity may vary significantly between the two interfaces[73]. Either interface can be operated in the positive- or negative-ion mode, resulting in the generation of positively or negatively charged sample ions[73].

Two designs of mass filters are predominant for LC/MS (single-stage) applications: quadrupoles and ion traps. Quadrupoles use a set of four rods and a carefully controlled electric field to isolate selected ions from the sample. Ions of a selected mass-to-charge ratio (m/z) are then passed to an electron multiplier for detection, providing a selective response for the desired analyte[73]. Additional selectivity can be provided when using a triple-quadrupole MS detector.

Ion traps use a ring electrode in combination with end-cap electrodes to accomplish the same isolation of desired ions, followed by detection. Multiple-stage fragmentation and the isolation of a preferred product ion occurs in the same physical space (vs. in different parts of the detector as in the triple quadrupole). The ions are sent to the electron multiplier for detection or the fragmentation process can be performed over and over, isolating and breaking ion fragments into successively smaller fragments[73]. This is useful for structural identification, but historically the ion trap has not been as good for quantitative work as the quadrupole because of space-charge effects (ion interactions within the detector) and variability in the output signal intensity. Thus quadrupoles (single and triple) tend to be more widely used for routine quantitative work, whereas ion traps are preferred when structural identification is needed[73], as is the case in historical artefacts.

Flame Atomic Absorption Spectroscopy (FAAS)

Atomic absorption is the process that occurs when a ground-state atom absorbs energy in the form of electromagnetic radiation at a specific wavelength and is elevated to an excited state[74]. In flame atomic absorption spectroscopy (FAAS) an aqueous solution of the sample is aspirated in a flame at high temperature and atomised by the process. The flame employs a premix burner in which the fuel, oxidant and sample are mixed before being introduced into the flame. The most common fuel-oxidiser combination is acetylene and air, producing a flame temperature of 2400–2700 K. A nebuliser creates an aerosol of the liquid sample by passing the liquid through a capillary[78].

Radiation characteristic of a particular element is passed through an atomic vapour of the sample[79]. The cathode of the hollow cathode lamp is made from the same element that is being determined[78]. The amount of light absorbed by the sample is proportional to the concentration of the element in the solution. Each element present in the sample has to be measured separately, so the technique is relatively slow.

Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Analysis by optical emission spectroscopy (OES) constitutes a general method for measuring elements on the study of the radiation emitted by their atoms present in an excited state, usually following ionization. The light source of the spectrometer is nothing

other than the sample of which all the atoms have been excited[80]. This marks an important difference from atomic absorption spectroscopy (AAS) in which measurements can only be made for elements for which the instrument has been customized (choice of the hollow cathode lamp). From a single run, a multielement analysis can be obtained in strict contrast to AAS[80].

When a chemical element in the atomic state is exposed to appropriate conditions of excitation, it emits a characteristic radiation. On this observation is based a general form of elemental analysis, both qualitative and quantitative. This optical emission spectroscopy issued from samples is very complex and leads to spectra with thousands of spectral lines accompanied by a continuum background. The instruments designed for these analyses comprise several parts: the device responsible for bringing the sample in the form of excited or/and ionized atoms (based on gas plasmas, sparks or lasers), an high quality optical bench which conditions the analytical performances, a detector with a sensor and finally a computerized section essential to control the entire instrument[80].

Several procedures are used to break down the samples into their constitutive elements, most notably by the effect of high temperature plasmas, spark sources or glow discharges. The emission instruments require very high quality optics to resolve interferences from both spectral lines and matrix effects[80].

The most common instruments for OES contain a plasma source that can reach temperatures of up to 8000 K. A plasma is a low pressure gas which is neutral overall, but which contains unbound negative electrons and positive ions. It is the fourth state of matter, along with solids, liquids, and gases. More than 99% of matter in the universe is thought to exist as plasma[81]. Many different plasma sources exist, but by far the most common is the inductively coupled plasma (ICP). The ICP is generated by coupling the energy from a radiofrequency generator into a suitable gas via a magnetic field which is induced through a two- or three-turn, water-cooled copper coil. Two gas flows, usually argon, flow in a tangential manner through the outer tubes of a concentric, three-tube quartz torch which is placed axially in the copper coil (*Figure 20*). Because the outer and intermediate gases flow tangentially (i.e. they swirl around as they pass through the torch), the plasma is continually revolving and has a ‘weak spot’ at the centre of its base, through which the carrier gas, containing the sample, can be introduced. When the gas is seeded with electrons, usually by means of a spark, the electrons accelerate in the magnetic field and reach energies sufficient to ionize gaseous atoms in the field. Subsequent collisions with other gaseous atoms causes

further ionization and so on, so that the plasma becomes self-sustaining. This occurs almost instantaneously. The magnetic field causes the ions and electrons to flow in the horizontal plane of the coil, thereby heating the neutral argon by collisional energy exchange, and a hot fireball is produced[82].

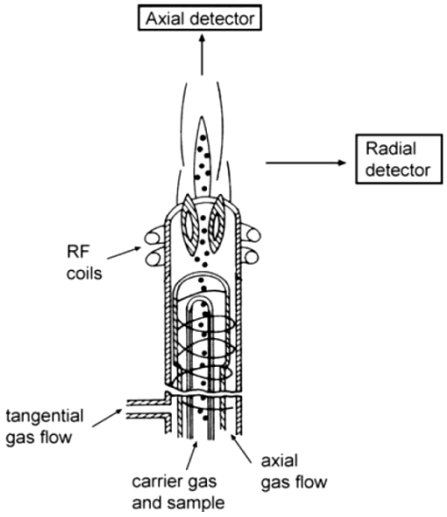


Figure 20. *Schematic diagram of an ICP torch[81].*

Inductively coupled plasma mass spectrometry (ICP-MS) is the association of two well established techniques, namely the inductively coupled plasma and mass spectrometry. The ICP has been described as an ideal ion source for inorganic mass spectrometry. The high temperature of the ICP ensures almost complete decomposition of the sample into its constituent atoms, and the ionization conditions within the ICP result in highly efficient ionization of most elements in the Periodic Table[82]. ICP of high energy generates charged ions from the atoms of the elements present in the sample. The ions generated are directed onto a mass spectrometer, separated, and measured according to their mass-to-charge ratio. The method is highly sensitive, and the detection limits for some metals may be 100 times lower than those obtained by graphite furnace atomic absorption technique[74].

Scanning Electron Microscopy (SEM)

In modern materials science, structural characterisation is basic for a thorough understanding of the nature of a material. The microscope has played an important part in formulating structural concepts in materials[83]. The scanning electron microscope (SEM) is one of the most widely used instruments in materials research laboratories today. SEM

provides information relating to topographical features, morphology, phase distribution, compositional differences, crystal structure, crystal orientation, and the presence and location of electrical defects[84].

The strength of the SEM lies in its inherent versatility due to the multiple signals generated, simple image formation process, wide magnification range, and excellent depth of field. SEM technique involves irradiating a sample with a fine beam of electrons. When the electron beam impinges on the specimen, the primary electrons can be reflected (backscattered electrons) or, alternatively, can produce secondary electrons or X-rays. Backscattered and secondary electrons produce the image of the sample, whereas the characteristic X-rays can be used to determine the nature and concentration of the elements present in the specimen[85]. The SEM electron beam is a focused probe of electrons accelerated to moderately high energy and positioned onto the sample by electromagnetic fields. An optical column is utilized to ensure that the incoming electrons are of similar energy and trajectory. These beam electrons interact with atoms in the specimen by a variety of mechanisms when they impinge on a point on the surface of the specimen[84].

The SEM is also capable of determining elemental composition of samples if coupled with an X-ray or electron spectrometer. When the surface of a sample is bombarded by the SEM's electron beam, electrons are ejected from the atoms orbital shells. The resulting electron vacancies are filled by electrons from higher energy orbitals, and an X-ray photon with characteristic energy and wavelength is emitted. Energy-dispersive X-ray spectroscopy (EDS) allows the measurement of that characteristic energy, thereby providing information about the chemical elements present in the surface of the sample[86].

Particle-Induced X-Ray Emission (PIXE)

Particle-induced X-ray emission (PIXE) is an elemental analysis technique that employs a Mega-electron-Volt energy beam of charged particles to induce characteristic X-ray emission from the inner shells of atoms in the sample[87]. The highly focused beam of protons is produced by a van de Graaff accelerator, which can be focused and steered just like an electron beam. A beam diameter of the order of microns is possible, giving spatial resolution similar to that obtainable by electron beam microanalysis[81]. The proton beam strikes the sample, producing inner shell vacancies, which, as before, may de-excite via emission of characteristic X-rays, which are almost always detected in the energy-dispersive mode using a Si(Li) spectrometer. The major advantage of this instrument over the electron microprobe

is that protons, being heavier and accelerated to a higher energy than electrons, tend to suffer less energy loss on their passage through the sample, giving rise to less *bremsstrahlung* ('braking radiation') and therefore producing a lower X-ray background[81].

In the proton microprobe, magnetic or electrostatic quadrupole lenses are employed to focus the beam to micrometer spot size; this makes it possible to perform μ-PIXE analysis of very small features and, also, by sweeping this microbeam along a preselected line or over an area of the specimen, to determine element distributions in one or two dimensions in a fully quantitative manner[87].

Colourimetry

Colour is a subjective phenomenon that makes the description of colour differences or comparisons between colours a complicated issue from a quantitative point of view[88]. However, much effort has been given to the development of colourimetric analysis, which allows a quantitative description of colour. Colourimetry provides a more precise way to define colour rather than spectrophotometry, as the human eye's sensitivity to light across the visible region is measured and a numerical description of colour is given[88].

There are three attributes that are used to describe colour. The first identifies a colour by the wavelength associated to it, and is known as hue. The second attribute relates to the level of white and/or black and is known as saturation. The third attribute is the brightness of the colour[88].

Many colour models have been developed to monitor some specific task in colour processing. Comission Internationale d'Eclairage (CIE) proposed, in 1931, a worldwide standard model for colour measurement, the CIE XYZ, based in the colour coordinates of primary colours. The model was revised in 1976, and the designation CIE $L^*a^*b^*$ was adopted. This model has been universally used to measure colour parameters and uses the following spatial coordinates in the Cartesian system: L^* for brightness, ranging from 0 (black) to 100 (white) and a^* and b^* coordinates, in which a^* ranges from green (-a) to red (+a) and b^* from blue (-b) to yellow (+b)[89].

Literature review on methods for the analysis of wool fibres, mordants and natural dyes

Wool fibres

The classical approach to analyse textiles is via optical and electron microscopy. Optical microscopy readily reveals the handedness of spun yarns. The shape of fibres allows for the discrimination between animal and plant fibres. Some characteristics of particular fibre types are also optically visible with the help of polarised light. The next step is a shape investigation in more detail using scanning electron microscopy (SEM) images[90].

Microscopy techniques have been essentially used for morphology studies and damage assessment in wool fibres. One of the first published studies concerning the use of SEM is the work by Hock and McMurdie[91], in 1943, in which the wool fibre structure under electron microscopy is presented. In the following years, several studies were published regarding the use of microscopy in wool fibre morphology. Wortmann *et al.*[92-94] and Langley and Kennedy[95] have used SEM to distinguish between wool and specialty fibres (often blended with wool). Haly[96], using optical microscopy (OM) and SEM, investigated transverse striations in extended wool fibres, which could be related to the unfolding of α-keratin into β-keratin during mechanical yield. Light transmission microscopy was used by Zhao *et al.*[97] to investigate structural modification and damage to the cuticle layer of wool fibres. Transmission electron microscopy (TEM) was used for cortex analysis and examination of longitudinal sections of wool merino fibres[98,99].

Regarding chemical composition of wool, energy-dispersive spectroscopy (EDS) and X-ray photoelectron spectroscopy (XPS) have been the preferred techniques. Carr *et al.*[85] used EDS combined with SEM and TEM to investigate cystine and tyrosine distribution in the morphological components of the wool fibre, showing that paracortical cells were richer in sulfur than orthocortical cells, with the opposite result for tyrosine content. Tillin *et al.*[100] used XPS to compare the concentration of available reaction sites between the epicuticle and the cortex of wool fibres, concluding that the surface of intact wool fibres is more hydrophobic than the bulk of the fibre. Carr *et al.*[101,102] used XPS to determine the sulfhydril content at the surface and epicuticle of wool fibres.

For structural information, laser Raman microscopy was used for investigation of the constituent proteins of several keratins, including wool[103].

Wool fibre investigations in cultural heritage studies are essentially about fibre identification and damage assessment.

D'Orazio *et al.*[104] have used SEM for the morphological analysis of textile fibres from textile artefacts recovered in the ancient cities around Vesuvius. Joosten *et al.*[105] have employed SEM for accessing the condition of textile fibres from archaeological textile fragments collected in the pre-historic salt mine of Hallstatt. In most of the samples, typical wool scales were severely degraded. Archaeological fibres from textile fragments excavated in the Cave of Letters, in the Dead Sea region, were investigated by a combined approach using microscopy (OM and SEM) and synchrotron radiation microbeam diffraction and microfluorescence. Most of the fibres were identified as wool[106]. OM, SEM and thermogravimetric analysis were used by Abdel-Kareem and El-Nagar to evaluate the deterioration extent of Coptic Egyptian textiles[107].

Raman spectroscopy has been used for fibre identification and damage assessment in highly degraded wool fibres from the Qumran textiles[108].

Thermoanalytical techniques like differential scanning calorimetry (DSC) and dynamic mechanical thermal analysis (DMTA) have also been used to evaluate the mechanical properties and thermal stability of wool threads from historical tapestries from European royal palaces and museums[109].

Mordants

For mordant characterisation in textile samples, scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDS) remains the most commonly used technique. A series of works by Indictor and Koestler concerning mordant detection in textiles has been published[110-113]. Contemporary wool[111,112], silk[112] and cotton[112] samples, and historical silk threads[110,113] were analysed for mordant identification. Atomic absorption quantification of the mordants in historical samples was also reported[111]. SEM-EDS has been used for mordant identification in several historical textile samples including the Coptic textiles[107], wool fibres from Hallstatt[105] and even in wool fibres from ancient Arraiolos tapestries[2].

Williams and Indictor have used particle-induced X-ray emission (PIXE) to detect Al, Fe, Cu, Sn and Cr on cotton and wool dyed using alum, iron (II) sufate, copper (II) sulfate, tin (II) chloride and potassium dichromate as mordants. The results were generally equivalent to

those obtained by SEM-EDS[114]. Vodopivec and co-workers also used PIXE to determine elemental composition in a lace fragment found in a 17th century Slovenian tomb[115].

The use of X-ray fluorescence (XRF) technique for mordant identification has also been proposed[116,117].

The extremely low detection limits of induced coupled plasma optical emission spectroscopy (ICP-OES) and mass spectrometry (ICP-MS) make these two techniques extremely suitable for the quantification of metallic ions in historical textile fibres. Aydin quantified several elements in natural undyed Turkish wool samples by ICP-OES[118], whereas Pranaytitė[119] and Dussubieux[120] reported the use of ICP-MS technique for the quantification of metals in contemporary Chinese textiles and in archaeological textile fibres, respectively.

Natural dyes

Over the past decades, several analytical techniques and procedures have been developed for the characterisation and identification of organic dyes in textiles, but the analysis of dyestuffs in historical samples remains a challenging task, mainly due to the complexity of their chemical composition and the possible presence of mixtures of chromophores and degradation products[121].

The first step in the analysis of an historical textile piece is a visual and photographic inspection; this approach is particularly useful when dealing with coloured objects. Colourimetric techniques have been extensively used for monitoring and evaluating the rates of fading of organic dyes[121-123]. Spectrophotometric techniques such as visible, UV-reflectance and UV-fluorescence spectroscopy, and infrared photography may guide selective sampling for further analysis, thus enhancing critical data acquisition while minimizing any damage to the artefact[124,125].

UV-Vis spectroscopy has been used for dye identification in heritage textiles[117,126-129]. UV-Vis reflectance techniques (such as fibre optics reflectance spectroscopy, FORS) allowed in some cases for the non-invasive identification of dyestuffs[130,131].

Fluorescence spectroscopy offers higher sensitivity and selectivity in a non-destructive way. Fluorescence and three-dimensional fluorescence have been used as tools for the study of natural dyes used in historical samples of dyed textiles[132-134].

It is also possible to analyse dyes on historic textile fibres using Fourier transform infrared (FTIR) spectroscopy. FTIR and micro-FTIR spectroscopy have rarely been applied to the identification of specific dyestuffs in textiles[135-137].

Conventional dispersive Raman spectroscopy has been a popular technique for studying dyes on textiles[125,138-146]. Many dye chromophores produce resonance Raman effects so even very low concentrations of dye can be detected on textile fibres. Dyes including indigo, purpurin, alizarin and madder, have been successfully detected using Raman techniques. It has also been possible to characterise indigo from different sources[142,145,146].

The increase in Raman signals through surface-enhanced Raman scattering (SERS) has opened up new possibilities and enhanced the interest in the Raman study of organic materials in cultural heritage[121,147]. SERS offers the opportunity to obtain Raman spectra from highly fluorescent materials, thus making the application of Raman to pigments and dyestuffs extremely promising[148,149].

Because organic dyes are a mixture of chromophores, their identification in the field of cultural heritage is mainly based on the application of micro-destructive methods entailing a solvent extraction of the chromophores from the textile fibres, followed by separation techniques[60,121].

The most commonly used chromatographic technique for the identification of dyes in textiles is definitely high-performance liquid chromatography (HPLC), although there are several studies on the use of thin-layer chromatography (TLC) for the identification of dyes in textiles of historical interest[117,150-157]. For many years, UV-Vis spectrophotometry was the main detector used for HPLC of textile dyes. More recently, diode-array (DAD) and mass spectrometry (MS) detectors have been introduced, thus significantly improving the detection limits of the procedures and leading to the identification of previously unknown components in complex dyes[69,158-184].

Information available through DAD detection can be improved by the use of complexation agents, as shown in Surowiec *et al.*[185]. Fluorimetric detection has also been considered[165,186].

Recent works have pointed out the need for a thoughtful optimization of mass spectrometric and chromatographic parameters in order to allow for better ionization yields and ultimately for lower detection limits[2,181]. An example of the optimization of instrumental parameters is given by Rafaëlly *et al.*[181], who studied anthraquinoid compounds. The optimization was carried out by adjusting source-dependent parameters, mobile-phase composition, and testing post-column additives. An example of the optimization of detection modes is given by the work of Surowiec *et al.*[187], who compared selected ion monitoring (SIM) and multiple reaction monitoring (MRM) acquisition modes, in both positive and negative

ion modes. MS detection is also able to provide a fragmentation pattern, essential for the identification of natural dyes in historical artefacts.

Very few reports have been published concerning the utilization of gas chromatography (GC) for natural dyes identification purposes. GC/MS has been used in the study of natural dyes, by derivatisation of the components of the fibre extract[188-190]. Pyrolysis GC/MS (Py–GC–MS) has also been applied to the analysis of dyes[191,192].

Capillary electrophoresis (CE) has seldom been used for the determination of organic dyes. The separation of anthraquinones is described in Puchalska *et al.*[193], and an analysis of anthraquinones, flavonoids, and gallic acid is discussed in Surowiec *et al.*[194] and Trojanowicz *et al.*[195].

Few studies have been conducted on natural dyes using secondary ion mass spectroscopy (SIMS)[196,197]. Lee *et al.*[197] employed time-of-flight SIMS (ToF-SIMS) in the study of some reference molecules, contemporary dyed silk and samples from historical textiles.

5. Experimental

Materials and reagents

Table 4 presents the list of reagents used in the entire work along with the specifications from the suppliers.

Table 4. *List of reagents and specifications.*

Reagent	CAS Registry Number	Purity (% or grade)	Supplier
Acetone	67-64-1	p.a.	Vaz Pereira (Portugal)
Acetonitrile	75-05-8	For LC-MS	Merck (Darmstaadt, Germany)
Alizarin	72-48-0	For microscopy	Fluka (Buchs, Switzerland)
Alum (Aluminium potassium sulfate dodecahydrate)	7784-24-9	98.0	Kremer Pigmente (Aichstetten, Germany)
Aluminium standard	-	For ICP	Fluka (Buchs, Switzerland)
Aluminium standard	-	For ICP	Merck (Darmstaadt, Germany)
Apigenin	520-36-5	99.0	Fluka (Buchs, Switzerland)
Brazilin	474-07-7	For microscopy	Fluka (Buchs, Switzerland)
Carminic acid	1260-17-9	96.0	Fluka (Buchs, Switzerland)
Copper (II) sulfate pentahydrate	7758-99-8	99.0	Himedia Laboratories (Mumbai, India)
Copper standard	-	For AAS	Panreac (Barcelona, Spain)
Copper standard	-	For ICP	Merck (Darmstaadt, Germany)
EDTA disodium salt dihydrate	6381-92-6	99.0	Sigma-Aldrich (Milwaukee, WI, USA)
Formic acid	64-18-6	98.0	Merck (Darmstaadt, Germany)
Haematein	475-25-2	For microscopy	Fluka (Buchs, Switzerland)
Haematoxylin	517-28-2	For microscopy	Sigma (St. Louis, MO, USA)
Hydrochloric acid	7647-01-0	37.0	Panreac (Barcelona, Spain)
Indigotin	482-89-3	95.0	Fluka (Buchs, Switzerland)

Reagent	CAS Registry Number	Purity (% or grade)	Supplier
Iron (II) sulfate heptahydrate	7782-63-0	99.0	Scharlau Chemie (Barcelona, Spain)
Iron standard	-	For ICP	Fluka (Buchs, Switzerland)
Iron standard	-	For ICP	Merck (Darmstaadt, Germany)
Luteolin	491-70-3	98.0	Sigma (St. Louis, MO, USA)
Luteolin 7-*O*-glucoside	5373-11-5	98.0	Extrasynthése (Genay, France)
Methanol	67-56-1	For LC-MS	Merck (Darmstaadt, Germany)
N,N-dimethylformamide	68-12-2	99.8	Panreac (Barcelona, Spain)
Nitric acid	7697-37-2	65.0	Panreac (Barcelona, Spain)
Oxalic acid	144-67-2	99.0	Riedel-de-Haën (Seelze, Germany)
Purpurin	81-54-9	90.0	Eastman Organic Chemicals (Rochester, NY, USA)
Pyridine	110-86-1	99.0	Fluka (Buchs, Switzerland)
Quercitrin	522-12-3	98.5	Extrasynthése (Genay, France)
Sodium dithionite	7775-14-6	85.0	Panreac (Barcelona, Spain)
Sodium hydroxide	1310-73-2	97.0	Akzo Nobel (Amsterdam, The Netherlands)
Zinc standard	-	For ICP	Merck (Darmstaadt, Germany)

Water purified by a Millipore Simplicity UV system (Billerica, MA, USA) was used for sample preparation and all the analyses. Weld (*Reseda luteola* L.), cochineal (*Dactylopius coccus* Costa), woad (*Isatis tinctoria* L.), brazilwood (*Caesalpinia* spp.), madder (*Rubia tinctorum* L.) and logwood (*Haematoxylum campechianum* L.) were purchased from Kremer Pigmente (Aichstetten, Germany). Undyed industrial Arraiolos sheep wool was acquired from Rosarios4 (Mira de Aire, Portugal).

Arraiolos tapestries sampling

Thirteen Arraiolos historical carpets were selected from the National Museum of Ancient Art (NMAA, Lisboa, Portugal) collection (17^{th}-19^{th} century period). Different portions of embroidery wool yarns between 3 and 5 mm with the same hue were sampled in distinct parts of the carpets with fine point tweezers and spring bow scissors from thread ends in damaged areas or in the back side. Samples were collected and individually stored in E*ppendorf* tubes protected from the light.

Figure 21 and *Table 5* show fibre sampling location, identification and colour for the 17^{th}-18^{th} century Arraiolos carpets. *Figure 22* and *Table 6* show fibre sampling location, identification and colour for the 18^{th}-19^{th} century Arraiolos carpets.

Figure 21. *Arraiolos carpets from 17th-18th century period (Tapestries A, E, F, G, I, J and L) with fibre sampling location.*

Table 5. *Fibre identification and colour for the Arraiolos carpets from 17th-18th century period.*

ID	Sample	Colour
Tapestry A (NMAA 88)	A1	Bl
	A2	Gr
	A3	Gr
	A4	Rs
	A5	R
	A6	R
	A7	Y
	A8	Bl
	A9	W
Tapestry F (NMAA 25)	F1	Y
	F2	Y
	F3	Bl
	F4	Rs
	F5	Br
	F6	Bg
	F7	Bg
	F8	Bl
	F9	Bl
	F10	Gr
Tapestry I (NMAA 114)	I1	Y
	I2	W
	I3	Bl
	I4	Bl
	I5	Gr
	I6	Bl
	I7	O
	I8	Gr
	I9	Y
	I10	Br
	I11	Br
	I12	Br
	I13	Gy
Tapestry L (NMAA 89)	L1	W
	L2	Gr
	L3	Bg
	L4	Y
	L5	O
	L6	O
	L7	R
	L8	R
	L9	Rs
	L10	Rs
	L11	Gr
	L12	Gr
	L13	Gr
	L14	Br
	L15	Bl
	L16	Bl
	L17	Bl
	L18	Bl
	L19	Bl

ID	Sample	Colour
Tapestry E (NMAA 74)	E1	Bl
	E2	Gr
	E3	Gr
	E4	Y
	E5	Y
	E6	R
	E7	Bl
	E8	O
	E9	Rs
	E10	Bk
	E11	Gy
	E12	Gy
	E13	O
	E14	Bg
Tapestry G (NMAA 55)	G1	OBr
	G2	Y
	G3	Bg
	G4	O
	G5	Bl
	G6	Gr
	G7	Y
	G8	Bl
	G9	Bg
Tapestry J (NMAA 19)	J1	Y
	J2	W
	J3	Bl
	J4	Bl
	J5	Bl
	J6	Br
	J7	Gr
	J8	Br

Bg beige, *Bk* black, *Bl* blue, *Br* brown, *Gr* green, *Gy* grey, *O* orange, *OBr* orange-brown, *R* red, *Rs* rose, *W* white, *Y* yellow

Figure 22. *Arraiolos carpets from 18th-19th century period (Tapestries B, C, D, H, M and N) with fibre sampling location.*

Table 6. *Fibre identification and colour for the Arraiolos carpets from 18th-19th century period.*

ID	Sample	Colour
Tapestry B (NMAA 38)	B1	Br
	B2	Y
	B3	Y
	B4	Bg
	B5	Bg
	B6	Gr
	B7	Bg
	B8	Gr
	B9	Bl
	B10	Bl
	B11	O
	B12	Gr
Tapestry D (NMAA 36)	D1	Bk
	D2	Br
	D3	Y
	D4	LBr
	D5	Gr
	D6	W
	D7	Gr
	D8	Gr
	D9	Bl
	D10	Bl
	D11	Bl
	D12	Y
	D13	Bg
	D14	Y
Tapestry M (NMAA 24)	M1	Y
	M2	Bl
	M3	Gr
	M4	Bl
	M5	Y
	M6	W
	M7	Bl
	M8	Bl

ID	Sample	Colour
Tapestry C (NMAA 29)	C1	Y
	C2	Y
	C3	W
	C4	Bg
	C5	Gr
	C6	Bl
	C7	Gr
	C8	Bl
	C9	Bl
	C10	Bl
	C11	Gr
Tapestry H (NMAA 40)	H1	Y
	H2	Br
	H3	Bl
	H4	Gr
	H5	Gr
	H6	Gr
	H7	Bl
	H8	Bl
	H9	Rs
	H10	Br
	H11	Rs
	H12	Bg
Tapestry N (NMAA 30)	N1	Y
	N2	W
	N3	Y
	N4	Bl
	N5	Bl
	N6	Bl
	N7	Bl
	N8	Gr
	N9	Bg

Bg beige, *Bk* black, *Bl* blue, *Br* brown, *Gr* green, *LBr* light-brown, *O* orange, *W* white, *Y* yellow

Contemporary dyed wool samples

Samples for evaluation of dye extraction procedures

A sample (4.0 g) of sheep wool previously spun and scoured was mordanted for 30 min. in 200 mL of boiling water containing 1.0 g of alum (adapted from[198,199]). The wool was removed, rinsed and left to dry protected from light. Except for woad, all wool samples were dyed in the following way: each dye bath was prepared with 0.5 g of dried material (weld, brazilwood, logwood or madder) immersed in 50 mL H_2O and heated at 90 °C for 2 h and vacuum filtered. After this, the previously mordanted wool was added to the dye solution

and kept at 90 °C for 30 min. In the case of cochineal, the dye bath was prepared by soaking 0.5 g of dried insects in 50 mL H_2O for 24 h, after which, the above procedure was followed.

For woad, 0.5 g of dye was mixed in a small amount of warm water, obtaining a blue opaque solution. Two millilitres of NaOH 4.25 mol.dm^{-3} plus 2.0 mL of $Na_2S_2O_4$ 0.86 mol.dm^{-3} was added, and after 15 min., the solution turned to a translucent green–yellow colour. Woad solution was diluted to 50 mL (pH adjusted to 10). Non-mordanted wool was dyed for 30 min. at 50 °C and then removed and oxidized by exposure to air. After the dyeing procedure, all wool samples were removed, rinsed and left to dry protected from light.

Samples for evaluation of the mordant effect, dyeing technique and photodegradation of red natural dyes

Two natural red dyes were selected for wool dyeing, namely madder (*Rubia tinctorum* L.) and brazilwood (*Caesalpinia* spp.). Two methods were used for wool wool dyeing, which included a pre-mordanting procedure (MD procedure) and a simultaneous mordanting procedure (M+D procedure).

MD procedure

One gram of sheep wool was mordanted for 30 min. in 50 mL of boiling water containing different amounts of mordant salts (concentration and sample notation in *Table 7*). Afterwards, the wool was removed and rinsed with cool water. The dye baths were prepared with 2.0 g of madder immersed in 50.0 mL H_2O and heated at about 90 °C for 30 min. The solution was allowed to cool and after simple filtration of the plant material, the previously mordanted wool was added to the dye solution bath and reheated at 90 °C for 30 min.

M+D procedure

The dye bath was prepared as described for the MD procedure. After plant material filtration, the different amounts of mordant salts were added (concentration and sample notation in *Table 7*) to each solution. When the mordant salts were dissolved, 2.0 g of sheep wool were immersed in each solution that was kept at 90 °C for 30 min. After the dyeing procedure, all wool samples were thoroughly rinsed with ultrapure water and left to dry in the open air protected from light.

Table 7. *Mordant salt bath concentration (mol.dm^{-3}) and corresponding mass of metal ion available in the dyeing baths per gram of wool (mg g^{-1}). Sample notation presented was used throughout this work.*

Mordant metal ion	Mordant salt bath concentration (mol.dm^{-3})	Mass of metal ion available in the dyeing bath per gram of wool (mg g^{-1})	Sample notation	
			M+D procedure	MD procedure
Al^{3+}	0.1000	135	AlM+D1	AlMD1
	0.0085	11	AlM+D85	AlMD85
	0.0030	4	AlM+D3	AlMD3
Cu^{2+}	0.0400	128	CuM+D4	CuMD4
	0.0016	5	CuM+D16	CuMD16
Fe^{2+}	0.0400	112	FeM+D4	FeMD4
	0.0016	5	FeM+D16	FeMD16

Accelerated ageing

A UV-Vis Solarbox 3000E (Co.Fo.Me.Gra, Milan, Italy) equipped with a Xe lamp and a 310 nm filter was used. The ageing conditions were as follows – temperature: 55 °C, irradiance: 400 W/m^2.

Contemporary samples dyed with madder were collected after 48, 120, 360, 480, 600, 720 and 960 h of light exposure.

Contemporary samples dyed with brazilwood were collected after 48, 120, 360, 480, 600, 720, 840 and 960 h of light exposure.

Colourimetry

Colourimetric analyses were performed in all historical and contemporary samples. A Datacolor International Mercury portable spectrophotometer (Zurich, Switzerland) was used for colourimetric measurements (L^*, a^* and b^* coordinates, CIELab space defined by Commission Internationale de l'Eclairage, in 1976). The spectrophotometer was equipped with a Xenon lamp and a photodiode sensitive to the 360-750 nm spectral range. Black and white standards were used for calibration. Illuminant CIE D65; 10° of observation angle and specular component excluded.

For each sample, analyses were performed in three different parts, with the average value used for data interpretation.

Analysis of fibres and mordants

Scanning electron microscopy - energy dispersive spectroscopy (SEM-EDS)

SEM-EDS analyses were accomplished in an Hitachi S-3700N variable pressure scanning electron microscope coupled with a Bruker X-ray energy dispersion spectrometer (Hitachi High-Techologies Europe – Krefeld, Germany).

SEM-EDS was used for morphological characterisation, point and map chemical analysis of wool dyed fibres. Samples were mounted in aluminium sample holders with double-sided adhesive carbon tape and coated with an Au or Au-Pd layer. Acceleration voltage of 15.0 kV and 20.0 kV was used, respectively, for SEM and EDS analyses. Surface distribution maps were collected at 90 kcps. For punctual analysis, five measurements were made in distinct parts of the selected fibres and the averaged values were used.

Micro-Particle induced X-Ray emission (μ-PIXE)

μ-PIXE analysis was performed using an Oxford Microbeams type nuclear microprobe[200] installed in one of the beam lines of the 2.5 MV Van de Graaff accelerator of Instituto Tecnológico e Nuclear (ITN, Sacavém, Portugal). A 2 MeV proton beam with 200 pA beam current was focused down to 3 × 4 μm^2 and used during sample irradiation. The produced X-rays were recorded with a 80 mm^2 Si(Li) detector of 155 eV resolution located at a backward angle of 45°. Scanning the focused beam over the sample allowed to register the characteristic elemental X-rays as a function of proton beam position so that two-dimensional elemental distribution maps could be obtained. From those maps, some spots were chosen to perform μ-PIXE point analysis. Basic data acquisition, handling and mapping were done with the OMDAQ program[201], while spectra fitting and quantitative results were obtained with the GUPIX computer package[202].

Inductively coupled plasma mass spectrometry (ICP-MS)

Measurements were carried out using a Thermo Scientific Element XR ICP-MS instrument (Bremen, Germany), equipped with a sector field mass spectrometer of reverse Nier-Johnson geometry, which allows measurements to be carried out at higher mass resolution in order to avoid spectral interferences. Sample introduction was accomplished by

means of a Micro Mist nebuliser (Glass Expansion – West Melbourne, Australia), mounted onto a cyclonic spray chamber from Glass Expansion.

For sample preparation, historical fibres of 0.1–2.0 mg were placed in polypropylene tubes and digested with 0.5 mL of concentrated HNO_3 in an ultrasound bath without temperature control. Acidic digestion proceeded until complete fibre dissolution (approximately 2 h). The solution was then diluted to 5 mL using ultrapure water.

Typical instrument settings and data acquisition parameters used for the determination of the elements Al, Fe, Cu and Zn are summarized in *Table 8*.

Concentrations for all nuclides were determined via external calibration versus a standard solution, containing 35 μg L^{-1} of the analyte elements. This standard solution was prepared by dilution of commercially available 1000 mg L^{-1} single element standard solutions with 0.14 M HNO_3.

Table 8. *ICP-MS instrument settings and data acquisition parameters.*

Parameter (units)	Value
Resolution (m/Δm)	4000 (Medium)
Cool gas (L min^{-1})	16.0
Auxiliary gas (L min^{-1})	0.80
Sample gas (L min^{-1})	0.993
RF power (W)	1250
Sample uptake rate (mL min^{-1})	0.20
Scan type	E-scan
Nuclides monitored	$^{27}Al^+$ - $^{56}Fe^+$ - $^{57}Fe^+$ - $^{63}Cu^+$ - $^{65}Cu^+$ - $^{64}Zn^+$ - $^{66}Zn^+$ - $^{67}Zn^+$ - $^{68}Zn^+$
Sample time (ms)	10.0
Samples per peak	20.0
Segment duration (ms)	200
Sweeps	36.0
Total measurement time per sample (s)	126

Inductively coupled plasma optical emission spectroscopy (ICP-OES)

Analyses were performed in an ICP-OES model Ultima from Horiba Jobin Yvon (Longjumeau, France) equipped with a radio frequency (RF) generator of 40.68 MHz and a type Czerny-Turner monochromator with 1.00 m (sequential).

ICP-OES was used for the evaluation of Al and Fe in the contemporary dyed wool fibres. For sample preparation, 1.5 mg of wool samples and 1.0 mL of concentrated HNO_3 were added

to an *Eppendorf* tube and digested in an ultrasound bath, without temperature control, until sample solubilisation (30 to 60 min.). The solution was then transferred to a 25 mL flask and the volume was adjusted with ultrapure water. For each mordant metal ion concentration, three unaged wool samples were digested. Replicate analyses were performed to evaluate analytical methodology reproducibility. Three analyses were done for each sample at the conditions presented in *Table 9*. Fe and Al calibration standards were prepared by diluting the respective 1000 mg L^{-1} stock solutions.

Table 9. *ICP-OES instrument settings.*

Parameter (units)	Value
Potency (W)	1200
Argon flow (L min^{-1})	12.0
Mira Mist nebulizer pressure (bar)	3.0
Pump velocity (rpm)	15.0

Flame atomic absorption spectroscopy (FAAS)

Analyses were carried out on a Perkin Elmer 3100 FAAS (Perkin Elmer – Norwalk, CT, USA) equipped with a Cu cathode lamp (resonance line at λ= 324.8 nm, 0.7 nm slit width and 15 mA lamp current intensity) and an air/acetylene flame.

FAAS was used for the evaluation of Cu in the contemporary dyed wool fibres. For sample preparation, 50.0 mg of each wool sample were heated (without boiling) on a hot plate with 4.0 mL of concentrated HNO_3 until the wool fibre was digested. The sample was then transferred to a 50 mL volumetric flask and the volume was adjusted with ultrapure water. For each mordant metal ion concentration, three unaged wool samples were digested. Three replicate analyses were performed on each sample for analytical methodology reproducibility evaluation. Cu calibration standards were prepared from a 1000 mg L^{-1} stock solution.

Analysis of natural dyes

Extraction methods

Contemporary dyed wool samples for evaluation of dye extraction procedures

For wool dyed with cochineal, weld, madder, brazilwood and logwood, six extraction methods were evaluated (HCl-1, HCl-2, Formic acid, EDTA-1, EDTA-2 and Oxalic acid). Woad dyed wool was extracted using two additional procedures (Pyridine and DMF). Glass vials of 1.5 mL capacity were used in all extractions.

HCl-1 method

Two milligrams of dyed wool were placed in capped vials with 400 μL of 37% HCl/MeOH/H_2O (2:1:1, *v/v/v*) solution and heated at 100 °C for 10 min[203]. Vials were cooled to room temperature, and the solvent was evaporated under vacuum.

HCl-2 method

The procedure was the same as in HCl-1 method, but after the drying process, 400 μL of MeOH/DMF (1:1, *v/v*) solution was added to the residue, and the solution was heated at 100 °C for 5 min[169]. Vials were cooled to room temperature, and the solvent was evaporated under vacuum.

Formic acid method

Two milligrams of dyed wool were placed in capped vials with 400 μL of HCOOH/MeOH (1:19, *v/v*) solution and heated at 40 °C for 30 min[177]. After being cooled to room temperature, the solvent was evaporated under vacuum.

EDTA-1 method

Samples of 2 mg of dyed wool were placed in capped vials, and 400 μL of 0.001 mol.dm^{-3} aqueous Na_2EDTA/acetonitrile/MeOH (1:5:44, *v/v/v*) solution was added (adapted from[177]). The vials were kept at 60 °C for 30 min. After being cooled to room temperature, the solvent was evaporated under vacuum.

EDTA-2 method

Two milligrams of dyed wool were placed in vials, and 1.0 mL of 0.1% Na_2EDTA in H_2O/DMF (1:1, *v/v*) solution was added[204]. The vials were capped and kept at 100 °C for 30 min. Vials were cooled to room temperature, and the solvent was evaporated under vacuum.

Oxalic acid method

Two milligrams of dyed wool were placed in capped vials with 400 µL of 2 $mol.dm^{-3}$ oxalic acid/MeOH/acetone/H_2O (1:30:30:40, *v/v/v/v*) solution and heated at 60 °C for 30 min[205]. Vials were cooled to room temperature, and the solvent was evaporated under vacuum.

Pyridine method

Two milligrams of woad dyed wool was placed in capped vials with 0.5 mL of pyridine and heated at 100 °C for 15 min[167]. Vials were then cooled and solvent evaporated under vacuum.

DMF method

Two milligrams of woad dyed wool were placed in capped vials with 0.5 mL of DMF and heated at 100 °C for 15 min[156]. Vials were then cooled and solvent evaporated under vacuum.

Five samples of each dyed fibre were extracted per method, and three replicate HPLC/DAD analyses were performed on each extract. All dried residues were dissolved in 500 µL of MeOH/H_2O (1:1, *v/v*) solution, except for those of woad and HCl-2 methods which were reconstituted in 500 µL of MeOH/DMF (1:1, *v/v*) solution. All solutions were filtered through a 0.45-µm PTFE prior to LC analysis.

Contemporary dyed wool samples for evaluation of the mordant effect, dyeing technique and photodegradation of red natural dyes

Twenty milligrams of dyed wool were placed in vials and 1 mL of 0.1% EDTA in water/DMF (1:1, *v/v*) solution was added. The vials were capped and kept at 100 °C in liquid paraffin for 30 min. After it, they were cooled to room temperature and the samples were

lyophilized. The dried residues were dissolved in 1.0 mL of MeOH/H_2O (1:1, *v/v*). All solutions were filtered through a 0.45 μm PTFE filter prior to analysis. For each mordant metal ion concentration, three wool samples were extracted. Three replicate analyses were performed on each sample to evaluate analytical methodology reproducibility.

Arraiolos historical samples

Samples of approximately 2.0 mg were extracted following the EDTA-2 methodology[204]. All samples were vacuum-dried, dissolved in 250 μL MeOH/H_2O (1:1, *v/v*) and filtered. The remaining threads of blue and green samples were redissolved in 250 μL MeOH/DMF (1:1, *v/v*) and filtered.

High-performance liquid chromatography (HPLC)

HPLC-DAD analysis

HPLC-DAD analyses were performed in an Agilent 1100 system (Agilent Technologies – Waldbronn, Germany) equipped with a 1100 Agilent series diode-array detector. This technique was used in the evaluation of extraction procedures using contemporary dyed wool samples.

The analytical column used was a Lichrocart Purospher Star RP-18 (250 × 4.6 mm, 5 μm particle size) from Merck (Darmstadt, Germany), set at a temperature of 30 °C. The mobile phase consisted of acetonitrile (A) and 2.5% of aqueous acetonitrile (*v/v*) containing 0.5% HCOOH (*v/v*) (B). A flow of 1.0 mL min^{-1} was used with the following gradient: 0–100% A from 0 to 10 min., 100% A from 10 to 15 min. The injection volume was 20 μL, and DAD detection was set between 200 and 700 nm. Chromatographic profiles were recorded at 600, 265, 450, 445, 495 and 290 nm for indigo, weld, brazilwood, logwood, cochineal and madder, respectively.

HPLC-DAD-MS analysis

MS analysis was carried out in an LCQ Fleet mass spectrometer (Thermo Finnigan – San Jose, CA, USA) equipped with an electrospray ionization (ESI) source and using an ion trap mass analyzer. The mass spectrometry equipment was coupled to a Surveyor Thermo Finnigan HPLC system with autosampler and DAD detector.

Arraiolos historical samples

The conditions of MS analysis were: capillary temperature of 300 °C, source voltage of 5.0 kV, source current of 100.0 μA and capillary voltage of -20.0 V in negative ion mode and 22.0 V in positive ion mode. Analytes were detected in full MS mode (*m/z* 100–800): in negative ion mode, two segments were used, 10% CID from 0 to 15 min. and 30% CID from 15 to 30 min.; in positive ion mode, 30% CID was used from 0 to 30 min. All samples were injected in negative and positive ion modes. Column temperature was set at 30 °C, and tray temperature was set at 24 °C. DAD detector was set at 200–800 nm. The analytical column was a Fortis-C18 (150 × 2.1 mm, 3 μm particle size) from Fortis Technologies (Cheshire, UK). A flow of 0.2 mL min^{-1} was used with a mobile phase consisting of acetonitrile (A) and water acidified with 0.1% formic acid (B). The gradient used was 0–90% A from 0 to 20 min., then 90% A from 20 to 30 min. Injection volume was set to 10 μL.

Contemporary dyed wool samples

Madder

The conditions of the MS analysis were: capillary temperature of 300 °C, source voltage of 5.0 kV, source current of 100.0 μA and capillary voltage of -15.0 V in negative ion mode. Analytes were detected in the selected reaction monitoring (SRM) mode and the transitions used were the following: alizarin 239→211 and purpurin 255→227. The analytical column was a Zorbax Eclipse XDB-C_{18} (150 × 2.1 mm, 3.5 μm particle size). Column temperature was set at 30 °C and tray temperature was set at 24 °C. The mobile phase used was 100% methanol with a flow of 0.2 mL min^{-1} and the injection volume was 25 μL.

Brazilwood

The analytical column was a Fortis-C18, used with a 0.2 mL min^{-1} flow and sample injection volumes of 10 μL. Column temperature was set at 30 °C and tray temperature was set at 24 °C.

For brazilein identification in brazilwood dyed samples before and after light exposure the following conditions were used: capillary temperature of 300 °C, source voltage of 5.0 kV, source current of 100.0 μA and capillary voltage of -20.0 V, in negative full MS mode (*m/z* 100-600). DAD detector was set at 200-800 nm. The mobile phase consisted of acetonitrile (A)

and water acidified with 0.1% formic acid (B). The gradient used was 0-90% A from 0-20 min., then 90% A from 20-30 min. For brazilein MS quantification, the same capillary temperature, source voltage and source current were used. A capillary voltage of -25.0 V was used in negative ion mode. Brazilein was detected in selected ion monitoring (SIM) mode at *m/z* 283 and 100% methanol was used as mobile phase.

Statistical data analysis

Statistical treatment (one-way ANOVA, $P<0.05$, SPSS® 15.0 for Windows) was performed on the data to determine significant differences whenever they occurred.

6. Results and discussion

Extraction of natural dyes from wool

The analysis and identification of dyes used in historical textiles are of extreme importance in conservation science as they can help in elucidating the textile's place of origin and time of production. Moreover, they will provide information that can be used for choosing the appropriate conditions for textiles conservation or restoration. The organic natural colourants used to dye textiles are among one of the most degradable materials used in the works of art. Chromophore identification in ancient textiles can be a challenging task mainly due to the low amounts of intact dye that can be extracted from the usually small historical samples. Extra difficulties arise from the different rates at which the chromophores suffer light and wear degradation[189,203] and the common use of different dyes to obtain the fibres desired hues.

Dye analysis is usually performed by liquid chromatography with a diode-array detector[164,165,168,169,176,206] but nowadays, mass detection is becoming increasingly important[60,158,159,170,177,181,184,207,208]. A successful approach to identify natural dyes requires that, prior to the chromatographic analysis, an adequate analytical methodology is used for their extraction from the textile fibres. The chemical structure and properties of the different dye chromophores suggest that probably no single procedure is adequate for the dye´s extraction. Despite the obvious importance of the sample preparation methodology for the ultimate relevance of the analytical data collected, few systematic studies on the recovery rates of the different chromophores with the available extraction methods have been published[169,177,209]. One of the most commonly used extraction methods involves the use of hydrochloric acid (HCl) and methanol (MeOH) at high temperature[159,164,169,170,176,177,203]. After solvent evaporation, different solvents have been used to solubilise the resulting dried

residue. Methanol (MeOH) is usually used for the yellow and red dyes solubilisation, while dimethylformamide (DMF) is preferred for the indigoids.

Wouters and Verhecken[203] proposed, several years ago an HCl based method for dye extraction from wool dyed with several coccid insect dyes. Wool dyed fibres were hydrolysed in a mixture of 37% HCl/MeOH/H_2O (2:1:1, *v/v/v*) placed in a boiling water bath for 10 min. and after several procedures the residue was redissolved in an appropriate volume of H_2O/MeOH (1:1, *v/v*). Although losses during manipulations are avoided and good extractions yields can be obtained, the use of an HCl based extraction procedures leads to information loss (fibre destruction and chromophore glycosidic bonds break) and some mild procedures have been developed. Some years later, Tiedemann and Yang[204] presented a milder procedure which consisted in heating the yarn with 0.1% Na_2EDTA in H_2O/DMF (1:1, *v/v*) for 30 min. in a boiling bath and then cooled rapidly. The extraction method was successfully applied on red contemporary wool dyed samples and on red wool historical Peruvian samples. Concentrations of the yarn extracts were measured with the photometry function on the UV-Vis spectrophotometer and the authors found that although being as powerful as the conventional HCl method, the Na_2EDTA/DMF extraction protocol preserves the fibre structure for further investigation. In the same period, Kirby and White[69] suggested a method involving the use of a boron trifluoride/MeOH mixture for the extraction of lake pigment dyestuffs from paintings and the same procedure was used a few years later[210] to extract dyestuffs from purple samples in a 15th century velvet panel. As Sanyova and Reisse[211] pointed out, one drawback of this method is the esterification of carboxyl-containing colourants, such as pseudopurpurin and munjistin. Milder extraction methods which would minimize unwanted effects such as acid hydrolysis, decarboxylation or methylation, but still extract alizarin, purpurin and other colourants as quantitatively as possible were desirable. The use of hydrofluoric acid solutions for the extraction of anthraquinones from their aluminium complexes in madder lakes fulfilled the hope of non-destructive extraction of pseudopurpurin, glycosides and other labile molecules and it proved to be at least as efficient as HCl for alizarin and purpurin[211].

Strong acid procedures are particularly disadvantageous for yellow dyes, the majority of which are flavonoids, often with glycoside groups. In a way to preserve glycosidic linkages, Zhang and Laursen[177] presented two different extraction protocols: an H_2EDTA/Acetonitrile/MeOH (1:5:44, *v/v/v*) mixture kept at 60 °C for 30 min. and a formic acid/MeOH (1:19, *v/v*) mixture kept at 40 °C for 30 min., being the residues redissolved in

MeOH/H_2O (1:1, *v/v*) . The methods were tested on silk dyed with yellow and red natural dyes. For the flavonoid dyes on silk, EDTA method seems to be more efficient, while formic acid procedure is more adequate for the anthraquinone-type dyes. The formic acid and EDTA extraction methods not only gave higher extraction yields than did HCl procedure but also more information about the nature of the original dyestuff can be obtained. With the same goal, Guinot and Andary[205] proposed a mild method for dye extraction from wool dyed with weld based on the use of oxalic acid.

When indigoid dyestuffs are present in the fibre, the use of DMF[156], hot pyridine[167], MeOH/DMF[169] or dimethylsulfoxide (DMSO)[209] for the residue solubilisation is recommended instead of MeOH or MeOH/H_2O, since indigotin and indirubin are insoluble in these solvents[169,209].

Long ago, Schweppe[156] identified the natural dyes on the fibres without taking them up into solution, based on the fact that natural mordant dyes form lakes of various colours with different mordant ions. Concerning the fibres dyed with mixtures of natural dyes and blue indigoid dyes, Schweppe[156] pointed out that the indigo should be removed from the fibre by repeated boiling with dimethylformamide (DMF) until the solvent remains colourless. Once the indigo has been removed, the yellow or the red dyes are left behind and could be identified by forming the various coloured lakes. Surowiec *et al.*[167] in a study of archaeological Coptic textiles proposed an extraction procedure of alkaline hydrolysis, for samples containing blue dyestuffs (indigotin or its derivatives), based on hot pyridine, while yellow and red dyes were extracted by acidic hydrolysis with a mixture of 3 mol.dm^{-3} HCl solution in ethanol (1:1), heated in a water bath at 90 °C for about 30 min. Later, Surowiec *et al.*[169] based on the acidic extraction with a mixture of 37% hydrochloric acid:MeOH:water (2:1:1, *v/v/v*) in a water bath at 100 °C for 10 min., proposed an additional DMF/MeOH (1:1, *v/v*) extraction step to enhance the extraction of the vat dye indigotin, a component of the blue dyes woad and indigo, and mordant dyes released during hydrolysis step. The recovery efficiencies for flavonoids, plant-derived anthraquinones, ellagic acid and indigotin were improved with this additional MeOH/DMF extraction step and, particularly, the recovery of indigotin offered a notable improvement.

Finally, in a recent work, Valianou *et al.*[209] presented a systematic comparative study of five different dye extraction procedures based on hydrochloric, citric, oxalic and trifluoroacetic acids (TFA) and an HCOOH/H_2EDTA mixture. All five extraction methods were tested on dyed wool with red and yellow mordant dyes (madder, cochineal, weld and young

fustic), a yellow direct dye (turmeric) and a blue vat dye (woad) and also on historical textile samples. Particular working conditions were established after a vast study, but in general, wool samples were treated in a boiling water bath with a mixture in the adequate proportions of the respective acid:MeOH:H_2O until dryness, being the residue reconstituted with DMSO. In the case of HCOOH/H_2EDTA mixture, EDTA solution was added to the initial mixture after wool extraction of 5 min. with formic acid. The upper solution was then isolated in a second tube and dried and the procedure was repeated to treat the residue of the first tube. TFA method provided elevated extraction yields for most of the studied dye components and was selected to be used on historical samples, where it was successfully applied in the identification of several colouring compounds, some of which could not have been detected if the HCl method was used.

The purpose of the study presented here was to make a systematic evaluation on the efficiency of several currently available extraction procedures used to extract different dye components from wool dyed fibres. Contemporary dyed samples were used in order to evaluate the different analytical methodologies. Studied dyes cover a wide range of chemical structures (*Figure 23*) and have been historically used to obtain a wide array of different hues.

Evaluation of dye extraction procedures

In this work, six extraction procedures have been evaluated (referred as HCl-1, HCl-2, Formic acid, EDTA-1, EDTA-2 and Oxalic acid methods) to extract wool samples dyed with madder, weld, cochineal, logwood, brazilwood and woad, and two additional extraction procedures (Pyridine and DMF methods), which were only used to extract wool dyed with woad.

Five samples of each dyed fibre were extracted per extraction method and three replicate analyses were performed on each extract. The chromatographic analysis of the wool extracts was done using HPLC/DAD. The identification of the dye chromophores was done based on their UV spectra and by comparison with authentic standards. Representative chromatograms of all the wool extracts obtained with the EDTA-2 extraction method are shown in *Figure 24*. The evaluation of the extraction methods efficiency was based on the comparison of the chromatographic peak areas obtained from HPLC/DAD analyses for the main chromophores in each dye (*Table 10*). One-way ANOVA statistics was used to identify significant differences among the extraction procedures whenever they occurred.

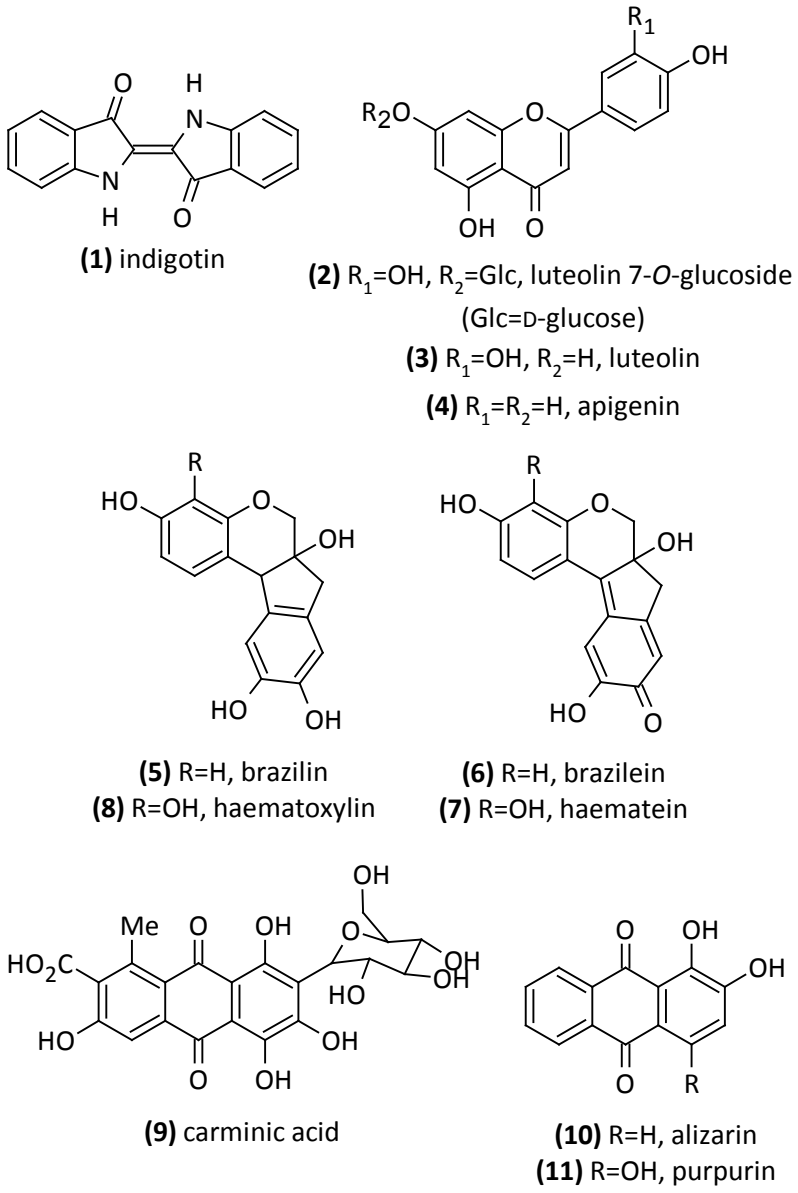


Figure 23. *Chemical structures of the major chromophores of the dyes studied in the evaluation of extraction procedures: (1) woad, (2, 3, 4) weld, (5, 6) brazilwood, (7, 8) logwood, (9) cochineal and (10, 11) madder.*

The HCl-1 and HCl-2 methods are the most aggressive extraction methods available, leading to the almost complete destruction of the fibre structure. SEM analysis performed on fibres subjected to HCl based extraction procedures showed that the main wool fibre structure is almost completely lost and, whenever maintained, the fibres lose their characteristic scale structure (*Figure 26*). The main difference between the tested HCl methods is the addition of an extraction step with MeOH/DMF on the HCl-2 method. Contrary to previously reported results[169], with the exception of brazilein (6, *Table 10*) and

haematein (7, *Table 10*), no significant improvement in the natural dyes extraction yields were obtained with the HCl-2 procedure when compared to those obtained with HCl-1.

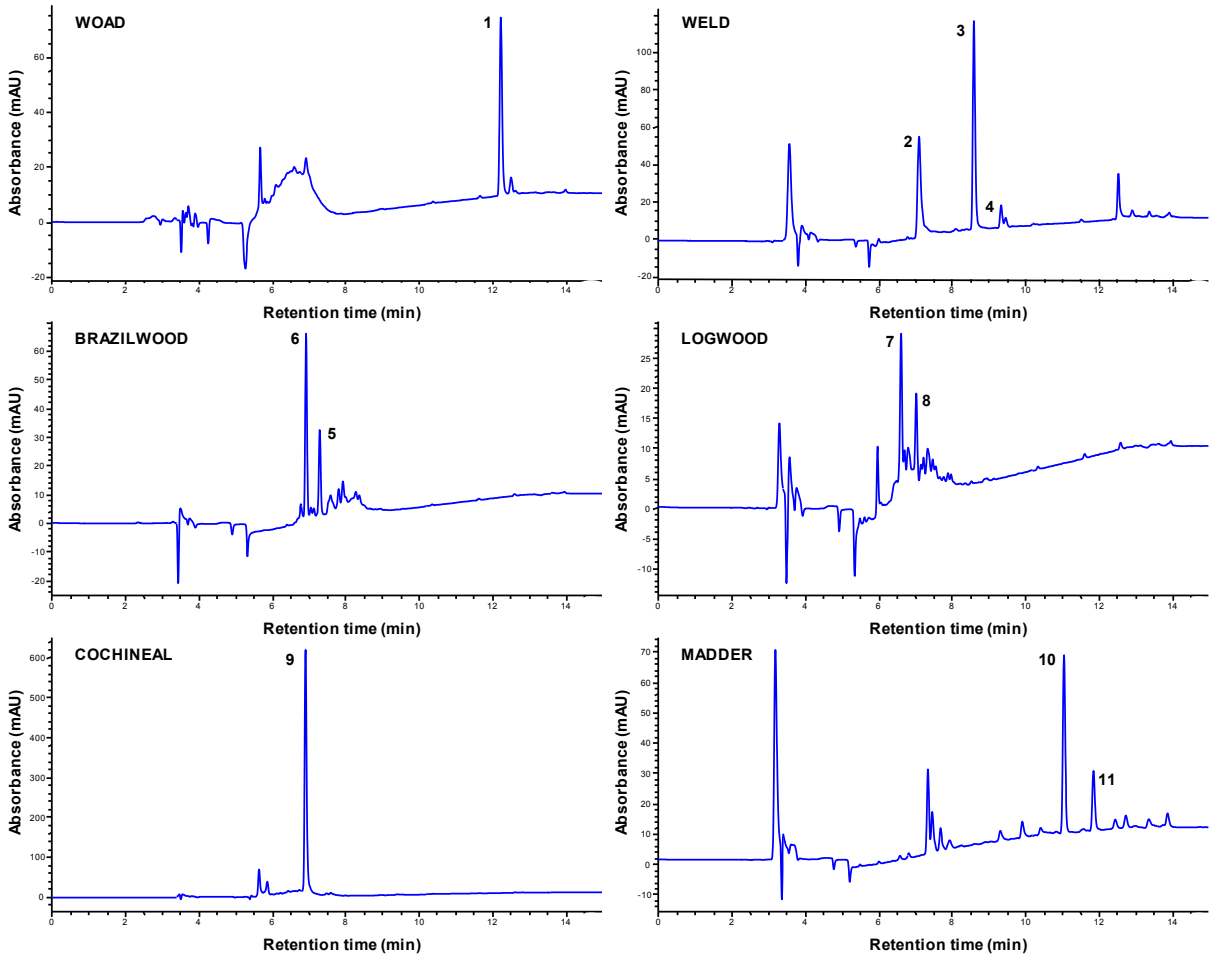


Figure 24. *HPLC/DAD chromatograms of the dyed wool extracts obtained with the EDTA-2 extraction method. Chromatographic profiles recorded at 600, 265, 450, 445, 495 and 290 nm for woad, brazilwood, logwood, cochineal and madder, respectively. For peak identification, see Figure 23.*

Hot pyridine and DMF are described in the literature for the extraction of indigo dyes from textile samples[156,167]. Nevertheless, indigotin (1, *Table 10*) was not observed in the DMF extract and only a small chromatographic peak with r_t = 12.5 min. was detected, likely indirubin, a compound which is normally present at variable concentration in the indigo dyes.

HCl-1 was the most effective method to extract indigotin. However, yellow flavonoid dyes were commonly used together with indigo dyes to obtain green hues, and these originally green colours tend to become bluish when subjected to light due to the faster photodegradation of the flavonoid dyes. HCl based methods are not suitable for the

extraction of the yellow flavonoid dyes due to hydrolysis of the *O*-glycosidic bonds[177,209]. Being so, caution is needed when they are used to extracted bluish samples since relevant information regarding the source of other natural dyes could be lost.

Table 10. *HPLC/DAD peak areas (mAU × min) of the natural dyes major chromophores extracted from wool dyed samples by the different extraction procedures (see Figure 23 for peak identification).*

Peak	Extraction method: peak area[§]														
	HCl-1		HCl-2		Formic		EDTA-1		EDTA-2		Oxalic		Pyridine		DMF
	Mean†	(SD)‡	Mean†	(SD)‡	Mean†	(SD)‡	Mean†	(SD)‡	Mean†	(SD)‡	Mean†	(SD)‡	Mean†	(SD)‡	Mean†
1	26.80^{a}	(0.44)	17.51^{b}	(0.86)	10.50^{c}	(0.64)	5.95^{d}	(0.44)	15.73^{b}	(0.15)	10.26^{c}	(0.14)	15.20^{b}	(1.44)	n.d.
2	n.d.		n.d.		14.76^{a}	(1.24)	22.95^{b}	(0.64)	293.71^{c}	(25.79)	115.12^{d}	(2.53)	-		-
3	352.10^{ab}	(66.51)	290.25^{ab}	(33.21)	32.83^{c}	(4.10)	55.63^{d}	(5.00)	213.66^{a}	(24.44)	326.26^{b}	(22.62)	-		-
4	24.40^{a}	(3.06)	21.23^{a}	(2.09)	n.d.		n.d.		13.71^{b}	(1.72)	22.42^{a}	(0.80)	-		-
5	n.d.		n.d.		n.d.		n.d.		26.80^{a}	(2.25)	41.92^{b}	(6.05)	-		-
6	n.d.		31.19^{a}	(3.28)	57.81^{b}	(4.15)	18.80^{c}	(0.48)	327.72^{d}	(8.22)	418.21^{e}	(40.66)	-		-
7	57.71^{a}	(7.29)	141.08^{b}	(18.03)	48.86^{a}	(2.00)	17.04^{c}	(0.35)	1257.31^{d}	(40.34)	1107.92^{e}	(18.73)	-		-
8	n.d.		n.d.		n.d.		n.d.		40.98^{a}	(7.37)	43.34^{a}	(4.75)	-		-
9	1528.24^{a}	(251.42)	144.82^{b}	(23.44)	68.60^{c}	(8.89)	16.21^{d}	(1.64)	1613.93^{a}	(43.90)	412.83^{e}	(12.22)	-		-
10	2109.94^{a}	(82.02)	1982.76^{a}	(134.37)	608.53^{b}	(59.43)	236.96^{c}	(8.52)	1538.50^{d}	(107.48)	728.16^{b}	(16.22)	-		-
11	2886.50^{a}	(151.18)	3307.43^{a}	(181.48)	293.77^{bc}	(156.08)	103.16^{b}	(2.91)	1087.59^{d}	(71.17)	436.73^{c}	(13.52)	-		-

n.d. not detected

§ Normalized to milligrams of dyed wool extracted by each method, samples dried and redissolved in 500 µL of solvent, 20 µL injection. Chromatographic profiles recorded at (nanometres): 600 (woad: 1), 265 (weld: 2, 3, 4), 450 (brazilwood: 5, 6), 445 (logwood: 7, 8), 495 (cochineal: 9) and 290 (madder: 10, 11).

† The values represent the mean of three replicate measurements on five different extracts. For each compound means followed by different index letters are significantly different (one-way ANOVA, $P < 0.05$).

‡ Standard deviation.

For the indigoids extraction, one-way ANOVA analysis showed that the results obtained with the milder method EDTA-2 are not significantly different from those obtained with pyridine ($P < 0.05$, *Table 10*). The former method enables efficient extraction of other dye components. In fact, when the EDTA-2 procedure is used, residual amounts of yellow glycosilated chromophores from weld can be extracted and identified together with indigo in samples which present nowadays a blue hue (historical samples analyses).

The yellow chromophores of weld behaved slightly differently when the different extraction procedures were applied. Luteolin (3, *Table 10*) was extracted with a similar efficiency by the HCl-1, HCl-2, EDTA-2 and oxalic acid methods, while significantly lower yields were obtained with the EDTA-1 and formic acid procedures. Apigenin (4, *Table 10*) amounts are, as expected, much lower and could not be detected when the EDTA-1 and formic acid methodologies were used, while the other tested methods performed equally well. Surowiec *et al.*[169] compared the two HCl procedures for the extraction of weld dye

aglycones, concluding also that they result in very similar HPLC peak areas. However, the known hydrolysis of the flavonoid glycosides should be taken into consideration for the apparent good yields obtained with the HCl methods for the studied aglycones[209]. In fact, luteolin-7-*O*-glucoside (2, *Table 10*) is absent in chromatograms of the HCl methods, being only detected when milder extraction procedures were used. Weld was reportedly used in Arraiolos carpets to obtain yellow hues[1,12] and, in fact, it has already been detected in another carpet[2]. Usually, not all the compounds identified in weld plant extracts can be identified in the historical textile samples. However, and despite the widely distribution of these compounds in the plant kingdom, weld is frequently pointed out in the literature as the yellow colour source in the historical textiles, sometimes based only in the identification of very few flavone derivatives[178,212,213].

HPLC/DAD/MS analysis of the extracts obtained from an Arraiolos historical yellow sample (I1, see Chapter 5 for fibre sampling details) with the EDTA-2 procedure enabled the identification of up to nine flavone derivatives (*Figure 25* and *Table 11*), based on the comparison of their UV-Vis and mass spectra data with those reported in the literature[2,184,214] (full discussion of the UV-Vis and mass data are presented in section '*Analysis of Arraiolos historical tapestries from the National Museum of Ancient Art (NMAA) collection*').

The chromatographic profile of the historical sample presented in *Figure 25* is unusually comprehensive for an historical sample, proving the effectiveness of the EDTA-2 extraction procedure.

Brazilwood and logwood chromophores (5-8, *Table 10*) are homoisoflavonoids which were overall poorly recovered with exception for EDTA-2 and oxalic acid extraction methods. Statistical analysis indicates that both methods are not equally effective in the extraction of the homoisoflavonoid dyes. In fact, oxalic acid yields slightly higher peak areas for the brazilwood chromophores (5 and 6, *Table 10*), while EDTA-2 is more effective in the extraction of haematein (7, *Table 10*). Homoisoflavonoids are very sensitive to light degradation[215] and only very small amounts of intact chromophores are expected to be present in historical textiles requiring, therefore, very effective extraction methods for their recovery.

The mild methods formic acid, EDTA-1 and oxalic acid, performed very poorly in the extraction of the anthraquinone dyes (9-11, *Table 10*). Unexpectedly, carminic acid (9, *Table 10*) was very poorly extracted by the HCl-2 method, but no significant differences were observed between the extraction yields obtained with EDTA-2 and HCl-1 methods. Valianou

et al.[209] evaluated both HCl-1 and oxalic acid methods in the extraction of carminic acid but did not report a considerable difference between them.

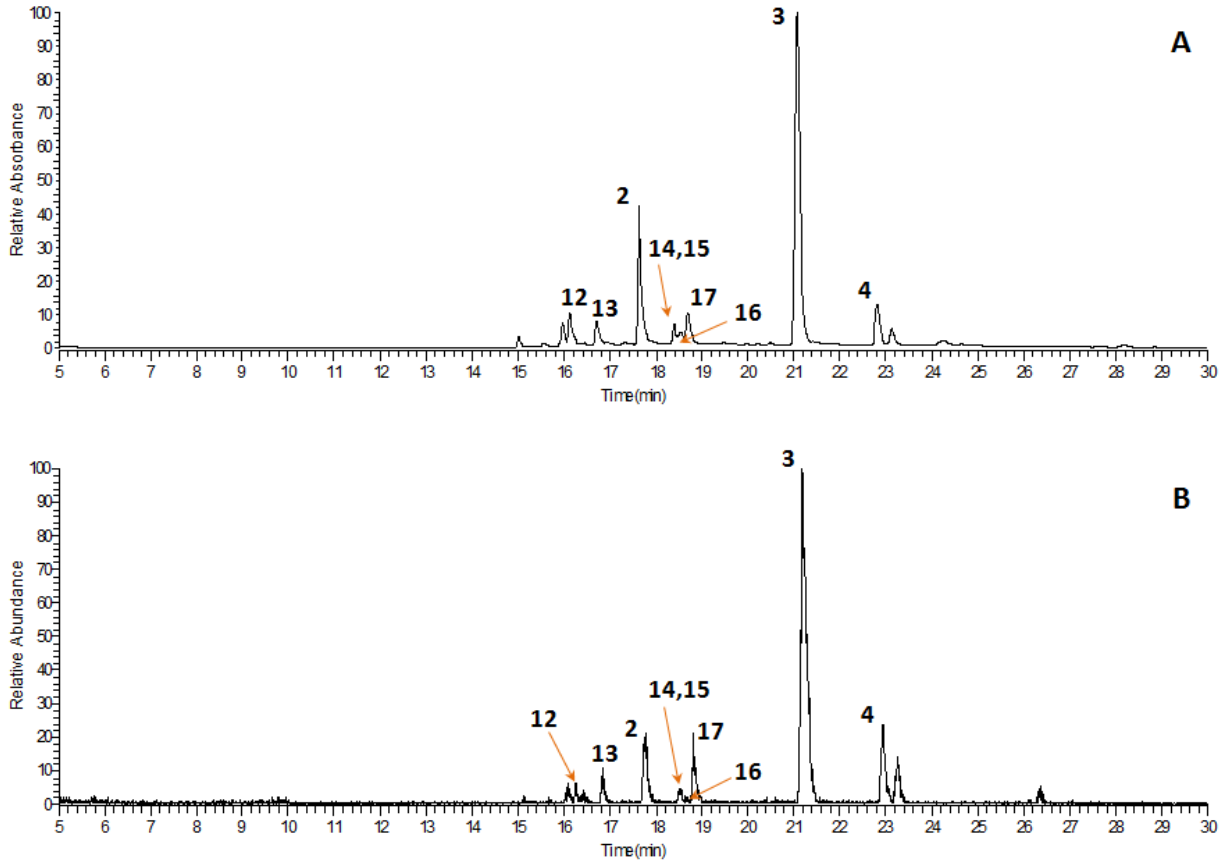


Figure 25. *HPLC/DAD/MS chromatograms of weld dyed wool sample I1 extracted using method EDTA-2: (A) DAD chromatogram (265 nm); (B) Total ion current chromatogram. Identification of the chromatographic peaks is based on UV-Vis and mass data (Table 11).*

Table 11. *Identification of chromophores in weld dyed wool sample I1 extracted using method EDTA-2 (chromatographic peaks refer to Figure 25).*

Peak	LC/DAD r_t (min.)	LC/DAD data (nm)	LC/MS data (*m/z*)*	Identification
12	16.13	245, 267, 335	**609 [M-H]$^-$**, 447, 285	Luteolin di-*O*-glucoside
13	16.71	245, 267, 335	**609 [M-H]$^-$**, 447, 285	Luteolin 3,7'-di-*O*-glucoside
2	17.63	254, 267, 348	**447 [M-H]$^-$**, 285	Luteolin 7-*O*-glucoside
14	18.37	245, 268, 335	**447 [M-H]$^-$**, 285	Luteolin 4'-*O*-glucoside
15	18.46	246, 263, 328	**431 [M-H]$^-$**, 269	Apigenin 7-*O*-glucoside
16	18.53	247, 269, 341	**461 [M-H]$^-$**, 299	Chrysoeriol 7-*O*-glucoside
17	18.69	246, 267, 339	**447 [M-H]$^-$**, 285	Luteolin 3'-*O*-glucoside
3	21.07	253, 266, 348	**285 [M-H]$^-$**, 243, 241, 217, 199, 175, 151, 133	Luteolin
4	22.81	249, 267, 336	**269 [M-H]$^-$**, 225, 201, 181, 149, 117	Apigenin

* In bold: major ions

Madder dye components (10 and 11, *Table 10*) were extracted with higher efficiency by both HCl methods. The EDTA-2 extraction procedure also performed reasonable well and, for the reasons presented above, this method should be considered whenever the presence of yellow flavonoid dyes is suspected. Surowiec *et al.*[169] reported higher extraction yields for the madder dye components when HCl-2 was used compared to HCl-1, which we could not confirm. Valianou *et al.*[209] also observed that milder extraction methods, like oxalic acid, were less effective in the extraction of the madder anthraquinones.

One of the most striking differences observed in this study is the difference in the peak areas for the same dye obtained with the two procedures involving EDTA (*Table 10*). EDTA is a very good chelating agent and it is expected to displace the dye molecule, binding the mordant metal ion which is attached to the fibre[177,204]. An important difference between the two tested methods is the solvent used to solubilise the displaced dye. According to the results presented in *Table 10*, and for all the chromophores investigated, the solvent DMF appears to be a much more effective solvent than the more polar mixture of acetonitrile/MeOH. The differences in EDTA concentration and temperature between the two methods might also play a role in the different extraction yields obtained.

A main feature of the EDTA methods is their mildness. In fact, SEM images confirmed that the wool fibre scale structure is practically intact after the extraction procedure with Na_2EDTA/DMF (*Figure 26*).

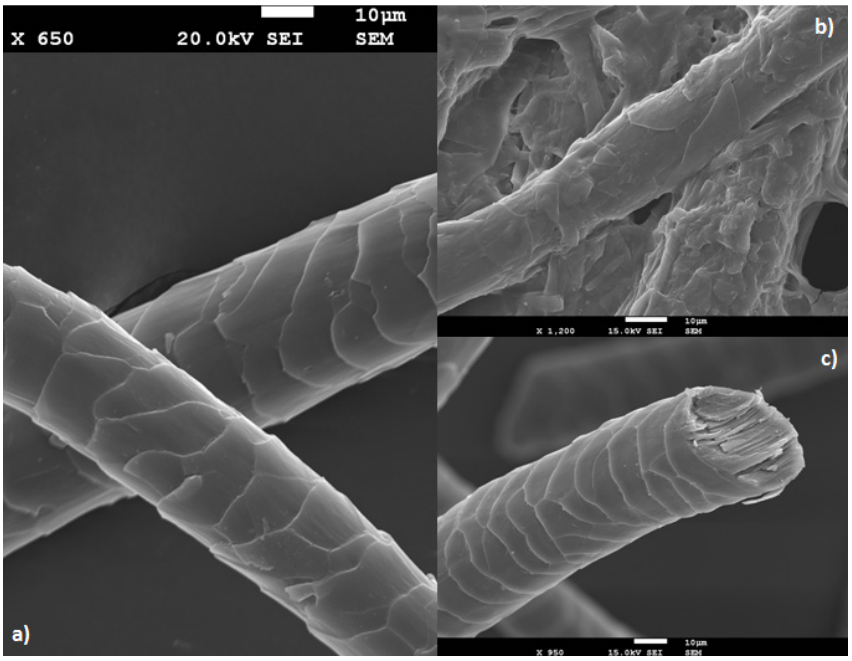


Figure 26. *SEM micrographs of a) untreated, undyed wool; b) and c) dyed wool extracted with HCl-1 and EDTA-2 methods, respectively.*

An overall appreciation of the tested extraction methods indicates that the most effective procedures were the HCl-1, EDTA-2 and oxalic acid. Considering the already referred shortcomings of the HCl methods and the poor anthraquinone extraction yields obtained with the oxalic acid procedure, authors believe that, among the tested methods, EDTA-2 is the most suitable method to extract unknown dyes from an historical sample.

Evaluation of the mordant effect, dyeing technique and photodegradation in contemporary wool samples dyed with red natural dyes

The 20th century was dominated by the synthetic bright and stable colours. However, increased public awareness on product safety and concerns on the sustainability and replacement of oil-based products explains the recent interest on natural dyes[57,216]. Textile dyers have mastered for millennia the use of natural dyes and mordants to get the array of colours seen in museums worldwide. Most of that knowledge was never written down as it was passed orally throughout generations and nowadays we know very little about the use of mordants and how they influence the colour and photodegradation of textiles dyed with natural dyes.

The main goal of this study was to get information on the role that the mordant ion nature, concentration and its mode of application have on the final hue and chromophore photodegradation of wool fibres dyed with two natural dyes: madder and brazilwood. Alum [$KAl(SO_4)_2.12H_2O$], copper (II) and iron (II) sulfates were used as mordants, at different concentrations, and were applied by two different procedures (pre-mordanting, MD, and simultaneous mordanting, M+D) to sheep wool samples (sample notation in *Table 7*). The lower values of the mordant concentrations used (0.0085 and 0.0030 mol.dm^{-3}, for alum, and 0.0016 mol.dm^{-3}, for copper and iron sulfates) were chosen based on dyeing recipes either used by local artisans or reported in natural dyeing books[57,217], while the higher values (0.1000 mol.dm^{-3}, for alum, and 0.0400 mol.dm^{-3}, for copper and iron sulfates) were chosen for comparison. The same amount of dye was employed for all mordant salts, bath concentrations and dyeing procedures.

Samples were artificially aged to identify the influence of the mordant on the dye's chromophores photodegradation. A set of analytical techniques was used for complete characterisation of the dyed fibres before and after light exposure, which included colour and chromophore analysis (colourimetry and HPLC/MS analysis), determination of mordant

ions amounts in the fibres (FAAS and ICP-OES analysis), morphological characterisation of the fibres and punctual chemical analysis (SEM-EDS studies).

Wool samples dyed with madder

Madder (*Rubia* spp.) has been cultivated in large amounts as a source for red plant dyes in Europe, Asia and America for centuries[47,58,187,218]. The dyestuff is extracted from the dried roots of the plant, being the bark much richer in anthraquinone derivatives than the wooden parts. The composition of the extracted anthraquinones differs between the varieties of Rubia. For example, the major component forming the natural dye of the European madder (*Rubia tinctorum* L.) is alizarin (1,2-dihydroxyanthraquinone, *Table 3*), while purpurin (1,2,4-trihydroxyanthraquinone, *Table 3*) is the major component of Indian madder (*Rubia cordifolia* L.), although it is also found in considerable amounts in the European madder. Anthraquinone glycosides are also present in the plant´s roots but, during storage, hydrolysis of the glycosides occurs, which is completed under the acidic conditions established in the dyeing procedure[218].

Analysis of natural dyes is an active area of research in cultural heritage studies[58,60,207]. A major problem associated with the identification of the original natural source of the historical textiles colour arises from the fact that, due to the lightfastness, some chromophores become mostly degraded and only minute amounts can be extracted from the samples. It is therefore of utmost importance to shed some light into the effect of the metal ion and dyeing method on the fibres final hue and chromophore photodegradation. Clementi *et al.*[218] have already studied the lightfastness of wool dyed with madder and alum under different conditions, concluding that the dyeing procedure influenced the fibre final hue and, when compared to the un-mordanted samples, the metal ion protected the madder chromophores from photodegradation.

Colourimetric studies

Colour is a complex phenomenon. In the light–sample interaction different physical phenomena are observed: transmission, absorption, scattering, refraction, etc. One way of describing sample colour is to use numerical terms, which can be converted to CIE (Commission Internationale de l'Eclairage) colour specifications. The principal attributes of sample colours are lightness or value (L*), chroma or saturation (C*) and hue (h°)[219,220].

Lightness considers colour as a source of reflected light ranging from black to white. Chroma is correlated to the degree of grey tone of the colour and is an indicator of colourfulness, so it accounts for the effects of discolouration[51]. Hue is expressed in degrees and defines the tonality that we normally identify with the name of a colour (red, yellow, green or blue)[51,220].

Table 12 presents the colour coordinates of the CIELab/Ch colour space measured by colourimetry for dyed samples preserved from light and after light exposure. As it can be seen, for samples not subjected to artificial ageing (t = 0 h), not only the metal ion chemical nature, but also the mordant bath concentration and dyeing procedure have strong influence on the wool fibre hue. Empirically, brighter and more pinkish colours were obtained with Al, which is probably why alum was the most popular mordant in the natural dyeing industry[47,218]. In fact, the colourimetric studies showed that, for mordant bath solutions with similar amounts of mass of metal ion (Al, Cu or Fe, *Table 7*), dyed fibres mordanted with Al present higher values of the luminance parameter (L*). In general, for all samples, luminance tends to increase during light exposure. As for the chroma value, the most systematic decrease observed during the ageing process is presented by the Al mordanted samples, especially the ones dyed by M+D procedure.

Differences in colour are likely due to differences in the complexes that are formed under the different dyeing conditions used, namely, the nature and concentration of the metal ion (existence of *d* electrons), the nature of the chromophore (existence of conjugated systems and substitution of C, H, O atoms by electron donors or acceptors atoms) and the solution pH. At the pH values of 3–5 obtained for the mordant solutions used in this work (data not shown), metal ions can bind to the carboxylate groups in the wool fibre, while also binding the chromophore molecules and, in some cases, water molecules as well[15,196,221-224].

According to the ligand field theory, the formation of the complexes leads to a loss of degeneracy of the *d* electrons of the metal ion, dividing them in two groups separated by an energy gap, which is dependent on the chemical nature of the ligands and their space distribution and on the nature and oxidation state of the metal ion[225]. Colour arises when some photons of the white light (400-720 nm) have enough energy to promote a transition of the electrons across that energy gap (absorption of radiation of some wavelengths), leaving the other wavelengths to be seen by the human eye. Some metals like Al, that do not have *d* electrons, can also form coloured complexes and their colour also arises from electronic transitions, but involving other valence electrons[225]. The dyeing conditions used are likely to have generated different complex structures with different energy gaps, leading to the

colours observed in the dyed wool. The reddish colours should correspond to complexes with larger energy gaps, while bluish colours correspond to complexes with shorter energy gaps, and in between are the violet colours[225].

Table 12. *Colourimetric data for madder dyed samples preserved from light (t = 0 h) and after light exposure (t = 48, 120, 360, 480, 600, 720 and 960 h).*

		Al						Fe				Cu			
		M+D			MD			M+D		MD		M+D		MD	
Time in the solar box (h)	CIELab/Ch colour parameters	AlM+D1	AlM+D85	AlM+D3	AlMD1	AlMD85	AlMD3	FeM+D4	FeM+D16	FeMD4	FeMD16	CuM+D4	CuM+D16	CuMD4	CuMD16
0	L*	54.52	57.23	51.85	33.24	36.37	40.99	40.44	43.57	26.09	27.27	50.04	43.76	51.89	28.87
	a*	30.33	24.21	30.78	38.19	38.40	37.64	7.89	9.67	11.80	13.10	11.36	22.54	16.25	21.50
	b*	36.93	35.39	36.96	23.02	27.11	32.80	17.11	18.33	6.81	8.07	18.96	23.06	27.24	8.65
	C*	47.79	42.88	48.10	44.59	47.00	49.93	18.84	20.73	13.62	15.39	22.10	32.25	31.72	23.17
	h°	50.60	55.62	50.21	31.09	35.22	41.07	65.24	62.18	29.98	31.64	59.07	45.66	59.18	21.91
48	L*	55.54	59.43	54.58	36.99	42.71	44.07	45.75	47.53	27.31	27.58	53.66	47.01	53.92	30.15
	a*	30.35	24.34	30.17	35.97	40.76	36.87	8.35	9.21	11.08	11.63	10.12	23.14	15.51	21.20
	b*	33.43	31.83	34.62	22.59	29.66	32.54	15.49	16.99	7.32	7.80	19.68	23.35	23.60	8.84
	C*	45.15	40.07	45.93	42.47	50.40	49.18	17.60	20.73	13.28	14.01	22.13	32.87	28.24	22.96
	h°	47.77	52.60	48.93	32.13	36.04	41.43	61.67	62.78	33.45	33.85	62.79	45.26	56.69	22.64
120	L*	57.00	60.76	57.47	36.96	45.15	46.11	51.79	50.34	30.41	29.82	54.29	47.85	58.52	33.47
	a*	28.99	22.37	27.22	38.30	37.42	34.10	8.92	9.06	11.32	11.42	10.64	22.99	16.20	22.21
	b*	31.62	29.13	31.63	23.68	26.97	30.31	18.11	15.89	8.30	8.08	20.00	22.15	24.09	9.39
	C*	43.05	36.73	41.73	45.03	46.12	45.63	20.19	18.30	14.04	13.99	22.66	31.92	29.03	24.11
	h°	47.67	52.48	49.29	31.73	35.78	41.64	63.77	60.31	36.24	35.28	61.99	43.94	56.07	22.92
360	L*	59.78	64.24	62.81	41.19	47.98	51.09	52.21	59.48	28.41	33.75	54.86	53.49	60.72	32.21
	a*	22.88	17.71	21.11	36.09	35.59	29.90	6.34	6.74	11.55	9.84	9.60	21.15	13.31	23.34
	b*	27.88	25.27	26.64	22.84	27.22	28.41	16.83	16.11	7.69	9.81	20.07	21.65	21.90	9.10
	C*	36.07	30.86	33.99	42.71	44.81	41.25	17.98	17.46	13.88	13.90	22.25	30.23	25.62	25.06
	h°	50.63	54.98	51.60	32.33	37.41	43.54	69.35	67.29	33.67	44.93	64.43	45.62	58.71	21.30
480	L*	62.14	65.78	64.85	42.56	51.83	54.94	56.73	60.50	35.82	37.19	56.90	56.27	62.73	35.49
	a*	21.29	15.09	17.96	35.85	32.35	27.27	6.06	6.18	9.80	9.06	9.32	19.95	12.87	22.25
	b*	27.36	23.05	23.98	21.91	24.56	26.86	17.63	15.62	10.66	9.85	19.33	21.21	21.16	8.87
	C*	34.67	27.55	29.96	42.01	40.61	38.28	18.64	16.80	14.48	13.38	21.46	29.11	24.77	23.95
	h°	52.11	56.78	53.17	31.43	37.21	44.57	71.04	68.40	47.40	47.39	64.27	46.75	58.70	21.73
600	L*	64.92	67.43	65.26	45.71	55.10	58.69	58.43	62.07	37.36	38.24	55.49	54.56	63.68	37.07
	a*	19.52	14.31	17.64	34.74	31.57	26.52	5.25	5.72	9.57	8.57	9.14	19.15	11.78	22.62
	b*	27.67	22.88	23.62	21.83	25.31	27.16	17.72	16.49	11.70	10.02	19.09	19.85	21.38	9.73
	C*	33.86	26.99	29.49	41.03	40.46	38.00	18.48	17.45	15.11	13.19	21.17	27.58	24.41	24.63
	h°	54.80	57.97	53.24	32.15	38.72	45.62	73.48	70.88	50.73	49.47	64.41	46.04	61.15	23.28
720	L*	64.94	68.59	66.12	43.43	54.99	56.10	59.86	62.72	37.72	40.54	58.69	55.90	63.06	38.00
	a*	17.47	12.77	15.04	33.03	29.29	22.86	5.28	5.62	9.07	8.17	9.12	18.86	10.76	22.59
	b*	26.28	21.94	22.02	20.37	23.62	24.45	18.43	16.45	12.17	12.39	19.71	19.89	20.75	10.00
	C*	31.56	25.39	26.66	38.81	37.63	33.47	19.17	17.39	15.18	14.84	21.72	27.41	23.37	24.70
	h°	56.39	59.80	55.67	31.67	38.88	46.92	74.02	71.13	53.30	56.61	65.16	46.51	62.59	23.88
960	L*	63.64	64.65	64.78	46.24	55.81	59.69	57.40	63.34	39.06	38.38	55.77	55.04	62.24	33.00
	a*	13.39	11.02	10.42	32.33	27.33	20.31	4.55	4.41	8.88	7.86	9.56	17.03	9.97	22.58
	b*	23.08	18.86	17.96	19.55	22.62	22.59	16.15	15.31	12.71	10.18	20.13	18.31	20.14	8.10
	C*	26.68	21.84	20.76	37.78	35.48	30.37	16.78	15.93	15.50	12.86	22.29	25.01	22.47	23.99
	h°	59.87	59.69	59.88	31.16	39.61	48.04	74.28	73.94	55.06	52.30	64.60	47.08	63.65	19.73

Poor lightfastness is a major drawback of the natural dyes and textile conservators worldwide know that not all the chromophores behave in a similar fashion under light. Despite the fact that the chemical nature of the mordant is known to influence photodegradation of dyed wool samples, very few studies on the subject have been reported in the literature[123,226-228].

The colour loss observed on the dyed textiles subjected to light can be attributed to the degradation of the natural dyes chromophores which is accomplished by photo-oxidative degradation, yielding small molecules[122,189].

Several studies have been published on the photodegradation of yellow dyes[122,189,226] and the identification of their degradation products[189], as well as on the photodegradation of red dyes, namely madder, with the identification of alizarin degradation products[206]. Cox Crews[122], in a study on the fading of the yellow dyes used by the American Indians, showed that the nature of the mordant ion was more important than the dye itself or the length of light exposure in predicting the coloured textiles lightfastness, with dyes applied with alum mordants fading significantly more than when applied with chromium, copper or iron salts. Yoshizumi *et al.*[226] have verified that, when used with alum (the only mordant tested), madder chromophores are more photostable than the common yellow chromophores, but less stable than some synthetic dyes. Changes in tensile strength and elasticity of the wool fibre were also shown to be influenced by the nature of the mordant ion and, in this case, alum mordants seem to diminish the wool phototendering, occurring when dyed fabrics are exposed to sunlight[227].

Padfield and Landi[123] have studied the lightfastness of natural dyes in wool and cotton dyed fibres. Several mordants were used in the dyeing procedures and madder was found to have poor lightfastness for alum mordanted wool samples. In iron mordanted wool fibres, light fading was not so distinct. Zarkogianni *et al.*[228] also dyed cotton and wool fabrics with several natural sources, including madder, and used a variety of mordants. Wash, light and rub fastness were tested and colour measurements were made[228]. The authors concluded that, in general, pre-mordanting the fibres enhances wash fastness for madder wool dyed samples. Light fastness wasn't improved, except when using copper, zinc and iron-based mordants. Rub fastness didn't improve after mordanting[228].

The dyed wools prepared on this study were subjected to artificial ageing, with samples being collected at fixed time intervals and their colour evaluated by colourimetry. The fading

of the original colours can be evaluated in terms of the colour difference calculated using the following formula of CIE Committee in 1976:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + \left(\Delta b^*\right)^2}$$, where:

ΔL* = lightness–darkness difference, Δa* = redness–greenness difference, and Δb* = yellowness–blueness difference[226].

The results presented in *Figure 27* show that fading is overall more pronounced in the first hours and tends to stabilize after 480–600 h of light exposure. Colour difference was more pronounced for the samples mordanted with alum, which has already been observed for the yellow dyes[122] and it is an extreme relevant aspect considering the historical usage of this mordant and dye.

Independent on the mordant bath concentration, the dyeing technique seems to have a small impact in the ΔE variation for the samples mordanted with Al salt. As for the Fe salt, samples dyed by the M+D procedure are more affected by photofading. For the same mordant bath concentration, the Cu mordanted samples present the higher relative variation on the ΔE parameter among the two tested dyeing methods.

In general, samples mordanted by the M+D procedure in smaller bath concentrations of alum and copper (II) sulfate tend to suffer higher colour differences when subjected to light. On the other hand, in the MD procedure the behaviour changes: while for the Al mordanted samples the smaller bath concentration produces higher variation on the ΔE parameter, for the Cu mordanted fibres the opposite behaviour is observed. The ΔE variation for the samples mordanted with the Fe salt proved to be somehow independent on the concentration.

Mordant evaluation and wool morphological characterisation

In order to evaluate the actual amounts of mordant metal ion present in the wool fibres, wool samples (t = 0 h) were analyzed by ICP-OES (Al and Fe) and FAAS (Cu). Both methods were found to be highly reproducible, with an RSD ≤ 5% (data not shown).

Despite the large mass of metal ion available in the dyeing baths (*Table 7*), the actual amounts of mordant metal ion present in the wool fibres proved to be overall much lower (*Figure 28*). Clementi *et al.*[218] also referred that the aluminium-sulfur ratios detected by EDS analyses in wool samples dyed with alum did not reflect the composition of the mordant bath.

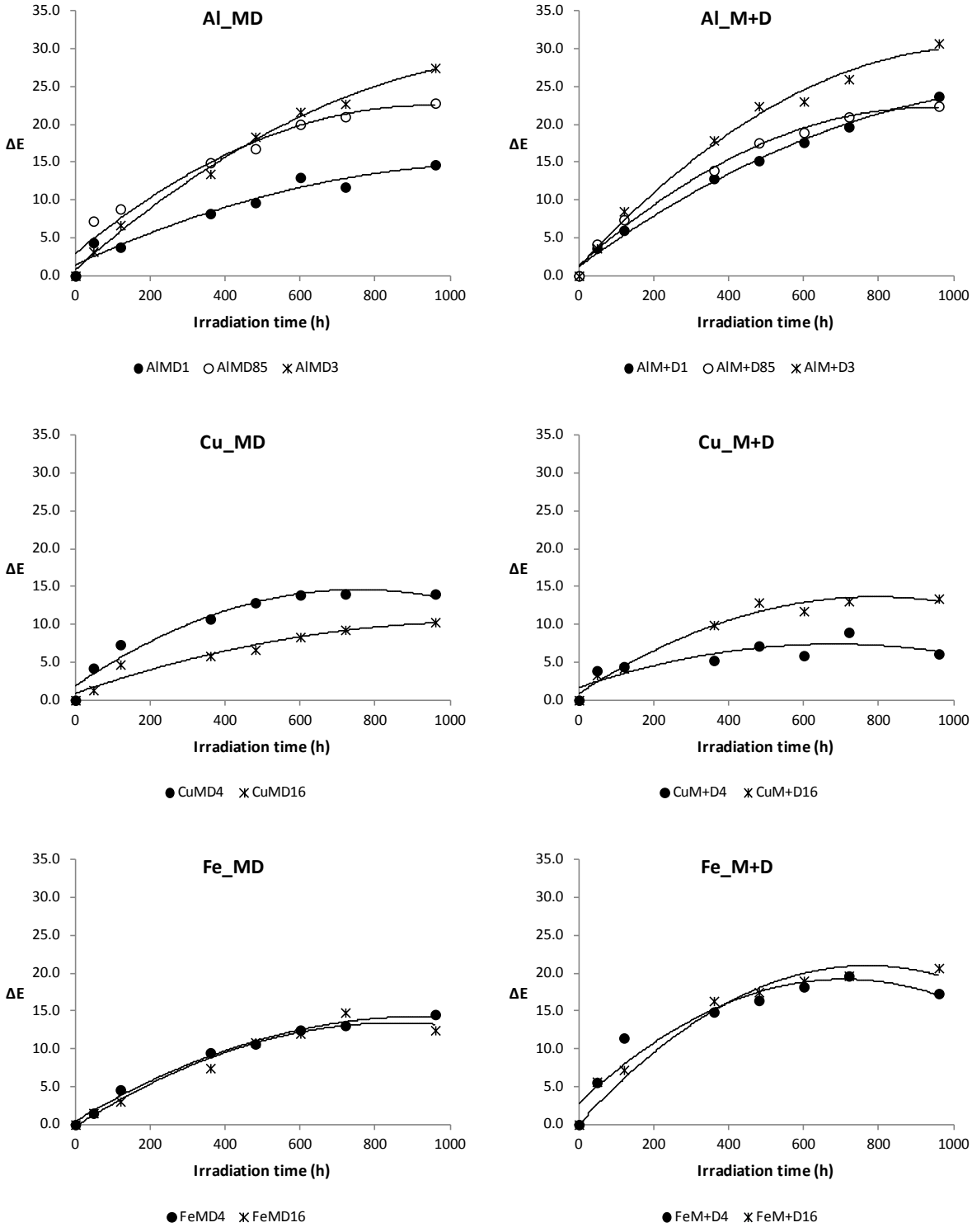


Figure 27. *Fading characteristics of madder dyed wool by the MD and M+D procedures in different bath concentrations of alum (0.1000, 0.0085 and 0.0030 mol.dm^{-3}), copper (II) sulfate and iron (II) sulfate (0.0400 and 0.0016 mol.dm^{-3} for both mordants) after t = 48, 120, 360, 480, 600, 720 and 960 h of light exposure.*

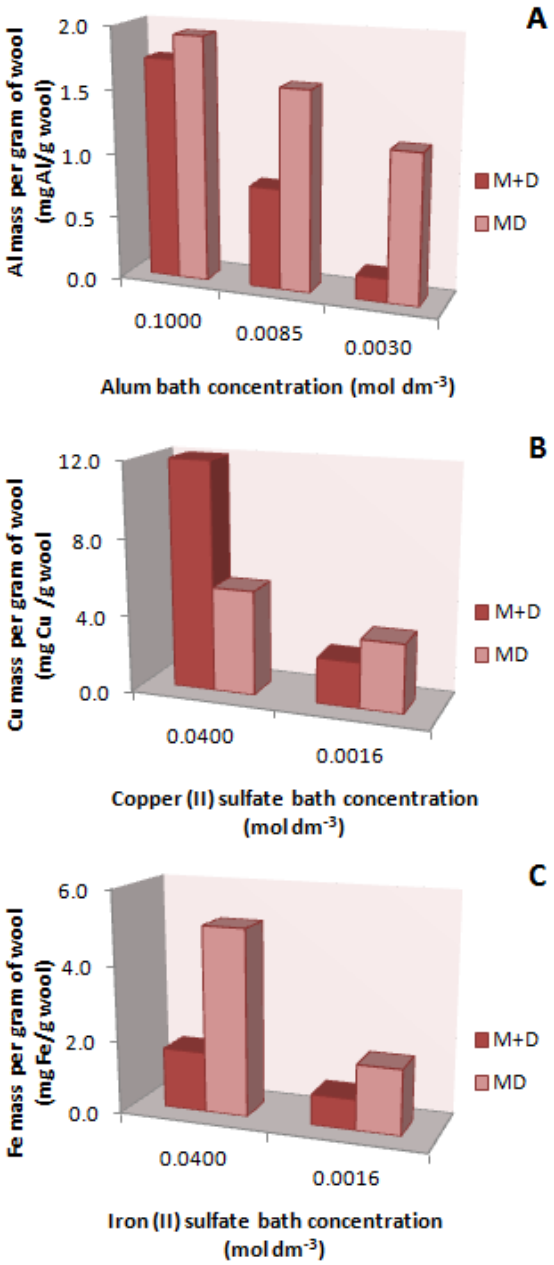


Figure 28. *Quantification of mordant metal ion (Al, Cu, Fe) in wool samples dyed with madder at different concentrations of mordant bath: alum (A), copper (II) sulfate (B) and iron (II) sulfate (C), by the MD and M+D dyeing methods.*

Increasing mordant bath concentration leads to a small increase in the fibre metal ion content. Cu ion seems to have a higher affinity for the wool fibres, since it was detected in higher amounts in fibres mordanted with the copper salt than in those mordanted with equivalent concentrations of iron or aluminium ions.

In general, and for a similar mordant concentration, the MD method yields samples with higher amounts of metal ion. The binding of the metal ion to the fibre is likely to proceed in a

different way depending on the dyeing procedure used. In the M+D method, the binding between the metal ion and the chromophore molecule occurs before the wool is even introduced in the dyeing bath, making it more difficult for the metal to bind the wool protein structure due to the bulkiness of the chemical structure formed. In the MD procedure the binding of the mordant to the wool occurs beforehand, probably explaining the higher amount of metal ions on these fibres.

Point microchemical analysis by EDS was performed in samples mordanted with Al, Fe or Cu (t = 0 h) in order to assess the metal ion distribution on the fibres. Analyses have shown that the metal ions were not evenly distributed: newly fractured zones presented relatively higher amounts (% weight) of the metal ion than the surface, pointing out the existence of some metal lixiviation (*Table 13*, RSD ≤ 5%). This is likely due to the washing procedure on both dyeing methods. Non-homogeneity in the Al distribution was also observed by Clementi *et al.*[218]. Overall, fibres dyed using MD procedure tend to yield higher concentrations (% weight) of mordant metal ions than M+D dyed fibres.

Table 13. *EDS concentrations of mordant metal ion in madder dyed wool samples on the interior (In) and surface (Out) of the wool fibres.*

Sample	Mordant metal ion concentration (% weight)	
	In	Out
AlM+D1	1.48	0.24
AlMD1	2.90	0.90
AlM+D85	0.77	0.29
AlMD85	1.80	0.30
AlM+D3	0.41	0.27
AlMD3	0.56	0.36
CuM+D4	1.92	1.06
CuMD4	5.25	3.56
CuM+D16	1.78	1.26
CuMD16	2.12	1.58
FeM+D4	0.78	0.52
FeMD4	3.20	1.81
FeM+D16	0.51	0.44
FeMD16	0.89	0.21

Morphological characterisation by SEM analysis showed that the fibre surfaces displayed the typical scale structure of wool with, in some cases, scale loss and roughened surfaces (*Figure 29*).

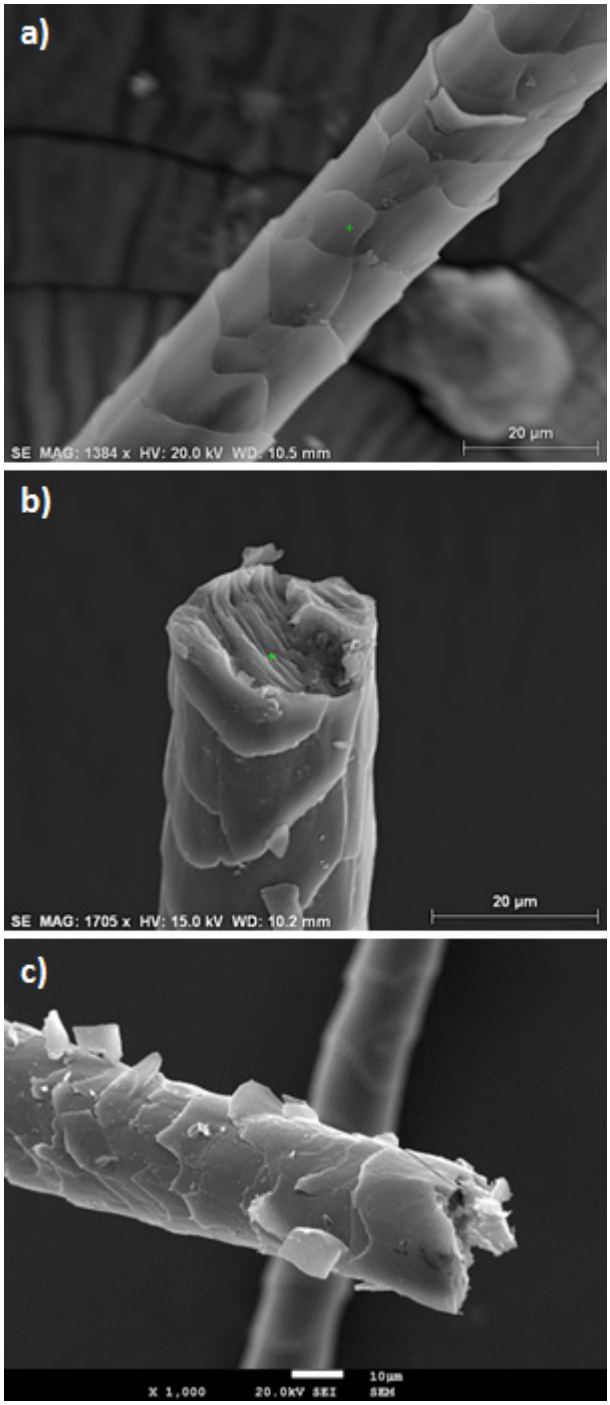


Figure 29. *SEM micrographs of madder dyed wool samples: a) AlM+D1, b) FeM+D4 and c) CuMD16.*

Chromophore evaluation

In order to assess the amounts of alizarin and purpurin in the wool samples before and along the light ageing process, an analytical methodology based on the wool extraction with EDTA/DMF[204] followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis was established. In *Figure 30* bar graphs corresponding to the chromatographic peak areas of alizarin and purpurin in wool samples before irradiation (t = 0 h) and after light exposure (t = 480 and 960 h) are presented.

Considerable differences in the chromophore contents of the wool samples before light exposure are observed for the same mordant, different mordant bath concentration and dyeing procedure, partially explaining the observed colour differences (*Table 12*). Overall, for the same mordant, samples dyed by the MD procedure (t = 0 h) present higher amounts of chromophores. When alum was used, the MD method yielded samples with a stronger pink hue (higher a* values, *Table 12*) while the samples dyed by the M+D method were more orange (higher b* values, *Table 12*).

Chromophore analysis (*Figure 30*, t = 0 h) showed that samples dyed by the MD method, using Al or Fe, have alizarin/purpurin peak areas <1, while all the samples dyed by the M+D method have alizarin/purpurin peak areas >1. Clementi *et al.*[218] observed the same behaviour in samples dyed with madder and 20% of alum by the MD procedure.

Within the same dyeing procedure, the colour of the samples mordanted with the two tested concentrations for copper (II) sulfate differ considerably, with the CuM+D16 and the CuMD16 samples always presenting a much darker colour (smaller L* parameter, *Table 12*). Chromophore extraction showed that the peak areas of alizarin and purpurin in the Cu_MD or Cu_M+D samples are very different, with the samples mordanted in the 0.0016 $mol.dm^{-3}$ bath presenting higher peak areas, especially those dyed by the MD procedure (*Figure 30*).

The use of iron mordant and the M+D procedure yielded samples with less intense reddish colour (lower values of a* and b*, *Table 12*) and chromophore analysis showed that the peak areas for alizarin and purpurin are 5 to 10 times smaller than those measured in the MD samples (*Figure 30*).

Chromophore analysis done on the artificially aged samples (*Figure 30*) corroborated the colourimetric study (*Table 12* and *Figure 27*), showing that the colour fading could be correlated to the degradation of the madder chromophores. Overall, increasing light exposure leads to a decrease in the alizarin and purpurin chromatographic peak areas.

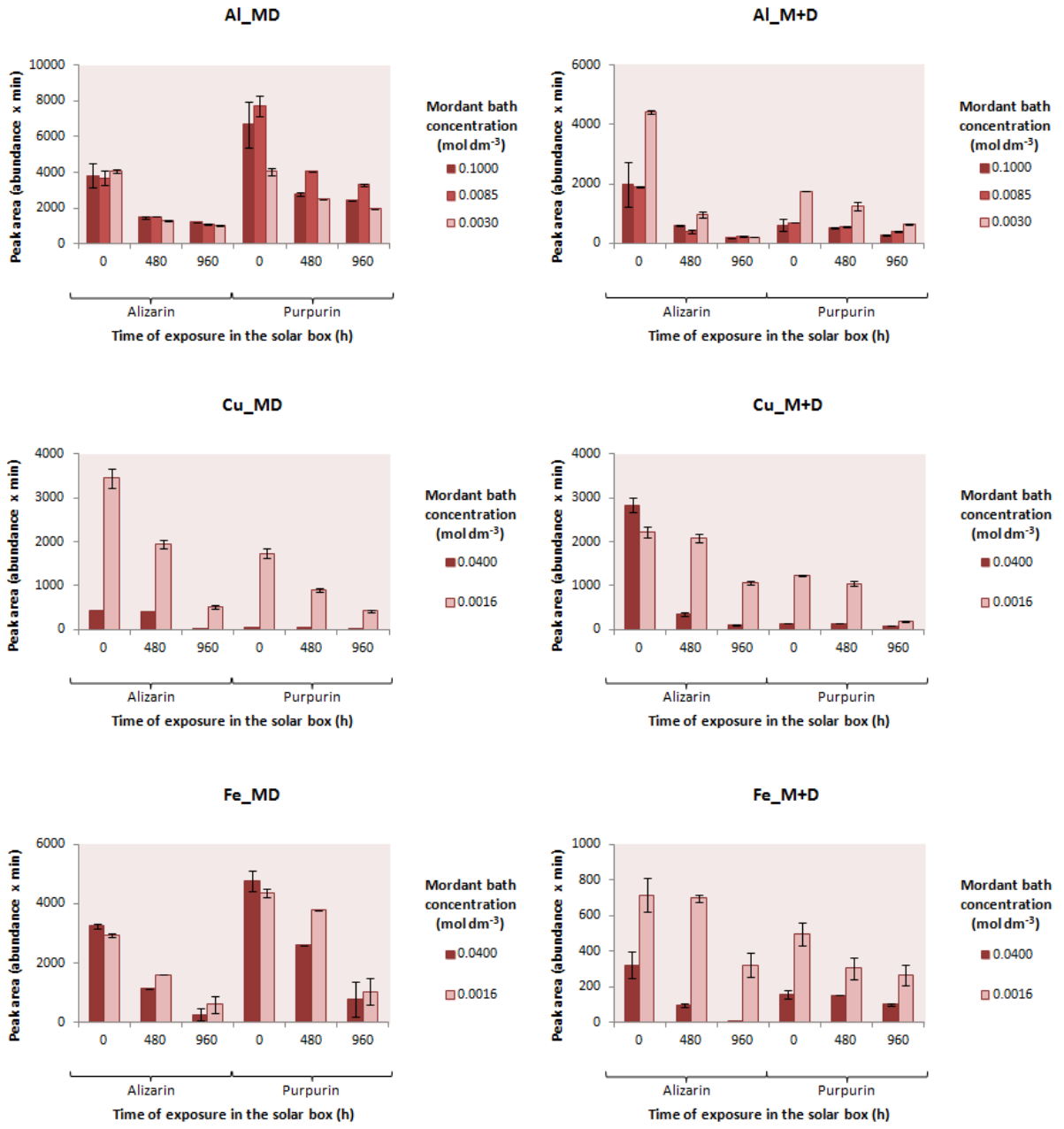


Figure 30. *LC-MS/MS peak areas of alizarin and purpurin in madder wool samples dyed at different concentrations of alum, copper (II) sulfate and iron (II) sulfate by the MD and M+D dyeing methods before (t = 0 h) and after (t = 480 and 960 h) light exposure.*

The alizarin and purpurin chromophores degradation rates were not constant, being in general more severe in the first 480 hours of light exposure (*Figure 30* and *Figure 31*) for samples mordanted with Al than with Cu or Fe. This fact comes in agreement with the more pronounced ΔE variation observed in the same period of time for the Al_MD and Al_M+D samples (*Figure 27*), confirming the poor lightfastness for samples dyed with madder and Al as Cox Crews[122] observed when natural yellow dyes were used with Al. Additionally, alizarin

degraded more extensively than purpurin in these samples. An opposite behaviour was observed by Clementi *et al.*[218] but for naturally aged samples mordanted with 20% alum and dyed with madder. When those samples were artificially aged in the presence of air, the authors observed an improvement on the purpurin lightfastness.

Overall, the chromophores (alizarin and purpurin) degradation rates are different and dependent on the mordant nature and dyeing procedure. These differences are likely to be responsible for hue changes observed along with the colour fading, although the presence of minor components could also affect the final hue.

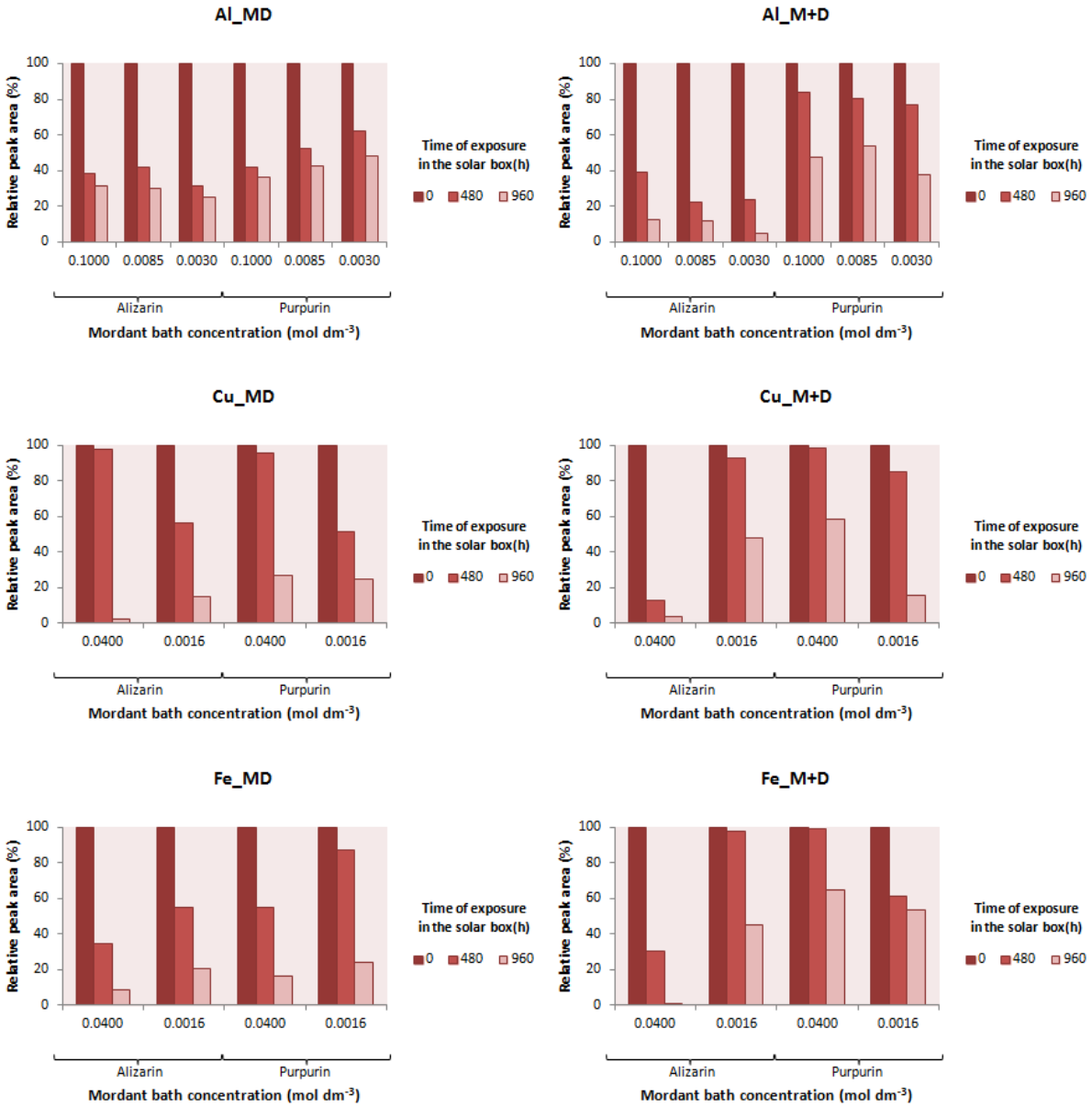


Figure 31. *Relative LC-MS/MS peak areas of alizarin and purpurin in wool samples dyed with madder at different concentrations of alum, copper (II) sulfate and iron (II) sulfate by the MD and M+D dyeing methods before (t = 0 h) and after (t = 480 and 960 h of light exposure).*

Wool samples dyed with brazilwood

Soluble redwood dyes are extracted from various species of the genus *Caesalpinia*. The principal varieties are Brazilwood, Peachwood, Sappanwood, Limawood and Pernambuco wood, though they are frequently collectively known as brazilwood[47,59,60]. The main colouring constituents of brazilwood are the homoisoflavonoids brazilin (*Figure 14*) and brazilein (*Figure 14*). Brazilin has been isolated, which by oxidation gives brazilein, the main chromophore in brazilwood[47,229]. The tree is indigenous to the East Indies, Central and South America and also to Africa. Brazilwood was known in Europe long before the discovery of South America because it was imported as a dyewood from the East Indies. Soon after the discovery of South America, large quantities of the valuable redwood were found in the forests along the Amazon and the newly discovered country was then given the name 'Brasil'[47,56,230].

The soluble brazilwood dyes wool and cotton with the assistance of a mordant. Different dye-mordant combinations can produce a wide range of colours. However, when exposed to light these colours easily fade or even change. Minimizing those fading changes has become crucial in conservation science[231]. Since lighting conditions carry the risk of causing irreversible colour changes, it is reasonable to seek a deeper understanding of the process of light fading. Few studies have been published concerning the influence of light exposure on brazilwood dyed textiles. Padfield and Landi[123] have studied the lightfastness of natural dyes in wool and cotton dyed fibres. Several mordants were used in the dyeing procedures and brazilwood was found to fade extremely fast when exposed to light. Zarkogianni *et al.*[228] also dyed cotton and wool fabrics with several natural sources, including brazilwood, and used a variety of mordants. Wash, light and rub fastness were tested and colour measurements were made[228]. The authors concluded that, in general, pre-mordanting the fibres improves wash fastness for brazilwood wool dyed samples. Light fastness wasn't enhanced, except when using iron-based mordants. Rub fastness didn't improve after mordanting[228].

Colourimetric studies

Table 14 presents the CIELab/Ch colour parameters for dyed samples preserved from light and after light exposure. As it can be seen, mordant metal ion, mordant bath concentration and dyeing procedure have strong influence in the wool fibre hues. For t=0 h and using the MD procedure, red colours were observed when using Al as mordant. When using Cu and Fe as mordants, wool fibre hues ranged from red-brown to grey hues. In the

case of the M+D methodology, at t=0 h, purple, green or bluish hues can be observed when using Al, Cu and Fe as mordants, respectively. In general, the lightness of the samples increases during light exposure. As for the chroma value, the most systematic decrease observed during the ageing process is presented by the Al mordanted samples dyed by MD procedure.

For the results presented in *Figure 32* it can be observed that, in general, fading is more pronounced in the first 400 h of light exposure and tends to stabilize beyond 600 h of light exposure. Colour variation was more pronounced for the alum dyed samples, as already observed by Crews[122], in wool dyed with natural yellow dyes, and in the present work, for madder dyed wool samples[232].

Apart from the mordant bath concentration, the dyeing technique had influence in the ΔE difference for the samples mordanted with Al and Cu. Samples dyed by MD procedure are more affected by photofading. As for the Fe salt at a concentration of 0.0016 $mol.dm^{-3}$, the colour variation is more accentuated in the first 48 h for the M+D procedure.

For Al mordanted samples, ΔE variation showed little dependence from the salt concentration. In the case of Fe mordanted samples, for the MD procedure, colour variation is independent from the concentration. Colour variation is also independent from the dyeing procedure for 0.0400 $mol.dm^{-3}$ Fe salt bath concentration.

As for Cu mordanted samples, no stabilisation of the ΔE parameter was observed up to 960 h of light exposure for samples prepared by the M+D method.

Mordant evaluation

Brazilwood dyed wool samples were digested with nitric acid and analysed by ICP-OES (Al and Fe) and FAAS (Cu). These techniques were found to be highly reproducible with an RSD ≤ 5% (data not shown).

Although larger mass values of metal ions were available in the dyeing baths (*Table 7*), the actual amounts of mordant metal ions found in the wool samples after HNO_3 digestion were, in general, much lower.

Table 14. *Colourimetric data for brazilwood dyed samples preserved from light (t = 0 h) and after light exposure (t = 48, 120, 240, 360, 480, 600, 720, 840 and 960 h).*

		Al						Fe				Cu			
		M+D			MD			M+D		MD		M+D		MD	
Time in the solar box (h)	**CIELab/Ch colour parameters**	AlM+D1	AlM+D85	AlM+D3	AlMD1	AlMD85	AlMD3	FeM+D4	FeM+D16	FeMD4	FeMD16	CuM+D4	CuM+D16	CuMD4	CuMD16
0	L*	37.47	34.29	34.00	34.80	38.76	39.51	35.40	53.62	22.96	25.54	20.58	24.62	23.42	21.50
	a*	10.72	11.21	9.93	45.55	43.27	38.52	1.47	1.49	6.41	5.90	0.54	-0.55	17.37	12.13
	b*	-9.61	-8.62	-7.48	21.33	19.69	18.40	-7.29	-8.19	0.10	1.15	-4.55	-3.73	5.72	1.86
	C*	14.40	14.14	12.43	50.29	47.54	42.69	7.44	8.33	6.41	6.01	4.58	3.77	18.29	12.27
	hº	318.13	322.44	323.01	25.09	24.47	25.53	281.43	280.29	0.88	11.05	276.73	261.62	18.24	8.73
48	L*	40.43	35.81	37.64	37.08	42.10	46.58	35.64	41.58	22.93	26.16	21.02	23.63	22.78	25.09
	a*	9.80	8.91	8.13	38.49	31.80	26.06	0.82	0.62	5.78	5.09	-0.59	-0.61	17.42	12.59
	b*	4.19	2.71	3.38	20.76	19.07	19.05	1.85	5.03	1.81	3.51	-2.46	-1.41	6.58	3.70
	C*	10.65	9.31	8.81	43.73	37.08	32.28	2.02	5.07	6.05	6.18	2.53	1.54	18.62	13.12
	hº	23.15	16.91	22.56	28.34	30.95	36.17	65.95	82.93	17.40	34.58	256.51	246.71	20.69	16.36
120	L*	43.31	38.29	39.45	43.25	50.84	54.64	37.11	42.33	25.97	31.52	20.58	27.05	26.70	27.69
	a*	8.70	8.51	7.72	33.06	24.55	20.55	0.90	0.92	5.50	5.24	-0.95	-1.32	19.45	16.09
	b*	9.08	5.09	6.14	22.01	20.07	20.96	4.30	6.98	3.64	4.95	-1.97	0.43	8.30	6.22
	C*	12.58	9.91	9.86	39.71	31.71	29.35	4.40	7.04	6.59	7.21	2.18	1.39	21.14	17.25
	hº	46.21	30.89	38.52	33.65	39.26	45.57	78.21	82.46	33.49	43.33	244.19	161.94	23.12	21.14
240	L*	51.05	44.50	48.20	53.08	58.20	62.46	42.97	47.92	30.70	36.79	22.56	30.81	32.23	36.22
	a*	7.58	7.04	6.23	22.99	17.08	13.21	0.93	0.69	5.28	4.87	-1.01	-1.00	17.48	14.42
	b*	13.87	8.03	9.78	22.44	19.95	21.26	7.66	9.36	6.71	8.79	-0.75	1.94	10.45	10.36
	C*	15.80	10.68	11.60	32.13	26.26	25.03	7.72	9.39	8.54	10.05	1.26	2.18	20.36	17.76
	hº	61.36	48.76	57.49	44.30	49.43	58.14	83.07	85.81	51.79	60.98	216.58	117.33	30.88	35.70
360	L*	53.25	49.91	53.44	58.15	61.41	67.26	45.20	48.07	34.78	38.81	21.66	31.77	34.01	39.19
	a*	5.88	5.11	4.13	16.43	11.82	8.91	0.89	0.79	4.82	4.10	-1.87	-1.64	14.65	11.22
	b*	15.95	9.14	11.61	22.27	18.90	20.67	9.49	10.18	9.66	9.95	0.35	5.11	12.18	12.02
	C*	17.00	10.47	12.33	27.67	22.29	22.51	9.53	10.21	10.80	10.76	1.90	5.36	19.05	16.44
	hº	69.76	60.78	70.44	53.58	57.98	66.69	84.61	85.56	63.51	67.59	169.52	107.81	39.75	46.99
480	L*	54.14	50.53	55.73	64.07	63.54	69.90	48.00	53.02	39.79	43.11	23.66	35.52	39.38	47.57
	a*	6.25	4.29	2.89	12.57	9.16	6.53	1.01	0.78	4.70	3.98	-1.18	-1.61	12.93	9.24
	b*	15.86	10.37	11.88	22.43	18.39	19.13	9.96	10.98	11.34	11.39	0.90	6.48	13.62	14.81
	C*	17.05	11.22	12.23	25.71	20.54	20.21	10.01	11.01	12.27	12.06	1.48	6.48	18.78	17.45
	hº	68.51	67.53	76.35	60.74	63.52	71.15	84.19	85.93	67.47	70.75	142.86	103.93	46.49	58.03
600	L*	58.61	53.43	61.39	66.26	65.83	68.38	49.80	52.87	41.03	43.29	25.05	36.73	42.59	49.78
	a*	4.04	3.55	2.11	10.36	7.92	4.86	1.23	0.85	4.34	3.68	-1.69	-1.84	11.46	7.92
	b*	17.36	10.50	12.65	22.82	18.67	17.72	11.21	11.43	12.71	12.72	2.00	6.90	15.09	15.92
	C*	17.82	11.09	12.82	25.06	20.28	18.38	11.28	11.46	13.43	13.24	2.62	7.14	18.96	17.78
	hº	76.90	71.35	80.54	65.57	67.01	74.68	83.73	85.74	71.15	73.88	130.18	104.95	52.78	63.54
720	L*	58.57	55.10	61.68	67.85	64.48	69.58	50.18	55.78	43.53	44.77	27.82	40.23	45.11	53.01
	a*	3.26	2.94	1.56	8.72	5.50	4.29	0.86	0.41	4.15	3.34	-1.81	-1.54	10.40	5.82
	b*	15.78	10.38	12.07	21.11	16.58	17.22	10.58	11.45	14.10	12.81	3.32	7.77	16.84	15.67
	C*	16.12	10.79	12.17	22.84	17.47	17.74	10.61	11.46	14.70	13.24	3.78	7.92	19.79	16.72
	hº	78.34	74.20	82.63	67.55	71.65	76.02	85.35	87.95	73.58	75.40	118.65	101.18	58.29	69.61
840	L*	59.25	58.72	62.08	69.12	67.17	68.74	46.58	54.39	39.06	45.05	25.45	43.14	43.95	52.25
	a*	2.72	2.16	1.32	7.92	4.89	3.36	0.94	0.65	3.92	3.49	-1.40	-0.88	9.78	6.15
	b*	14.67	10.85	11.76	21.06	15.55	15.10	10.06	11.29	12.09	13.22	4.26	9.43	15.99	16.32
	C*	14.92	11.06	11.84	22.50	16.30	15.47	10.10	11.31	12.71	13.67	4.48	9.47	18.75	17.44
	hº	79.51	78.74	83.59	69.39	72.54	77.45	84.68	86.70	72.05	75.21	108.23	95.31	58.55	69.35
960	L*	61.71	63.34	62.96	70.33	64.72	66.79	49.62	55.84	39.81	45.98	25.74	45.21	45.09	54.27
	a*	2.47	1.66	1.00	7.01	3.34	2.46	1.30	0.62	3.97	3.39	-1.18	-0.53	9.22	5.48
	b*	15.14	11.69	11.34	21.00	14.11	13.85	11.34	11.88	11.34	12.77	3.96	11.27	16.06	16.40
	C*	15.34	11.81	11.38	22.14	14.50	14.07	11.41	11.90	12.01	13.21	4.13	11.29	18.52	17.29
	hº	80.75	81.90	84.95	71.53	76.67	79.91	83.48	87.01	70.68	75.14	106.63	92.70	60.15	71.52

An increase in the mordant bath concentration results only in a small increase in the fibre metal ion content (*Figure 33*). Nevertheless, the ratio between the metal ion content in the fibre and the mass of metal ion present in the dyeing bath is larger for the smaller bath concentrations of mordant salts. Cu ions seem to have greater affinity for the wool fibres and, in the case of CuMD16 samples, the mordant was almost fully bound to the fibre. Copper salts are well known for their ability to form complex amines[30]. Moreover, it has been previously established that Cu ions react not only with the carboxylate groups in wool but also with the products of the cystine hydrolysis of wool keratin that occurs at high temperatures[233,234]. The high copper uptake may be attributed to the formation of copper sulfide and to the carboxylate reactions[233,234].

Overall, for equivalent mordant concentrations, the MD dyeing procedure results in samples with higher amounts of metal ions. Similar results were obtained in the present work for madder dyed wool[232]. This is probably explained by the differences between both dyeing methods. In the M+D method, metal ion and chromophore bind before the wool is added to the dyeing bath, making more difficult for the metal to bind to the wool protein structure. In the MD procedure, metal ion binds to wool beforehand, thus resulting in higher amounts of metal ions in the fibres.

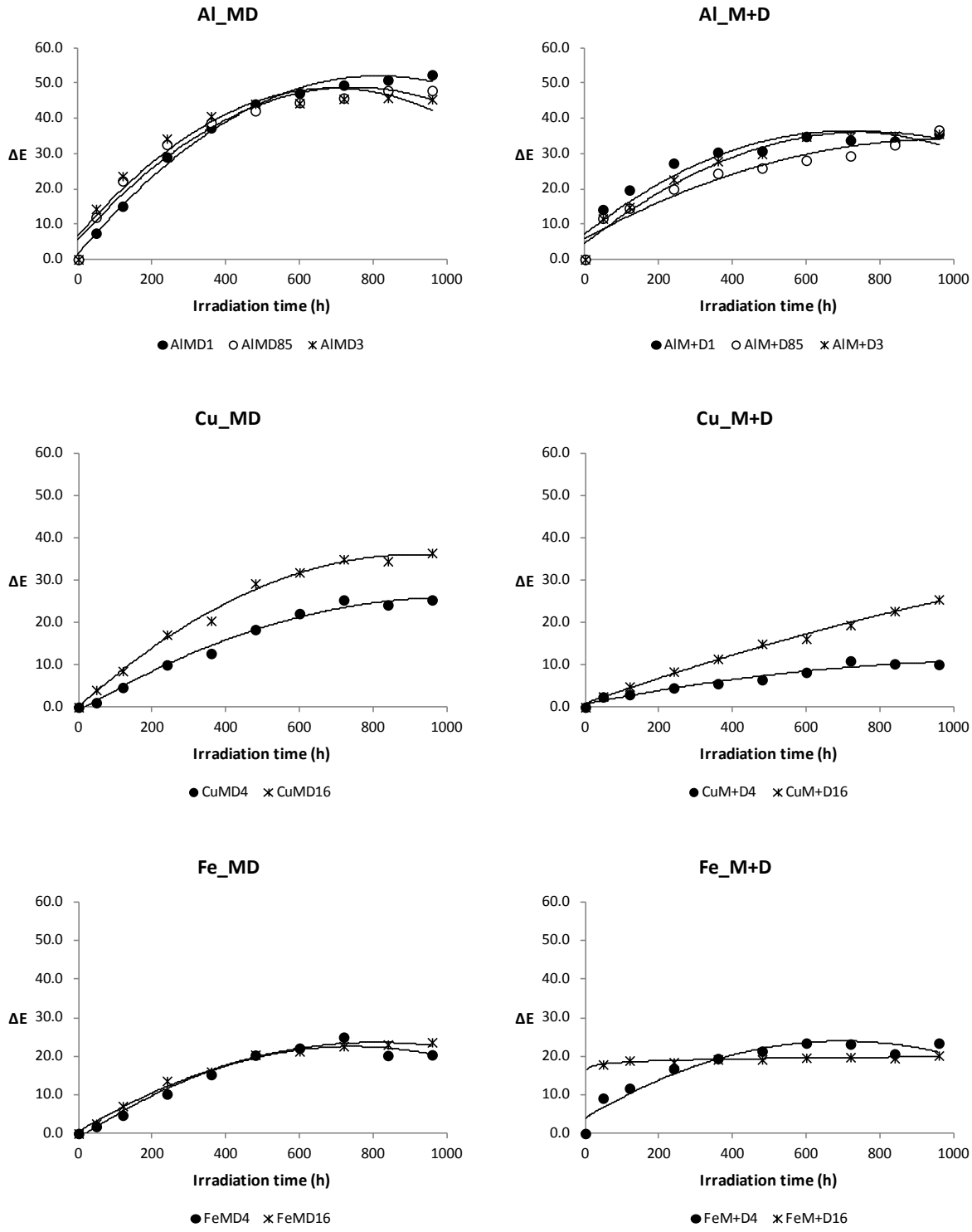


Figure 32. *Fading characteristics of brazilwood dyed wool by the MD and M+D procedures in different bath concentrations of alum (0.1000, 0.0085 and 0.0030 mol.dm^{-3}), copper (II) sulfate and iron (II) sulfate (0.0400 and 0.0016 mol.dm^{-3} for both mordants) after t = 48, 120, 360, 480, 600, 720 and 960 h of light exposure.*

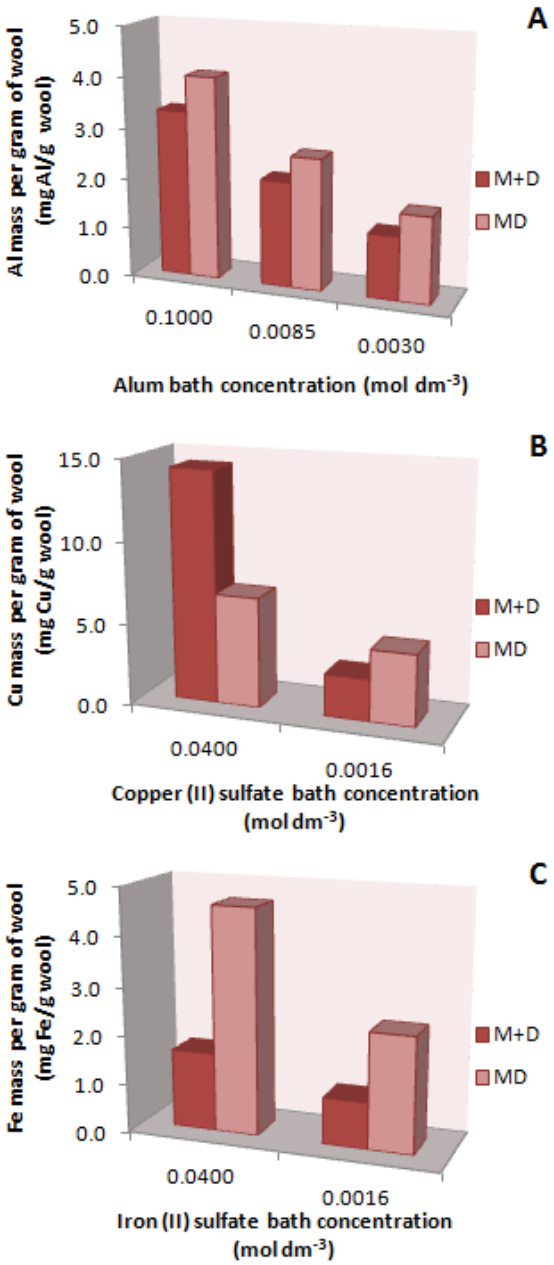


Figure 33. *Quantification of mordant metal ion (Al, Cu, Fe) in wool samples dyed with brazilwood at different concentrations of mordant bath: alum (A), copper (II) sulfate (B) and iron (II) sulfate (C), by the MD and M+D dyeing methods.*

Chromatographic studies

Although brazilwood is well-known for its poor light fastness[56], no study on the chromophore variation through light ageing has yet been published.

For the evaluation of brazilein in the wool before and during the light ageing process, LC-MS/MS technique was used. Samples were first subjected to a mild extraction procedure

using Na_2EDTA/DMF[204]. *Figure 34* represents the chromatographic peak areas of brazilein dyed wool before (t = 0 h) and after (t = 240, 600 and 960 h) light exposure.

For the same mordant, samples dyed by the MD procedure yielded higher brazilein peak areas (*Figure 34*). In the MD dyeing procedure, higher amounts of metal ions are bound to the fibres, probably resulting in a higher rate of metal-chromophore complexes attached to the wool fibres.

Brazilein degradation rate was not constant for both dyeing procedures, being overall more accentuated in the first 240 h for the MD dyeing method (*Figure 35*), which is in agreement with the colour fading behaviour observed in the colourimetric studies.

For the same dyeing procedure, the use of copper (II) sulfate as mordant results in an extraction of higher amounts of brazilein (*Figure 34*). As already stated, this mordant seems to have greater affinity for the wool fibres (*Figure 33*), probably allowing for a larger number of available sites for the formation of the brazilein-copper complexes.

The chromatographic profiles of brazilwood dyed wool AlMD1 before (t = 0 h) and after (t = 960 h) light exposure are shown in *Figure 36*. An alum mordanted sample was chosen because of the historical importance of this mordant. Peak identification was done using UV-Vis and mass spectra (negative mode). As expected, at t = 0 h, the major compound identified in the brazilwood dyed wool sample was brazilein ($[M-H]^-$ *m/z* 283). After 960 h of light exposure, brazilein was still identified as the major compound in brazilwood dyed wool, but a new peak eluted at r_t=18.8 min., yielding a deprotonated molecular ion $[M-H]^-$ at *m/z* 243 and UV-Vis characteristic bands at 257, 307 and 337 nm was detected. This compound, whose structure is not yet determined, is generally known as Type C compound and was first described by Nowik[66], after acid hydrolysis of several species of soluble redwoods, collectively known as brazilwood. Type C compound has also been described by Karapanagiotis *et al.* in samples collected from Cretan icons[235] and post-Byzantine textiles[179], but no explanation was given for the nature of this compound. In the present work, no acidic extraction method was used for the chromophore extraction from dyed wool, thus the resulting Type C compound cannot be considered a product of an acid hydrolysis. Therefore, Type C compound is likely a photodegradation product of brazilwood dye and the detection of this analyte in historical textile samples can be used to identify brazilwood as dye source.

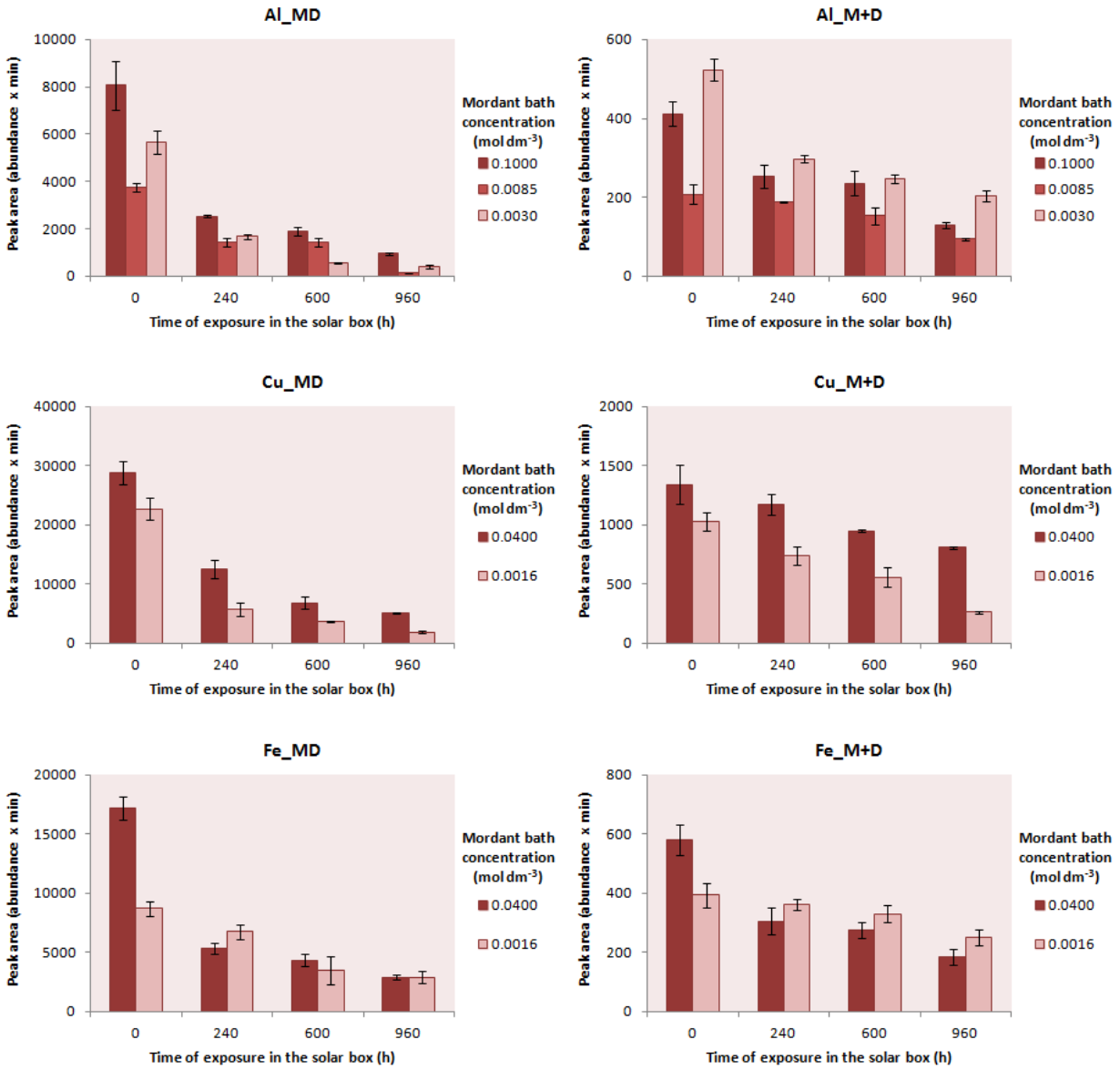


Figure 34. *LC/MS peak areas of brazilein in brazilwood wool samples dyed at different concentrations of alum, copper (II) sulfate and iron (II) sulfate by the MD and M+D dyeing methods before (t = 0 h) and after (t = 240, 600 and 960 h) light exposure.*

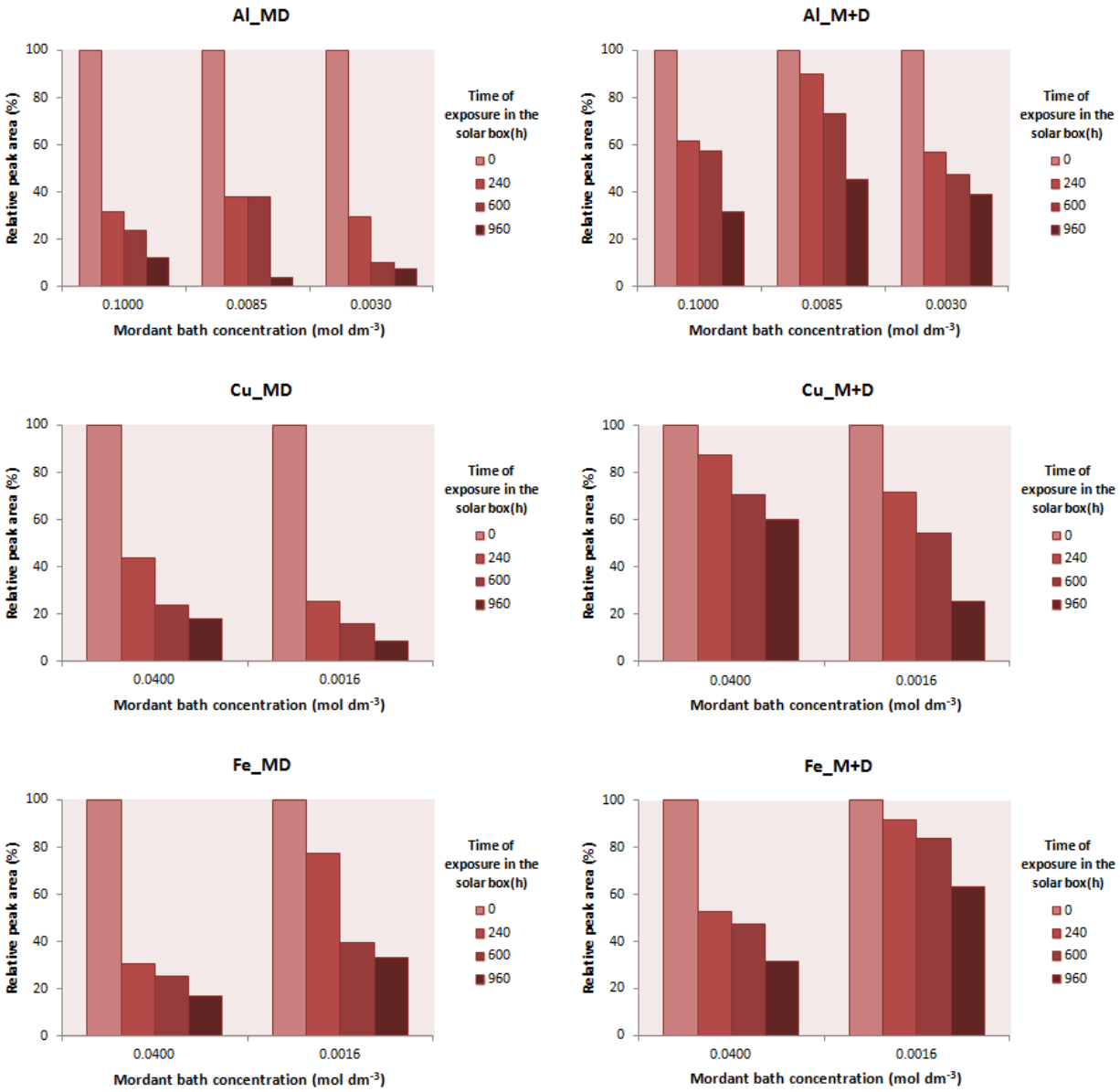


Figure 35. *Relative LC/MS peak areas of brazilein in wool samples dyed with different concentrations of alum, copper (II) sulfate and iron (II) sulfate by the MD and M+D dyeing methods before (t = 0 h) and after (t = 240, 600 and 960 h of light exposure).*

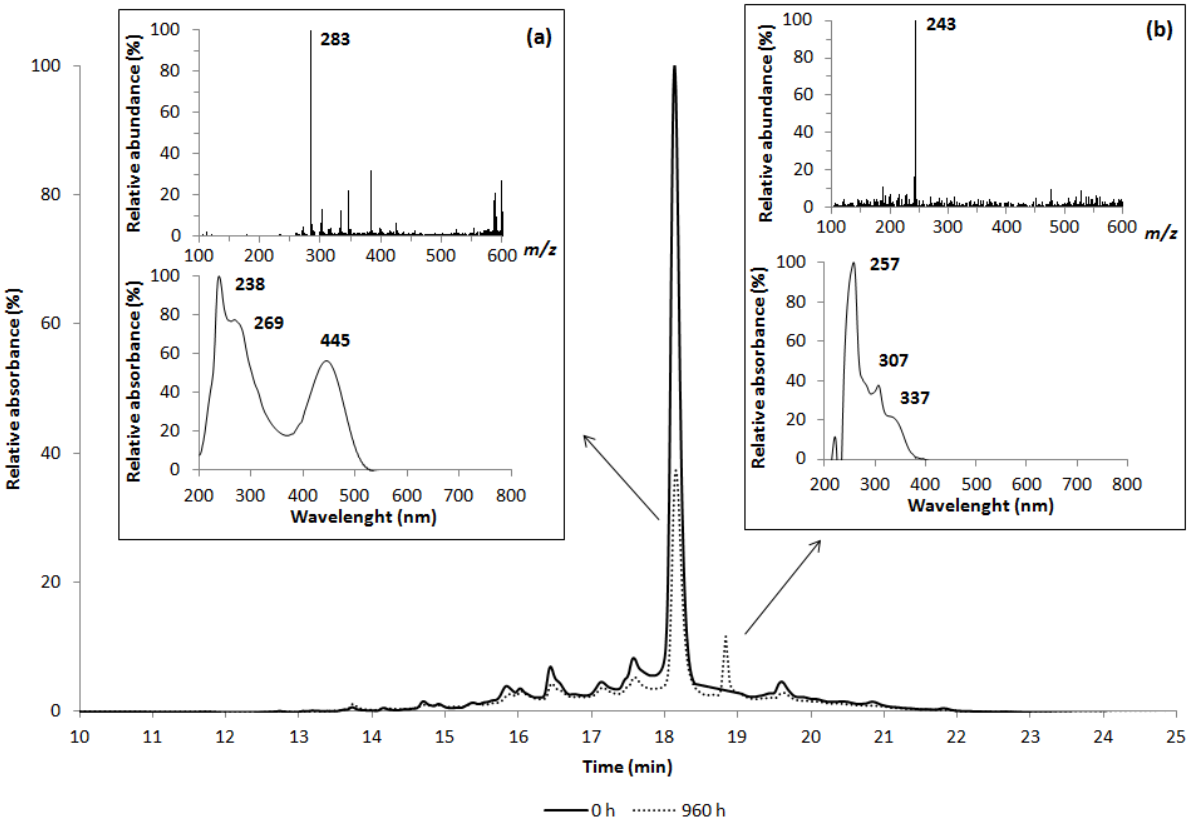


Figure 36. *Chromatographic profiles (DAD detection at 400 nm) of brazilwood dyed wool before (t = 0 h) and after (t = 960 h) light exposure. Brazilein (a) was identified in both samples and type C compound (b) was only detected after light ageing.*

Analysis of Arraiolos historical tapestries from the National Museum of Ancient Art (NMAA) collection

Despite being very popular and present in various Portuguese museum's collections, there is only a single published study concerning the material study of two tapestries belonging to the Museum Machado de Castro[2].

For the study presented here, thirteen Arraiolos historical tapestries were selected by the museum textile curator from the NMAA (Lisboa, Portugal) collection. The tapestries were selected based on their stylistic features deemed representative of the different periods of production between the 17th and the 19th centuries (for details, see Chapter 2).

A total of 148 samples were collected in the thirteen tapestries and information regarding sample collection and nomenclature is presented in the experimental section (Chapter 5).

Wool fibre analysis by scanning electron microscopy (SEM)

SEM micrographs of wool fibre surfaces display, in general, the typical scale structure (*Figure 37*, modern wool). However, in most cases, the historical samples presented roughened surfaces, with some damage of the scale structure due to wear and natural ageing of the fibres (*Figure 37*, B1 and A7).

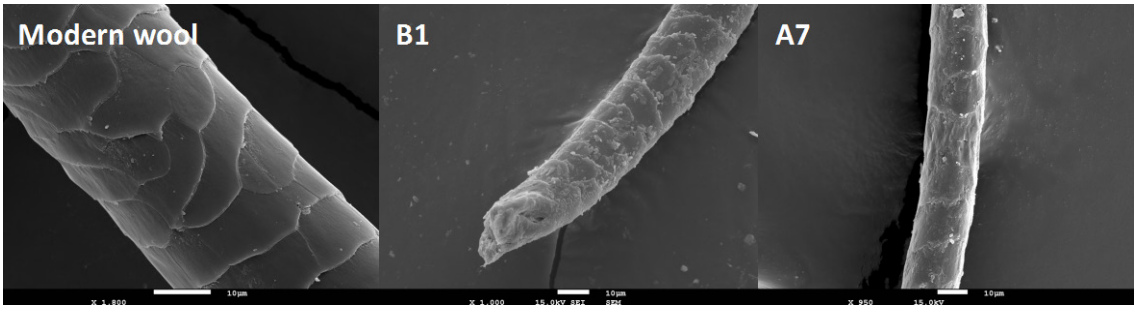


Figure 37. *SEM micrographs of the surface of modern and historical wool fibres.*

Fracture morphologies were investigated on the fibres taken from the historical Arraiolos tapestries (*Figure 38*). Fracture morphology was assessed according to Hearle *et al.*[236] classification. The presence of transverse cracks and longitudinal splitting are indicative of embrittlement due to loss of flexibility and elasticity, causing cracks when the fibre is subjected to bending or stretching. Other types of fractures were observed in the analysed fibres, being the most commonly found a combination of granular and radial fractures. Some fibrillar multiple split ends were also found, but it was likely that they are naturally occurring ends, with fibrillation due to wear[196,236]. In some cases, biological colonization was found (mould and bacteria) which may also contribute to loss of tensile strength of the wool fibres (*Figure 39*).

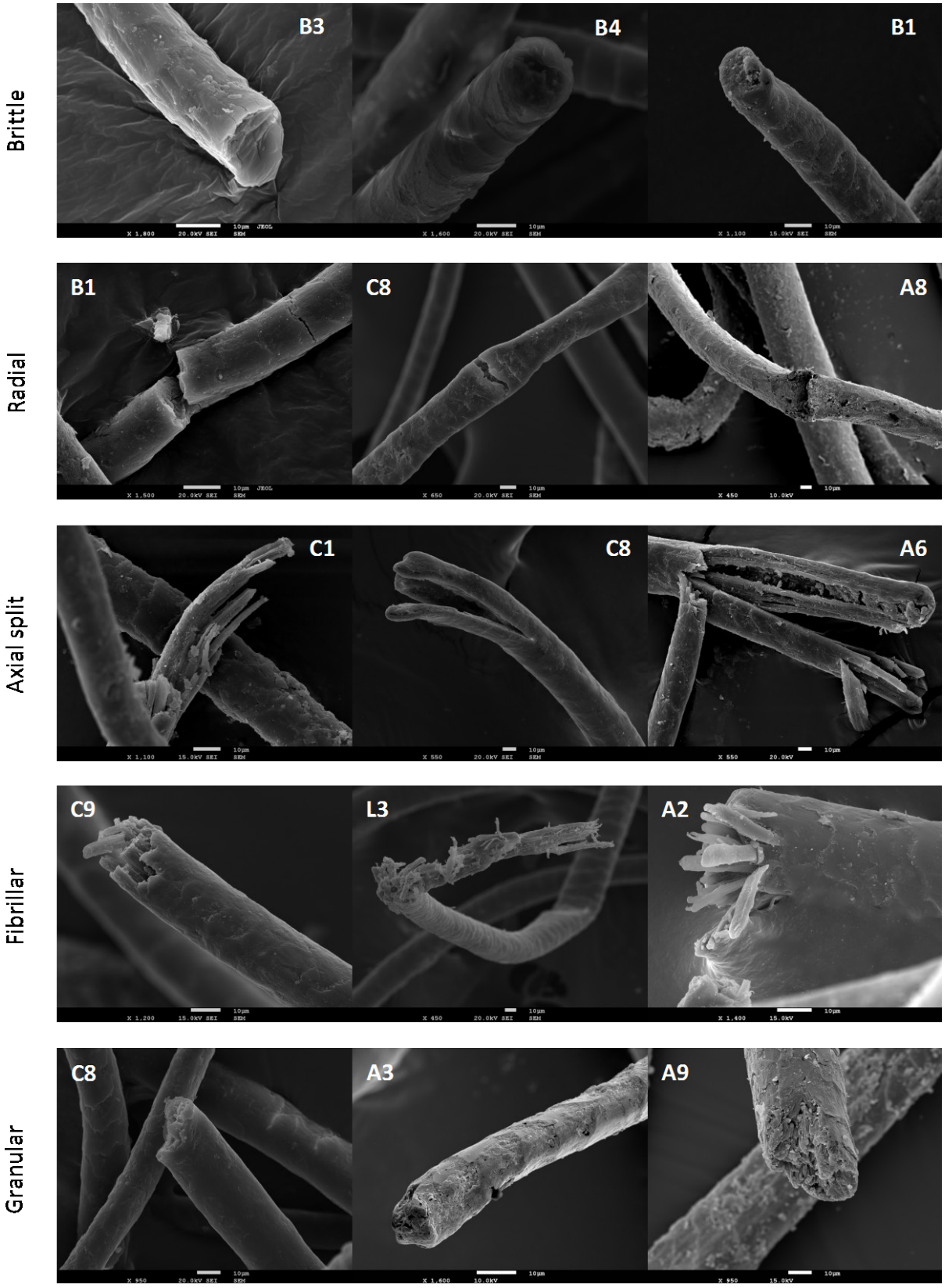


Figure 38. *SEM micrographs of the historical fibres exhibiting distinct types of fractures.*

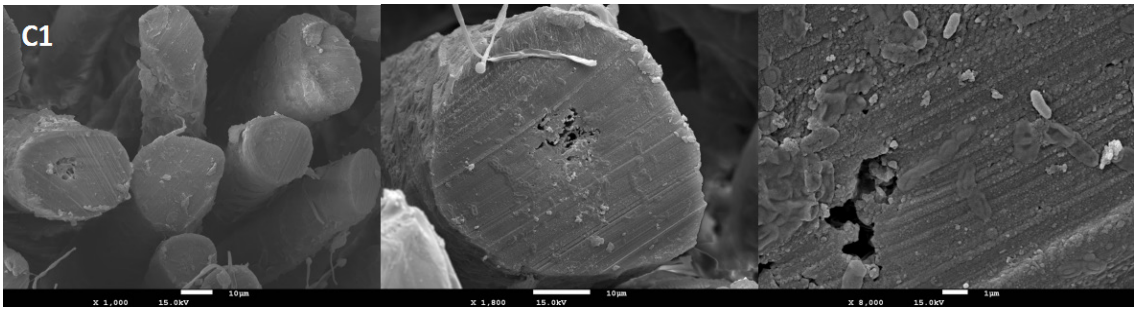


Figure 39. *Biological colonization in historical fibre C1.*

Analysis and identification of natural dyes and mordants

For analysis and identification of natural dyes, the thirteen tapestries were divided in two groups. Rugs produced in the 17th century and in the 17th-18th century transition (Tapestries A, E, F, G, I, J and L) are discussed in subchapter '*17th-18th century tapestries*'. Rugs produced in the 18th century, 18th-19th century transition and 19th century (Tapestries B, C, D, H, M and N) are discussed in subchapter '*18th-19th century tapestries*'. Full description of all carpets is given in Chapter 2 and information regarding sample collection and notation is presented in Chapter 5 (*Figure 21* and *Table 5*, *Figure 22* and *Table 6*). Colourimetric and chromatographic data for each sample is presented in *Table 15* to *Table 21* and *Table 22* to *Table 27*. Mordant analysis is discussed for all the carpets in subchapter '*Analysis and quantification of mordants in Arraiolos tapestries*'.

Analysis and identification of natural dyes in Arraiolos tapestries

17th-18th century tapestries

The previous study, on the available methods for the extraction of natural dyes from wool, allowed the establishment of EDTA-2 as the most suitable method for the extraction of natural dyes from historical wool samples. HPLC/DAD/MS was used for dye identification, as presented in *Table 15* to *Table 21*.

Table 15. *Colourimetric data and identification of natural dyes in wool samples collected from Arraiolos tapestry A.*

ID	Colour†	CIEL*a*b* coordinates			LC/DAD r_t (min.)	LC/DAD data (nm)	LC/MS data (*m/z*)‡		Identification	Possible dye source
		L*	a*	b*			ESI⁻	ESI⁺		
A1	Bl	22.33	-2.66	-8.73	16.49	245, 276, 313, 487	**491 [M-H]⁻**, 447, 357, 327	-	Carminic acid	Cochineal + Indigo or Woad
					26.58	251, 286, 319, 607	-	**263 [M+H]⁺**, 235, 219	Indigotin	
A2	Gr	48.51	-1.27	24.18	15.99	246, 267, 327	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Weld + Indigo or Woad
					16.42	244, 268, 339	**609 [M-H]⁻**, 447, 285	-	Luteolin 3',7-di-*O*-glucoside	
					17.33	248, 266, 348	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
					18.09	245, 266, 331	**431 [M-H]⁻**, 269	-	Apigenin 7-*O*-glucoside	
A3	Gr	33.72	-6.95	10.72	18.22	247, 269, 341	**461 [M-H]⁻**, 299	-	Chrysoeriol 7-*O*-glucoside	
					20.57	252, 265, 348	**285 [M-H]⁻**, 241, 217, 199, 175, 151, 133	-	Luteolin	
					26.60	252, 285, 320, 605	-	**263 [M+H]⁺**, 235, 219	Indigotin	
A4	Rs	46.10	14.42	10.35	16.67	240, 276, 308, 493	**491 [M-H]⁻**, 447, 357, 327, 299	-	Carminic acid	Cochineal + Madder
					18.02	245, 277, 315, 491	**491 [M-H]⁻**, 447, 357	-	dcIV	
					18.42	245, 277, 315, 491	**491 [M-H]⁻**, 447, 357	-	dcVII	
					24.74	252, 274, 423	-	**241 [M+H]⁺**, 213, 185, 157	Alizarin	
					26.31	256, 288, 479	-	**257 [M+H]⁺**, 229	Purpurin	
A5	R	44.23	21.12	15.86	18.02	247, 266, 407	**269 [M-H]⁻**, 254, 223	-	Morindone	Madder (*Morinda* spp.)
					18.21	259, 284, 415	**239 [M-H]⁻**, 195	-	Xanthopurpurin	
					24.73	249, 278, 426	-	**241 [M+H]⁺**, 213, 185, 157	Alizarin	
					26.30	256, 294, 479	-	**257 [M+H]⁺**, 229	Purpurin	
A6	R	40.01	31.18	17.91	24.72	250, 278, 426	-	**241 [M+H]⁺**, 213, 185, 157	Alizarin	Madder
					26.29	256, 293, 479	-	**257 [M+H]⁺**, 229	Purpurin	
A7	Y	51.42	3.78	30.08	16.41	247, 268, 340	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	Weld
					17.32	249, 268, 347	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
A8	Bl	45.98	-5.66	-0.38	17.34	243, 269, 348	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	Weld? + Indigo or Woad
					26.61	253, 285, 319, 607	-	**263 [M+H]⁺**, 235, 219	Indigotin	
A9	W	60.27	2.78	16.57	-	-	-	-	-	No dye source identified

† *Bl* blue, *Gr* green, *R* red, *Rs* rose, *W* white, *Y* yellow

‡ In bold: major ions

Table 16. *Colourimetric data and identification of natural dyes in wool samples collected from Arraiolos tapestry E.*

ID	Colour†	CIEL*a*b* coordinates			LC/DAD r_t (min.)	LC/DAD data (nm)	LC/MS data (*m/z*)‡		Identification	Possible dye source
		L*	a*	b*			ESI⁻	ESI⁺		
E1 E7 E10	Bl Bl Bk	25.93 49.27 18.11	-4.40 -5.77 -0.75	-7.04 7.09 -0.33	26.55	245, 286, 331, 606	-	**263 [M+H]⁺**, 235, 219	Indigotin	Indigo or Woad
E2 E3	Gr Gr	41.39 33.03	-6.99 -8.27	25.11 12.15	16.04	245, 267, 335	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Weld + Indigo or Woad
					16.43	245, 267, 335	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					17.33	254, 267, 348	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
					18.07	246, 263, 328	**431 [M-H]⁻**, 269	-	Apigenin 7-*O*-glucoside	
					20.58	253, 266, 348	**285 [M-H]⁻**, 199, 175, 133	-	Luteolin	
					26.55	245, 286, 331, 606	-	**263 [M+H]⁺**, 235, 219	Indigotin	
E4 E5	Y Y	55.73 56.43	9.27 9.43	37.87 47.62	16.04	245, 267, 335	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Weld
					16.43	245, 267, 335	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					17.33	254, 267, 348	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
					20.58	253, 266, 348	**285 [M-H]⁻**, 199, 175, 133	-	Luteolin	
E6 E13	R O	35.11 42.31	32.91 25.56	21.91 26.74	15.99	243, 277, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Weld + Madder
					16.45	243, 277, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					17.37	250, 267, 346	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
					20.62	250, 267, 347	**285 [M-H]⁻**, 217, 199, 175	-	Luteolin	
					24.74	252, 274, 423	-	**241 [M+H]⁺**, 213, 185, 157	Alizarin	
					26.31	256, 288, 479	-	**257 [M+H]⁺**, 229	Purpurin	
E8	O	52.41	15.04	24.40	24.74	252, 274, 423	-	**241 [M+H]⁺**, 213, 185, 157	Alizarin	Madder
					26.31	256, 288, 479	-	**257 [M+H]⁺**, 229	Purpurin	
E9	Rs	45.02	20.72	15.65	16.47	240, 276, 308, 493	**491 [M-H]⁻**, 447, 357, 327, 299	-	Carminic acid	Cochineal
E11 E12	Gy Gy	32.58 42.89	2.37 2.41	4.85 12.71	24.74	252, 274, 423	-	**241 [M+H]⁺**, 213, 185, 157	Alizarin	Madder + Indigo or Woad
					26.31	256, 288, 479	-	**257 [M+H]⁺**, 229	Purpurin	
					26.55	245, 286, 331, 606	-	**263 [M+H]⁺**, 235, 219	Indigotin	
E14	Bg	56.56	9.41	26.91	-	-	-	-	-	No dye source identified

† *Bg* beige, *Bk* black, *Bl* blue, *Gr* green, *Gy* grey, *O* orange, *R* red, *Rs* rose, *Y* yellow

‡ In bold: major ions

Table 17. *Colourimetric data and identification of natural dyes in wool samples collected from Arraiolos tapestry F.*

ID	Colour†	CIEL*a*b* coordinates			LC/DAD r_t (min.)	LC/DAD data (nm)	LC/MS data (*m/z*)‡		Identification	Possible dye source
		L*	a*	b*			ESI⁻	ESI⁺		
F1	Y	36.67	4.69	22.69	24.74	252, 274, 423	-	**241 [M+H]⁺**, 213, 185, 157	Alizarin	Madder
					26.31	256, 288, 479	-	**257 [M+H]⁺**, 229	Purpurin	
F2	Y	51.49	3.32	24.39	15.99	243, 277, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Weld
					16.45	243, 277, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					17.37	250, 267, 346	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
					18.12	244, 265, 324	**431 [M-H]⁻**, 269	-	Apigenin 7-*O*-glucoside	
					20.62	250, 267, 347	**285 [M-H]⁻**, 199, 175, 151	-	Luteolin	
F3	Bl	24.02	-4.25	-11.77	26.55	245, 286, 331, 606	-	**263 [M+H]⁺**, 235, 219	Indigotin	Indigo or Woad
F9	Bl	42.78	-6.33	-2.94						
F4	Rs	36.81	31.41	13.25	16.47	240, 276, 308, 493	**491 [M-H]⁻**, 447, 357, 327, 299	-	Carminic acid	Cochineal + Madder + Weld
					17.37	250, 267, 346	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
					24.74	252, 274, 423	-	**241 [M+H]⁺**, 213, 185, 157	Alizarin	
					26.31	256, 288, 479	-	**257 [M+H]⁺**, 229	Purpurin	
F5	Br	19.90	3.87	5.00	-	-	-	-	-	No dye source identified
F6	Bg	50.70	4.62	24.38						
F7	Bg	53.29	4.15	16.44						
F8	Gr	28.20	-10.80	-1.13	15.99	243, 277, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Weld + Indigo or Woad
					16.45	243, 277, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					17.37	250, 267, 346	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
F10	Gr	33.30	-7.11	16.04	18.12	244, 265, 324	**431 [M-H]⁻**, 269	-	Apigenin 7-*O*-glucoside	
					20.62	250, 267, 347	**285 [M-H]⁻**, 199, 175, 151	-	Luteolin	
					26.55	245, 286, 331, 606	-	**263 [M+H]⁺**, 235, 219	Indigotin	

† *Bg* beige, *Bl* blue, *Br* brown, *Gr* green, *Rs* rose, *Y* yellow

‡ In bold: major ions

Table 18. *Colourimetric data and identification of natural dyes in wool samples collected from Arraiolos tapestry G.*

ID	Colour†	CIEL*a*b* coordinates			LC/DAD r_t (min.)	LC/DAD data (nm)	LC/MS data (*m/z*)‡		Identification	Possible dye source
		L*	a*	b*			ESI⁻	ESI⁺		
G1	OBr	29.24	22.90	18.94	15.99	243, 277, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Weld + Brazilwood + Madder
					16.45	243, 277, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					17.37	250, 267, 346	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
					18.80	259, 307, 333	**243 [M-H]⁻**	**245 [M+H]⁺**	Type C compound	
					20.62	250, 267, 347	**285 [M-H]⁻**, 241, 217, 199, 175, 133	-	Luteolin	
					24.74	249, 278, 429	**239 [M-H]⁻**, 211	-	Alizarin	
					26.31	256, 290, 479	**255 [M-H]⁻**, 227	-	Purpurin	
G2	Y	56.08	4.28	33.18	15.99	243, 277, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Weld
					16.45	243, 277, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					17.37	250, 267, 346	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
					18.12	244, 265, 324	**431 [M-H]⁻**, 269	-	Apigenin 7-*O*-glucoside	
					20.62	250, 267, 347	**285 [M-H]⁻**, 241, 217, 199, 175, 133	-	Luteolin	
G3	Bg	60.54	3.02	17.00	-	-	-	-	-	No dye source identified
G4	O	44.51	14.30	26.32	15.99	243, 277, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Weld + Madder
					16.45	243, 277, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					17.37	250, 267, 346	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
					18.12	244, 265, 324	**431 [M-H]⁻**, 269	-	Apigenin 7-*O*-glucoside	
					20.62	250, 267, 347	**285 [M-H]⁻**, 241, 217, 199, 175, 151, 133	-	Luteolin	
					24.74	249, 278, 429	**239 [M-H]⁻**, 211	-	Alizarin	
					26.31	256, 290, 479	**255 [M-H]⁻**, 227	-	Purpurin	
G5	Bl	39.38	-7.47	-5.12	26.63	253, 282, 327, 606	-	**263 [M+H]⁺**, 235, 219	Indigotin	Indigo or Woad
G8	Bl	18.17	0.60	-10.48						
G6	Gr	28.26	-7.57	-1.40	15.99	243, 277, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Weld + Indigo or Woad
					16.45	243, 277, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					17.37	250, 267, 346	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
					18.12	244, 265, 324	**431 [M-H]⁻**, 269	-	Apigenin 7-*O*-glucoside	
					20.62	250, 267, 347	**285 [M-H]⁻**, 241, 217, 199, 175, 151, 133	-	Luteolin	
					26.63	253, 282, 327, 606	-	**263 [M+H]⁺**, 235, 219	Indigotin	
G7	Y	53.19	6.46	22.19	18.80	259, 307, 333	**243 [M-H]⁻**	**245 [M+H]⁺**	Type C compound	Brazilwood
G9	Bg	63.34	8.79	25.22						

† *Bg* beige, *Bl* blue, *Gr* green, *O* orange, *OBr* orange-brown, *Y* yellow

‡ In bold: major ions

Table 19. *Colourimetric data and identification of natural dyes in wool samples collected from Arraiolos tapestry I.*

ID	Colour†	CIEL*a*b* coordinates			LC/DAD r_t (min.)	LC/DAD data (nm)	LC/MS data (*m/z*)‡		Identification	Possible dye source
		L*	a*	b*			ESI⁻	ESI⁺		
I1	Y	59.95	5.01	47.12	16.13	245, 267, 335	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Weld
					16.71	245, 267, 335	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					17.63	254, 267, 348	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
					18.37	245, 268, 335	**447 [M-H]⁻**, 285	-	Luteolin 4'-*O*-glucoside	
					18.46	246, 263, 328	**431 [M-H]⁻**, 269	-	Apigenin 7-*O*-glucoside	
					18.53	247, 269, 341	**461 [M-H]⁻**, 299	-	Chrysoeriol 7-*O*-glucoside	
					18.69	246, 267, 339	**447 [M-H]⁻**, 285	-	Luteolin 3'-*O*-glucoside	
					21.07	253, 266, 348	**285 [M-H]⁻**, 257, 199, 175, 151, 133	-	Luteolin	
					22.81	249, 267, 336	**269 [M-H]⁻**, 225, 151, 149	-	Apigenin	
I2	W	67.19	1.64	14.25	-	-	-	-	-	No dye source identified
I13	Gy	42.03	-0.22	13.35						
I3	Bl	38.24	-5.03	-5.42						
I4	Bl	31.36	-4.83	-10.94	26.55	245, 286, 331, 606	-	**263 [M+H]⁺**, 235, 219	Indigotin	Indigo or Woad
I6	Bl	44.67	-5.61	2.60						
I5	Gr	31.77	-5.82	8.36	16.01	224, 253, 329	-	**611 [M+H]⁺**, 449, 287	Luteolin di-*O*-glucoside	Spurge flax + Indigo or Woad
					16.44	243, 267, 336	-	**611 [M+H]⁺**, 449, 287	Luteolin 3,7'-di-*O*-glucoside	
					16.89	260, 324	**177 [M-H]⁻**, 133	-	Daphnetin derivative	
					17.37	244, 269, 337	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
I8	Gr	45.44	-6.10	18.57	18.08	244, 267, 324	**431 [M-H]⁻**, 269	-	Apigenin 7-*O*-glucoside	
					20.62	250, 267, 347	**285 [M-H]⁻**, 241, 217, 175	-	Luteolin	
					22.19	255, 317	**351 [M-H]⁻**, 307	-	Daphnoretin	
					26.55	245, 286, 331, 606	-	**263 [M+H]⁺**, 235, 219	Indigotin	
I7	O	42.62	15.87	25.94	16.01	224, 253, 319	-	**611 [M+H]⁺**, 449, 287	Luteolin di-*O*-glucoside	Weld + Brazilwood + Madder
					16.44	243, 267, 334	-	**611 [M+H]⁺**, 449, 287	Luteolin 3,7'-di-*O*-glucoside	
					17.37	244, 269, 337	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
					18.02	244, 267, 324	**431 [M-H]⁻**, 269	-	Apigenin 7-*O*-glucoside	
					18.80	259, 307, 333	**243 [M-H]⁻**	**245 [M+H]⁺**	Type C compound	
					20.62	250, 267, 347	**285 [M-H]⁻**, 267, 243, 241, 213, 199, 175, 151, 133	-	Luteolin	
					22.24	249, 267, 336	**269 [M-H]⁻**, 225, 201	-	Apigenin	
					24.74	252, 274, 423	-	**241 [M+H]⁺**, 213, 185, 157	Alizarin	
					26.31	256, 288, 479	-	**257 [M+H]⁺**, 229	Purpurin	
I9	Y	52.59	5.40	24.08	16.01	224, 253, 319	-	**611 [M+H]⁺**, 449, 287	Luteolin di-*O*-glucoside	Weld + Brazilwood
					16.44	243, 267, 334	-	**611 [M+H]⁺**, 449, 287	Luteolin 3,7'-di-*O*-glucoside	
I12	Br	50.06	7.46	24.80	17.37	244, 269, 337	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
					18.02	244, 267, 324	**431 [M-H]⁻**, 269	-	Apigenin 7-*O*-glucoside	
					18.80	259, 307, 333	**243 [M-H]⁻**	**245 [M+H]⁺**	Type C compound	
					20.62	250, 267, 347	**285 [M-H]⁻**, 241, 217, 175	-	Luteolin	
I10	Br	19.65	3.31	6.27	17.17	254, 367	**301 [M-H]⁻**, 257, 229, 185, 145	**303 [M+H]⁺**	Ellagic acid	Unknown
I11	Br	25.87	4.60	9.82	17.72	252, 345	**447 [M-H]⁻**, 301	**449 [M+H]⁺**, 303	Quercitrin	

† *Bl* blue, *Br* brown, *Gr* green, *Gy* grey, *O* orange, *W* white, *Y* yellow
‡ In bold: major ions

Table 20. *Colourimetric data and identification of natural dyes in wool samples collected from Arraiolos tapestry J.*

ID	Colour†	CIEL*a*b* coordinates			LC/DAD r_t (min.)	LC/DAD data (nm)	LC/MS data (*m/z*)‡		Identification	Possible dye source
		L*	a*	b*			ESI⁻	ESI⁺		
J1	Y	55.76	3.11	30.67	16.45	243, 277, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	Weld + Brazilwood
					17.37	250, 267, 346	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
					18.78	222, 258, 307, 340	**243 [M-H]⁻**	-	Type C compound	
					20.62	250, 267, 347	**285 [M-H]⁻**, 243, 217, 199, 151, 133	-	Luteolin	
J2	W	64.53	1.53	13.72	-	-	-	-	-	No dye source identified
J3	Bl	22.65	-3.46	-10.94	26.55	245, 286, 331, 606	-	**263 [M+H]⁺**, 235, 219	Indigotin	Indigo or Woad
J4	Bl	38.27	-6.60	-2.81						
J5	Bl	52.66	-3.91	8.02						
J6	Br	17.94	0.94	5.11	15.95	245, 268, 334	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Weld
					16.47	245, 268, 334	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					17.38	249, 255, 348	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
J8	Br	30.72	2.71	15.81	18.12	246, 263, 328	**431 [M-H]⁻**, 269	-	Apigenin 7-*O*-glucoside	
					18.22	247, 269, 341	**461 [M-H]⁻**, 299	-	Chrysoeriol 7-*O*-glucoside	
					20.60	251, 267, 347	**285 [M-H]⁻**, 241, 217, 199, 175, 151, 133	-	Luteolin	
J7	Gr	34.79	-8.86	5.61	15.95	245, 268, 334	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Weld + Indigo or Woad
					16.47	245, 268, 334	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					17.38	249, 255, 348	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
					18.07	245, 268, 335	**447 [M-H]⁻**, 285	-	Luteolin 4'-*O*-glucoside	
					18.12	246, 263, 328	**431 [M-H]⁻**, 269	-	Apigenin 7-*O*-glucoside	
					18.22	247, 269, 341	**461 [M-H]⁻**, 299	-	Chrysoeriol 7-*O*-glucoside	
					20.60	251, 267, 347	**285 [M-H]⁻**, 217, 199, 175, 151	-	Luteolin	
					26.55	245, 286, 331, 606	-	**263 [M+H]⁺**, 235, 219	Indigotin	

† *Bl* blue, *Br* brown, *Gr* green, *W* white , *Y* yellow
‡ In bold: major ions

Table 21. *Colourimetric data and identification of natural dyes in wool samples collected from Arraiolos tapestry L.*

ID	Colour†	CIEL*a*b* coordinates			LC/DAD r_t (min.)	LC/DAD data (nm)	LC/MS data (*m/z*)‡		Identification	Possible dye source
		L*	a*	b*			ESI⁻	ESI⁺		
L1	W	49.17	3.54	18.38	-	-	-	-	-	No dye source identified
L3	Bg	52.03	4.83	20.39						
L14	Br	17.65	5.39	10.02						
L2	Gr	37.56	0.97	15.66	15.99	243, 277, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Weld + Indigo or Woad
					16.45	243, 277, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					17.37	250, 267, 346	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
L11	Gr	25.62	-7.84	4.21	18.12	244, 265, 324	**431 [M-H]⁻**, 269	-	Apigenin 7-*O*-glucoside	
L12	Gr	43.34	-5.76	20.88	20.62	250, 267, 347	**285 [M-H]⁻**, 199, 175, 133	-	Luteolin	
L13	Gr	37.63	1.96	18.90	26.55	245, 286, 331, 606	-	**263 [M+H]⁺**, 235, 219	Indigotin	
L4	Y	44.25	3.87	26.67	15.99	243, 277, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Weld
					16.45	243, 277, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					17.37	250, 267, 346	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
					18.12	244, 265, 324	**431 [M-H]⁻**, 269	-	Apigenin 7-*O*-glucoside	
					18.22	247, 269, 341	**461 [M-H]⁻**, 299	-	Chrysoeriol 7-*O*-glucoside	
					20.62	250, 267, 347	**285 [M-H]⁻**, 199, 175, 133	-	Luteolin	
L5	O	40.00	11.82	25.99	15.99	243, 277, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Weld + Madder
					16.45	243, 277, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					17.37	250, 267, 346	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
L6	O	35.79	20.11	24.92	20.62	250, 267, 347	**285 [M-H]⁻**, 199, 175, 133	-	Luteolin	
L7	R	30.28	31.64	18.89	24.74	252, 274, 423	-	**241 [M+H]⁺**, 213, 185, 157	Alizarin	
					26.31	256, 288, 479	-	**257 [M+H]⁺**, 229	Purpurin	
L8	R	25.13	22.58	15.07	24.74	252, 274, 423	-	**241 [M+H]⁺**, 213, 185, 157	Alizarin	Madder
					26.31	256, 288, 479	-	**257 [M+H]⁺**, 229	Purpurin	
L9	Rs	35.97	18.11	8.69	16.67	240, 276, 308, 493	**491 [M-H]⁻**, 447, 357, 327, 299	-	Carminic acid	Cochineal + Madder
L10	Rs	43.29	13.52	12.15	24.74	252, 274, 423	-	**241 [M+H]⁺**, 213, 185, 157	Alizarin	
					26.31	256, 288, 479	-	**257 [M+H]⁺**, 229	Purpurin	
L15	Bl	59.33	-2.49	9.67	26.55	245, 286, 331, 606	-	**263 [M+H]⁺**, 235, 219	Indigotin	Indigo or Woad
L16	Bl	17.77	-3.68	-9.53						
L17	Bl	26.00	-5.31	-5.80						
L18	Bl	35.06	-9.68	5.02						
L19	Bl	17.30	0.56	-1.90	24.74	252, 274, 423	-	**241 [M+H]⁺**, 213, 185, 157	Alizarin	Madder + Indigo or Woad
					26.31	256, 288, 479	-	**257 [M+H]⁺**, 229	Purpurin	
					26.55	245, 286, 331, 606	-	**263 [M+H]⁺**, 235, 219	Indigotin	

† *Bg* beige, *Bl* blue, *Br* brown, *Gr* green, *O* orange, *R* red, *Rs* rose, *W* white, *Y* yellow
‡ In bold: major ions

Historical references concerning the use of natural dyes in Arraiolos carpets are scarce and indicate only the use of indigo, weld, spurge flax, logwood and brazilwood as the colour sources[1,3,4,12] (see *Table 1*).

Weld was reportedly used in Arraiolos carpets to obtain yellow hues[1,12] and, in fact, it has already been detected in two other carpets from the Museum Machado de Castro collection[2].

The analysis of the samples collected in the NMAA carpets enabled the identification of weld in several yellow, green, brown, blue and reddish samples (see *Table 14* to *Table 21*). The phenolic compounds extracted from these samples absorb in the region of spectra below 350 nm, confirming their flavone structure, and their elution pattern and mass spectra correspond to the luteolin, apigenin and chrysoeriol derivatives reported in weld extracts[2,184,214]. Usually, not all the compounds identified in weld plant extracts can be identified in the historical textile samples, and weld is frequently pointed out in the literature as the yellow colour source in the historical textiles, sometimes based solely in the identification of a few flavone derivatives[178,212,213].

Mass spectrometry fragment nomenclature throughout this work is in accordance to that proposed by Ma *et al.*[237] and Fabre *et al.*[238] for flavonoids, and by Dommon and Costelo[239] for glycoconjugates.

The negative ESI mass spectra of luteolin-di-*O*-glucosides yielded a deprotonated ion, $[M-H]^-$, at *m/z* 609 which gives rise to Y_1^- (*m/z* 447) and Y_0^- (*m/z* 285), ions formed by the sequential loss of two hexose residues (162 Da). The compounds with with r_t = 16.00 min. and 16.45 min. were identified as luteolin-di-*O*-glucoside and luteolin-3,7´-di-*O*-glucoside based on their UV spectra and elution order[214,240].

Several flavone-*O*-glucosides could be identified in I1 sample (*Table 19*) (please see *Figure 25* for chromatographic profile). The presence of luteolin-*O*-glucosides was suggested by the loss of an hexose residue (162 Da) from the deprotonated ion, $[M-H]^-$ (*m/z* 447), with the formation of Y_0^- at *m/z* 285. Compounds with r_t = 17.33 min., 18.07 min. and 18.47 min. were identified as luteolin-7-*O*-glucoside, luteolin-4´-*O*-glucoside, and luteolin-3´-*O*-glucoside, respectively, based on their UV spectra and elution order[214,240]. The chromatographic peak with r_t = 18.12 min. was attributed to apigenin-7-*O*-glucoside as $[M-H]^-$ (*m/z* 431) and was detected along with the Y_0^- ion at *m/z* 269, which results from the loss of an hexose unit from the original deprotonated ion.

The chromatographic peak at r_t = 18.22 min. was attributed to the methoxyflavone glycoside chrysoeriol 7-*O*-glucoside which has already been detected in historical samples dyed with weld[184]. The $[M-H]^-$ (*m/z* 461) was detected along with the Y_0^- ion at *m/z* 299, which results from the loss of an hexose unit from the original deprotonated ion. Besides samples dyed only with weld, chrysoeriol 7-*O*-glucoside was also identified in samples dyed with weld and indigo or woad (A2, A3 - *Table 15*; J7 - *Table 20*).

Flavone aglycones luteolin and apigenin were identified in several weld-dyed samples (*Table 15* to *Table 21*). The deprotonated molecular ion of luteolin was detected at *m/z* 285 along with the fragments derived from the retro-Diels-Alder (RDA) reaction, $^{1,3}A^-$ at *m/z* 151 and $^{1,3}B^-$ at *m/z* 133, which provide information on the number and substituents of rings A and B of the flavonoid structure[60,238]. Further fragmentation yielded fragments at *m/z* 243 [M-H-C_2H_2O]$^-$, *m/z* 217 [M-H-C_3O_2]$^-$, *m/z* 199 [M-H-C_2H_2O-CO_2]$^-$, and *m/z* 175 [M-H-C_3O_2-C_2H_2O]$^-$ [238]. *Figure 40* depicts the luteolin fragmentation pattern. Similar fragmentation was observed for apigenin and the deprotonated molecular ion was detected at *m/z* 269 along with the RDA fragments $^{1,3}B^-$ at *m/z* 117 and $^{1,4}B^-$+2H at *m/z* 149. Further fragmentation yielded ions detected at *m/z* 181 [M-H-2CO_2]$^-$, *m/z* 201 [M-H-C_3O_2]$^-$, and *m/z* 225 [M-H-CO_2]$^-$ [238].

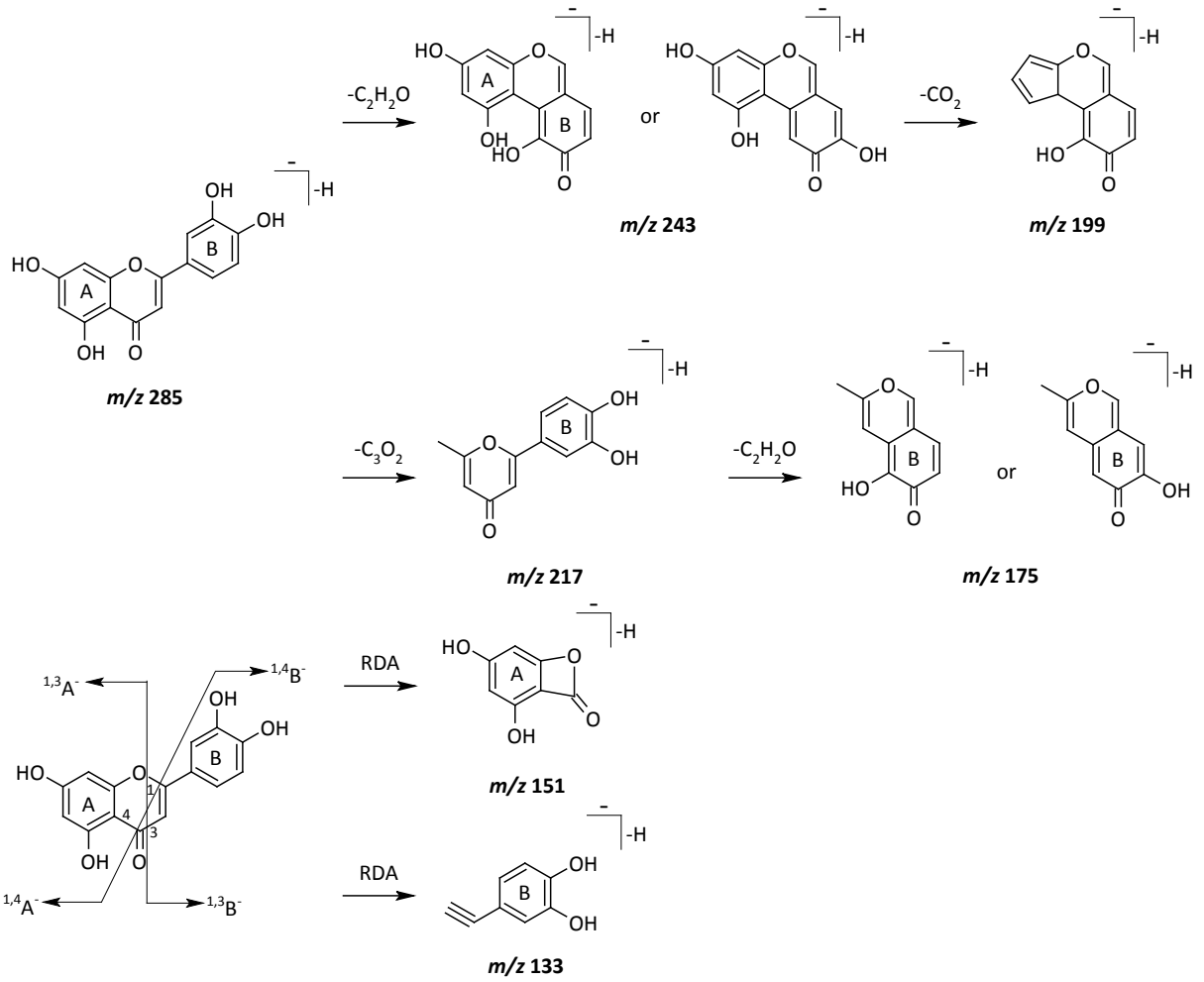


Figure 40. *Proposed fragmentation for flavone luteolin, showing the different retrociclisation cleavages. (Adapted from [238])*

Indigo was identified in several green and blue samples (*Table 15* to *Table 21*). It was also identified in black sample E10 (*Table 16*), probably resulting from a very concentrated dyeing bath. Green dyes are rare[47] and green hues were usually obtained by sequentially dyeing the fibre with blue and yellow dyes. Arraiolos recipes refer the use of weld and indigo to dye wool fibres in green hues[1,12]. The identification of indigotin as the chromatographic peak eluting at approximately 26.50 min. was done based on its characteristic UV-Vis and mass spectra[60,64,166]. As reported in the literature[60,166], indigoids are generally detected in the ESI positive mode, presenting low intensity signals. The samples yielded the protonated molecular ion $[M+H]^+$ at *m/z* 263 and the characteristic fragment ions $[M+H-28]^+$ at *m/z* 235 and $[M+H-44]^+$ at *m/z* 219, which correspond to the subsequent loss of CO and NH_2 (*Figure 41*).

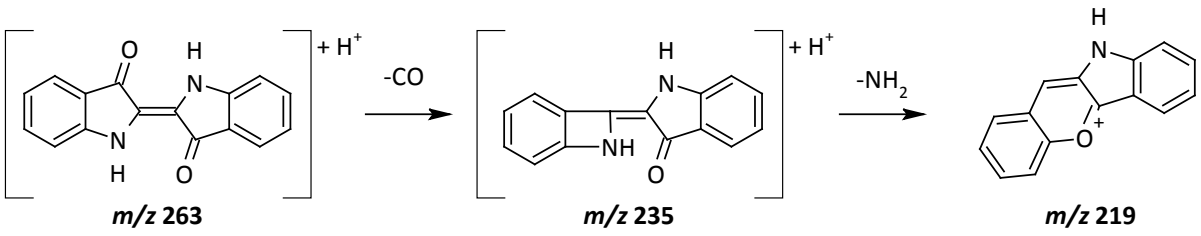


Figure 41. *Fragmentation pattern of indigotin. (Adapted from [60])*

Indigo is obtained throughout a fermentation process of *Isatis tinctoria* L. (commonly known as woad and widely available in Southern and central Europe, North Africa and West Asia) or *Indigofera tinctoria* L. (a native of tropical Asia)[47]. The indigo made from *Indigofera tinctoria* was of superior quality and was widely available in Europe in the 18th century[47]. Despite the historical interest, it is not possible to identify the plant species based solely on the indigotin identification in the textile fibres.

Despite being referred in the traditional Arraiolos recipes, the combination of indigo and spurge flax to dye in green hues is not described in the historical recipes. Green hues were reportedly made with indigo and weld, and that combination has already been detected previously in Arraiolos tapestries from the 17th century[2]. Spurge flax and indigo were found together in green samples I5 and I8 (*Table 19*). Spurge flax chemical composition is very similar to weld, the major difference between these two dyes are the coumarins daphnetin, daphnin and daphnoretin that are found in spurge flax[2,55]. Di-coumarin daphnoretin was identified at 22.19 min., based on its UV-Vis and mass spectra; the $[M-H]^-$ (*m/z* 351) was detected along with the fragment at *m/z* 307 $[M-H-CO_2]^-$. At r_t = 16.89 min., fragments *m/z* 177

and 133 were detected along with the deprotonated molecular ion at *m/z* 515; fragment *m/z* 177 is characteristic of daphnetin derivatives[2] and fragment *m/z* 133 corresponds to a subsequent loss of CO_2.

Red anthraquinone dyes were, for the first time, detected in Arraiolos tapestries in this work, and these dye sources are not described in Arraiolos available literature[1,12]. The main colouring component for cochineal was identified alone in sample E9 (*Table 16*), and together with other chromophores in samples A1, A4 (*Table 15*), L9, L10 (*Table 21*), and F4 (*Table 17*), as the chromatographic peak eluting at approximately 16.50 min., carminic acid, giving rise to the deprotonated molecular ion at *m/z* 491. Fragmentation of the molecular ion yields fragments at *m/z* 447 $[M\text{-}H\text{-}CO_2]^-$, *m/z* 357 $[M\text{-}H\text{-}CO_2\text{-}90]^-$, *m/z* 327 $[M\text{-}H\text{-}CO_2\text{-}120]^-$ and *m/z* 299 $[M\text{-}H\text{-}CO_2\text{-}CO\text{-}120]^-$, which are characteristic of the anthraquinones fragmentation pattern[60]. The ions with *m/z* 357 and 327 arise due to a fragmentation pattern characteristic of a C-glycoside molecule (*Figure 42*).

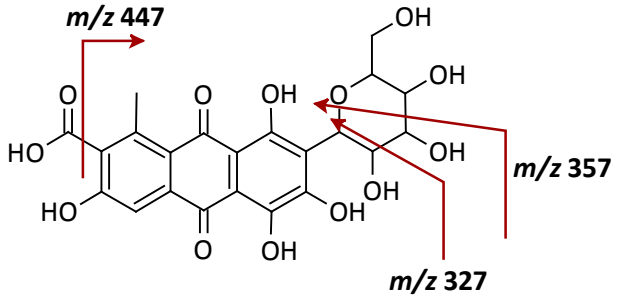


Figure 42. *Proposed fragmentation of carminic acid.*

Two minor compounds, dcIV and dcVII, were found in cochineal dyed sample A4 (*Table 15*). Both UV-Vis and mass spectra are very similar to carminic acid. These two compounds have already been described by other authors[65,173], being likely isomers from the carminic acid, but no chemical structure has been proposed so far. The identification of these two additional peaks in the samples does not allow the determination of the scale insect species used in the dyeing process.

Madder is a scarlet dye extracted from perennial herbaceous plants of the *Rubiacea* family, of which there are about 35 species[39]. The composition of the extracted anthraquinones differs between the varieties of *Rubiaceae*. In general, it is very difficult to determine which madder source was used in the dyeing process. Alizarin and purpurin are found in most of madder species, and have been identified in the present work as the

chromatographic peaks eluting at approximately 24.70 and 26.30 min., respectively (A4, A5, A6 - *Table 15*; E6, E8, E11, E12, E13 - *Table 16*; F1, F4 - *Table 17*; G1, G4 - *Table 18*; I7 - *Table 19*; L5, L6, L7, L8, L9, L10, L19 - *Table 21*) . Alizarin ($[M+H]^+$ = 241 Da) yielded fragments at *m/z* 213, 185 and 157, corresponding to subsequent losses of CO (*Figure 43*). The molecular protonated ion at *m/z* 257 was assigned to purpurin and yielded a $[M+H-CO]^+$ fragment. Besides alizarin and purpurin, red sample A5 presented two other chromatographic peaks. At r_t = 18.02 min., a molecular deprotonated ion of *m/z* 269, yielding two fragments at *m/z* 254 ($[M-H-CH_3]^-$) and *m/z* 223 ($[M-H-CO-H_2O]^-$) was assigned as morindone, an anthraquinone colouring matter that is only found in *Morinda* spp[59]. Xanthopurpurin, also found in *Morinda* spp., was identified at r_t = 18.21 min., with a molecular deprotonated ion of *m/z* 239 that yielded the fragment $[M-H-CO_2]^-$ = 195 Da. *Figure 44* depicts the chromatogram for sample A5, together with the mass spectra for morindone.

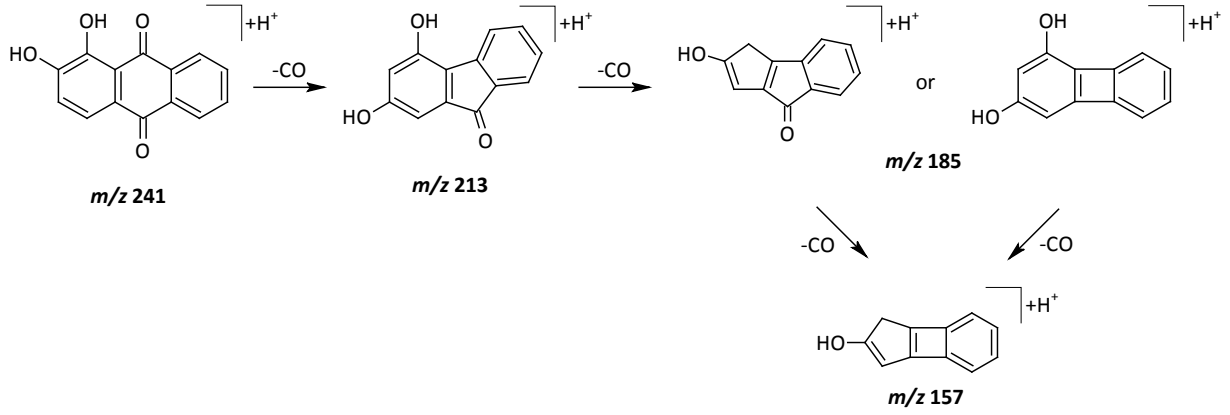


Figure 43. *Proposed fragmentation pattern for alizarin. (Adapted from [170])*

Despite the fact that samples I9 (*Table 19*) and J1 (*Table 20*) present nowadays a yellowish hue, they are thought to have been originally dyed in an orange/reddish hue. This conclusion is supported by the fact that the base of the embroidery stitches, which is less exposed to the effects of light, still presents a faded orange/reddish hue. No anthraquinone molecule, nor the homoisoflavonoids brazilin or brazilein were detected in these samples. Nevertheless, a compound with r_t = 18.80 min. in the chromatographic profile of these samples extracts was tentatively identified as Type C compound, an analyte previously described by Karapanagiotis *et al.* in samples collected from Cretan icons[235] and post-Byzantine textiles[179]. The authors[179,235] didn't suggest an explanation for the nature of this compound but indicate that they were likely a photodegradation product of brazilein, which

has already been proved in subchapter '*Evaluation of the mordant effect, dyeing technique and photodegradation in contemporary wool samples dyed with red natural dyes*'.

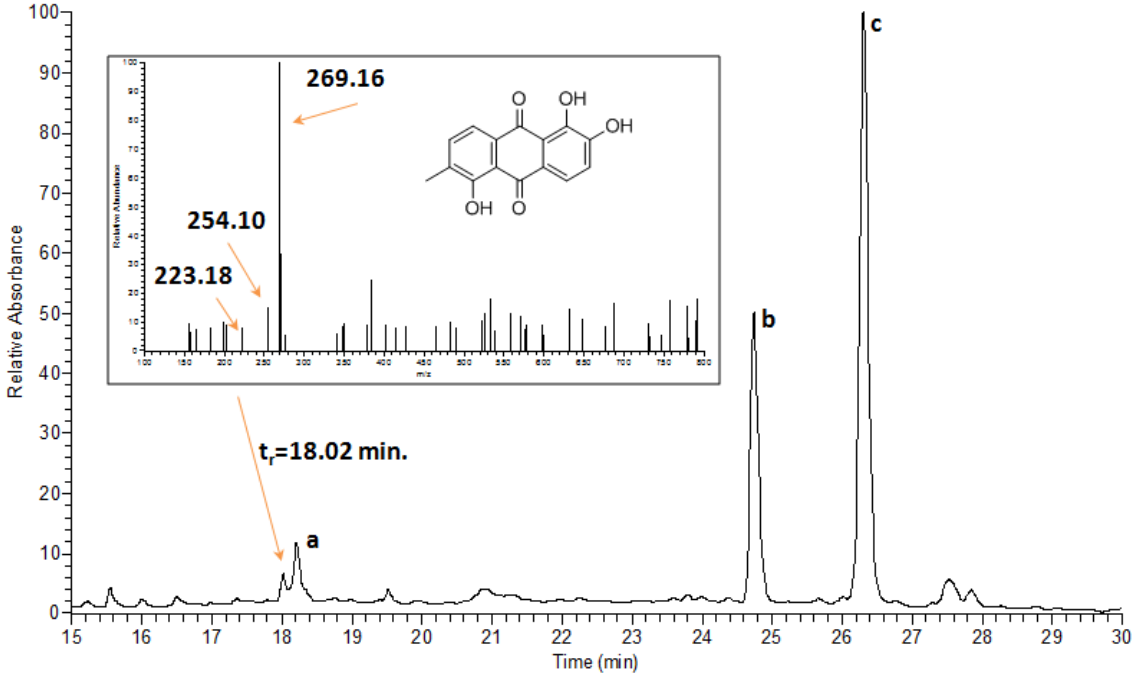


Figure 44. *HPLC/DAD chromatogram of sample A5 (recorded at 254 nm) and mass spectrum of morindone. Identification of the chromatographic peaks is based on UV-Vis and mass data (Table 15) as follows: (a) xanthopurpurin; (b) alizarin; (c) purpurin.*

The presence of both weld and brazilwood in samples I9 and J1 could explain the orange/redish colour observed in the base of the embroidery stitches. Type C compound was identified together with several weld chromophores also in brown sample I12. Brazilwood is known for its fast light degradation[215] and the colour fading in Arraiolos carpets has already been described by Pessanha[11], who stated that some areas originally dyed in red hues became brownish with time. Samples I9 and J1 were taken from the filling colour of the studied carpets (*Figure 21*). Brazilwood dye photodegradation has a tremendous impact in the actual perception of these carpets when compared to what it might have originally been intended by its makers. Despite the referred use of brazilwood in the Arraiolos historical dyeing recipes[1,12], it is now for the first time tentatively identified in these carpets.

Although fibres A1 (*Table 15*), E11, E12 (*Table 16*) and L19 (*Table 21*) present nowadays a blue/grey hue, they were probably originally dyed in purple, since red chromophores from insect and plant anthraquinone dyes were detected along with indigotin in these samples. Carminic acid, the main chromophore in cochineal dye was identified in sample A1, and the

major chromophores in madder dye, alizarin and purpurin were detected in samples E11, E12 and L19. Red dyes most likely underwent light degradation and only the more stable blue dye remained visible. A similar situation likely occurred in the case of blue sample A8 (most likely originally dyed in green), the majority of yellow chromophores degraded (only a luteolin derivative remains detectable), thus resulting in a visible blue hue.

Extraction of white and beige samples (A9, E14, F5, F6, F7, G3, I2, I13, J2, L1, L3 and L14, *Table 15* to *Table 21*) didn't yield any recognizable dye on the chromatogram.

Ellagic acid and quercitrin were both identified in brown samples I10 and I11 (*Table 19*), but no dye source was suggested. Ellagic acid was detected at 17.17 min., with the deprotonated molecular ion at *m/z* 301, and fragments *m/z* 257, 229 and 185 corresponding to the subsequent losses of CO_2 and CO. The chromatographic peak at r_t = 17.72 min. was attributed to the quercetin rhamnoside, quercitrin. The $[M-H]^-$ (*m/z* 447) was detected along with the Y_0^- ion at *m/z* 301, which results from the loss of a deoxyhexose unit (146 Da) from the original deprotonated ion. Ellagic acid and quercitrin can be found in quercitron bark and sumac[47], but further dyeing studies are required for the identification of a dye source for samples I10 and I11.

18^{th}-19^{th} century tapestries

Table 22 to *Table 27* present colourimetric data for all 18^{th}-19^{th} century tapestries, along with the HPLC/DAD/MS data used for the identification of the dye sources.

Table 22. *Colourimetric data and identification of natural dyes in wool samples collected from Arraiolos tapestry B.*

ID	Colour†	CIEL*a*b* coordinates			LC/DAD r_t (min.)	LC/DAD data (nm)	LC/MS data (*m/z*)‡		Identification	Possible dye source
		L*	a*	b*			ESI⁻	ESI⁺		
B1	Br	20.76	3.67	4.49	-	-	-	-	-	No dye source identified
B4	Bg	59.68	1.77	16.12						
B2 B11	Y O	53.68 55.50	5.34 11.45	28.20 37.62	16.04	245, 267, 335	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Weld + Brazilwood
					16.52	244, 265, 340	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					17.39	251, 268, 348	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
					18.22	247, 269, 341	**461 [M-H]⁻**, 299	-	Chrysoeriol 7-*O*-glucoside	
					18.19	248, 268, 336	**431 [M-H]⁻**, 269	-	Apigenin 7-*O*-glucoside	
					18.80	259, 307, 333	**243 [M-H]⁻**	**245 [M+H]⁺**	Type C compound	
B3	Y	58.48	2.54	26.84	16.03	242, 264, 333	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Weld
					16.48	244, 265, 340	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					17.41	251, 268, 348	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
					18.03	248, 268, 336	**431 [M-H]⁻**, 269	-	Apigenin 7-*O*-glucoside	
					20.58	249, 270, 346	**285 [M-H]⁻**, 241, 217, 199, 175	-	Luteolin	
B5	Bg	60.60	3.62	25.15	18.80	259, 307, 333	**243 [M-H]⁻**	**245 [M+H]⁺**	Type C compound	Brazilwood
B7	Bg	57.34	4.31	25.79						
B6 B8 B12	Gr Gr Gr	30.86 43.16 40.09	-7.19 -5.45 -8.86	0.72 14.40 6.23	16.04	245, 267, 335	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Weld + Indigo or Woad
					16.43	245, 267, 335	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					17.33	254, 267, 348	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
					18.07	246, 263, 328	**431 [M-H]⁻**, 269	-	Apigenin 7-*O*-glucoside	
					18.22	247, 269, 341	**461 [M-H]⁻**, 299	-	Chrysoeriol 7-*O*-glucoside	
					20.58	253, 266, 348	**285 [M-H]⁻**, 241, 217, 199, 175, 133	-	Luteolin	
					22.24	249, 267, 336	**269 [M-H]⁻**, 227, 225, 201, 183, 181	-	Apigenin	
					26.60	245, 286, 332, 606	-	**263 [M+H]⁺**, 235, 219	Indigotin	
B9	Bl	28.09	-4.85	-10.62	26.60	245, 286, 332, 606	-	**263 [M+H]⁺**, 235, 219	Indigotin	Indigo or Woad
B10	Bl	37.94	-7.33	-4.59						

† *Bg* beige, *Bl* blue, *Br* brown, *Gr* green, *O* orange, *Y* yellow
‡ In bold: major ions

Table 23. *Colourimetric data and identification of natural dyes in wool samples collected from Arraiolos tapestry C.*

ID	Colour†	CIEL*a*b* coordinates			LC/DAD r_t (min.)	LC/DAD data (nm)	LC/MS data (*m/z*)‡		Identification	Possible dye source
		L*	a*	b*			ESI⁻	ESI⁺		
C1	Y	53.76	4.68	26.96	15.99	243, 277, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Weld + Brazilwood
					16.45	243, 277, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					17.37	250, 267, 346	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
C2	Y	60.25	3.76	33.75	18.12	244, 265, 324	**431 [M-H]⁻**, 269	-	Apigenin 7-*O*-glucoside	
					18.22	247, 269, 341	**461 [M-H]⁻**, 299	-	Chrysoeriol 7-*O*-glucoside	
					18.80	259, 307, 333	**243 [M-H]⁻**	**245 [M+H]⁺**	Type C compound	
					20.62	250, 267, 347	**285 [M-H]⁻**, 241, 217, 199, 175	-	Luteolin	
C3	W	62.23	3.35	20.83	-	-	-	-	-	No dye source identified
C4	Bg	55.44	4.67	24.42	18.80	259, 307, 333	**243 [M-H]⁻**	**245 [M+H]⁺**	Type C compound	Brazilwood
C5	Gr	55.44	4.67	24.42	15.95	245, 268, 334	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Weld + Indigo or Woad
					16.47	245, 268, 334	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					17.38	249, 255, 348	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
C7	Gr	37.97	-7.94	5.96	18.12	244, 267, 335	**431 [M-H]⁻**, 269	-	Apigenin 7-*O*-glucoside	
					18.22	247, 269, 341	**461 [M-H]⁻**, 299	-	Chrysoeriol 7-*O*-glucoside	
C11	Gr	43.99	-4.42	17.53	20.60	251, 267, 347	**285 [M-H]⁻**, 241, 217, 199, 151, 133	-	Luteolin	
					26.55	245, 286, 331, 606	-	**263 [M+H]⁺**, 235, 219	Indigotin	
C6	Bl	35.38	-6.19	-7.04	16.50	243, 276, 311, 493	**491 [M-H]⁻**, 447, 357, 327	-	Carminic acid	Cochineal + Indigo or Woad
					26.60	249, 286, 333, 606	-	**263 [M+H]⁺**, 235, 219	Indigotin	
C8	Bl	28.22	-6.88	-3.34	26.60	245, 286, 332, 606	-	**263 [M+H]⁺**, 235, 219	Indigotin	Indigo or Woad
C9	Bl	47.60	-5.62	5.15						
C10	Bl	21.04	-3.23	-6.72	18.80	259, 307, 333	**243 [M-H]⁻**	**245 [M+H]⁺**	Type C compound	Brazilwood + Indigo or Woad
					26.60	245, 286, 332, 606	-	**263 [M+H]⁺**, 235, 219	Indigotin	

† *Bg*-Beige, *Bl*-blue, *Gr*-green, *W*-white, *Y*-yellow
‡ In bold: major ions

Table 24. *Colourimetric data and identification of natural dyes in wool samples collected from Arraiolos tapestry D.*

ID	Colour†	CIEL*a*b* coordinates			LC/DAD r_t (min.)	LC/DAD data (nm)	LC/MS data (*m/z*)‡		Identification	Possible dye source
		L*	a*	b*			ESI⁻	ESI⁺		
D1	Bk	15.77	0.74	1.20	15.99	243, 277, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Weld
					16.45	243, 277, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					17.37	250, 267, 346	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
					18.12	244, 265, 324	**431 [M-H]⁻**, 269	-	Apigenin 7-*O*-glucoside	
					20.62	250, 267, 347	**285 [M-H]⁻**, 243, 217, 199, 151, 133	-	Luteolin	
D2	Br	26.29	3.91	10.51	-	-	-	-	-	No dye source identified
D6	W	60.39	3.48	20.21						
D3	Y	53.90	7.39	32.66	15.59	243, 277, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Weld + Brazilwood
					16.47	242, 280, 324	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					17.32	250, 267, 347	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
D4	LBr	38.61	9.57	24.78	18.12	246, 263, 329	**431 [M-H]⁻**, 269	-	Apigenin 7-*O*-glucoside	
D14	Y	57.97	11.47	38.09	18.80	259, 307, 333	**243 [M-H]⁻**	**245 [M+H]⁺**	Type C compound	
					20.62	250, 267, 347	**285 [M-H]⁻**, 243, 217, 199, 151, 133	-	Luteolin	
D5	Gr	39.01	-6.40	17.21	15.95	245, 268, 334	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Weld + Indigo or Woad
					16.47	245, 268, 334	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					17.38	249, 255, 348	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
D7	Gr	37.64	-7.68	8.47	18.12	244, 267, 335	**431 [M-H]⁻**, 269	-	Apigenin 7-*O*-glucoside	
D8	Gr	31.07	-7.25	1.40	18.25	247, 269, 341	**461 [M-H]⁻**, 299	-	Chrysoeriol 7-*O*-glucoside	
					20.60	251, 267, 347	**285 [M-H]⁻**, 199, 175, 133	-	Luteolin	
					26.55	245, 286, 331, 606	-	**263 [M+H]⁺**, 235, 219	Indigotin	
D9	Bl	24.41	-3.79	-9.78	26.53	245, 286, 332, 606	-	**263 [M+H]⁺**, 235, 219	Indigotin	Indigo or Woad
D10	Bl	40.62	-6.81	0.39						
D11	Bl	29.80	-5.93	-10.88						
D12	Y	56.61	7.67	42.44	15.94	245, 267, 335	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Weld
					16.43	245, 267, 335	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					17.33	254, 267, 348	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
					18.07	245, 268, 335	**447 [M-H]⁻**, 285	-	Luteolin 4'-*O*-glucoside	
					18.19	246, 263, 328	**431 [M-H]⁻**, 269	-	Apigenin 7-*O*-glucoside	
					18.30	247, 269, 341	**461 [M-H]⁻**, 299	-	Chrysoeriol 7-*O*-glucoside	
					18.47	246, 267, 339	**447 [M-H]⁻**, 285	-	Luteolin 3'-*O*-glucoside	
					21.58	253, 266, 348	**285 [M-H]⁻**, 243, 241, 217, 199, 175, 151, 133	-	Luteolin	
					22.24	249, 267, 336	**269 [M-H]⁻**, 225, 201, 181, 149, 117	-	Apigenin	
D13	Bg	52.45	6.78	27.77	18.80	259, 307, 333	**243 [M-H]⁻**	**245 [M+H]⁺**	Type C compound	Brazilwood

† *Bg* beige, *Bk* black, *Bl* blue, *Br* brown, *Gr* green, *LBr* light brown, *W* white, *Y* yellow

‡ In bold: major ions

Table 25. *Colourimetric data and identification of natural dyes in wool samples collected from Arraiolos tapestry H.*

ID	Colour†	CIEL*a*b* coordinates			LC/DAD r_t (min.)	LC/DAD data (nm)	LC/MS data (*m/z*)‡		Identification	Possible dye source
		L*	a*	b*			ESI⁻	ESI⁺		
H1	Y	65.04	1.39	49.66	16.00	224, 253, 319	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Weld
					16.49	243, 267, 334	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					17.31	244, 269, 337	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
					18.14	245, 268, 335	**447 [M-H]⁻**, 285	-	Luteolin 4'-*O*-glucoside	
					18.20	243, 268, 332	**431 [M-H]⁻**, 269	-	Apigenin 7-*O*-glucoside	
					18.42	246, 267, 339	**447 [M-H]⁻**, 285	-	Luteolin 3'-*O*-glucoside	
					20.68	250, 267, 347	**285 [M-H]⁻**, 243, 241, 217, 199, 175, 151, 133	-	Luteolin	
H2	Br	23.41	6.67	10.07	16.01	223, 253, 320	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Weld + Brazilwood
					16.42	244, 265, 332	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					17.39	243, 266, 348	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
					18.04	243, 268, 332	**431 [M-H]⁻**, 269	-	Apigenin 7-*O*-glucoside	
					18.82	246, 267, 331	**243 [M-H]⁻**	**245 [M+H]⁺**	Type C compound	
H3	Bl	48.34	-7.51	-6.55	18.80	246, 267, 331	**243 [M-H]⁻**	**245 [M+H]⁺**	Type C compound	Brazilwood + Indigo or Woad
H7	Bl	28.45	-3.53	-15.22	26.58	253, 282, 606	-	**263 [M+H]⁺**, 235, 219	Indigotin	
H4	Gr	33.89	-9.38	11.97	16.01	224, 255, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Spurge flax + Indigo or Woad
					16.42	243, 268, 329	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					16.84	225, 261, 324	**515 [M-H]⁻**, 339, 177	-	Daphnetin derivative	
					17.42	243, 266, 348	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
					18.08	243, 268, 332	**431 [M-H]⁻**, 269	-	Apigenin 7-*O*-glucoside	
					20.57	250, 267, 347	**285 [M-H]⁻**, 243, 175, 151, 133	-	Luteolin	
					26.51	245, 286, 607	-	**263 [M+H]⁺**, 235, 219	Indigotin	
H5	Gr	39.82	-7.27	23.37	15.03	224, 256, 309	**339 [M-H]⁻**, 177	-	Daphnin	Spurge flax + Indigo or Woad
H6	Gr	24.06	-9.12	4.49	15.70	223, 244, 322	**339 [M-H]⁻**, 177	-	Daphnetin 8-*O*-glucoside	
					16.01	224, 255, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	
					16.42	243, 268, 329	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					16.83	225, 261, 324	**515 [M-H]⁻**, 339, 177	-	Daphnetin derivative	
					17.41	243, 266, 348	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
					18.06	245, 268, 335	**447 [M-H]⁻**, 285	-	Luteolin 4'-*O*-glucoside	
					18.18	243, 268, 332	**431 [M-H]⁻**, 269	-	Apigenin 7-*O*-glucoside	
					20.55	250, 267, 347	**285 [M-H]⁻**, 243, 175, 151, 133	-	Luteolin	
					26.50	245, 286, 607	-	**263 [M+H]⁺**, 235, 219	Indigotin	
H8	Bl	36.67	-6.44	-10.28	26.50	245, 286, 607	-	**263 [M+H]⁺**, 235, 219	Indigotin	Indigo or Woad
H9	Rs	56.52	11.65	25.84	17.41	243, 266, 348	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	Weld + Brazilwood
H10	Br	38.53	8.06	17.40	18.12	224, 243, 445	**283 [M-H]⁻**, 265, 173	-	Brazilein	
					18.80	246, 267, 331	**243 [M-H]⁻**	**245 [M+H]⁺**	Type C compound	
H11	Rs	44.67	18.52	27.20	18.14	224, 243, 445	**283 [M-H]⁻**, 265, 173	-	Brazilein	Brazilwood
					18.80	246, 267, 331	**243 [M-H]⁻**	**245 [M+H]⁺**	Type C compound	
H12	Bg	53.68	9.23	30.79	18.80	246, 267, 331	**243 [M-H]⁻**	**245 [M+H]⁺**	Type C compound	Brazilwood

† *Bg* beige, *Bl* blue, *Br* brown, *Gr* green, *Rs* rose, *Y* yellow
‡ In bold: major ions

Table 26. *Colourimetric data and identification of natural dyes in wool samples collected from Arraiolos tapestry M.*

ID	Colour†	CIEL*a*b* coordinates			LC/DAD r_t (min.)	LC/DAD data (nm)	LC/MS data (*m/z*)‡		Identification	Possible dye source
		L*	a*	b*			ESI$^-$	ESI$^+$		
M1	Y	49.40	4.30	25.70	16.01	224, 253, 319	-	**611 [M+H]$^+$**, 449, 287	Luteolin di-*O*-glucoside	Spurge flax + Brazilwood
					16.44	243, 267, 334	-	**611 [M+H]$^+$**, 449, 287	Luteolin 3,7'-di-*O*-glucoside	
					16.89	260, 323	**177 [M-H]$^-$**, 149		Daphnetin derivative	
					17.37	244, 269, 337	**447 [M-H]$^-$**, 285	-	Luteolin 7-*O*-glucoside	
					18.02	244, 267, 324	**431 [M-H]$^-$**, 269	-	Apigenin 7-*O*-glucoside	
					18.80	259, 307, 333	**243 [M-H]$^-$**	**245 [M+H]$^+$**	Type C compound	
					20.57	250, 267, 347	**285 [M-H]$^-$**, 243, 175, 151, 133	-	Luteolin	
					22.21	255, 317	**351 [M-H]$^-$**, 307	-	Daphnoretin	
M2 M4 M7	Gr Gr Bl	33.41 37.19 18.23	-8.88 -1.46 -4.78	-0.38 16.96 -2.45	16.03	223, 253, 320	-	**611 [M+H]$^+$**, 449, 287	Luteolin di-*O*-glucoside	Weld + Indigo or Woad
					16.48	244, 265, 332	-	**611 [M+H]$^+$**, 449, 287	Luteolin 3,7'-di-*O*-glucoside	
					17.30	243, 266, 348	**447 [M-H]$^-$**, 285	-	Luteolin 7-*O*-glucoside	
					18.05	243, 268, 332	**431 [M-H]$^-$**, 269	-	Apigenin 7-*O*-glucoside	
					18.22	247, 269, 341	**461 [M-H]$^-$**, 299	-	Chrysoeriol 7-*O*-glucoside	
					20.51	250, 267, 347	**285 [M-H]$^-$**, 243, 175, 151, 133	-	Luteolin	
					22.25	241, 266, 335	**269 [M-H]$^-$**, 225, 201	-	Apigenin	
					26.62	247, 286, 331, 607	-	**263 [M+H]$^+$**, 235, 219	Indigotin	
M3 M8	Bl Bl	38.54 23.97	-4.56 -3.29	2.05 -8.92	26.63	253, 282, 327, 606	-	**263 [M+H]$^+$**, 235, 219	Indigotin	Indigo or Woad
M5	Y	50.73	3.60	21.16	18.80	259, 307, 333	**243 [M-H]$^-$**	**245 [M+H]$^+$**	Type C compound	Brazilwood
M6	W	51.14	2.72	16.13	-	-	-	-	-	No dye source identified

† *Bl* blue, *Gr* green, *R* red, *W* white, *Y* yellow
‡ In bold: major ions

Table 27. *Colourimetric data and identification of natural dyes in wool samples collected from Arraiolos tapestry N.*

ID	Colour†	CIEL*a*b* coordinates			LC/DAD r_t (min.)	LC/DAD data (nm)	LC/MS data (*m/z*)‡		Identification	Possible dye source
		L*	a*	b*			ESI⁻	ESI⁺		
N1	Y	57.33	4.99	26.11	16.40	243, 267, 334	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	Weld + Brazilwood
					17.34	249, 268, 346	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
					18.79	259, 307, 333	**243 [M-H]⁻**	**245 [M+H]⁺**	Type C compound	
N2	W	64.36	2.85	18.02	-	-	-	-	-	No dye source identified
N3	Y	53.17	2.57	38.10	17.35	249, 266, 346	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	Weld
					20.62	251, 267, 346	-	**287 [M+H]⁺**, 219, 203, 153	Luteolin	
N4	Bl	37.62	-6.63	-3.68	26.59	249, 286, 607	-	**263 [M+H]⁺**, 235, 219	Indigotin	Indigo or Woad + Unknown
					29.00	260, 317, 577	**647 [M-H]⁻**	**649 [M+H]⁺**	Unknown	
N5	Bl	27.20	-5.30	-8.63	26.57	249, 286, 607	-	**263 [M+H]⁺**, 235, 219	Indigotin	Indigo or Woad
N7	Bl	50.88	-3.19	8.38						
N6	Bl	28.80	-7.86	-2.03	16.00	224, 255, 323	**609 [M-H]⁻**, 447, 285	-	Luteolin di-*O*-glucoside	Weld + Indigo or Woad
					16.42	243, 268, 329	**609 [M-H]⁻**, 447, 285	-	Luteolin 3,7'-di-*O*-glucoside	
					17.35	243, 266, 348	**447 [M-H]⁻**, 285	-	Luteolin 7-*O*-glucoside	
N8	Gr	45.76	-3.82	21.50	20.61	250, 267, 347	**285 [M-H]⁻**, 243, 175, 151, 133	-	Luteolin	
					26.54	249, 286, 607	-	**263 [M+H]⁺**, 235, 219	Indigotin	
N9	Bg	67.59	2.44	18.13	18.78	259, 307, 333	**243 [M-H]⁻**	**245 [M+H]⁺**	Type C compound	Brazilwood

† *Bg* beige, *Bl* blue, *Gr* green, *W* white, *Y* yellow

‡ In bold: major ions

Chromatographic profiles of the 18th-19th century Arraiolos samples are very similar to those observed in the 17th-18th century group. Weld, brazilwood, indigo and spurge flax remain as dye sources. Madder was not identified in the studied tapestries and cochineal was only detected in sample C6, which was most certainly originally dyed in purple, as blue indigotin was also identified in this fibre (*Table 23*).

By the end of the 18th century, it is known that the production of Arraiolos tapestries suffered a severe decrease[5]. The high prices of imported madder and cochineal dyes are likely to have contributed for the reduction in their use. In 1807, the Commercial Court issued an order in which brazilwood merchants were required not to obstruct to the supply of the brazilwood dye[5]. It is possible that, by that time, brazilwood began to be the only widely available dye source of red hues.

As stated before, brazilwood reds are extremely light fugitive, and that can be observed in wool samples B5 and B7 (*Table 22*), C4 (*Table 23*), D13 (*Table 24*), H12 (*Table 25*), M5(*Table 26*) and N9 (*Table 27*). Instead of presenting the red hues characteristic of brazilwood dyeings, samples present nowadays beige and yellow hues. Brazilwood-weld dyed samples, which present today a yellow hue (B2 - *Table 22*; C1, C2 - *Table 23*; D3, D14 - *Table 24*; N1 - *Table 27*) as a

result of brazilwood photodegradation are thought to have been originally dyed in an orange hue, characteristic of the mixture of weld and brazilwood chromophores. Samples C10 (*Table 23*), H3 and H7 (*Table 25*), which show currently a blue hue on the fibres, were probably originally dyed in purple, as brazilwood and indigo were detected as dye sources. Only the blue hue remained visible, as brazilwood dye suffered photodegradation. In all these samples, the identification of brazilwood dye was based on the detection of Type C compound, already identified in the 17th-18th century samples.

Unexpectedly, in the rose and brown samples H9, H10 and H11 (*Table 25*) the chromophore molecule of brazilwood, brazilein, was detected at r_t = 18.12 min., with the deprotonated molecular ion at *m/z* 283 yielding an *m/z* 265 fragment ($[M-H-H_2O]^-$). Major fragment ion is found at *m/z* 173 and a fragmentation mechanism has been proposed by Hulme *et al.*[215] (*Figure 45*).

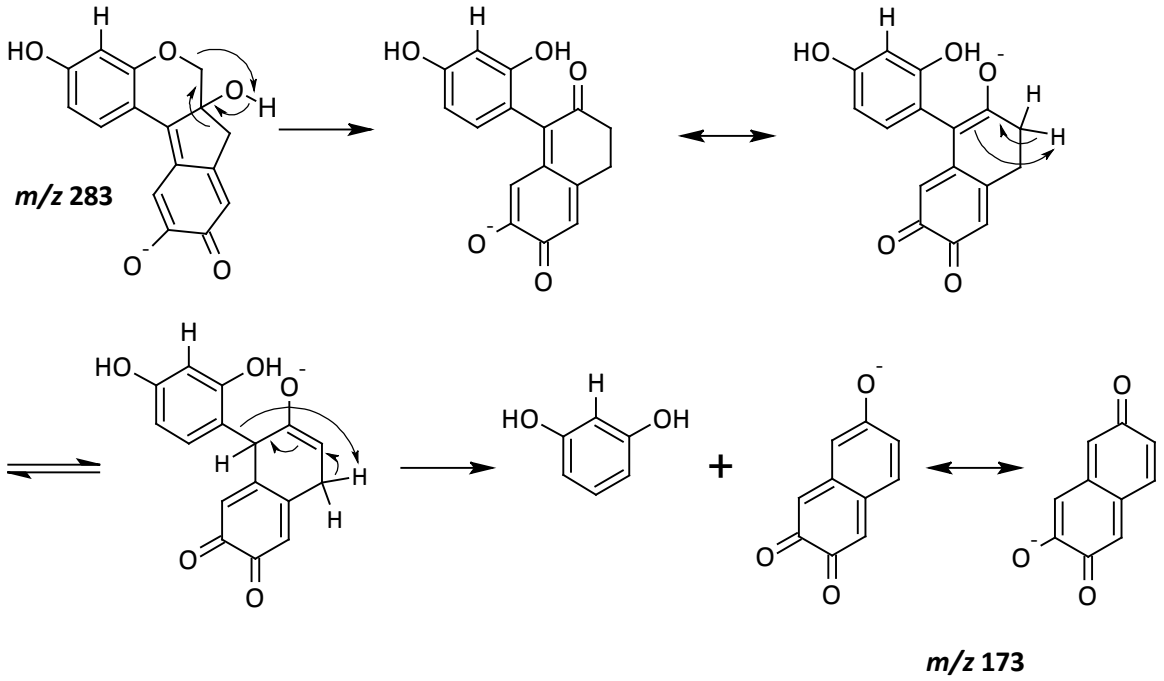


Figure 45. *Fragmentation mechanism proposed for brazilein. (Adapted from [215])*

The combination of weld and indigo (B6, B8, B12 - *Table 22*; C5, C7, C11 - *Table 23*; D5, D7, D8 - *Table 24*; M2, M4, M7 - *Table 26*; N6, N8 - *Table 27*) or spurge flax and indigo (H4, H5, H6 - *Table 25*) was again used to obtain green hues in the wool fibres. However, wool samples M7 and N6 present nowadays a blue hue, as a consequence of the degradation of the weld yellow chromophores.

Some chromophores of spurge flax were already identified in a tapestry from the 17th-18th period (green samples I5 and I8, *Table 19*), but in samples H5 and H6 (*Table 25*) two additional spurge flax chromophores were identified. Daphnetin 7-*O*-glucoside (daphnin) was detected at 15.03 min. and daphnetin 8-*O*-glucoside was detected at 15.70 min. The $[M-H]^-$ (*m/z* 339) was detected along with the Y_0^- ion at *m/z* 177, which results from the loss of an hexose unit (162 Da) from the original deprotonated ion. The identification of the chemical nature of the two glycosides is based on a literature report where spurge flax extracts are eluted using a chromatographic system to the one used here[2].

Spurge flax was also used in combination with brazilwood to dye sample M1, which currently presents a yellow hue instead of the orange hue that would be expected when dyeing with a yellow dye source and a red dye source. Poor lightfastness of brazilwood dye is again responsible for this effect.

Extraction of white, beige and brown samples B1 and B4 (*Table 22*), C3 (*Table 23*), D2 and D6 (*Table 24*), M6 (*Table 26*) and N2 (*Table 27*) did not yield any recognizable dye on the chromatogram.

Apart from the recognizable chromophore indigotin, at r_t = 26.59 min., another colouring compound was detected in sample N4, at r_t = 29.00 min (*Table 27*). The compound presents a molecular weight of 648 and is detectable both in positive and negative ion mode. The UV-Vis spectra of the compound presents a strong absorption band at a wavelength of 577 nm, characteristic of the absorption of yellow-green radiation and transmission of a violet visible colour[241]. No identification was assigned to this compound, and further work is needed to identify this chromophore.

Analysis and quantification of mordants in Arraiolos tapestries

Despite the reported use of other metal salts, alum has always been the most used mordant for the red and yellow flavonoid dyes, recognized for the brilliance of the colours obtained[242]. Alum and 'caparosa' (sulfate of iron, copper or zinc) are the mordants referred in the Arraiolos historical dyeing recipes[1,12] (*Table 1*).

In order to account for the mordant effect in the colours seen in the studied Arraiolos carpets, the contents of fibres mordant metal ion were analysed in the collected samples by particle-induced X-ray emission (PIXE), scanning electron microscopy using an energy-dispersive X-ray spectroscopy detector (SEM-EDS) and inductively coupled plasma mass spectrometry (ICP-MS).

PIXE and SEM-EDS were used in selected samples to obtain information on the mordant chemical nature and its distribution on the dyed fibres, while ICP-MS was performed in all the samples to quantify the mordant ions.

SEM–EDS technique is a powerful technique for it provides high magnification (SEM) with the ability to generate localized chemical information (EDS). The major problem of this technique is that it presents relatively poor sensitivity to trace elements and to elements lighter than Na[83]. In PIXE the sample is exposed to an ion beam and the sensitivity of the method at trace element level is significantly better than that of the EDS methods. The PIXE method offers maximum sensitivity when atomic number *Z* of a detected element is in the range 18–40[243], which include the major elements composing the mordants normally used by the dyers.

An important feature available in both the SEM-EDS and the PIXE techniques is the ability to obtain element distribution mapping which is a powerful tool to investigate the homogeneity of the mordant metal distribution in the dyed wool samples.

SEM-EDS was performed in a total of 67 wool samples (data not shown). The presence of carbon, oxygen and sulphur was detected in all samples, as natural constituents of wool fibres. Aluminium was also detected in most of the fibres confirming the widespread use of alum as mordant in the Arraiolos dyeing process. *Figure 46* shows a representative EDS spectra of a sample likely mordanted with Al and *Figure 47* shows the elemental mapping where it can be seen that the Al ion is evenly distributed among the fibre surface. Calcium (observed in *Figure 46*) and sometimes magnesium were found in relative high amounts in the fibres, probably a result of the insoluble calcium and magnesium salts contained in the soaps used for wool scouring[244].

Relative high contents of Al and sometimes Fe and Zn were detected in analysed blue samples where only indigotin had been detected (A8 - *Table 15*; C8, C9 - *Table 23*; E1, E7 - *Table 16*; L15-L18 - *Table 21*; M8 - *Table 26*) (*Figure 48*). This was a surprising result because vat dyes do not require mordant, and their use results in an increase in the costs of the dyeing process without obvious advantages for the fibre final hue.

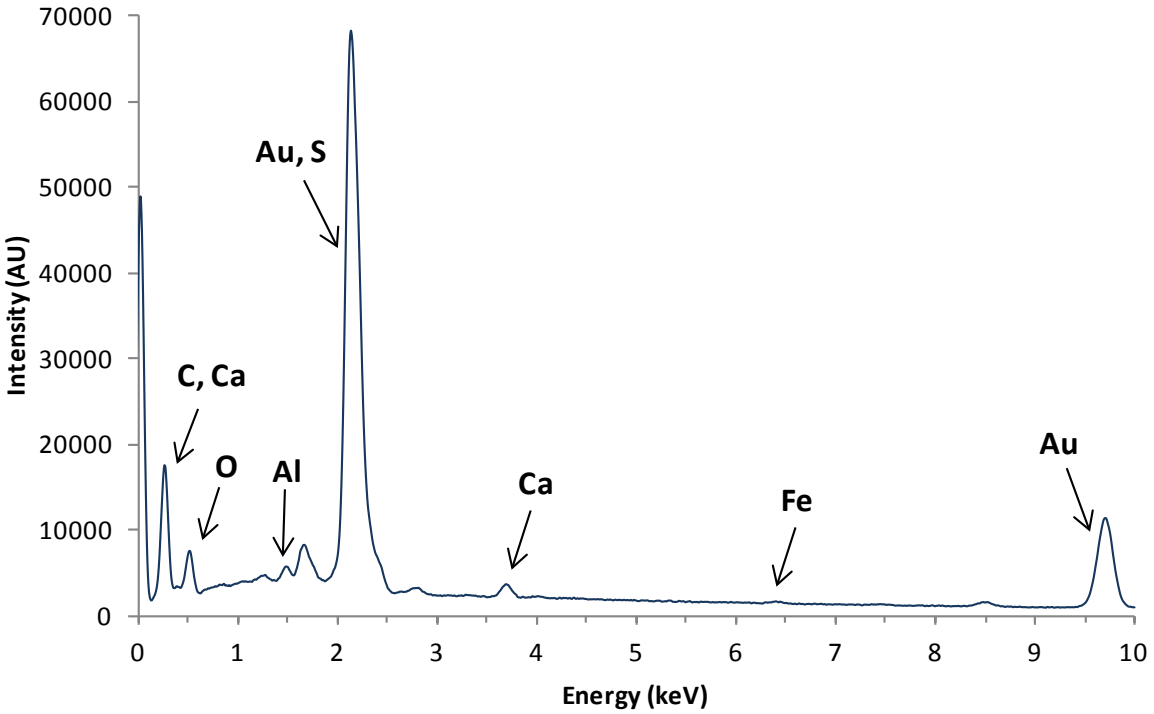


Figure 46. *EDS spectra of red coloured historical sample A6.*

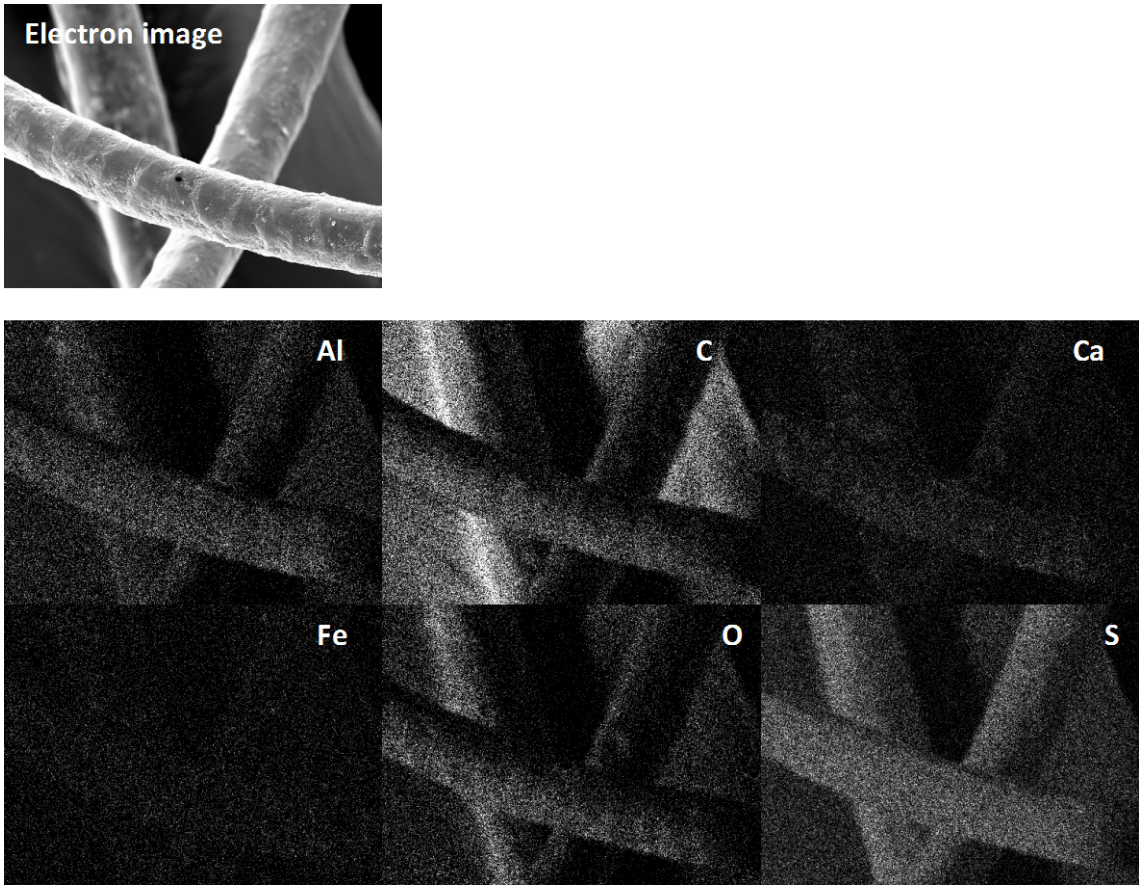


Figure 47. *EDS elemental mapping of historical sample A6.*

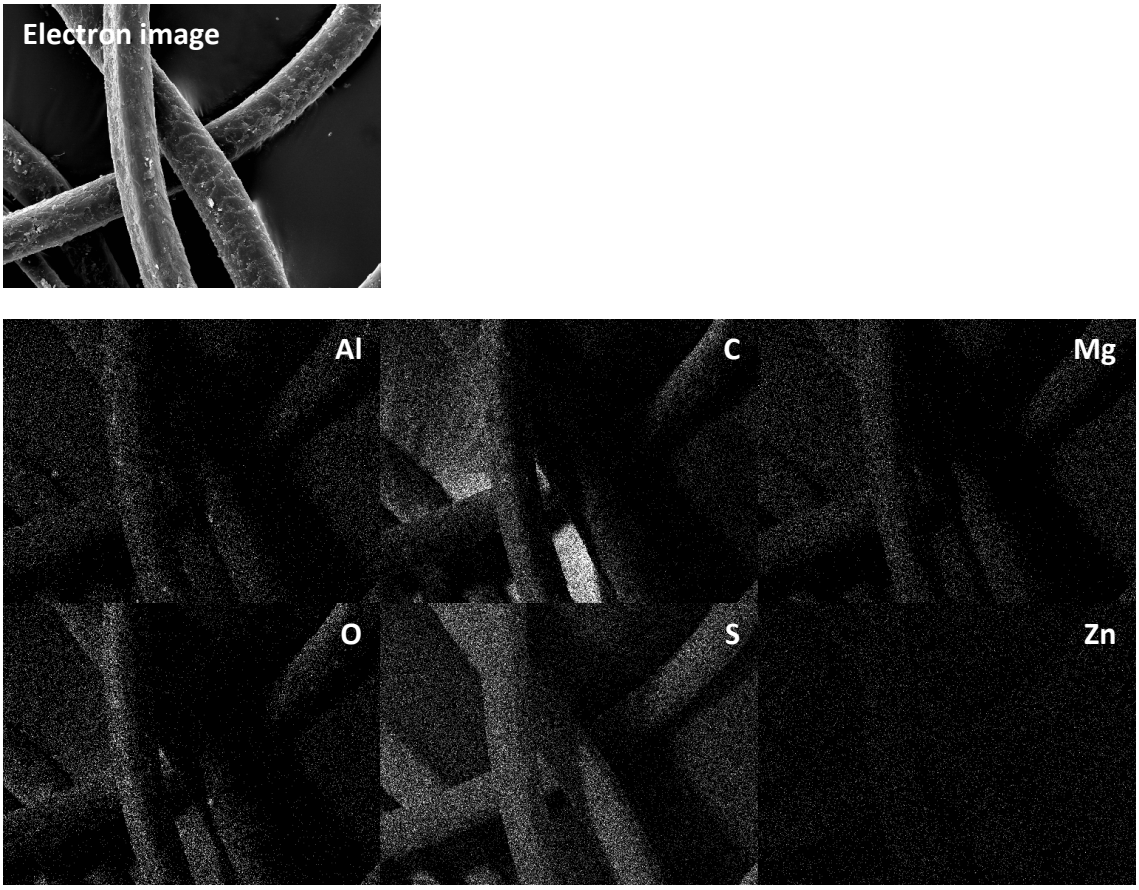


Figure 48. *EDS elemental mapping of blue coloured historical sample A8.*

As previously referred, in some regions of Europe it is reported the use of mud or silt rich in iron salts in the dyeing process to obtain darker hues[46]. Some dark coloured samples presented also a relative high amount of iron (B1, C9, E1, E2, E12, L3, L14, N7). *Figure 49* and *Figure 50* present the EDS spectra and elemental mapping of black sample B1, respectively. EDS spectra shows an almost absence of Al and the distinct presence of Fe in sample B1; EDS mapping shows an evenly distribution of the selected elements, especially of Fe.

μ-PIXE analysis of 22 wool fibres samples (data not shown) overall confirmed the results obtained by SEM-EDS. Aluminium was found again in relative high amounts in most of the studied fibres, while the presence of iron was more important in some blue and dark coloured samples. For comparison with the SEM-EDS data, *Figure 51* and *Figure 52* present the μ-PIXE element mapping for red coloured historical sample A6 and black sample B1, respectively. The remaining high levels of Ca and Mg in the samples were likely due to the wool scouring process, while the Si was likely due to accumulated dust (data not shown).

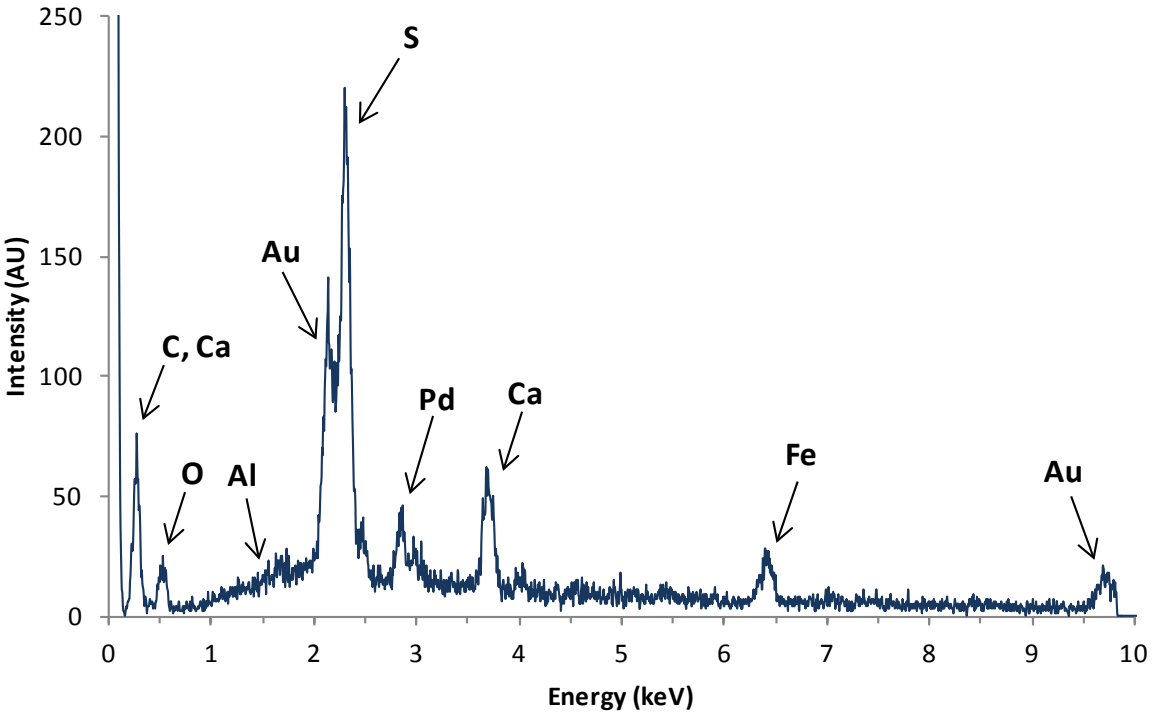


Figure 49. *EDS spectra of historical black sample B1.*

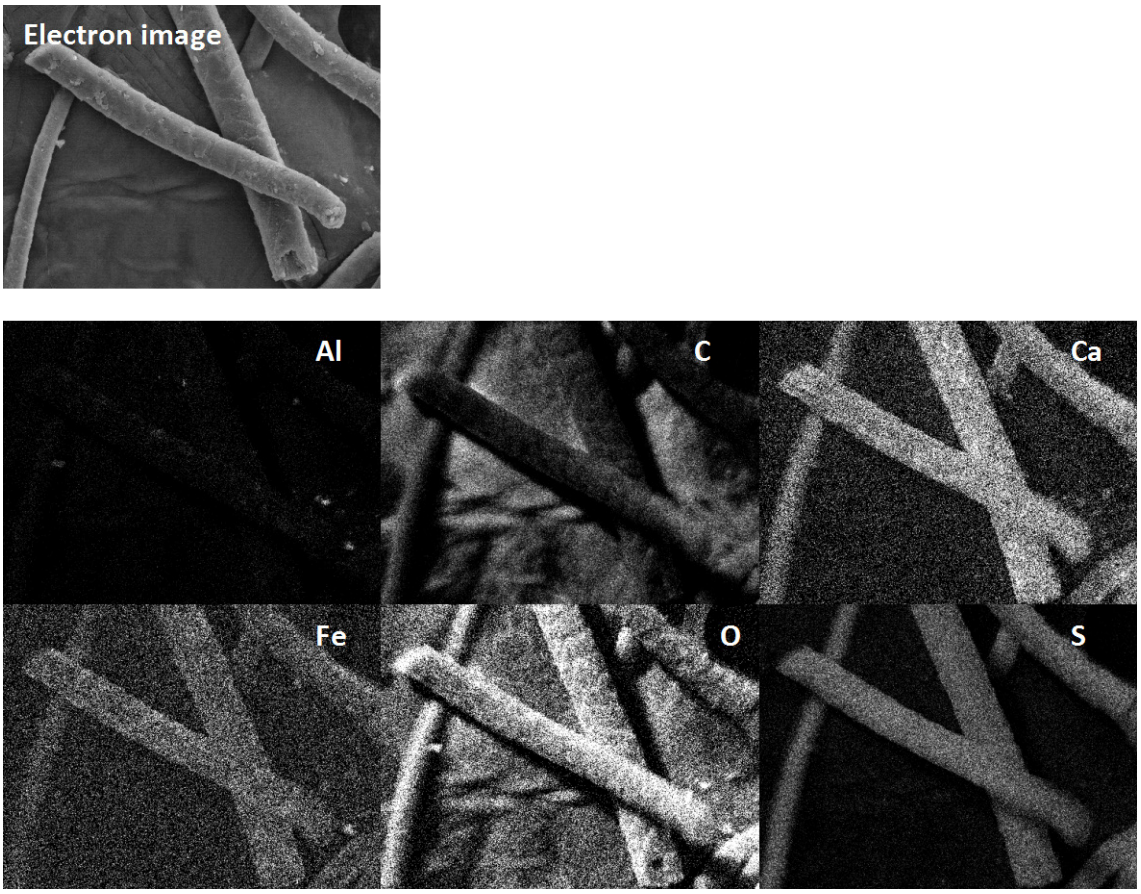


Figure 50. *EDS elemental mapping of historical sample B1.*

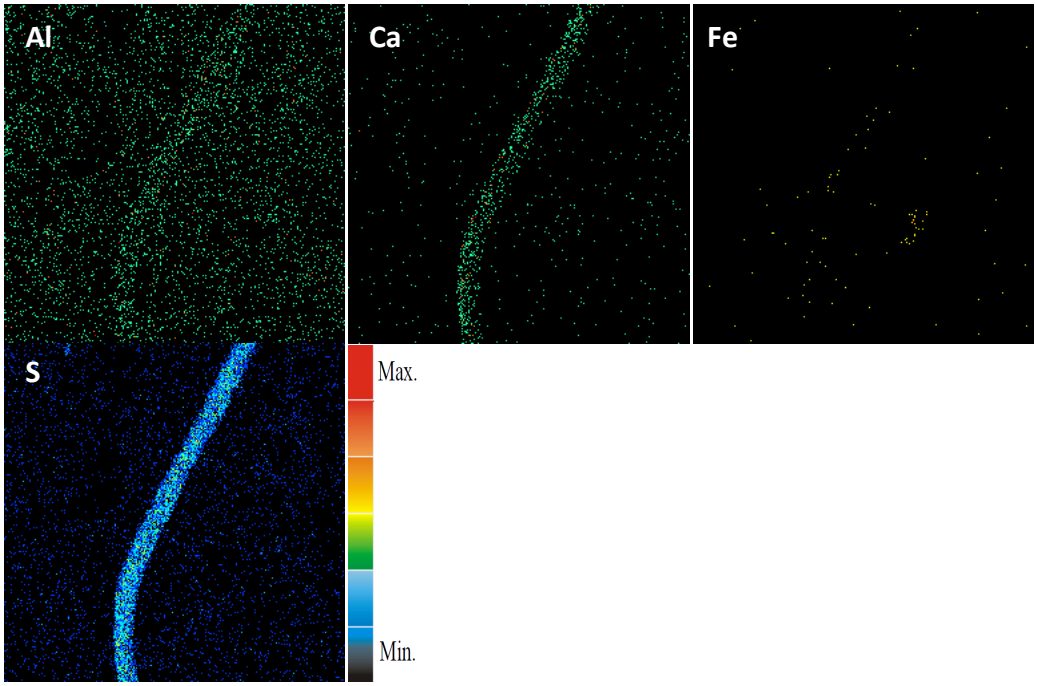


Figure 51. *μ-PIXE elemental mapping of red coloured historical sample A6.*

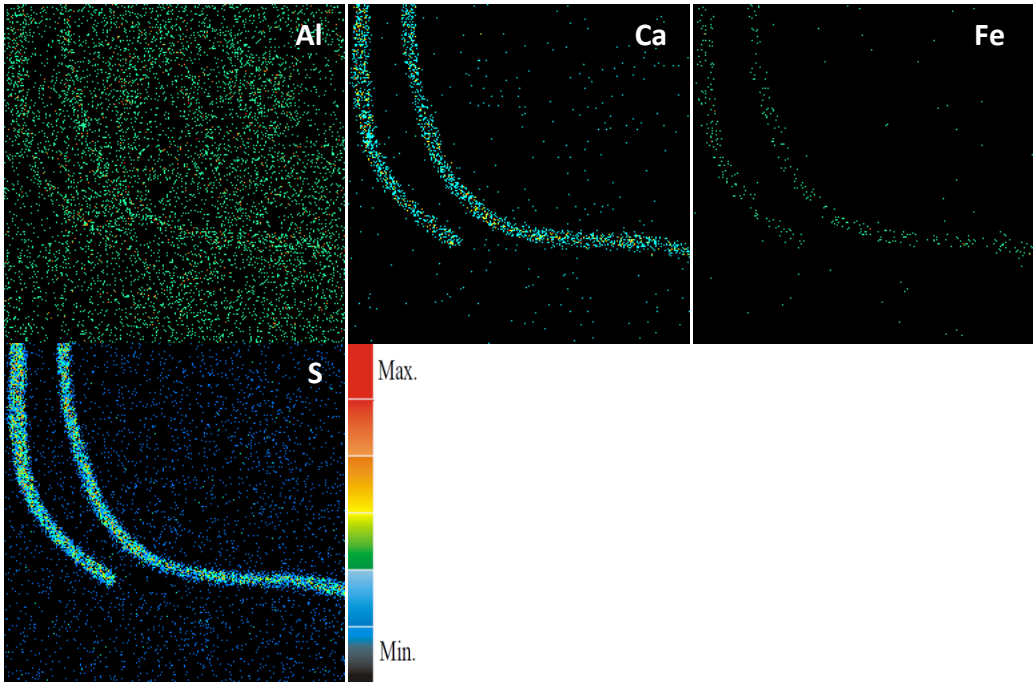


Figure 52. *μ-PIXE elemental mapping of black coloured historical sample B1.*

ICP-MS analysis was performed in all the wool samples in order to quantify the amount of the different metallic ions (Al, Fe, Cu and Zn) present in the fibres. *Figure 53* and *Figure 54* present the ICP-MS mordant metal ion quantification in wool samples collected from the 17^{th}-18^{th} century and from the 18^{th}-19^{th} century Arraiolos tapestries, respectively.

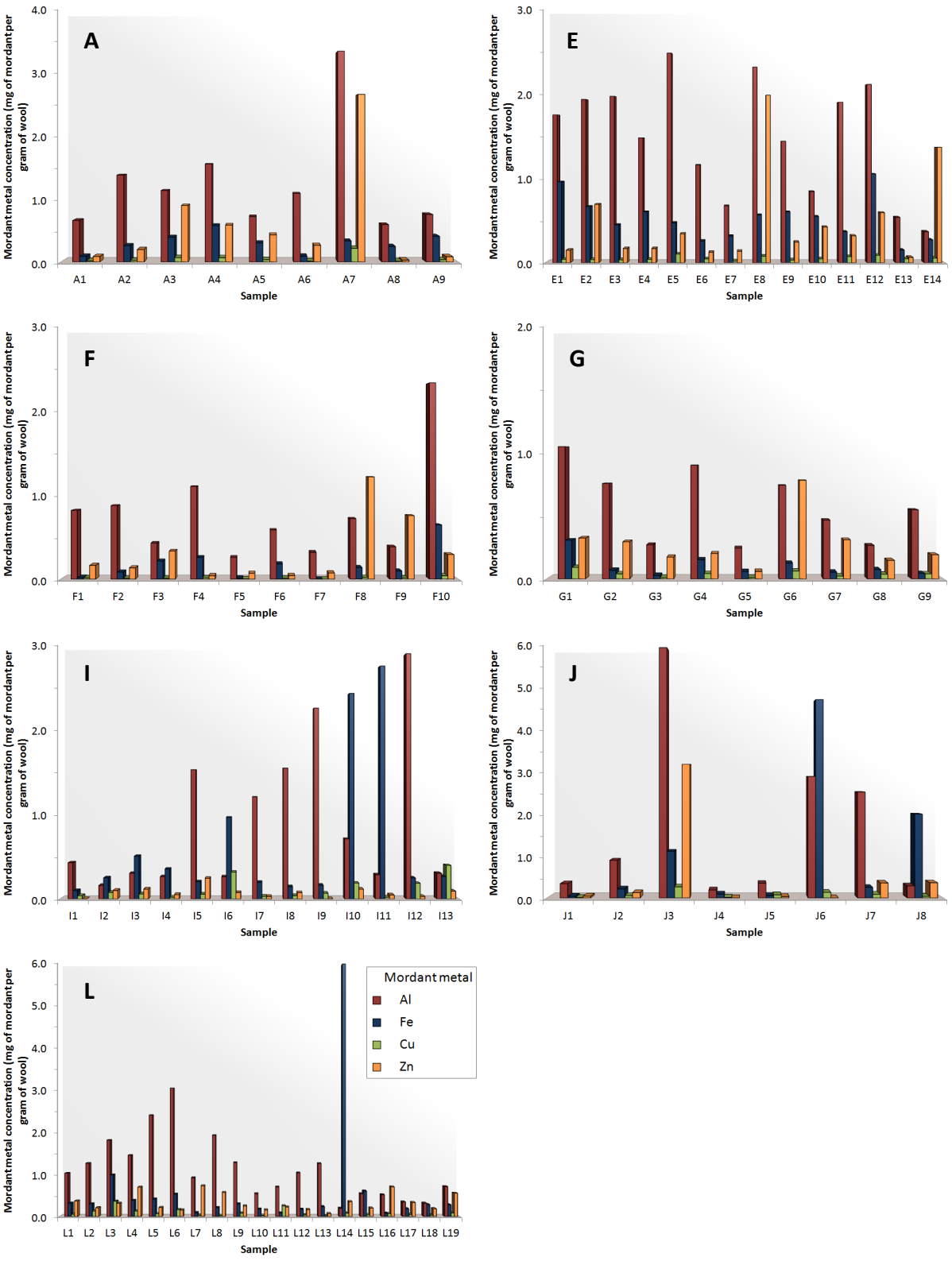


Figure 53. *ICP-MS mordant metal ion quantification in wool samples collected from the 17th-18th century Arraiolos tapestries.*

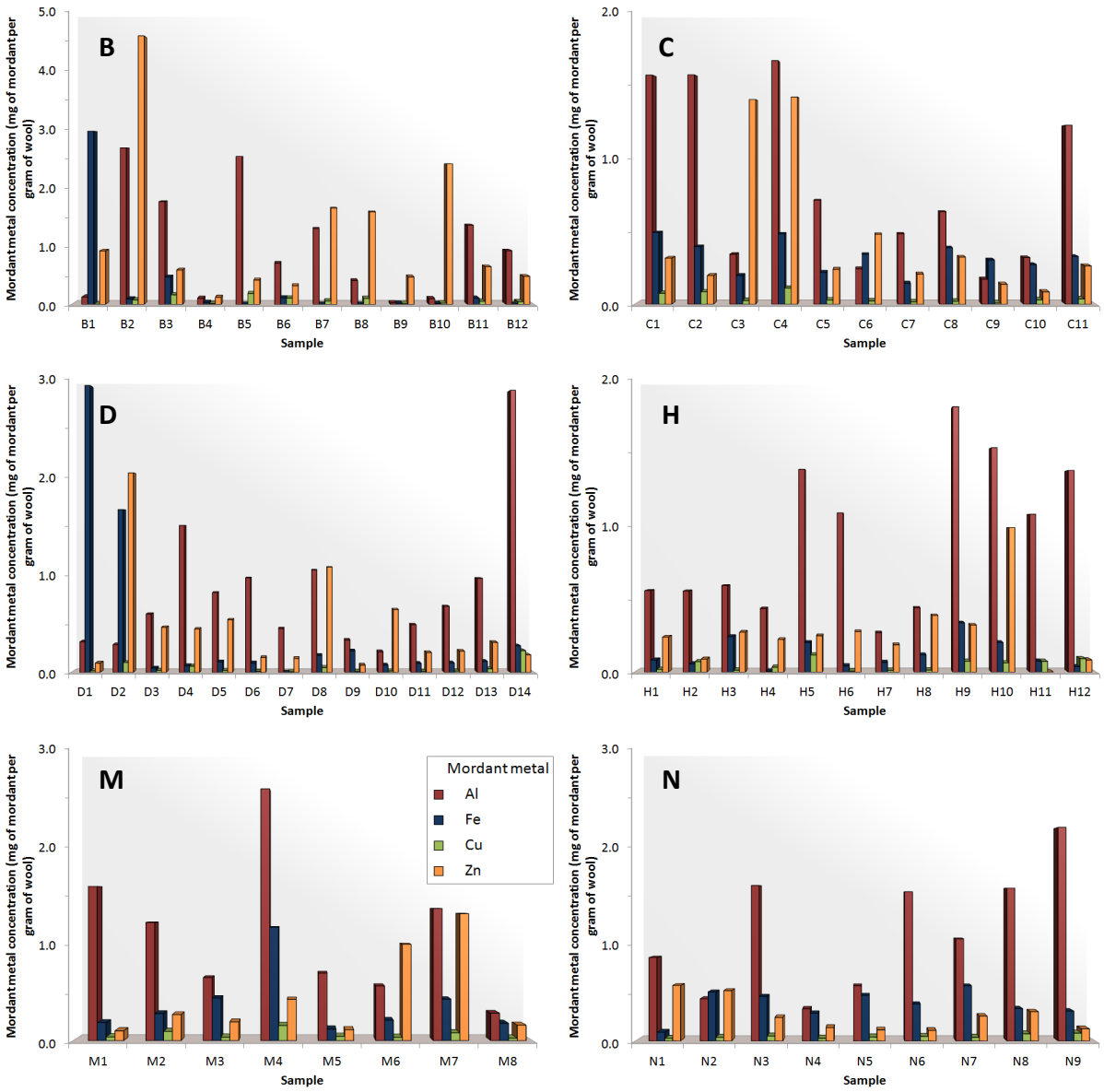


Figure 54. *ICP-MS mordant metal ion quantification in wool samples collected from the 18th-19th century Arraiolos tapestries.*

Analysis of Al, Fe, Cu and Zn contents in blank solutions were below the limit of detection (LOD) of the analytical method (1 µg/L). Metal ion quantification was also performed on several samples of actual untreated sheep wool and the amounts detected were much lower than those detected in the samples of the historical carpets (data not shown).

The order of magnitude of the aluminium and iron concentrations detected in the historical fibres is similar to those found in the contemporary red dyed fibres (compare *Figure 53* and *Figure 54* with *Figure 28* and *Figure 33*). Overall, the results showed that

concentrations of Al and Fe are much higher than Cu, which is only detected in residual amounts. Whenever copper salts are used as mordant, the concentrations detected in the wool are normally higher than those of Al and Fe whenever these salts are used in equivalent concentrations (see *Figure 28* and *Figure 33*) and, therefore, it can be assumed that copper salts have not been used as main mordants in the studied carpets.

Al was identified as the most abundant metal ion in most of the samples, confirming the widespread use of alum as mordant in the Arraiolos tapestries and the results obtained by SEM-EDS and μ-PIXE. ICP-MS data confirmed the high amounts of Al, and sometimes Fe and Zn, previously found in samples where only indigotin had been detected (samples B9-10, C8-9, D9-11, E1, E7, E10, F3, F9, G5, G8, H8, I3-4, I6, J3-5, L15-18, M3, M8, N5, N7, see *Table 16* to *Table 27* for colorimetric data). Due to red and yellow dyes light fastness it is possible that some of these samples were also dyed with a currently undetectable mordant dye. However, this is unlikely to have occurred for all the samples, because originally blue wool was certainly used in the embroidery work.

Detectable amounts of Al, Fe and Zn were detected in wool samples without extractable chromophores (A9, B1, B4, C3, D2, D6, E14, F5-7, G3, I2, I13, J2, L1, L3, L14, M6, N2), with most of these samples having white, grey and brown or black hues (see *Table 15* to *Table 24*, *Table 26* and *Table 27* for colorimetric data). The white and brown or black colours could be attributed to the use of virgin wool, but the very high contents of metals in some of these samples (for example B1, D2, L14 for iron and L1, L3 for aluminium) suggest that these were likely originally dyed samples, where chromophore photodegradation prevents nowadays the identification of the dye source.

Some samples present high amounts of zinc, considering this metal ion alone or when its concentration is compared to the other metal ions in the sample. Comparing the two studied periods, fewer samples from the 17th-18th century present high amounts of Zn (A3, A7, E8, E14, F8, G6, J3, L7 and L16 in *Figure 53*) when compared with samples from the 18th-19th period (B2, B7-8, B10, C3-4, C6, D2-3, D5, D8, D10, H8, H10, M6-7 and N1-2 in *Figure 54*). The presence of zinc could not be associated with a certain dye or hue in the Arraiolos carpets. Zn salts have been seldom referred as mordants, with exception of some reports on their use together with weld to obtain yellow hues in Coptic textiles[171,245].

7. Conclusions

Natural dyes are chemically very different, making their effective extraction from textile fibres a complex and difficult task. Despite different extraction yields within the classes of natural dyes, the evaluation of eight extraction procedures available in the literature lead to the conclusion that the most adequate procedure to extract the tested natural dyes from dyed wool fibres was the use of a mixture of Na_2EDTA/DMF (EDTA-2 method)[204]. The procedure was used for natural dyes extraction from the studied collection of historical Arraiolos tapestries. The procedure proved to be very effective as several dye components could be recovered from most of the samples. The effectiveness of the extraction procedure was most evident in the extraction of up to nine different flavone derivatives from different yellow samples, enabling the identification, with an high degree of certainty, of the usage of weld in the making of these carpets.

For madder and brazilwood contemporary dyed wool samples, fibre colour hue was found to be highly dependent of mordant ion nature, mordant bath concentration and dyeing method, and this is probably a consequence of the different metal-chromophore complexes formed during the dyeing process.

Mordant quantification showed that the amount of metal ions found in fibres is very small when compared to the concentrations added to the dyeing bath. However, for the smaller concentrations of mordant salts, the ratio between the metal ion content in the fibre and the mass of metal ion present in the dyeing bath is higher. Cu ions seem to have greater affinity for the wool fibres than Al or Fe ions – for equivalent mordant bath concentrations, the uptake of Cu in the fibres is at least twice the amounts of Al or Fe. Pre-mordanting (MD) dyeing procedure yielded fibres with larger amounts of metal ions, which is likely due to the fact that metal ion binds to wool before complexing with the chromophore, thus resulting in

higher amounts of metal ions in the fibres. This behaviour can also be used to explain the larger chromophore peak areas presented by MD dyed samples – if higher amounts of metal are bound to the fibre, it is likely that a higher number of wool-metal-dye structures are formed and then extracted for chromatographic analysis.

When the madder and brazilwood dyed samples were subjected to accelerated ageing, the chromophore photodegradation rate was not constant along the assay, being overall more accentuated in the first hours of light exposure. The chromophore photodegradation was also more pronounced whenever alum was used as mordant, a result of uttermost importance considering the use of this mordant in the dyeing industry worldwide.

In the case of the madder dyed wool samples, degradation rate of alizarin and purpurin was not the same and alizarin degraded more extensively than purpurin when alum was used as mordant. This result could partially explain the change in hue occurring in the samples subjected to light ageing.

Chromatographic analysis on brazilwood dyed samples before and after light exposure detected the formation of Type C compound during the light ageing process. The latter had already been identified in historical samples[179,235] but no hypothesis for its origin was clearly presented. Unlike to what has been proposed, the compound seems to be more a photodegradation product of brazilwood dye than an artefact caused by acid hydrolysis of the samples, as a mild EDTA-based extraction procedure was used in the present work. The structure of Type C compound seems to be related to brazilein structure, but further fragmentation studies and isolation of this compound for NMR analysis are required in the future. The detection of this compound in the historical wool samples was used to identify, for the first time, brazilwood as a dye source used in the making of Arraiolos tapestries.

HPLC/DAD/MS technique combined with the information obtained from ICP-MS, SEM-EDS and μ-PIXE was fundamental to the tentative identification and characterisation of the natural dyes and mordants found in the wool samples collected from the studied thirteen Arraiolos tapestries from the NMAA collection. The use of a Na_2EDTA/DMF mild extraction method for the dye removal from wool was an essential step in the acquisition of extremely complete chromatographic profiles of the samples, enabling for a better understanding of dye source used in their making.

Weld, indigo, spurge flax and brazilwood were identified as natural dye sources, as already described in the Arraiolos historical dyeing recipes[1,12]. The anthraquinone-based

natural dye sources madder and cochineal were for the first time described in historical Arraiolos. Green hues in the fibres were obtained not only by dyeing with weld and indigo, as expected, but also with spurge flax and indigo. Ellagic acid and quercitrin were identified in two samples, but no dye source was suggested, and further dyeing studies using quercitron bark and sumac, two dye sources in which these compounds are normally found[47], are required in the future.

As observed in the photodegradation studies, brazilwood is an extremely fugitive dye. The colour fading in Arraiolos carpets has already been described by Pessanha[11], who stated that some areas originally dyed in red hues became brownish with time. Brazilwood dye photodegradation has a tremendous impact in the actual perception of these carpets when compared to what it might have originally been intended by its makers. This is particularly significant because the remnants of this dye were found in the embroidery stitches of the background field of several tapestries.

Less important in terms of embroidery area but no less important in terms of visual perception is the effect of the photodegradation of the yellow flavonoid dyes has on some originally green hues which today appear as blue coloured areas.

With this study it was possible to contribute for a better understanding of the materials and techniques used in the historical Arraiolos carpets production , which in turn will enable a better preservation of these important Portuguese textile treasures, in terms of restoration, storage and display conditions.

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Manhita A, Costa C, Ferreira T, Mirão J, Vargas H, Ribeiro I, Seruya I, Pacheco T, Alves L, Candeias A. **Rediscovering the materials of Arraiolos tapestries: fibre and mordant analysis by SEM-EDS and μ-PIXE.** *Microsc. Microanal.* 14 (**2008**) 91-94.

Manhita A, Ferreira V, Vargas H, Ribeiro I, Candeias A, Teixeira D, Ferreira T, Dias CB. **Enlightening the influence of mordant, dyeing technique and photodegradation on the colour hue of textiles dyed with madder - A chromatographic and spectrometric approach.** *Microchem. J.* 98 (**2011**) 82-90.

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Manhita A, Santos V, Vargas H, Ribeiro I, Ferreira T, Candeias A, Dias CB. **Ageing of brazilwood dye in wool – a chromatographic and spectrometric study.** *J. Cult. Herit.* (submitted for publication)

Microsc Microanal 14 (supp 3), 2008
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doi: 10.1017/S1431927608089484

Rediscovering the materials of Arraiolos tapestries: fibre and mordant analysis by SEM-EDS and μ-PIXE

Ana Manhita[*], Cristina Costa[*], Teresa Ferreira[*], José Mirão[**], Helena Vargas[***], Isabel Ribeiro[***], Isabel Seruya[****], Teresa Pacheco[*****], Luís Alves[******] and António Candeias[*]

[*]Universidade de Évora, Chemistry Department and Évora Chemistry Center, Rua Romão Ramalho 59, 7000-671 Évora, candeias@uevora.pt, Portugal.
[**]Universidade de Évora, Geosciences Department and Geophysics Center of Évora, Portugal
[***]Instituto dos Museus e da Conservação, R. Janelas Verdes 37, 1249-018 Lisboa, Portugal.
[****]Universidade Nova de Lisboa, Conservation and Restoration Department, 2819-516 Monte Caparica, Portugal
[*****]Museu Nacional de Arte Antiga (MNAA), Rua das Janelas Verdes, 1249-017 Lisboa, Portugal
[******]Instituto Tecnológico e Nuclear, Estrada Nacional 10, 2686-953 Sacavém, Portugal

Arraiolos tapestries are probably one of the richest artistic Portuguese expressions in terms of textile heritage. It is difficult to date the beginning of the production of rugs in Arraiolos (Southern Portugal), but they were already produced in the late 1600's as they are listed in the inventories of Portuguese aristocratic households in the beginning of the 18th century. Sensitive detection techniques play an increasing role in the chemical investigation of historical objects since the knowledge derived from the chemical composition of materials is of upmost importance for textile conservation and restoration purposes. Textiles deteriorate due to natural causes like heat, radiation, mechanical stress, moisture, microbiological and enzymatic attack. Deterioration of materials causes breakdown of the molecular structure and results in a loss of strength, extensibility and durability, discoloration and fading which affects the appearance of the textiles[1].

In this work we present results of microanalysis by SEM-EDS and μ-PIXE of fibres from Arraiolos rugs (Rug nr. 88, from the 17th century) of the Portuguese Ancient Art National Museum collection (MNAA). The purpose of the study was to identify and evaluate the nature and the conservation state of the fibres and employed mordants by the Arraiolos dyers. This work is integrated in project "REMATAR - Rediscovering the Materials of Arraiolos Tapestries" (PTDC/HAH/64045/2006) funded by Fundacao para a Ciencia Tecnologia which intends to obtain detailed and systematised information on the composition, structure and degradation processes that occur on these tapestries and to use these data to improve their conservation methodologies. Moreover, the collected data on the dyestuffs and mordants will hopefully allow a time scale of materials usage that could assist on the dating of other Arraiolos pieces and the understanding of the sociological and economical factors that influenced the production of these tapestries.

The rich coloration presented in the sampled rug (Fig.1) suggests the use of distinct dyes. Colour hues can be obtained by the use of different mordants with the same dye. Experienced dyers could also use different dye concentration and dyeing conditions to obtain the desired fibre colour. Moreover, the identification of the mordant is an important issue in the analysis of fibre degradation as its nature affects the acidity of mordanting and dyeing solutions.

Fibre samples were taken from the wool fibres used for embroidery (samples A1 to A9) and from supporting canvas (sample A10). SEM micrographs and EDS spectra and 2D maps were carried out using a JEOL JSM-7001F scanning electron microscope coupled with an OXFORD X-ray

energy dispersive spectrometer with a Si(Li) detector. The fibre samples were coated with a thin, conductive film of gold. The current used varied from 10 to 25 kV. The μ-PIXE analysis was performed using the proton beam from the 3 MV Van de Graff accelerator at the Instituto de Tecnologia Nuclear (ITN, Lisbon, Portugal).

The EDS and μ-PIXE results (Fig.2 and Fig.3), presented as representative data for all the samples (A1 to A9), showed the presence of sulphur, silicon, calcium and potassium belonging to the wool composition. Besides these, aluminium was found evenly distributed over the fibres, independently of their colour. The presence of aluminium in the blue fibres samples (samples A1 and A8) is likely an indication that a pre-mordanting procedure of the wool was done by the Arraiolos dyers since indigo, the blue dye identified in these samples, is a vat dye and hence does not require a metal mordant.

EDS analyses also revealed the existence of small traces of lead and tin. However, they were assumed to be a contamination from the vats employed in the wool treatment, as they were barely detected. Salts of copper and iron, along with alum (double sulphate of aluminium and other metal), are reported worldwide as mordants for the yellow and red hues. Historical data on Arraiolos tapestries dyeing techniques only refer the usage of alum [2]. Data on the Rug nr. 88 samples confirm the absence of copper and iron, while aluminium was found in all the analysed samples. The EDX and μ-PIXE results seem to confirm that alum was, in fact, the only mordant used in the dyeing process of wool fibres for the manufacture of Rug nr. 88.

SEM micrographs of fibre surfaces display, in general, the typical scale structure of wool (Fig.4). However, in some cases it was observed roughened surfaces with damage of this scale structure due to wear and natural ageing. The presence of transverse cracks and longitudinal splitting are indicative of embrittlement due to loss of flexibility and elasticity, causing cracks when subjected to bending or stretching. Several types of fractures were observed in the analysed fibres, being the most commonly found a combination of granular and radial fractures. Some fibrillar multiple split ends were also found, but it was likely that they are naturally occurring ends, with fibrillation due to wear [3, 4]. In some cases, biological colonization was found (mould and bacteria) which may also contribute to loss of tensile strength.

In relation to the canvas (rug support matrix) the morphological analysis of the fibres show that the warp threads of Rug nr. 88 are linen, which is in agreement with historical data [2]. Furthermore, in general, the canvas fibres are in good state of conservation presenting only small cracks (Fig.5).

In conclusion, SEM-EDS in combination with μ-PIXE results allowed an evaluation of the fibre surface, fracture morphology and mordant analysis. Alum was used as mordant which is in accordance with the available historical treaties and the rug fibres exhibit the expected degradation due to wear and ageing. These results together with data from the dye identification and their degradation products as well as tensile strength analysis are now being pursued in order to evaluate the overall state of conservation of the Arraiolos museum pieces.

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Microsc Microanal 14 (supp 3), 2008

Figure1. Sampling points in 17th century Rug nr. 88 from the MNAA collection.

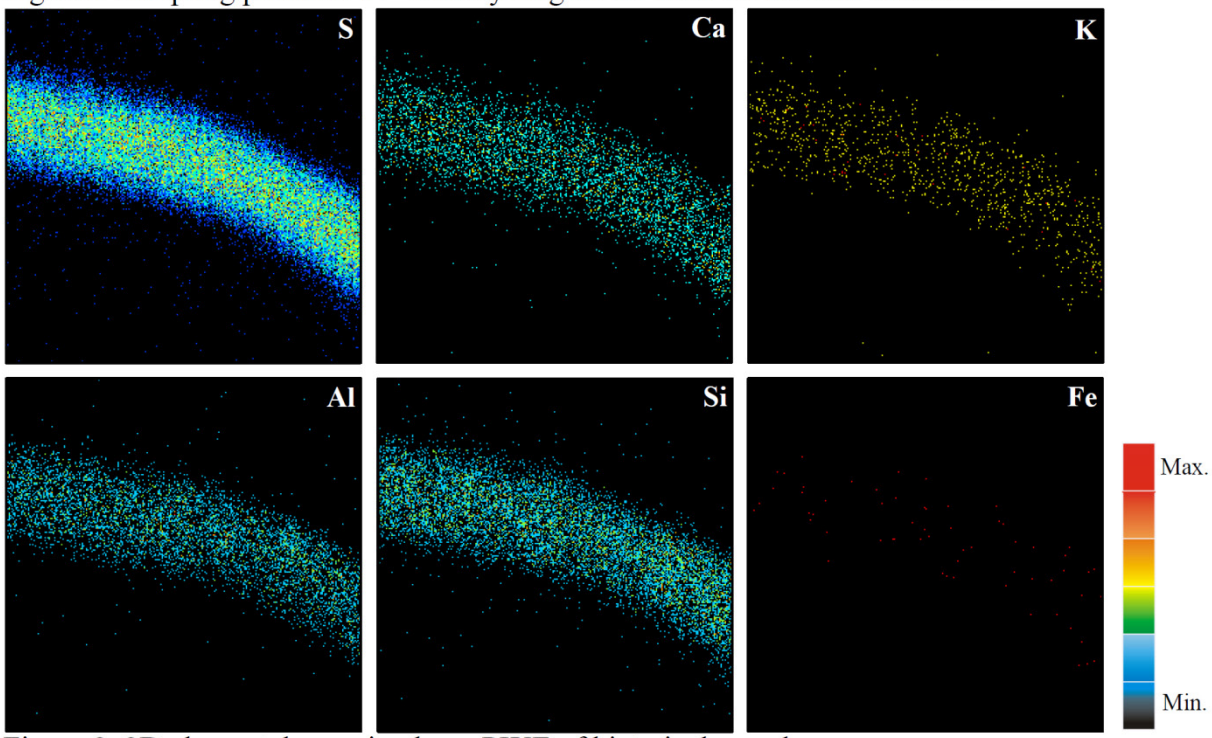

Figure 2. 2D elemental mapping by µ-PIXE of historical samples.

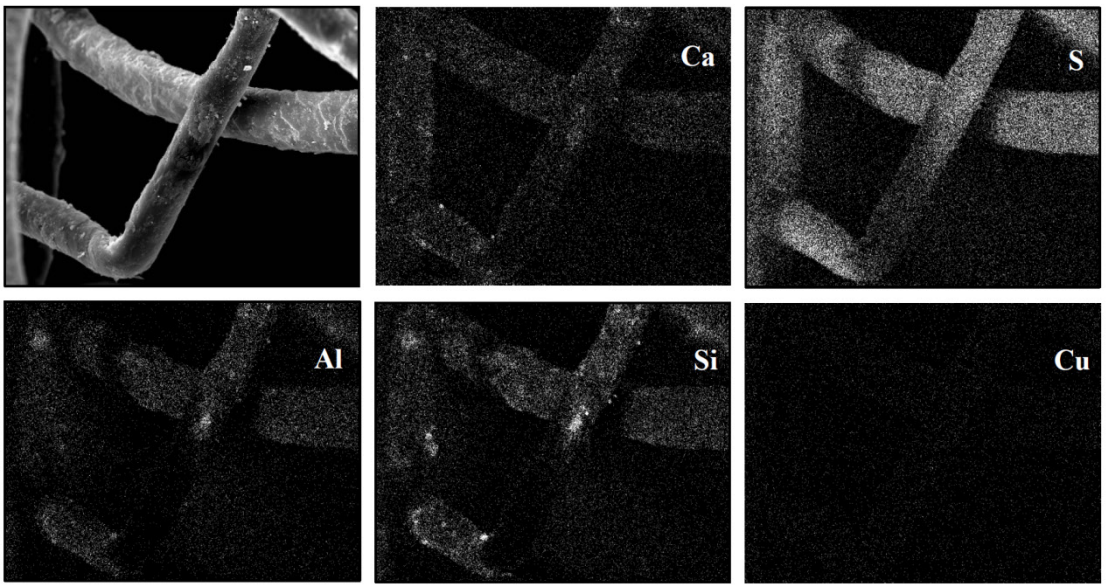

Figure 3. 2D elemental mapping by EDS for sample A1.

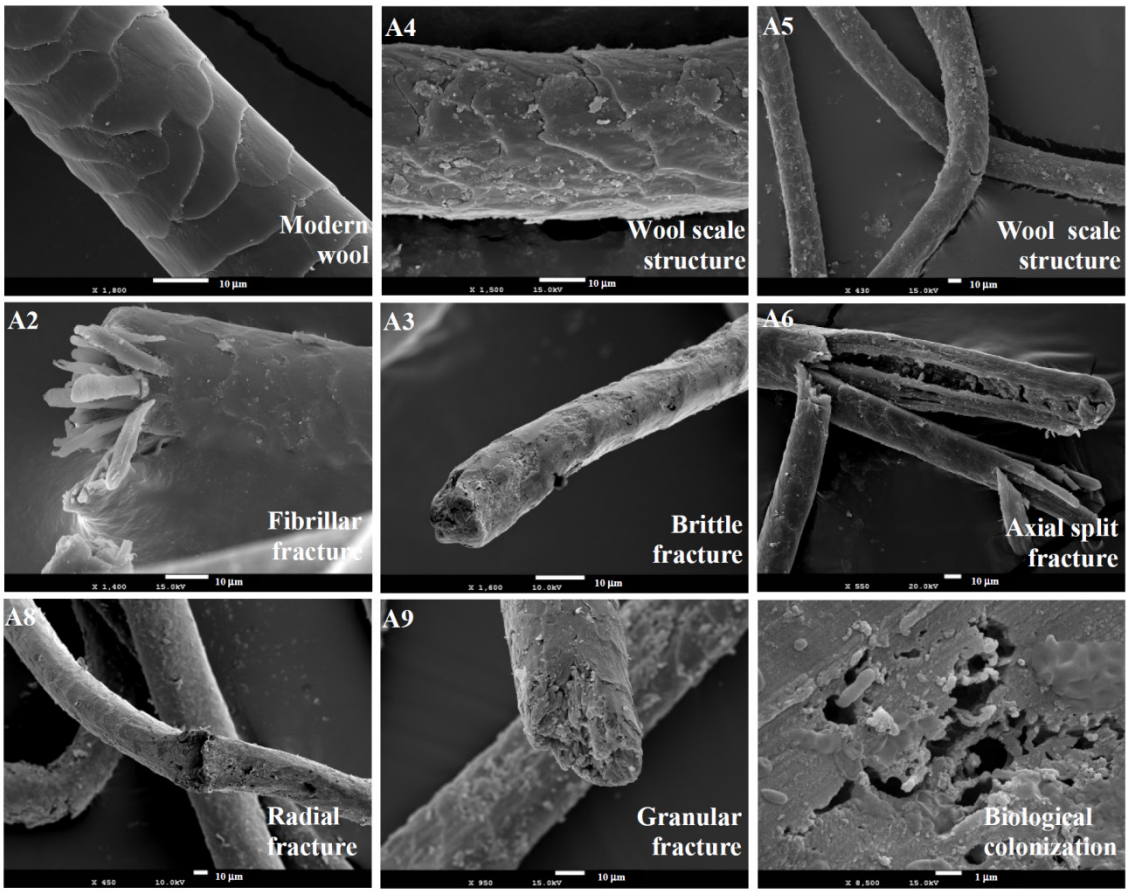

Figure 4. SEM micrographs of the historical fibres exhibiting distinct types of fractures and biological colonization.

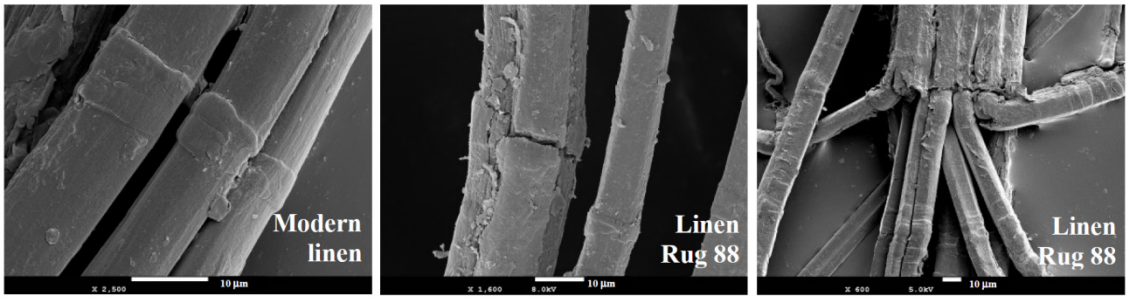

Figure 5. SEM micrographs of modern linen and warp threads from Rug 88 showing that linen was used as the support fibre.

Microchemical Journal 98 (2011) 82–90

Contents lists available at ScienceDirect

Microchemical Journal

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

Enlightening the influence of mordant, dyeing technique and photodegradation on the colour hue of textiles dyed with madder – A chromatographic and spectrometric approach

Ana Manhita [a,b], Vanda Ferreira [c], Helena Vargas [d], Isabel Ribeiro [d], António Candeias [a,d], Dora Teixeira [a,c,e], Teresa Ferreira [a,c], Cristina Barrocas Dias [a,c,*]

[a] HERCULES Laboratory, Largo do Marquês do Marialva, 8, 7000-554 Évora, Portugal
[b] Évora Chemistry Center, Rua Romão Ramalho 59, 7000-671 Évora, Portugal
[c] University of Évora, Chemistry Department, Rua Romão Ramalho 59, 7000-671 Évora, Portugal
[d] José de Figueiredo Conservation and Restoration Laboratory, Institute of Museums and Conservation, Rua das Janelas Verdes 37, 1249-018 Lisboa, Portugal
[e] ICAAM, Institute of Mediterranean Agricultural and Environmental Sciences, Núcleo da Mitra Apartado 94, 7002-774 Évora, Portugal

ARTICLE INFO

Article history:
Received 22 October 2010
Received in revised form 9 December 2010
Accepted 9 December 2010
Available online 16 December 2010

Keywords:
Madder (*Rubia tinctorum*)
Mordant
Photodegradation
LC-MS
ICP-OES
FAAS

ABSTRACT

Wool samples were dyed with madder and alum, copper, and iron salts at different concentration by pre-mordanting (MD) and simultaneous mordanting (M+D) procedures. Samples were artificially aged to identify the influence of the mordant on the madder chromophores photodegradation. A set of analytical techniques was used for complete characterisation of the dyed fibres before and after light exposure, which included colour and chromophore analysis (colourimetry and LC-ESI-MS/MS analysis), determination of mordant ions amounts in the fibres (FAAS and ICP-OES analysis), morphological characterisation of the fibres and punctual chemical analysis (SEM-EDS studies).

Fibre colour hue was found to be dependent on the mordant ion nature, mordant bath concentration and dyeing procedure. Mordant ion quantification showed that the uptake of metal ion by the fibres is relatively small, with the Cu ion presenting the highest affinity for the fibre. MD method yields fibres with higher amounts of metal ions and larger chromophore chromatographic peak areas corresponding, in general, to stronger colour hues. Photodegradation was more severe in alum mordant samples and in the first 480 h of light exposure. Chromophore degradation rates are unequal and dependent on the mordant nature, contributing for colour changes observed after light exposure.

1. Introduction

Dyeing fibres with materials collected from nature was done since pre-historic times until the development of synthetic dyes that occurred in the mid 19th century. Natural dyes are found in plants, lichens, fungi, insects and molluscs and their classification can be made based in several criteria, which include their chemical structure, colour or method by which they are applied to the textiles fibres [1–3].

Throughout the millennia, fibre dyeing was a complex task that required the skill and knowledge of specialized artisans to obtain the array of hues that we can see nowadays in museums worldwide. The dyeing process required the chromophores or chromogens obtained from the natural sources to be captured as strongly as possible by the textile fibres. For some natural dyes, including the red dyes, the binding to the fibre is considerably improved by the use of a metal ion salt (mordant) in the dyeing process [4]. Mordant metal ions like aluminium – alum was the most important mordant in ancient times – iron and copper are known to form a chemical bond with the acidic amino acid side chains (glutamic and aspartic acids) of the peptide wool backbone and the chromophore molecules [5–9]. Metal ions behave as Lewis acids in aqueous solutions. The positive charge of the central metal ion draws electron density from an oxygen–hydrogen bond on a water molecule coordinated to it and the departing proton will lower the mordant bath pH [10]. At pH values of 3.5–5, the deprotonation of the glutamic and aspartic acids side chains (pK_a around 4.0) of the wool backbone occurs, enabling the chemical bond between the wool and the metal cation.

Colours produced in this way stand fast against washing, while their lightfastness is also altered. The use of mordants also changes the hue of certain dyes, namely, different mordants used with the same dye may darken, brighten or drastically alter the final colour of the dyed fibre, but what is known nowadays about the use of mordants by the ancient artisans is much less than what is known about dyes [2].

Madder (*Rubia* spp.) has been cultivated in large amounts as a source for red plant dyes in Europe, Asia and America for centuries [1–3,11]. The dyestuff is extracted from the dried roots of the plant, being

* Corresponding author. University of Évora, Chemistry Department, Rua Romão Ramalho 59, 7000-671 Évora, Portugal. Tel.: +351 266745343; fax: +351 266745349.
E-mail address: cmbd@uevora.pt (C.B. Dias).

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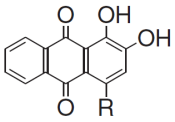
doi:10.1016/j.microc.2010.12.002



R=H, alizarin
R=OH, purpurin

Fig. 1. Chemical structure of alizarin and purpurin.

the bark much richer in anthraquinone derivatives than the wooden parts. The composition of the extracted anthraquinones differs between the varieties of *Rubia*. For example, the major component forming the natural dye of the European madder (*Rubia tinctorum*) is alizarin (1,2-dihydroxyanthraquinone, Fig. 1), while purpurin (1,2,4-trihydroxyanthraquinone, Fig. 1) is the major component of Indian madder (*Rubia cordifolia*), although it is also found in considerable amounts in the European madder. Anthraquinone glycosides are also present in the plant´s roots but, during storage, hydrolysis of the glycosides occurs, which is completed under the acidic conditions established in the dyeing procedure [3].

Analysis of natural dyes is an active area of research in cultural heritage studies [11–13]. A major problem associated with the identification of the original natural source of the historical textiles colour arises from the fact that, due to the lightfastness, some chromophores became mostly degraded and only minute amounts can be extracted from the samples. It is therefore of utmost importance to shed some light into the effect of the metal ion and dyeing method on the fibres final hue and chromophore photodegradation. Clementi et al. [3] have already studied the lightfastness of wool dyed with madder and alum under different conditions, concluding that the dyeing procedure influenced the fibre final hue and, when compared to the un-mordanted samples, the metal ion protected the madder chromophores from photodegradation.

In this work, the authors performed a systematic study where the dye bath concentration was kept constant while the mordant chemical nature (alum, copper and iron salts) and mordant bath concentration were changed. Two dyeing methods were used to dye the fibres: a pre-mordanting and a simultaneous mordanting procedure. Analyses of the dyed fibre metal ion content were performed by ICP-OES (inductively coupled plasma–optical emission spectrometry) and FAAS (flame atomic absorption spectrometry). Morphological characterisation and point X-ray microanalysis were carried out by SEM-EDS (scanning electron microscopy-energy dispersive X-ray spectrometry). Wool dyed samples were subjected to accelerated ageing studies under controlled conditions in order to evaluate the chromophore photodegradation. Analysis of the chromophore contents by LC-ESI-MS/MS (liquid chromatography coupled with mass spectrometry and electrospray ionization) and colourimetric studies were performed on the samples before and after artificial accelerated ageing.

2. Experimental

2.1. Materials and reagents

The following reagents (analytical grade) were used: N,N-dimethylformamide and nitric acid (65%) from Panreac (Barcelona, Spain), EDTA dissodium salt from Sigma-Aldrich (Milwaukee, WI, USA), iron (II) sulfate pentahydrate (99%) from Scharlau Chemie, S.A. (Barcelona, Spain) and copper sulfate pentahydrate (99%) from Himedia Laboratories (Mumbai, India). Methanol and formic acid (HPLC gradient grade) from Merck (Darmstadt, Germany) were used in LC-MS analysis. For mordant quantification, aluminium and iron standards were inductively coupled plasma grade or equivalent, from Fluka (Buchs, Switzerland), and copper standard was spectroscopic grade, also from Fluka (Buchs, Switzerland). Alum and madder (*Rubia tinctorum*) were purchased from Kremer Pigmente GmbH & Co. KG (Aichstetten, Germany).Water from a Millipore Simplicity UV system (Billerica, MA, USA) was used throughout this work, including for sample preparation. Undyed industrial Arraiolos sheep wool was acquired from Rosarios4 (Mira de Aire, Portugal).

2.2. Wool dyeing

Two methods were used for wool dyeing, which included a pre-mordanting procedure (MD procedure) and a simultaneous mordanting procedure (M + D procedure):

2.2.1. MD procedure

1.0 g of sheep wool was mordanted for 30 min in 50 mL of boiling water containing different amounts of mordant salts (concentration and sample notation in Table 1). Afterwards, the wool was removed and rinsed with cool water. The dye baths were prepared with 2.0 g of madder immersed in 50.0 mL H_2O and heated at about 90 °C for 30 min. The solution was allowed to cool and after simple filtration of the plant material, the previously mordanted wool was added to the dye solution bath and reheated at 90 °C for 30 min.

2.2.2. M + D procedure

The dye bath was prepared as described for the MD procedure. After plant material filtration, the different amounts of mordant salts were added (concentration and sample notation in Table 1) to each solution. When the mordant salts were dissolved, 2.0 g of sheep wool were immersed in each solution that was kept at 90 °C for 30 min.

After the dyeing procedure, all wool samples were thoroughly rinsed with ultrapure water and left to dry in the open air protected from light.

2.3. Colourimetric studies

Datacolor International Mercury portable spectrophotometer used for colourimetric studies (L*, a* and b* – CIELab space defined by Commission Internationale de l´Eclairage in 1976). The spectrometer

Table 1
Mordant salt bath concentration (mol/dm^3) and corresponding mass of metal ion available in the dyeing bath per gram of wool (mg/g). Sample notation presented was used throughout this manuscript.

Mordant metal ion	Mordant salt bath concentration (mol/dm^3)	Mass of metal ion available in the dyeing bath per gram of wool (mg/g)	Sample notation	
			M + D procedure	MD procedure
Al^{3+}	0.1000	135	AlM + D1	AlMD1
	0.0085	11	AlM + D85	AlMD85
	0.0030	4	AlM + D3	AlMD3
Cu^{2+}	0.0400	128	CuM + D4	CuMD4
	0.0016	5	CuM + D16	CuMD16
Fe^{2+}	0.0400	112	FeM + D4	FeMD4
	0.0016	5	FeM + D16	FeMD16

was equipped with a Xenon lamp and a photodiode sensitive to the 360–700 nm spectral range. Black and white standards were used for calibration. Iluminant CIE D65; 10º of observation angle and specular component excluded.

Analyses were performed in three different points of each wool sample, with the average value used for data interpretation.

2.4. Mordant quantification

2.4.1. ICP-OES analysis

Analyses were performed in an inductively coupled plasma-optical emission Horiba Jobin–Yvon spectrophotometer, model Ultima, equipped with a RF generator of 40.68 MHz and a type Czerny–Turner monochromator with 1.00 m (sequential). Three analyses were done for each sample and the conditions were the following – potency: 1200 kW, argon flow: 12 dm^3/min, nebulisator: Mira Mist with 3 bar pressure, pump velocity: 15 rpm. Fe and Al calibration standards were prepared by diluting the respective stock solution to obtain a 5 point calibration curve (0.01–1.00 mg/dm^3; $R^2 = 0.9999$).

For sample preparation, 1.5 mg of wool samples and 1.0 mL of concentrated HNO_3 were added to an *eppendorf* tube and digested in an ultrasound bath, without temperature control, until sample solubilisation (30 to 60 min). The solution was then transferred to a 25 mL flask and the volume was adjusted with ultrapure water. Replicate analyses were performed to evaluate analytical methodology reproducibility.

2.5. FAAS analysis

Analyses were performed on a Perkin Elmer 3100 flame atomic absorption spectrometer equipped with a Cu cathode lamp (resonance line at $\lambda = 324.8$ nm, 0.7 nm slit width and 15 mA lamp current intensity) and an air/acetylene flame.

Six working solutions (0.50–5.00 mg/dm^3; $R^2 = 0.9999$) for calibration curve construction were obtained with adequate dilution of a 100 mg/dm^3 solution prepared from a Panreac 1.000 ± 0.002 g/dm^3 Cu stock solution.

50.0 mg of each wool sample were heated (without boiling) on a hot plate with 4.0 mL of concentrated nitric acid until the wool fibre was digested. The sample was then transferred to a 50 mL volumetric flask and the volume was adjusted with ultrapure water. Replicate analyses were performed to evaluate analytical methodology reproducibility.

2.6. SEM-EDS studies

A Hitachi S-3700N variable pressure scanning electron microscope coupled with an Bruker X-ray energy dispersion spectrometer were used for morphological characterisation and point chemical analysis at the surface and in fractured zones of the wool dyed fibres for mordant assessment. Samples were mounted in aluminium sample holders with double-sided adhesive carbon tape and coated with an Au–Pd layer. Acceleration voltages of 15.0 kV and 20.0 kV were used, respectively, for SEM and EDS analyses.

2.7. Accelerated ageing studies

A UV–Vis Solarbox 3000E equipped with a Xe lamp and a 310 nm filter was used. The ageing conditions were as follows – temperature: 55 °C, irradiance: 400 W/m^2. Samples were collected after 48, 120, 360, 480, 600, 720 and 960 h of light exposure.

2.8. Chromophores analysis

2.8.1. Chromophore extraction procedure

20 mg of dyed wool were placed in vials and 1 mL of 0.1% EDTA in water/DMF (1:1, v/v) solution was added. The vials were capped and kept at 100 °C in liquid paraffin for 30 min. After it, they were cooled to room temperature and the samples were lyophilized. The dried residues were dissolved in 1.0 mL of MeOH/H_2O (1:1, v/v). All solutions were filtered through a 0.45 μm PTFE filter prior to analysis. Replicate analyses were performed to evaluate analytical methodology reproducibility.

2.9. LC-ESI-MS/MS analysis

LC-ESI-MS/MS analyses were carried out in a LCQ Advantage ThermoFinnigan mass spectrometer equipped with an electrospray ionization source and using an ion trap mass analyzer. The conditions of the MS analysis were: capillary temperature of 300 °C, source voltage of 5.0 kV, source current of 100.0 μA and capillary voltage of 15.0 V in negative ion mode. Analytes were detected in the SRM mode and the transitions used were the following: alizarin 239 → 211 and purpurin 255 → 227.

The mass spectrometer equipment was coupled to an HPLC system with autosampler (Surveyor ThermoFinnigan). The analytical column was a reversed phase Zorbax Eclipse XDB-C_{18}, (Agilent Technologies) (C_{18}, particle size 3.5 μm, 150 mm × 2.1 mm). The mobile phase used was 100% methanol with a flow of 0.2 mL/min and the injection volume was 25 μL.

3. Results and discussion

The 20th century was dominated by the synthetic bright and stable colours. However, increased public awareness on product safety and concerns on the sustainability and replacement of oil-based products explain the recent interest on natural dyes [2,11]. Textile dyers master for millennia the use of natural dyes and mordants to get the array of colours seen in museums worldwide. Most of that knowledge was never written down as it was passed orally throughout generations and nowadays we know very little about the use of mordants and how they influence the colour and photodegradation of textiles dyed with natural dyes.

The main goal of this work was to get information on the role that the mordant ion nature, concentration and its mode of application have on the final hue and chromophore photodegradation of wool fibres dyed with madder. Alum [$KAl(SO_4)_2.12H_2O$], copper (II) and iron (II) sulfates were used as mordants, at different concentrations, and were applied by two different procedures (pre-mordanting, MD, and simultaneous mordanting, M + D) to sheep wool samples (sample notation in Table 1). The lower values of the mordant concentrations used (0.0085 and 0.0030 mol/dm^3, for alum, and 0.0016 mol/dm^3, for copper and iron sulfates) were chosen based on dyeing recipes either used by local artisans or reported in natural dyeing books [14,15], while the higher values (0.1000 mol/dm^3, for alum, and 0.0400 mol/dm^3, for copper and iron sulfates) were chosen for comparison. The same amount of madder was employed for all mordant salts, bath concentrations and dyeing procedures.

3.1. Colourimetric studies of the wool dyed samples

Table 2 presents the L*a*b* coordinates of the CIELab colour space measured by colourimetry for dyed samples preserved from light and after light exposure. As it can be seen, for samples not subjected to artificial ageing ($t = 0$ h), not only the metal ion chemical nature, but also the mordant bath concentration and dyeing procedure have strong influence on the wool fibre hue. Empirically, brighter and more pinkish colours were obtained with Al, which is probably why alum was the most popular mordant in the natural dyeing industry [2,3]. In fact, the colourimetric studies showed that, for mordant bath solutions with similar amounts of mass of metal ion (Al, Cu or Fe, Table 1), dyed fibres mordanted with Al present higher values of the luminance parameter (L*).

Table 2
L*a*b* coordinates of the CIELab colour space measured for dyed samples preserved from light ($t = 0$ h) and after light exposure ($t = 48$, 120, 360, 480, 600, 720 and 960 h).

		Al						Fe				Cu			
		M + D			MD			M + D		MD		M + D		MD	
Time in the solar box (h)	CIELab colour parameters	AlM + D1	AlM + D85	AlM + D3	AlMD1	AlMD85	AlMD3	FeM + D4	FeM + D16	FeMD4	FeMD16	CuM + D4	CuM + D16	CuMD4	CuMD16
0	L*	54.52	57.23	51.85	33.24	36.37	40.99	40.44	43.57	26.09	27.27	50.04	43.76	51.89	28.87
	a*	30.33	24.21	30.78	38.19	38.40	37.64	7.89	9.67	11.80	13.10	11.36	22.54	16.25	21.50
	b*	36.93	35.39	36.96	23.02	27.11	32.80	17.11	18.33	6.81	8.07	18.96	23.06	27.24	8.65
48	L*	55.54	59.43	54.58	36.99	42.71	44.07	45.75	47.53	27.31	27.58	53.66	47.01	53.92	30.15
	a*	30.35	24.34	30.17	35.97	40.76	36.87	8.35	9.21	11.08	11.63	10.12	23.14	15.51	21.20
	b*	33.43	31.83	34.62	22.59	29.66	32.54	15.49	16.99	7.32	7.80	19.68	23.35	23.60	8.84
120	L*	57.00	60.76	57.47	36.96	45.15	46.11	51.79	50.34	30.41	29.82	54.29	47.85	58.52	33.47
	a*	28.99	22.37	27.22	38.30	37.42	34.10	8.92	9.06	11.32	11.42	10.64	22.99	16.20	22.21
	b*	31.62	29.13	31.63	23.68	26.97	30.31	18.11	15.89	8.30	8.08	20.00	22.15	24.09	9.39
360	L*	59.78	64.24	62.81	41.19	47.98	51.09	52.21	59.48	28.41	33.75	54.86	53.49	60.72	32.21
	a*	22.88	17.71	21.11	36.09	35.59	29.90	6.34	6.74	11.55	9.84	9.60	21.15	13.31	23.34
	b*	27.88	25.27	26.64	22.84	27.22	28.41	16.83	16.11	7.69	9.81	20.07	21.65	21.90	9.10
480	L*	62.14	65.78	64.85	42.56	51.83	54.94	56.73	60.50	35.82	37.19	56.90	56.27	62.73	35.49
	a*	21.29	15.09	17.96	35.85	32.35	27.27	6.06	6.18	9.80	9.06	9.32	19.95	12.87	22.25
	b*	27.36	23.05	23.98	21.91	24.56	26.86	17.63	15.62	10.66	9.85	19.33	21.21	21.16	8.87
600	L*	64.92	67.43	65.26	45.71	55.10	58.69	58.43	62.07	37.36	38.24	55.49	54.56	63.68	37.07
	a*	19.52	14.31	17.64	34.74	31.57	26.52	5.25	5.72	9.57	8.57	9.14	19.15	11.78	22.62
	b*	27.67	22.88	23.62	21.83	25.31	27.16	17.72	16.49	11.70	10.02	19.09	19.85	21.38	9.73
720	L*	64.94	68.59	66.12	43.43	54.99	56.10	59.86	62.72	37.72	40.54	58.69	55.90	63.06	38.00
	a*	17.47	12.77	15.04	33.03	29.29	22.86	5.28	5.62	9.07	8.17	9.12	18.86	10.76	22.59
	b*	26.28	21.94	22.02	20.37	23.62	24.45	18.43	16.45	12.17	12.39	19.71	19.89	20.75	10.00
960	L*	63.64	64.65	64.78	46.24	55.81	59.69	57.40	63.34	39.06	38.38	55.77	55.04	62.24	33.00
	a*	13.39	11.02	10.42	32.33	27.33	20.31	4.55	4.41	8.88	7.86	9.56	17.03	9.97	22.58
	b*	23.08	18.86	17.96	19.55	22.62	22.59	16.15	15.31	12.71	10.18	20.13	18.31	20.14	8.10

Differences in colour are likely due to differences in the complexes that are formed under the different dyeing conditions used, namely, the nature and concentration of the metal ion (existence of *d* electrons), the nature of the chromophore (existence of conjugated systems and substitution of C, H, O atoms by electron donors or acceptors atoms) and the solution pH. At the pH values of 3–5 obtained for the mordant solutions used in this work (data not shown), metal ions can bind carboxylate groups in the wool fibre, while also binding the chromophore molecules and, in some cases, water molecules as well [5,6,8–10,16].

According to the ligand field theory, the formation of the complexes leads to a loss of degeneracy of the *d* electrons of the metal ion, dividing them in two groups separated by an energy gap, which is dependent on the chemical nature of the ligands and their space distribution and on the nature and oxidation state of the metal ion [17]. Colour arises when some photons of the white light (400–720 nm) have enough energy to promote a transition of the electrons across that energy gap (absorption of radiation of some wavelengths), leaving the other wavelengths to be seen by the human eye. Some metals like Al, that do not have *d* electrons, can also form coloured complexes and their colour also arises from electronic transitions, but involving other valence electrons [17].

The dyeing conditions used are likely to have generated different complex structures with different energy gaps, leading to the colours observed in the dyed wool. The reddish colours should correspond to complexes with larger energy gaps, while bluish colours correspond to complexes with shorter energy gaps, and in between are the violet colours [17].

3.2. Mordant evaluation and wool morphological characterization

In order to evaluate the actual amounts of mordant metal ion present in the wool fibres, wool samples were analyzed by ICP-OES (Al and Fe) and FAAS (Cu). Samples were acid digested on a hot plate (Cu) and in an ultrasound system (Al and Fe). A microwave procedure was also tested (data not shown). Unlike to what was expected, the results were less reproducible and the procedure was more time consuming. Both methods, ICP-OES and FAAS, were found to be highly reproducible with an RSD ≤ 5% (data not shown).

Despite the large mass of metal ion available in the dyeing baths (Table 1), the actual amounts of mordant metal ion present in the wool fibres proved to be overall much lower (Fig. 2). Clementi et al. [3] also referred that the aluminium–sulphur ratios detected by EDS analyses in wool samples dyed with alum did not reflect the composition of the mordant bath.

Increasing mordant bath concentration leads to a small increase in the fibre metal ion content. Cu ion seems to have a higher affinity for the wool fibres, since it was detected in higher amounts in fibres mordanted with the copper salt than those mordanted with equivalent concentrations of iron or aluminium ions.

In general, and for a similar mordant concentration, the MD method yields samples with higher amounts of metal ion. The binding of the metal ion to the fibre is likely to proceed in a different way depending on the dyeing procedure used. In the M + D method, the binding between the metal ion and the chromophore molecule occurs before the wool is even introduced in the dyeing bath, making it more difficult for the metal to bind the wool protein structure due to the bulkiness of the chemical structure formed. In the MD procedure the binding of the mordant to the wool occurs beforehand, probably explaining the higher amount of metal ions on these fibres.

Point microchemical analysis by EDS was performed in some mordanted samples with Al, Fe or Cu in order to assess the metal ion distribution on the fibres. Analyses have shown that the metal ions were not evenly distributed: newly fractured zones present relatively higher amounts (wt.%) of the metal ion than the surface, pointing out the existence of some metal lixiviation (data not shown). This is likely due to the washing procedure on both dyeing methods. Non-homogeneity in the Al distribution was also observed by Clementi et al. [3].

Morphological characterisation by SEM analysis showed that the fibre surfaces displayed the typical scale structure of wool with, in some cases, scale loss and roughened surfaces (data not shown).

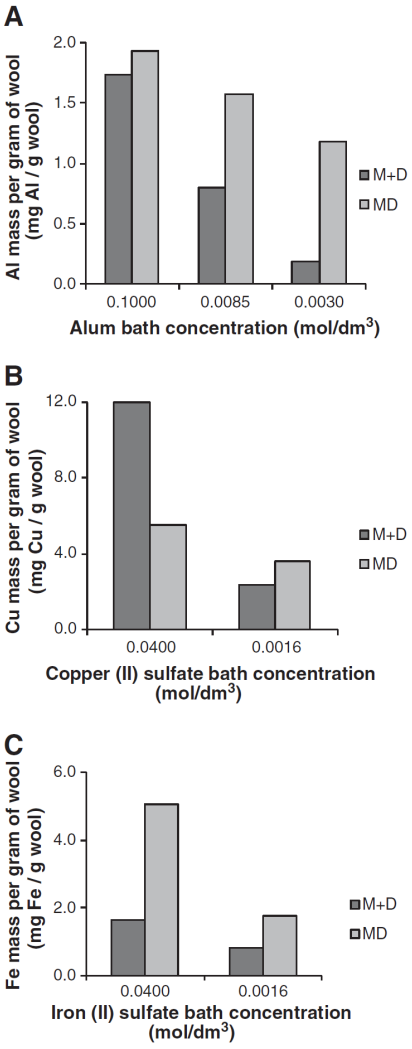


Fig. 2. Quantification of mordant metal ion (Al, Cu, Fe) in wool samples dyed with madder at different concentrations (in mol/dm^3) of mordant bath: alum (A), copper (II) sulfate (B) and iron (II) sulfate (C), by the MD and M + D dyeing methods (RSD ≤ 5%).

3.3. Colourimetric studies of the artificially aged dyed samples

Poor lightfastness is a major drawback of the natural dyes and textile conservators worldwide know that not all the chromophores behave in a similar fashion under light. Despite the fact that the chemical nature of the mordant is known to influence photodegradation of dyed wool samples, very few studies on the subject have been reported in the literature [18,19].

The colour loss observed on the dyed textiles subjected to light can be attributed to the degradation of the natural dyes chromophores which is accomplished by photo-oxidative degradation, yielding small molecules [20,21]. Several studies have been published on the photodegradation of yellow dyes [18,20,21] and the identification of their degradation products [20], as well as on the photodegradation of red dyes, namely madder, with the identification of alizarin degradation products [22]. Cox Crews [21], in a study on the fading of the yellow dyes used by the American Indians, showed that the nature of the mordant ion was more important than the dye itself or the length of light exposure in predicting the coloured textiles lightfastness, with dyes applied with alum mordants fading significantly more than when applied with chromium, copper or iron salts. Yoshizumi et al. [18] have verified that, when used with alum (the only mordant tested), madder chromophores are more photostable than the common yellow chromophores, but less stable than some synthetic dyes.

Changes in tensile strength and elasticity of the wool fibre were also shown to be influenced by the nature of the mordant ion and, in this case, alum mordants seem to diminish the wool phototendering, occurring when dyed fabrics are exposed to sunlight [19].

The dyed wools prepared on this study were subjected to artificial ageing, with samples being collected at fixed time intervals and their colour evaluated by colourimetry. The fading of the original colours can be evaluated in terms of the colour difference calculated using the following formula of CIE Committee in 1976: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$, where: ΔL^* = lightness–darkness difference, Δa^* = redness–greenness difference, and Δb^* = yellowness–blueness difference [18].

The results presented in Fig. 3 show that fading is overall more pronounced in the first hours and tends to stabilize after 480–600 h of light exposure. Colour difference was more pronounced for the samples mordanted with alum, which has already been observed for the yellow dyes [21] and it is an extreme relevant aspect considering the historical usage of this mordant and dye.

Independent on the mordant bath concentration, the dyeing technique seems to have a small impact in the ΔE variation for the samples mordanted with Al salt. As for the Fe salt, samples dyed by the M + D procedure are more affected by photofading. For the same mordant bath concentration, the Cu mordanted samples present the higher relative variation on the ΔE parameter among the two tested dyeing methods.

In general, samples mordanted by the M + D procedure in smaller bath concentrations of alum and copper (II) sulfate tend to suffer higher colour differences when subjected to light. On the other hand, in the MD procedure the behaviour changes: while for the Al mordanted samples the smaller bath concentration produces higher variation on the ΔE parameter, for the Cu mordanted fibres the opposite behaviour is observed. The ΔE variation for the samples mordanted with the Fe salt proved to be somehow independent on the concentration.

3.4. Chromophore evaluation

In order to assess the amounts of alizarin and purpurin in the wool samples before and along the light ageing process, an analytical methodology based on the wool extraction with EDTA/DMF [23] followed by LC-ESI-MS/MS analysis was established. In Fig. 4 bar graphs corresponding to the chromatographic peak areas of alizarin and purpurin in wool samples before irradiation ($t = 0$ h) and after light exposure ($t = 480$ and 960 h) are presented.

Considerable differences in the chromophore contents of the wool samples before light exposure are observed for the same mordant, different mordant bath concentration and dyeing procedure, partially explaining the observed colour differences (Table 2). Overall, for the same mordant, samples dyed by the MD procedure ($t = 0$ h) present higher amounts of chromophores. When alum was used, the MD method yielded samples with a stronger pink hue (higher a* values, Table 2) while the samples dyed by the M + D method were more orange (higher b* values, Table 2).

Chromophore analysis (Fig. 4, $t = 0$ h) showed that samples dyed by the MD method, using Al or Fe, have alizarin/purpurin peak areas <1, while all the samples dyed by the M + D method have alizarin/purpurin peak areas >1. Clementi et al. [3] observed the same behaviour in samples dyed with madder and 20% of alum by the MD procedure.

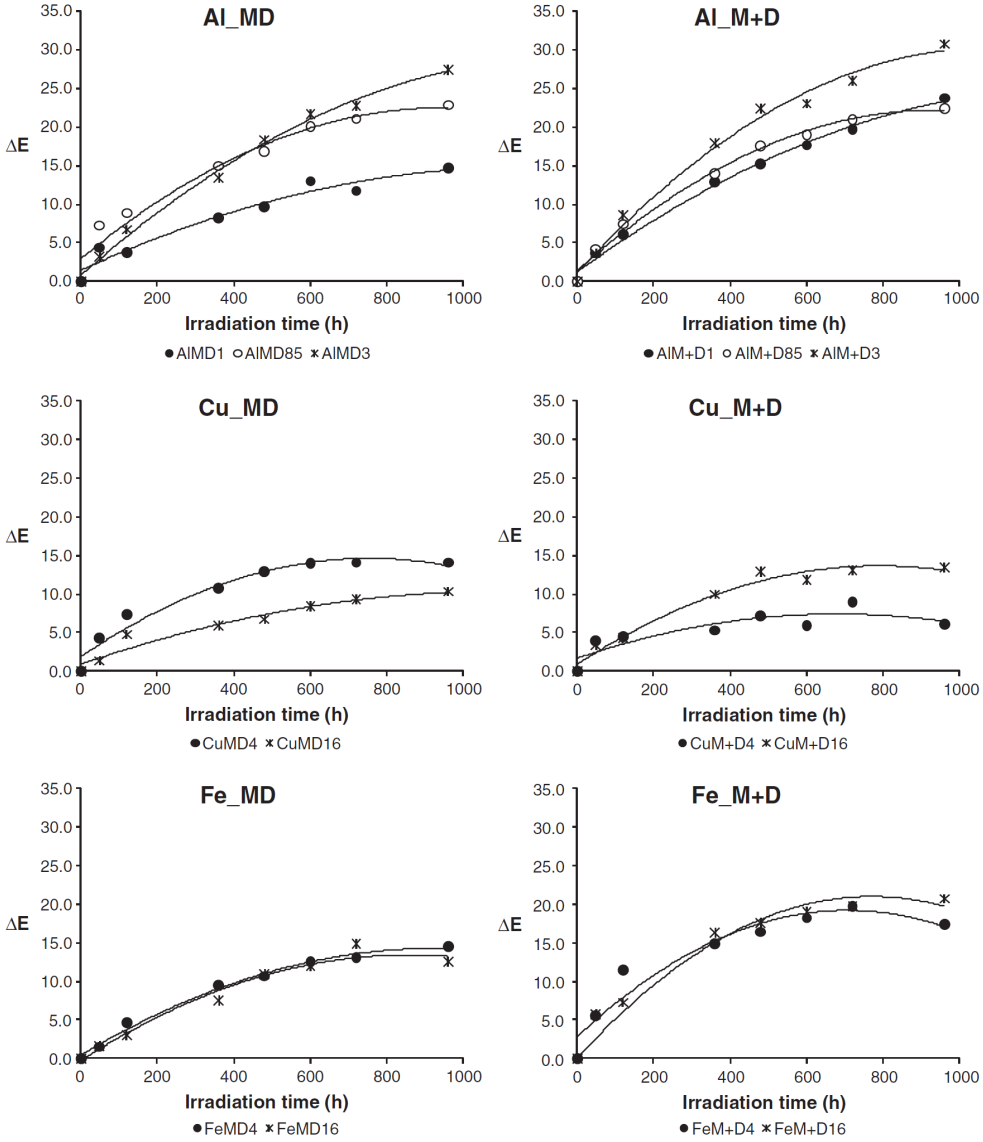


Fig. 3. Fading characteristics of wool dyed by the MD and M + D procedures in different bath concentrations of alum (0.1000, 0.0085 and 0.0030 mol/dm^3), copper (II) sulfate and iron (II) sulfate (0.0400 and 0.0016 mol/dm^3 for both mordants) after $t = 48$, 120, 360, 480, 600, 720 and 960 h of light exposure.

Within the same dyeing procedure, the colour of the samples mordanted with the two tested concentrations for copper (II) sulfate differ considerably, with the CuM + D16 and the CuMD16 samples always presenting a much darker colour (smaller L* parameter, Table 2). Chromophore extraction showed that the peak areas of alizarin and purpurin in the Cu_MD or Cu_M + D samples are very different, with the samples mordanted in the 0.0016 mol/dm^3 bath presenting higher peak areas, especially those dyed by the MD procedure (Fig. 4).

The use of iron mordant and the M + D procedure yielded samples with less intense reddish colour (lower values of a* and b*, Table 2) and chromophore analysis showed that the peak areas for alizarin and purpurin are 5 to 10 times smaller than those measured in the MD samples (Fig. 4).

Chromophore analysis done on the artificially aged samples (Fig. 4) corroborated the colourimetric study (Table 2 and Fig. 3), showing that the colour fading could be correlated to the degradation of the madder chromophores. Overall, increasing light exposure leads to a decrease in the alizarin and purpurin chromatographic peak areas.

The alizarin and purpurin chromophores degradation rates were not constant, being in general more severe in the first 480 h of light exposure (Figs. 4 and 5) for samples mordanted with Al than with Cu or Fe. This fact comes in agreement with the more pronounced ΔE variation observed in the same period of time for the Al_MD and Al_M + D samples (Fig. 3), confirming the poor lightfastness for samples dyed with madder and Al as Cox Crews [21] observed when natural yellow dyes were used with Al. Additionally, alizarin degraded more

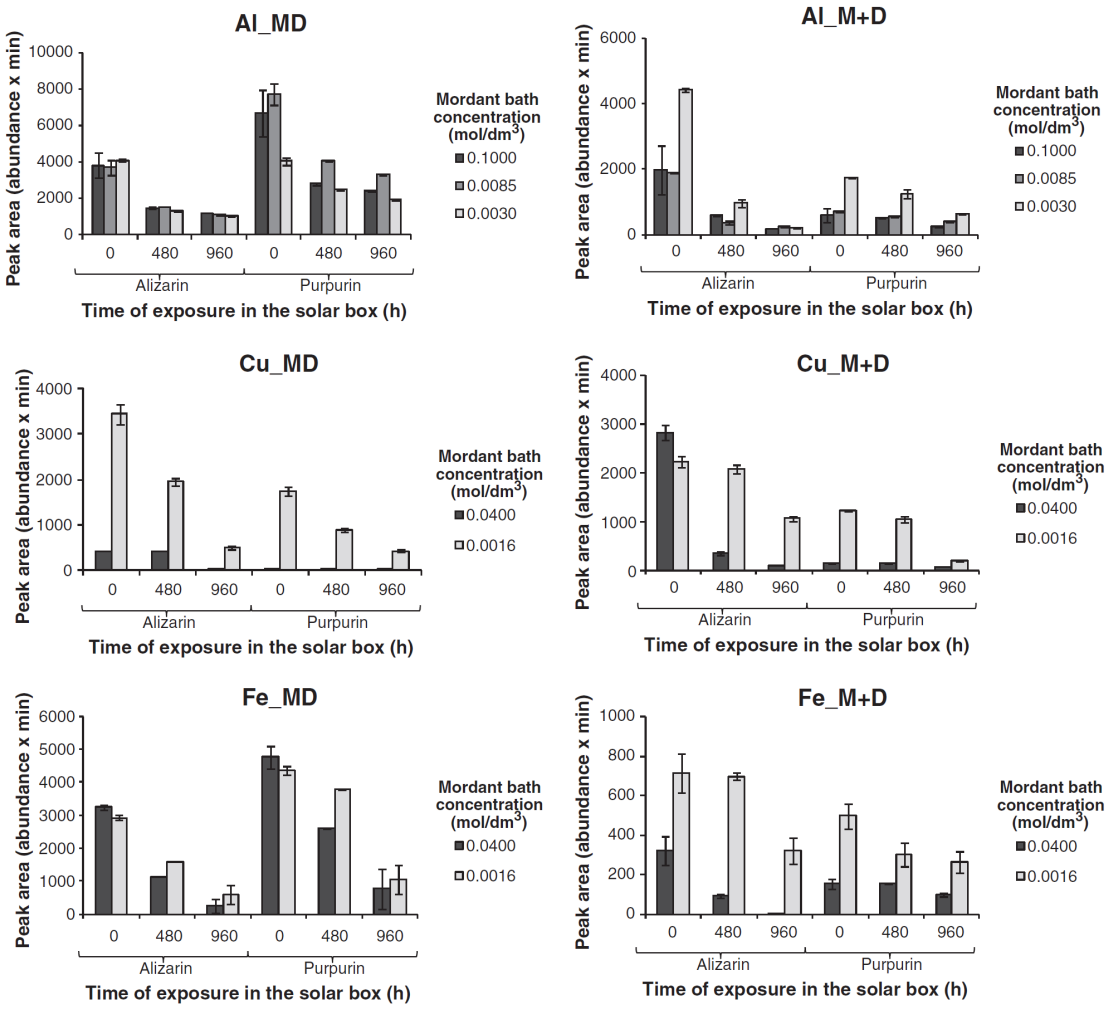


Fig. 4. LC-ESI-MS/MS peak areas of alizarin and purpurin in wool samples dyed at different concentrations of alum, copper (II) sulfate and iron (II) sulfate by the MD and M + D dyeing methods before ($t = 0$ h) and after ($t = 480$ h and $t = 960$ h) light exposure.

extensively than purpurin in these samples. An opposite behaviour was observed by Clementi et al. [3] but for naturally aged samples mordanted with 20% alum and dyed with madder. When those samples were artificially aged in the presence of air, the authors observed an improvement on the purpurin lightfastness.

Overall, the chromophores (alizarin and purpurin) degradation rates are different and dependent on the mordant nature and dyeing procedure. These differences are likely to be responsible for hue changes observed along with the colour fading, although the presence of minor components could also affect the final hue.

4. Conclusions

Mordant ion nature, mordant bath concentration and dyeing method have a strong impact in the fibre colour hue, which is likely due to the different complexes of fibre-mordant-chromophores formed under the different dyeing conditions.

Mordant ion quantification showed that when large amounts of metal ions are available in the mordant baths, the uptake by the fibres is relatively small, although for less concentrated baths the discrepancy is smaller. Cu ion has clearly a distinct behaviour from Al or even Fe ions since it presents a higher affinity to the fibre – the measured amounts of Cu in the fibres are in general more than twice the amounts of Al or Fe, for equivalent mordant baths concentrations. For the two dyeing methods tested, samples dyed by the MD procedure presented higher concentration of metal ions, which is likely due to the fact that, in this method, the mordant and the chromophore are introduced in sequential steps. The unexpected behaviour of CuM + D4 sample can be explained by the existence of heterogeneity in the distribution of the mordant ion, as the EDS analysis revealed.

Chromophore analysis showed that, overall, the samples dyed by the MD procedure also presented larger chromatographic peak areas which comes in agreement with the fact that they also have stronger colours. The peak areas ratio alizarin/purpurin changes with the dyeing method, also contributing for the observed colour differences.

Photodegradation was stronger in the first 480 h of light exposure and colour difference (ΔE) was more pronounced for the samples dyed with alum. Chromophore analysis in the samples subjected to artificial ageing showed that the observed fading is due to the chromophore degradation, being more severe in that period of time

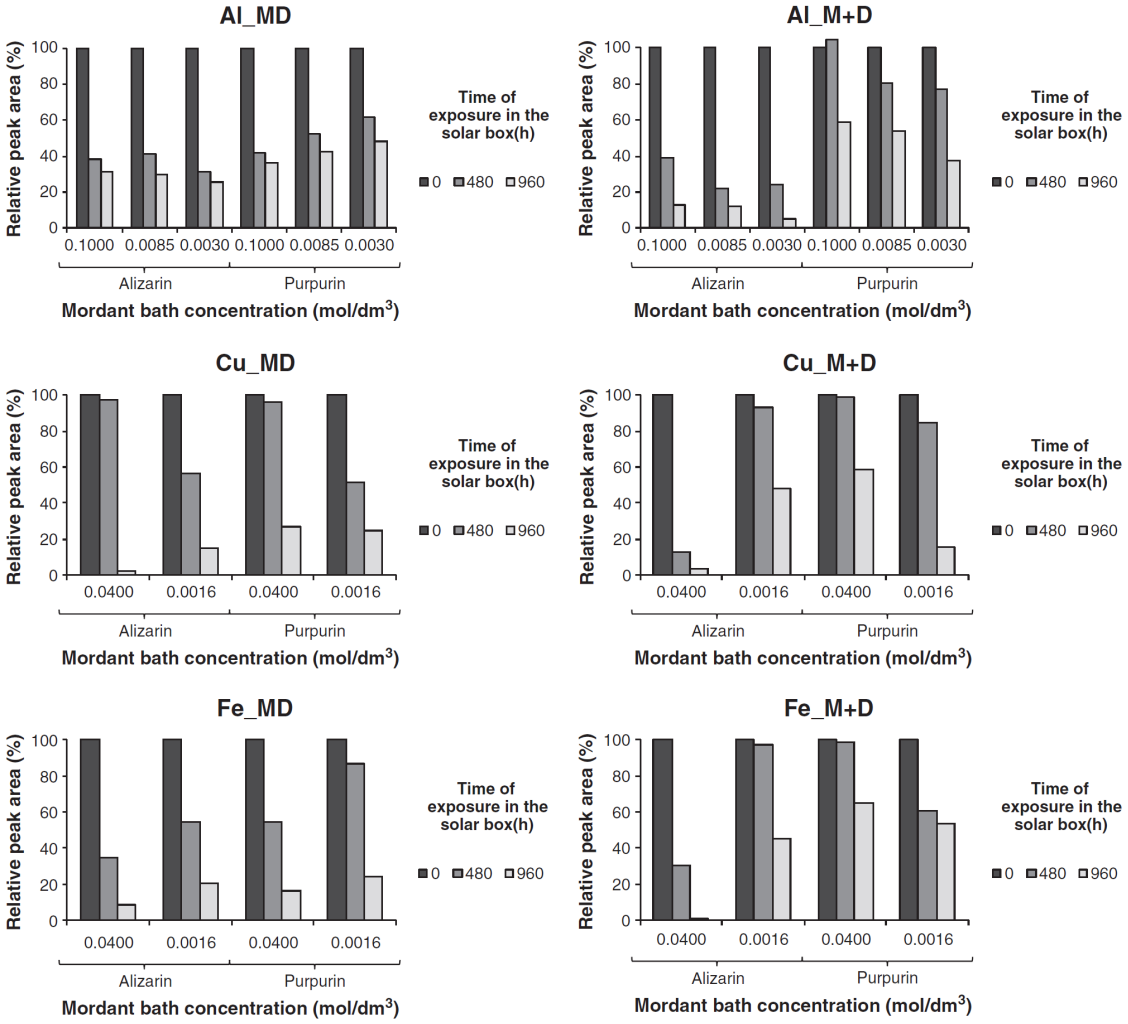


Fig. 5. Relative LC-ESI-MS peak areas of alizarin and purpurin in wool samples dyed with different concentrations of alum, cooper (II) sulfate and iron (II) sulfate by the MD and M + D dyeing methods before ($t = 0$ h) and after $t = 480$ h and 960 h of light exposure.

for samples mordanted with Al than with Cu or Fe. Additionally, alizarin degraded more extensively than purpurin in those conditions, contrary to what would be expected, and putting in evidence the important role of the mordant ion on the fading process. Overall, the different degradation rates of alizarin and purpurin can account for the observed changes in the colour hue of the fibres.

The multi-analytical approach used in this work allowed a deeper knowledge on the dyeing process as a whole and on the artificial ageing process, giving a contribution to the study and preservation of textiles artefacts.

Acknowledgements

The authors acknowledge the financial support of Project REMATAR, FCOMP-01-0124-FEDER-010482 (Ref[a] FCT PTDC/HAH/64045/2006) from the Portuguese Foundation for Science and Technology, FCT. A. Manhita also acknowledges FCT for the PhD fellowship (SFRH/BD/22411/2005).

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Anal Bioanal Chem (2011) 400:1501–1514
DOI 10.1007/s00216-011-4858-x

ORIGINAL PAPER

Extracting natural dyes from wool—an evaluation of extraction methods

Ana Manhita · Teresa Ferreira · António Candeias · Cristina Barrocas Dias

Received: 10 January 2011 / Revised: 21 February 2011 / Accepted: 26 February 2011 / Published online: 18 March 2011

Abstract The efficiency of eight different procedures used for the extraction of natural dyes was evaluated using contemporary wool samples dyed with cochineal, madder, woad, weld, brazilwood and logwood. Comparison was made based on the LC-DAD peak areas of the natural dye's main components which had been extracted from the wool samples. Among the tested methods, an extraction procedure with Na_2EDTA in water/DMF (1:1, *v/v*) proved to be the most suitable for the extraction of the studied dyes, which presented a wide range of chemical structures. The identification of the natural dyes used in the making of an eighteenth century Arraiolos carpet was possible using the Na_2EDTA/DMF extraction of the wool embroidery samples and an LC-DAD-MS methodology. The effectiveness of the Na_2EDTA/DMF extraction method was particularly observed in the extraction of weld dye components. Nine flavone derivatives previously identified in weld extracts could be identified in a single historical sample, confirming the use of this natural dye in the making of Arraiolos carpets. Indigo and brazilwood were also identified in the samples, and despite the fact that these natural dyes were referred in the historical recipes of Arraiolos dyeing, it is the first time that the use of brazilwood is confirmed. Mordant analysis by ICP-MS identified the widespread use of alum in the dyeing process, but in some samples with darker hues, high amounts of iron were found instead.

Keywords Natural dyes · Dye extraction · Historical textiles · Arraiolos carpets · LC-DAD-MS · ICP-MS

A. Manhita · T. Ferreira · A. Candeias · C. Barrocas Dias
Chemistry Department and Évora Chemistry Centre,
University of Évora,
Rua Romão Ramalho 59,
7000–671 Évora, Portugal

A. Manhita · T. Ferreira · A. Candeias · C. Barrocas Dias (✉)
HERCULES Laboratory,
Largo Marquês de Marialva, 8,
7000–554 Évora, Portugal
e-mail: cmbd@uevora.pt

A. Candeias
José de Figueiredo Conservation and Restoration Laboratory,
Institute of Museums and Conservation,
Rua das Janelas Verdes 37,
1249–018 Lisboa, Portugal

Introduction

Natural dyes have been used since prehistoric times until the mid nineteenth century to dye textiles. They can be found in a broad range of natural sources, such as plants, fungi (lichens and mushrooms), insects and molluscs [1–3]. Indigo, weld, brazilwood, logwood, cochineal and madder are among those natural dyes that were widely used and contain chromophores with a variety of chemical structures (Fig. 1).

Indigo blue dye is one of the oldest natural dyestuffs. It has been obtained from a variety of plant sources, such as *Indigofera tinctoria* L. or *Isatis tinctoria* L., being the latter of inferior quality and commonly named as woad [1–3]. The major constituent of this dye is indigotin (1, Fig. 1). Flavones and flavonols are the main chromophores in the majority of natural yellow dyes, many occurring in the plants as sugar derivatives (commonly glycosides). The main flavonoid yellow dye source mentioned in traditional European recipes is weld (*Reseda luteola* L.) [1–3]. The predominant chromophores found in this plant are the flavones luteolin (3, Fig. 1), apigenin (4, Fig. 1) and glycosides like luteolin 7-*O*-glucoside (2, Fig. 1). Weld was usually combined with red dyes to produce different shades

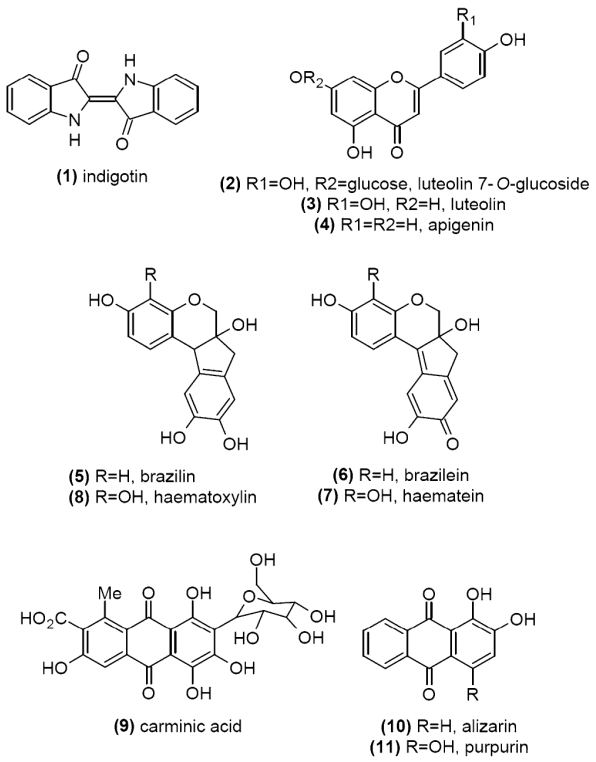

Fig. 1 Chemical structures of the major chromophores of the studied natural dyes: (*1*) woad, (*2*, *3*, *4*) weld, (*5*, *6*) brazilwood, (*7*, *8*) logwood, (*9*) cochineal and (*10*, *11*) madder

of orange and with indigo to produce green hues. Brazil was named after brazilwood (*Caesalpinia* spp.) due to the abundance of this redwood on its coasts [2]. Chemically, the major compound isolated from this redwood is brazilin (5, Fig. 1), which by oxidation forms the deep red brazilein (6, Fig. 1), the chromophore in brazilwoods [1–3]. Although belonging to the group of redwoods, logwood (*Haematoxylum campechianum* L.) is used to dye textiles in black. Haematein (7, Fig. 1) is the major colouring matter and derives from its precursor, haematoxylin (8, Fig. 1) [1–3]. Anthraquinone dyes represent the biggest group of natural quinone dyes [1–3] and can be of insect or plant origin. Carminic acid (9, Fig. 1) is the main constituent of cochineal, a plant parasite from Coccidea family used for dyeing textiles in red. Madder (*Rubia tinctorum* L.) is a plant anthraquinone red dye; alizarin (10, Fig. 1) and purpurin (11, Fig. 1) are the main chromophores of this plant. When combined with different mordants, madder can produce shades that vary from pink to black, purple and red [1–3].

The analysis and identification of dyes used in historical textiles are of extreme importance in conservation science as they can help in elucidating the textile's place of origin and time of production. Moreover, they will provide information that can be used for choosing the appropriate conditions for textiles conservation or restoration. The organic natural colourants used to dye textiles are among one of the most degradable materials used in the works of art. Chromophore identification in ancient textiles can be a challenging task mainly due to the low amounts of intact dye that can be extracted from the usually small historical samples. Extra difficulties arise from the different rates at which the chromophores suffer light and wear degradation [4, 5] and the common use of different dyes to obtain the fibres desired hues.

Dye analysis is usually performed by liquid chromatography with a diode array detector [6–11], but nowadays, mass detection is becoming increasingly important [12–20]. A successful approach to identify natural dyes requires that, prior to the chromatographic analysis, an adequate analytical methodology is used for their extraction from the textile fibres. The chemical structure and properties of the different dye chromophores suggest that probably no single procedure is adequate for the dye's extraction. Despite the obvious importance of the sample preparation methodology for the ultimate relevance of the analytical data collected, few systematic studies on the recovery rates of the different chromophores with the available extraction methods have been published [11, 16, 21]. One of the most commonly used extraction methods involves the use of hydrochloric acid (HCl) and methanol (MeOH) at high temperature [5–7, 11, 14, 16, 17]. After solvent evaporation, different solvents have been used to solubilise the resulting dried residue. Methanol (MeOH) is usually used for the yellow and red dyes solubilisation, while dimethylformamide (DMF) is preferred for the indigoids.

Wouters and Verhecken [5] proposed, several years ago, an HCl-based method for dye extraction from wool dyed with several coccid insect dyes. Wool dyed fibres were hydrolysed in a mixture of 37% HCl/MeOH/H_2O (2:1:1, *v/v/v*) placed in a boiling water bath for 10 min, and after several procedures, the residue was redissolved in an appropriate volume of H_2O/MeOH (1:1, *v/v*). Although losses during manipulations are avoided and good extraction yields can be obtained, the use of an HCl-based extraction procedures leads to information loss (fibre destruction and chromophore glycosidic bonds break), and some mild procedures have been developed. Some years later, Tiedemann and Yang [22] presented a milder procedure which consisted in heating the yarn with 0.1% H_2EDTA in H_2O/DMF (1:1, *v/v*) for 30 min in a boiling bath and then cooled rapidly. The extraction method was successfully applied on red contemporary wool dyed samples and on red wool historical Peruvian samples. Concentrations of the yarn extracts were measured with the photometry function on the UV–Vis spectrophotometer, and the authors found that although being as powerful as the conventional HCl method, the H_2EDTA/DMF extraction

protocol preserves the fibre structure for further investigation. In the same period, Kirby and White [23] suggested a method involving the use of a boron trifluoride/MeOH mixture for the extraction of lake pigment dyestuffs from paintings, and the same procedure was used a few years later [24] to extract dyestuffs from purple samples in a fifteenth century velvet panel. As Sanyova and Reisse [25] pointed out, one drawback of this method is the esterification of carboxyl-containing colourants, such as pseudopurpurin and munjistin. Milder extraction method which would minimize unwanted effects such as acid hydrolysis, decarboxylation or methylation, but still extract alizarin, purpurin and other colourants as quantitatively as possible were desirable. The use of hydrofluoric acid solutions for the extraction of anthraquinones from their aluminium complexes in madder lakes fulfilled the hope of non-destructive extraction of pseudopurpurin, glycosides and other labile molecules, and it proved to be at least as efficient as HCl for alizarin and purpurin [25].

Strong acid procedures are particularly disadvantageous for yellow dyes, the majority of which are flavonoids, often with glycoside groups. In a way to preserve glycosidic linkages, Zhang and Laursen [16] presented two different extraction protocols: an H_2EDTA/acetonitrile/MeOH (1:5:44, *v/v/v*) mixture kept at 60 °C for 30 min and a formic acid/MeOH (1:19, *v/v*) mixture kept at 40 °C for 30 min, being the residues redissolved in MeOH/H_2O (1:1, *v/v*) . The methods were tested on silk dyed with yellow and red natural dyes. For the flavonoid dyes on silk, EDTA method seems to be more efficient, while formic acid procedure is more adequate for the anthraquinone-type dyes. The formic acid and EDTA extraction methods not only gave higher extraction yields than did HCl procedure but also more information about the nature of the original dyestuff can be obtained. With the same goal, Guinot and Andary [26] proposed a mild method for dye extraction from wool dyed with weld based on the use of oxalic acid.

When indigoids dyestuffs are present in the fibre, the use of DMF [27], hot pyridine [28], MeOH/DMF [11] or dimethylsulfoxide (DMSO) [21] for the residue solubilisation is recommended instead of MeOH or MeOH/H_2O, since indigotin and indirubin are insoluble in these solvents [11, 21].

Long ago, Schweppe [27] identified the natural dyes on the fibres without taking them up into solution, based on the fact that natural mordant dyes form lakes of various colours with different mordant ions. Concerning the fibres dyed with mixtures of natural dyes and blue indigoid dyes, Schweppe [27] pointed out that the indigo should be removed from the fibre by repeated boiling with dimethylformamide (DMF) until the solvent remains colourless. Once the indigo has been removed, the yellow or the red dyes are left behind and could be identified by forming the various coloured lakes. Surowiec et al. [28] in a study of archaeological Coptic textiles proposed an extraction procedure of alkaline hydrolisis, for samples containing blue dyestuffs (indigotin or its derivatives), based on hot pyridine, while yellow and red dyes were extracted by acidic hydrolysis with a mixture of 3 mol dm^{-3} HCl solution in ethanol (1:1), heated in a water bath at 90 °C for about 30 min. Later, Surowiec et al. [11], based on the acidic extraction with a mixture of 37% hydrochloric acid: MeOH:water (2:1:1, *v/v/v*) in a water bath at 100 °C for 10 min, proposed an additional DMF/MeOH (1:1, *v/v*) extraction step to enhance the extraction of the vat dye indigotin, a component of the blue dyes woad and indigo, and mordant dyes released during hydrolysis step. The recovery efficiencies for flavonoids, plant-derived anthraquinones, ellagic acid and indigotin were improved with this additional MeOH/DMF extraction step and, particularly, the recovery of indigotin offered a notable improvement.

Finally, in a recent work, Valianou et al. [21] presented a systematic comparative study of five different dye extraction procedures based on hydrochloric, citric, oxalic and trifluoroacetic acids (TFA) and an HCOOH/H_2EDTA mixture. All five extraction methods were tested on dyed wool with red and yellow mordant dyes (madder, cochineal, weld and young fustic), a yellow direct dye (tumeric) and a blue vat dye (woad) and also on historical textile samples. Particular working conditions were established after a vast study, but in general, wool samples were treated in a boiling water bath with a mixture in the adequate proportions of the respective acid:MeOH:H_2O until dryness, being the residue reconstituted with DMSO. In the case of HCOOH/H_2EDTA mixture, EDTA solution was added to the initial mixture after wool extraction of 5 min with formic acid. The upper solution was then isolated in a second tube and dried, and the procedure was repeated to treat the residue of the first tube. TFA method provided elevated extraction yields for most of the studied dye components and was selected to be used on historical samples, where it was successfully applied in the identification of several colouring compounds, some of which could not have been detected if the HCl method was used.

The purpose of this work was to make a systematic evaluation on the efficiency of several currently available extraction procedures used to extract different dye components from wool dyed fibres. Contemporary dyed samples were used in order to evaluate the different analytical methodologies. The dyes investigated cover a wide range of chemical structures (Fig. 1), and they have been historically used to obtain a wide array of different hues.

The most effective extraction procedure was then used for the extraction of Arraiolos carpets historical samples. Arraiolos carpets are probably one of the richest artistic Portuguese expressions in terms of textile heritage, being

produced between the seventeenth and nineteenth centuries [29]. In this work, wool samples were collected from an eighteenth century carpet belonging to the National Museum of Ancient Arts (MNAA), and after extraction, the natural dyes were identified by LC-DAD-MS.

Experimental

Materials and reagents

The following analytical grade reagents were used: nitric acid 65%, *N*,*N*-dimethylformamide, 37% hydrochloric acid and sodium dithionite ($Na_2S_2O_4$) from Panreac (Barcelona, Spain); sodium hydroxide (NaOH) from Akzo Nobel (Amsterdam, The Netherlands); EDTA disodium salt from Sigma-Aldrich (Milwaukee, WI, USA); pyridine from Fluka (Buchs, Switzerland); oxalic acid from Riedel-de-Haën (Seelze, Germany) and acetone from Vaz Pereira (Lisbon, Portugal). Methanol, acetonitrile and formic acid (HPLC grade) were purchased from Merck (Darmstadt, Germany). Water purified by a Millipore Simplicity UV system (Billerica, MA, USA) was used for sample preparation and all the analyses. Alum, weld (*R. luteola* L.), cochineal (*Dactylopius coccus* Costa), woad (*I. tinctoria* L.), brazilwood (*Caesalpinia* spp.), madder (*R. tinctorum* L.) and logwood (*H. campechianum* L.) were purchased from Kremer Pigmente GmbH & Co. KG (Aichstetten, Germany). Standards of haematein, alizarin, carminic acid, brazilin, apigenin and indigotin were obtained from Fluka (Buchs, Switzerland); luteolin and haematoxylin were purchased from Sigma (St. Louis, MO, USA) and purpurin was acquired from Eastman Organic Chemicals (Rochester, NY, USA); luteolin 7-*O*-glucoside was obtained from Extrasynthèse (Genay, France). Undyed industrial Arraiolos sheep wool was acquired from Rosarios4 (Mira de Aire, Portugal).

Contemporary dyed wool samples

A sample (4.0 g) of sheep wool previously spun and scoured was mordanted for 30 min in 200 mL of boiling water containing 1.0 g of alum (adapted from [30, 31]). The wool was removed, rinsed and left to dry protected from light. Except for woad, all wool samples were dyed in the following way: each dye bath was prepared with 0.5 g of dried material (weld, brazilwood, logwood or madder) immersed in 50 mL H_2O and heated at 90 °C for 2 h and vacuum filtered. After this, the previously mordanted wool was added to the dye solution and kept at 90 °C for 30 min.

In the case of cochineal, the dye bath was prepared by soaking 0.5 g of dried insects in 50 mL H_2O for 24 h, after which, the above procedure was followed.

For woad, 0.5 g of dye was mixed in a small amount of warm water, obtaining a blue opaque solution. Two millilitres of NaOH 4.25 mol dm^{-3} plus 2.0 mL of $Na_2S_2O_4$ 0.86 mol dm^{-3} was added, and after 15 min, the solution turned to a translucent green–yellow colour. Woad solution was diluted to 50 mL (pH adjusted to 10). Non-mordanted wool was dyed for 30 min at 50 °C and then removed and oxidized by exposure to air.

After the dyeing procedure, all wool samples were removed, rinsed and left to dry protected from light.

Extraction procedures

For wool dyed with cochineal, weld, madder, brazilwood and logwood, six extraction methods were evaluated. Woad dyed wool was extracted using eight different procedures. Glass vials of 1.5 mL capacity were used in all extractions. Five samples of each dyed fibre were extracted per method, and three replicate LC-DAD analyses were performed on each extract. All dried residues were dissolved in 500 μL of MeOH/H_2O (1:1, *v/v*) solution, except for those of woad and HCl-2 methods which were reconstituted in 500 μL of MeOH/DMF (1:1, *v/v*) solution. All solutions were filtered through a 0.45-μm PTFE prior to LC analysis.

HCl-1 method

Two milligrammes of dyed wool was placed in capped vials with 400 μL of 37% HCl/MeOH/H_2O (2:1:1, *v/v/v*) solution and heated at 100 °C for 10 min [5]. Vials were cooled to room temperature, and the solvent was evaporated under vacuum.

HCl-2 method

The procedure was the same as in HCl-1 method, but after the drying process, 400 μL of MeOH/DMF (1:1, *v/v*) solution was added to the residue, and the solution was heated at 100 °C for 5 min [11]. Vials were cooled to room temperature, and the solvent was evaporated under vacuum.

Formic acid method

Two milligrammes of dyed wool was placed in capped vials with 400 μL of HCOOH/MeOH (1:19, *v/v*) solution and heated at 40 °C for 30 min [16]. After being cooled to room temperature, the solvent was evaporated under vacuum.

EDTA-1 method

Samples of 2 mg of dyed wool were placed in capped vials, and 400 μL of 0.001 mol dm^{-3} aqueous Na_2EDTA/acetonitrile/MeOH (1:5:44, *v/v/v*) solution was added

(adapted from [16]). The vials were kept at 60 °C for 30 min. After being cooled to room temperature, the solvent was evaporated under vacuum.

EDTA-2 method

Two milligrammes of dyed wool was placed in vials, and 1.0 mL of 0.1% Na_2EDTA in H_2O/DMF (1:1, *v/v*) solution was added [22]. The vials were capped and kept at 100 °C for 30 min. Vials were cooled to room temperature, and the solvent was evaporated under vacuum.

Oxalic acid method

Two milligrammes of dyed wool was placed in capped vials with 400 μL of 2 mol dm^{-3} oxalic acid/MeOH/acetone/H_2O (1:30:30:40, *v/v/v/v*) solution and heated at 60 °C for 30 min [26]. Vials were cooled to room temperature, and the solvent was evaporated under vacuum.

Pyridine method

Two milligrammes of woad dyed wool was placed in capped vials with 0.5 mL of pyridine and heated at 100 °C for 15 min [28]. Vials were then cooled and solvent evaporated under vacuum.

DMF method

Two milligrammes of woad dyed wool was placed in capped vials with 0.5 mL of DMF and heated at 100 °C for 15 min [27]. Vials were then cooled and solvent evaporated under vacuum.

LC-DAD analysis

An Agilent 1100 system (Agilent Technologies, Waldbronn, Germany) equipped with a diode-array detector and an HP Chem-Station (Agilent Technologies) was used for LC-DAD analyses. The analytical column was a reversed-phase Lichrocart Purospher Star RP-18, 250 mm length×4.6 mm i.d. and 5 μm particle size (Merck, Darmstadt, Germany), set at a temperature of 30 °C. The mobile phase consisted of acetonitrile (A) and 2.5% of aqueous acetonitrile (*v/v*) containing 0.5% HCOOH (*v/v*) (B). A flow of 1.0 mL min^{-1} was used with the following gradient: 0–100% A from 0 to 10 min, 100% A from 10 to 15 min. Between sample injections, a 5-min run of solvent B was used for column equilibration. The injection volume was 20 μL, and DAD detection was set between 200 and 700 nm. Chromatographic profiles were recorded at 600, 265, 450, 445, 495 and 290 nm for indigo, weld, brazilwood, logwood, cochineal and madder, respectively.

SEM analysis

A Hitachi S-3700N variable pressure scanning electron microscope (Tokyo, Japan) interfaced with a Bruker X-ray energy dispersive spectrometer was used for morphological characterization of the samples. Samples were mounted in aluminium holders with double-sided adhesive carbon tape and coated with an Au–Pd layer. The analyses were done using an acceleration voltage of 15.0 kV.

Statistical data analysis

Statistical treatment (one-way ANOVA, $P<0.05$, SPSS® 15.0 for Windows) was performed on the data to determine significant differences whenever they occurred.

Arraiolos historical samples

Sampling

An eighteenth century historical carpet was selected from the National Museum of Ancient Arts (MNAA, Lisbon, Portugal) collection (catalogue number 36). Different portions of embroidery wool yarns between 3 and 5 mm with the same hue were sampled in distinct parts of the carpet with fine point tweezers and spring bow scissors from thread ends in damaged areas or in the back side. Samples from 14 different hues (referred as S1 to S14, Fig. 2) were collected and individually stored in Eppendorf tubes protected from the light.

Colourimetric studies

A portable spectrophotometer equipped with a Xenon lamp and a photodiode sensitive to the 360–700-nm spectral

Fig. 2 Arraiolos carpet (MNAA collection, catalogue number 36) with fibre sampling (S1–S14) location

range from Datacolor (Zurich, Switzerland) was used for colourimetric studies (L*, a* and b*, CIELab space defined by Commission Internationale de l'Eclairage in 1976). Black and white standards were used for calibration, and analyses were done with standard illuminant/observer CIE D65/10° and specular component excluded. Analyses were performed on three different points of each wool sample, with the average value used for data interpretation.

Extraction procedure

Samples of approximately 2.0 mg were extracted following the EDTA-2 methodology. All samples were vacuum-dried, dissolved in 250 μL MeOH/H_2O (1:1, *v/v*) and filtered. The remaining threads of blue and green samples were redissolved in 250 μL MeOH/DMF (1:1, *v/v*) and filtered.

LC-DAD-MS analysis

An LCQ Advantage Thermo Finnigan mass spectrometer equipped with an ESI source, using anion trap mass analyzer and a PDA detector was used (San Jose, CA, USA). The conditions of MS analysis were capillary temperature of 300 °C, source voltage of 5.0 kV, source current of 100.0 μA, and capillary voltage of −20.0 V in negative ion mode and 22.0 V in positive ion mode. Analytes were detected in full MS mode (*m/z* 100–800): in negative ion mode, two segments were used, 10% CID from 0 to 15 min and 30% CID from 15 to 30 min; in positive ion mode, 30% CID was used from 0 to 30 min. All samples were injected in negative and positive ion modes. Column temperature was set at 30 °C, and tray temperature was set at 24 °C. PDA detector was set at 200–800 nm. The MS and PDA equipment were coupled to an LC system with auto sampler (Surveyor Thermo Finnigan). The analytical column was a reversed phase Fortis-C18 (Fortis Technologies; C_{18}, particle size 3.0 μm, 150× 2.1 mm). The mobile phase consisted of acetonitrile (A) and water acidified with 0.1% formic acid (B). The gradient used was 0–90% A from 0 to 20 min, then 90% A from 20 to 30 min. Injection volume was set to 10 μL.

ICP-MS analysis

Measurements were carried out using a ThermoScientific Element XR ICP-MS instrument, equipped with a sector field mass spectrometer of reverse Nier-Johnson geometry. Sample introduction was accomplished by means of a MicroMist nebulizer (sample uptake rate of 200 μL min^{-1}; Glass Expansion), mounted onto a cyclonic spray chamber (Glass Expansion). Typical instrument settings and data acquisition parameters used were Rf power, 1,250 W; auxiliary gas flow, 0.800 dm^3 min^{-1}; cooling gas flow, 16.000 dm^3 min^{-1}; sample gas flow, 0.993 dm^3 min^{-1}; resolution, 4,000 m/Δm; segment duration, 200 ms; sample time, 10 ms; E-scan type and 36 sweeps and total measurement time per sample, 126 s. Concentrations for all elements (Al, Fe, Cu and Zn) were determined via external calibration versus a standard solution containing 35 μg dm^{-3} of the analyte elements. This standard solution was prepared by dilution of commercially available 1 g dm^{-3} single element standard solutions (Merck) with 0.14 mol dm^{-3} HNO_3. High purity water (purified by means of a Milli-Q system (Millipore)) and HNO_3 (purified by sub-boiling distillation in a PFA equipment) were used for dilution.

For sample preparation, historical fibres of 0.1–2.0 mg were placed in polypropylene tubes and digested with 0.5 mL of concentrated HNO_3 in an ultrasound bath without temperature control. Acidic digestion proceeded until complete fibre dissolution (approximately 2 h). The solution was then diluted to 5 mL using ultrapure water.

Results and discussion

Evaluation of the extraction procedures

In this work, we have evaluated six extraction procedures (referred as HCl-1, HCl-2, formic acid, EDTA-1, EDTA-2 and oxalic acid methods) to extract wool samples dyed with madder, weld, cochineal, logwood, brazilwood and woad, and two additional extraction procedures (pyridine and DMF methods), which were only used to extract wool dyed with woad.

Five samples of each dyed fibre were extracted per extraction method, and three replicate analyses were performed on each extract. The chromatographic analysis of the wool extracts was done using reverse-phase liquid chromatography coupled with a DAD detector (LC-DAD). The identification of the dye chromophores was done based on their UV spectra and by comparison with authentic standards. Representative chromatograms of all the wool extracts obtained with the EDTA-2 extraction method are shown in Fig. 3. The evaluation of the extraction methods efficiency was based on the comparison of the chromatographic peak areas obtained from LC-DAD analyses for the main chromophores in each dye (Table 1). One-way ANOVA statistics was used to identify significant differences among the extraction procedures whenever they occurred.

The HCl-1 and HCl-2 methods are the most aggressive extraction methods available, leading to the almost complete destruction of the fibre structure. SEM analysis performed on fibres subjected to HCl-based extraction procedures showed that the main wool fibre structure is almost completely lost and, whenever maintained, the fibres lose their characteristic scale structure (Fig. 4a, b). The

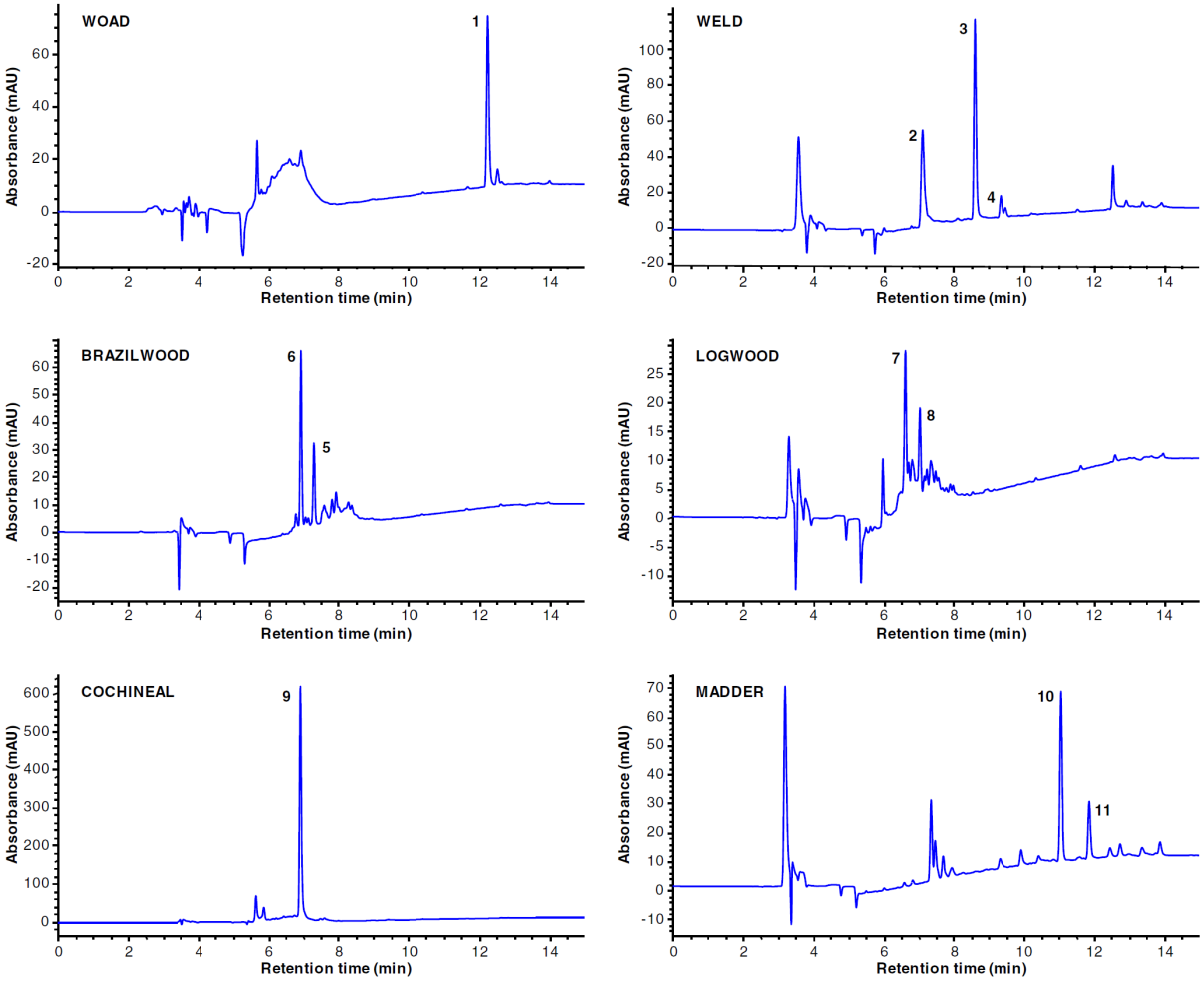

Fig. 3 LC-DAD chromatograms of the dyed wool extracts obtained with the EDTA-2 extraction method. Chromatographic profiles recorded at 600, 265, 450, 445, 495 and 290 nm for woad, weld, brazilwood, logwood, cochineal and madder, respectively. For peak identification, see Fig. 1

main difference between the tested HCl methods is the addition of an extraction step with MeOH/DMF on the HCl-2 method. Contrary to previously reported results [11], with the exception of brazilein (6, Table 1) and haematein (7, Table 1), no significant improvement in the natural dyes extraction yields was obtained with the HCl-2 procedure when compared to those obtained with HCl-1.

Hot pyridine and DMF are described in the literature for the extraction of indigo dyes from textile samples [27, 28]. Nevertheless, indigotin (1, Table 1) was not observed in the DMF extract, and only a small chromatographic peak with t_r=12.5 min was detected (data not shown), likely indirubin, a compound which is normally present at variable concentration in the indigo dyes.

HCl-1 was the most effective method to extract indigotin. However, researchers should be aware that yellow flavonoid dyes were commonly used together with indigo dyes to obtain green hues. These originally green colours tend to become bluish when subjected to light due to the faster photodegradation of the flavonoid dyes. HCl-based methods are not suitable for the extraction of the yellow flavonoid dyes due to hydrolysis of the *O*-glycosidic bonds [16, 21]. Being so, caution is needed when they are used to extract bluish samples since relevant information regarding the source of other natural dyes could be lost.

For the indigoids extraction, one-way ANOVA analysis showed that the results obtained with the milder method EDTA-2 are not significantly different from those obtained with pyridine (P<0.05, Table 1). The former method enables efficient extraction of other dye components. In fact, when the EDTA-2 procedure is used, residual amounts

Table 1 LC-DAD peak areas (mAU×min) of the natural dyes major chromophores extracted from wool dyed samples by the different extraction procedures (see Fig. 1 for peaks identification)

Peak	Extraction method: peak area*														
	HCl-1		HCl-2		Formic		EDTA 1		EDTA 2		Oxalic		Pyridine		DMF
	Mean†	(SD)‡	Mean†	(SD)‡	Mean†	(SD)‡	Mean†	(SD)‡	Mean†	(SD)‡	Mean†	(SD)‡	Mean†	(SD)‡	Mean†
1	26.8^{a}	(0.4)	18^{b}	(1)	10.5^{c}	(0.6)	6^{d}	(0.4)	16^{b}	(0.2)	10^{c}	(0.1)	15^{b}	(1)	n.d.
2	n.d.		n.d.		15^{a}	(1)	23^{b}	(0.6)	294^{c}	(26)	115^{d}	(2)	–		–
3	352^{ab}	(66)	290^{ab}	(33)	33^{c}	(4)	56^{d}	(5)	214^{a}	(24)	326^{b}	(23)	–		–
4	24^{a}	(3)	21^{a}	(2)	n.d.		n.d.		14^{b}	(2)	22.4^{a}	(0.8)	–		–
5	n.d.		n.d.		n.d.		n.d.		27^{a}	(2)	42^{b}	(6)	–		–
6	n.d.		31^{a}	(3)	58^{b}	(4)	19^{c}	(0.5)	328^{d}	(8)	418^{e}	(41)	–		–
7	58^{a}	(7)	141^{b}	(18)	49^{a}	(2)	17^{c}	(0.4)	1257^{d}	(40)	1108^{e}	(19)	–		–
8	n.d.		n.d.		n.d.		n.d.		41^{a}	(7)	43^{a}	(5)	–		–
9	$1{,}528^{a}$	(251)	145^{b}	(23)	69^{c}	(9)	16^{d}	(2)	1614^{a}	(44)	413^{e}	(12)	–		–
10	$2{,}110^{a}$	(82)	$1{,}983^{a}$	(134)	608^{b}	(59)	237^{c}	(8)	1538^{d}	(107)	728^{b}	(16)	–		–
11	$2{,}886^{a}$	(151)	$3{,}307^{a}$	(181)	294^{bc}	(156)	103^{b}	(3)	1088^{d}	(71)	437^{c}	(14)	–		–

n.d. not detected

* Normalized to milligrammes of dyed wool extracted by each method, samples dried and redissolved in 500 μL of solvent, 20 μL injection. Chromatographic profiles recorded at (nanometres): 600 (woad: 1), 265 (weld: 2, 3 and 4), 450 (brazilwood: 5 and 6), 445 (logwood: 7 and 8), 495 (cochineal: 9) and 290 (madder: 10 and 11)

† The values represent the mean of three replicate measurements on five different extracts. For each compound, means followed by different index letters are significantly different (one-way ANOVA, $P<0.05$)

‡ Standard deviation

of yellow glycosilated chromophores from weld can sometimes be extracted and identified together with indigo in samples which present nowadays a blue hue (historical samples analyses).

The yellow chromophores of weld behaved slightly differently when the different extraction procedures were applied. Luteolin (3, Table 1) was extracted with a similar efficiency by the HCl-1, HCl-2, EDTA-2 and oxalic acid

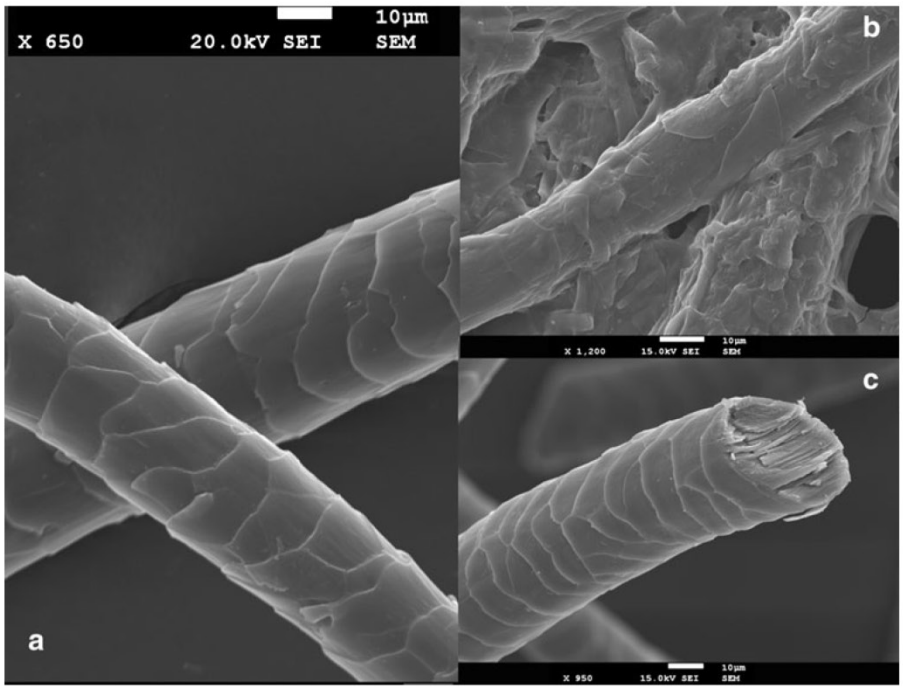

Fig. 4 SEM micrographs of **a** untreated, undyed wool; **b**, **c** dyed wool extracted with HCl-1 and EDTA-2 methods, respectively

methods, while significantly lower yields were obtained with the EDTA-1 and formic acid procedures. Apigenin (4, Table 1) amounts are, as expected, much lower and could not be detected when the EDTA-1 and formic acid methodologies were used, while the other tested methods performed equally well. Surowiec et al. [11] compared the two HCl procedures for the extraction of weld dye aglycones, concluding also that they result in very similar LC peak areas. However, the known hydrolysis of the flavonoid glycosides should be taken into consideration for the apparent good yields obtained with the HCl methods for the studied aglycones [21]. In fact, luteolin-7-*O*-glucoside (2, Table 1) is absent in chromatograms of the HCl methods, being only detected when milder extraction procedures were used. LC-DAD-MS analysis of the extracts obtained with the milder extraction methods enabled also the identification of up to seven flavone glycosides (data not shown), which have already been identified in weld [32], and it is of major importance for the historical samples analysis.

Brazilwood and logwood chromophores (5–8, Table 1) are neoflavonoids which were overall poorly recovered with exception for EDTA-2 and oxalic acid extraction methods. Statistic analysis indicates that both methods are not equally effective in the extraction of the neoflavonoid dyes. In fact, oxalic acid yields slightly higher peak areas for the brazilwood chromophores (5 and 6, Table 1), while EDTA-2 is more effective in the extraction of the haematein (7, Table 1). Neoflavonoids are very sensitive to light degradation [33], and only very small amounts of intact chromophores are expected to be present in historical textiles requiring, therefore, very effective extraction methods for their recovery.

The mild methods, formic acid, EDTA-1 and oxalic acid, perform very poorly in the extraction of the anthraquinone dyes (9–11, Table 1). Unexpectedly, carminic acid (9, Table 1) was very poorly extracted by the HCl-2 method, but no significant differences were observed between the extraction yields obtained with EDTA-2 and HCl-1 methods. Valianou et al. [21] evaluated both HCl-1 and oxalic acid methods in the extraction of carminic acid but did not report a considerable difference between them.

Madder dye components (10 and 11, Table 1) were extracted with higher efficiency by both HCl methods. The EDTA-2 extraction procedure also performed reasonable well and, for the reasons presented above, this method should be considered whenever the presence of yellow flavonoid dyes is suspected. Surowiec et al. [11] reported higher extraction yields for the madder dye components when HCl-2 was used compared to HCl-1, which we could not confirm. Valianou et al. [21] also observed that milder extraction methods, like oxalic acid, were less effective in the extraction of the madder anthraquinones.

One of the most striking differences observed in this study is the difference in the peak areas for the same dye obtained with the two procedures involving EDTA (Table 1). EDTA is a very good chelating agent, and it is expected to displace the dye molecule, binding the mordant metal ion which is attached to the fibre [16, 22]. An important difference between the two tested methods is the solvent used to solubilise the displaced dye. According to the results presented in Table 1, and for all the chromophores investigated, the solvent DMF appears to be a much more effective solvent than the more polar mixture of acetonitrile/MeOH. The differences in EDTA concentration and temperature between the two methods might also play a role in the different extraction yields obtained.

A main feature of the EDTA methods is their mildness. In fact, SEM images confirmed that the wool fibre scale structure is practically intact after the extraction procedure with Na_2EDTA/DMF (Fig. 4a, c).

An overall appreciation of the tested extraction methods indicates that the most effective procedures were the HCl-1, EDTA-2 and oxalic acid. Considering the already referred shortcomings of the HCl methods and the poor anthraquinone extraction yields obtained with the oxalic acid procedure, authors believe that, among the tested methods, EDTA-2 is the most suitable method to extract unknown dyes from an historical sample.

Arraiolos historical samples analyses

To test the previous assumption about EDTA-2 as the best extraction method, historical samples were collected from an Arraiolos carpet from the eighteenth century (MNAA collection, catalogue number 36). This is a large rectangular carpet (169×112 cm) with a yellow background and a typical animal pattern, and the blue border presents a design with oriental motifs (Fig. 2) [29].

Historical references concerning the usage of natural dyes in Arraiolos carpets are scarce and indicate only the use of indigo, weld, spurge flax (*Daphne gnidium* L.), logwood and brazilwood as the colour sources [29, 34–36].

Samples from 14 different hues were collected from the embroidery work, and their L*a*b* coordinates of the CIELab colour space measured by colourimetry (Table 2). The identification of the natural dyes was carried out by LC-DAD-MS analysis of wool samples extracts obtained using the EDTA-2 procedure (Table 2).

Weld was reportedly used in Arraiolos carpets to obtain yellow hues [34] and, in fact, it has already been detected in another carpet [37]. The analysis of the samples collected in the MNAA carpet enabled the identification of weld in yellow samples S3, S12 and S14, in green samples S5, S7 and S8, in light brown sample S4, and surprisingly, in black

Table 2 Identification of the natural dyes in wool samples collected from an Arraiolos carpet with catalogue number 36 from MNAA collection

Sample					R_t (min)	LC-DAD data (nm)	LC-MS data (*m/z*)		Identification	Possible dye source
ID	Colour	CIEL*a*b* coordinates					ESI^-	ESI^+		
		L*	a*	b*						
S1	Bk	15.77	0.74	1.20	15.99	243, 277, 323	609, 447, 285	–	Luteolin di-*O*-glucoside	Weld
					16.45	243, 277, 323	609, 447, 285	–	Luteolin 3,7′-di-*O*-glucoside	
					17.37	250, 267, 346	447, 285	–	Luteolin 7-*O*-glucoside	
					18.12	244, 265, 324	431, 269	–	Apigenin 7-*O*-glucoside	
					20.62	250, 267, 347	285, 243, 217, 199, 151, 133	–	Luteolin	
S2	Br	26.29	3.91	10.51	–	–	–	–	–	–
S3	Y	53.90	7.39	32.66	16.47	242, 280, 324	609, 447, 285	–	Luteolin 3,7′-di-*O*-glucoside	Weld+Brazilwood
					17.32	250, 267, 347	447, 285	–	Luteolin 7-*O*-glucoside	
					18.12	246, 263, 329	431, 269	–	Apigenin 7-*O*-glucoside	
					18.80	259, 307, 333	243	245	Type C compound	
S4	LBr	38.61	9.57	24.78						Same as S3
S5	Gr	39.01	−6.40	17.21	15.95	245, 268, 334	609, 447, 285	–	Luteolin di-*O*-glucoside	Weld+*Isatis* or *Indigofera tinctoria*
					16.47	245, 268, 334	609, 447, 285	–	Luteolin 3,7′-di-*O*-glucoside	
					17.38	249, 255, 348	447, 285	–	Luteolin 7-*O*-glucoside	
					18.12	244, 267, 335	431, 269	–	Apigenin 7-*O*-glucoside	
					18.25	247, 269, 341	461, 299	–	Chrysoeriol 7-*O*-glucoside	
					20.60	251, 267, 347	285, 199, 175, 133	–	Luteolin	
					26.55	245, 286, 331, 606	–	263, 235, 219	Indigotin	
S6	W	60.39	3.48	20.21	–	–	–	–	–	–
S7	Gr	37.64	−7.68	8.47						Same as S5
S8	Gr	31.07	−7.25	1.40						Same as S5
S9	Bl	24.41	−3.79	−9.78	26.53	245, 286, 332, 606	–	263, 235, 219	Indigotin	*Isatis* or *Indigofera tinctoria*
S10	Bl	40.62	−6.81	0.39						Same as S9
S11	Bl	29.80	−5.93	−10.88						Same as S9
S12	Y	56.61	7.67	42.44	15.94	245, 267, 335	609, 447, 285	–	Luteolin di-*O*-glucoside	Weld
					16.43	245, 267, 335	609, 447, 285	–	Luteolin 3,7′-di-*O*-glucoside	
					17.33	254, 267, 348	447, 285	–	Luteolin 7-*O*-glucoside	
					18.07	245, 268, 335	447, 285	–	Luteolin 4′-*O*-glucoside	
					18.19	246, 263, 328	431, 269	–	Apigenin 7-*O*-glucoside	
					18.30	247, 269, 341	461, 299	–	Chrysoeriol 7-*O*-glucoside	
					18.47	246, 267, 339	447, 285	–	Luteolin 3′-*O*-glucoside	
					21.58	253, 266, 348	285, 243, 241, 217, 199, 175, 151, 133	–	Luteolin	
					22.24	249, 267, 336	269, 225, 201, 181, 149, 117	–	Apigenin	
S13	Bg	52.45	6.78	27.77	18.80	259, 307, 333	243	245	Type C compound	Brazilwood
S14	Y	57.97	11.47	38.09	15.59	243, 277, 323	609, 447, 285	–	Luteolin di-*O*-glucoside	Weld+Brazilwood
					16.05	243, 277, 323	609, 447, 285	–	Luteolin 3,7′-di-*O*-glucoside	
					17.37	250, 267, 346	447, 285	–	Luteolin 7-*O*-glucoside	
					18.12	244, 265, 324	431, 269	–	Apigenin 7-*O*-glucoside	
					18.80	259, 307, 333	243	245	Type C compound	
					20.62	250, 267, 347	285, 243, 217, 199, 151, 133	–	Luteolin	

Bg beige, *Bk* black, *Bl* blue, *Br* brown, *Gr* green, *LBr* light brown, *W* white, *Y* yellow

sample S1. The phenolic compounds extracted from these samples absorb in the region of spectra below 350 nm, confirming their flavone structure, and their elution pattern and mass spectra correspond to the luteolin, apigenin and chrysoeriol derivatives reported in weld extracts [18, 32, 37]. Usually, not all the compounds identified in weld plant extracts can be identified in the historical textile samples. However, and despite the widely distribution of these compounds in the plant kingdom, weld is frequently pointed out in the literature [38–40] as the yellow colour source in the historical textiles, sometimes based only in the identification of very few flavone derivatives.

The chromatographic profile of sample S12 presented in Fig. 5 is unusually comprehensive for a historical sample, as nine flavone derivatives, which were previously identified in weld plant extracts, could be identified based on the comparison of their UV–Vis and mass spectra data with those reported in the literature (Table 2) [18, 32, 37]. The negative ESI mass spectra of luteolin-di-*O*-glucosides yielded a deprotonated ion, $[M–H]^-$, at *m/z* 609 which gives rise to Y_1^- (*m/z* 447) and Y_0^- (*m/z* 285), ions formed by the sequential loss of two hexose residues (162 amu). The compounds with r_t=15.94 and 16.43 min were identified as luteolin-di-*O*-glucoside and luteolin-3,7′-di-*O*-glucoside based on their UV spectra and elution order [32, 37].

Several flavone-*O*-glucosides could be identified in S12 sample (Fig. 5 and Table 2). The presence of luteolin-*O*-glucosides was suggested by the loss of a hexose residue (162 amu) from the deprotonated ion, $[M–H]^-$ (*m/z* 447), with the formation of Y_0^- at *m/z* 285. Compounds with r_t= 17.33, 18.07 and 18.47 min were identified as luteolin-7-*O*-glucoside, luteolin-4′-*O*-glucoside and luteolin-3′-*O*-glucoside, respectively, based on their UV spectra and elution order [32, 37]. The latter two compounds could only be identified on sample S12. The chromatographic peak with r_t=18.19 min was attributed to apigenin-7-*O*-glucoside, as $[M–H]^-$ (*m/z* 431) was detected along with the Y_0^- ion at *m/z* 269, which results from the loss of a hexose unit from the original deprotonated ion.

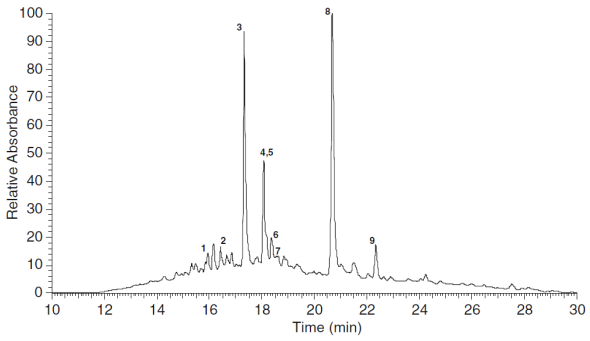

Fig. 5 LC-DAD-MS chromatogram of sample S12. Identification of the chromatographic peaks based on UV and mass data (Table 2) as follows: (*1*) luteolin di-*O*-glucoside; (*2*) luteolin 3,7′-di-*O*-glucoside; (*3*) luteolin 7-*O*-glucoside; (*4*) luteolin 4′-*O*-glucoside; (*5*) apigenin 7-*O*-glucoside; (*6*) luteolin 3′-*O*-glucoside; (*7*) chrysoeriol 7-*O*-glucoside; (*8*) luteolin; (*9*) apigenin

The chromatographic peak at r_t=18.30 min was attributed to the methoxyflavone glycoside chrysoeriol 7-*O*-glucoside, which has already been detected in historical samples dyed with weld [18]. The $[M–H]^-$ (*m/z* 461) was detected along with the Y_0^- ion at *m/z* 299, which results from the loss of a hexose unit from the original deprotonated ion. Chrysoeriol 7-*O*-glucoside was only identified in samples S5 and S12.

The flavone aglycones luteolin and apigenin were identified with r_t=21.58 and 22.24 min, respectively, in S12. The deprotonated molecular ion of luteolin was detected at *m/z* 285 along with the fragments derived from the retro-Diels-Alder (RDA) reaction, $^{1,3}A^-$ at *m/z*151 and $^{1,3}B^-$ at *m/z*133, which provide information on the number and substituents of rings A and B of the flavonoid structure [12, 41]. Further fragmentation yielded fragments at *m/z*243 $[M–H–C_2H_2O]^-$, *m/z*241 $[M–H–CO_2]^-$, *m/z*217 $[M–H–C_3O_2]^-$, *m/z*199 $[M–H–C_2H_2O–CO_2]^-$ and *m/z*175 $[M–H–C_3O_2–C_2H_2O]^-$ [41]. Similar fragmentation was observed for apigenin, and the deprotonated molecular ion was detected at *m/z*269 along with the RDA fragments $^{1,3}B^-$ at *m/z*117 and $^{1,4}B^-$ +2H at *m/z*149. Further fragmentation yields ions detected at *m/z*181 $[M–H–2CO_2]^-$, *m/z*201 $[M–H–C_3O_2]^-$ and *m/z*225 $[M–H–CO_2]^-$ [41].

Despite the fact that sample S3 presents nowadays a yellowish hue, it is thought to have been originally dyed in an orange/reddish hue. This conclusion is supported by the fact that the base of the embroidery stitches, which is less exposed to the effects of light, still presents a faded orange/reddish hue. Neither brazilin or brazilein were detected. Nevertheless, a compound with r_t=18.80 min in the chromatographic profile of this sample extract is tentatively identified as Type C compound (Table 2), an analyte previously described by Karapanagiotis et al. in samples collected from Cretan icons [42] and post-Byzantine textiles [43]. The authors [42, 43] did not suggest an explanation for the nature of this compound. However, preliminary results obtained by our group on light artificially aged wool samples dyed with brazilwood and extracted by the Na_2EDTA/DMF method suggest that Type C compound is likely to be a photodegradation product of this natural dye (unpublished data). The presence of both weld and brazilwood in sample S3 could explain the orange/reddish colour observed in the base of the embroidery stitches. Type C compound was identified together with several weld chromophores in the light brown sample S4 and in yellow sample S14. Brazilwood is known for its fast light degradation [33], and the colour fading in

Arraiolos carpets has already been described by Pessanha [35], who stated that some areas originally dyed in red hues became brownish with time. Sample S3 was taken from the filling colour of the studied carpet, and sample S14 belongs to the border and fringe (Fig. 2). Brazilwood dye photodegradation has a tremendous impact in the actual perception of this carpet when compared to what it might have originally been intended by its makers. Despite the referred use of brazilwood in the Arraiolos historical dyeing recipes [34], it is now for the first time tentatively identified in these carpets.

Indigo, together with weld, was identified in green samples S5, S7 and S8 and in blue samples S9, S10 and S11. Green dyes are rare [2], and green hues were usually obtained by sequentially dyeing the fibre with blue and yellow dyes. Arraiolos recipes refer the use of weld and indigo to dye wool fibres in green hues [34]. The identification of indigotin as the chromatographic peak eluting at approximately 26.50 min was done based on its characteristic UV–Vis and mass spectra [12, 44, 45]. As reported in the literature [12, 45], indigoids are generally detected in the ESI positive mode, presenting low intensity signals. The samples yielded the protonated molecular ion $[M+H]^+$ at *m/z*263 and the characteristic fragment ions $[M+H-28]^+$ at *m/z*235 and $[M+H-44]^+$ at *m/z*219, which correspond to the subsequent loss of CO and NH_2.

As stated before, indigo was obtained throughout a fermentation process of *I. tinctoria* L. (commonly known as woad and widely available in Southern and central Europe, North Africa and West Asia) or *I. tinctoria* L. (a native of tropical Asia) [2]. The indigo made from *I. tinctoria* was of superior quality and was widely available in Europe in the eighteenth century [2]. Despite the historical interest, it is not possible to identify the plant species based solely on the indigotin identification in the textile fibres.

Extraction of brown sample S2 and white sample S6 did not yield any recognizable dye on the chromatogram.

The colour hue of a fibre is known to be dependent on the overall dyeing process [1, 2]. Apart from the used natural dye and dyeing method, the concentration and chemical nature of the mordant strongly affects the fibre colour [46]. When the mordant used is an inorganic salt, the metal ion combines with the fibre and dye via a lasting chemical bond (forming a coordination compound), ensuring that the colour stand fast against washing but also having a great influence on the final colour obtained [1, 2]. Despite the reported use of other metal salts, alum, an aluminium sulphate, has always been the most used mordant for the red and yellow flavonoid dyes, recognized for the brilliance of the colours obtained [46]. Alum is also the only mordant referred in the Arraiolos historical dyeing recipes [34].

In order to account for the mordant effect in the colours seen in the Arraiolos carpet studied, mordant metal ion identification and quantification in the collected samples were accomplished by ICP-MS (Fig. 6). Analysis of Al, Fe, Cu and Zn contents in blank solutions was below the limit of detection (LOD) of the analytical method (data not shown). Metal ion quantification was also performed on several samples of actual untreated sheep wool, and the amounts detected were much lower than those detected in the samples of the historical carpet (data not shown).

Overall, the results showed that Al, Fe and Zn are detected in higher concentration than Cu, which is only detected in very residual amounts. The amounts of Zn are relatively high in several samples. Zn salts have been seldom referred as mordants, with exception of some reports on their use together with weld to obtain yellow hues in Coptic textiles [47, 48]. Sometimes the identification of moderate amounts of metallic ions other than Al on fibres has been attributed to several unintended factors, like the use of metallic containers in the dyeing process (the usual acidic conditions of the dye bath can lead to selective leaching), contaminations from the alum used or the presence of accumulated dirt [49–52]. We cannot know for sure the source of Zn in the Arraiolos samples but, considering the available literature and the historical sources on the making of these carpets, it is unlikely that zinc salts have been used as mordants.

Al was identified as the most abundant metal ion in most of the samples, confirming the wide use of alum as mordant. An unusual find was the detection of Al in the blue samples, S9–S11 (Fig. 6), along with indigo (Table 2). Indigo is a vat dye which does not require the use of mordant in the dyeing process [1, 2] and, as expected, Arraiolos historical recipes also do not refer the use of mordants for the blue hues [34].

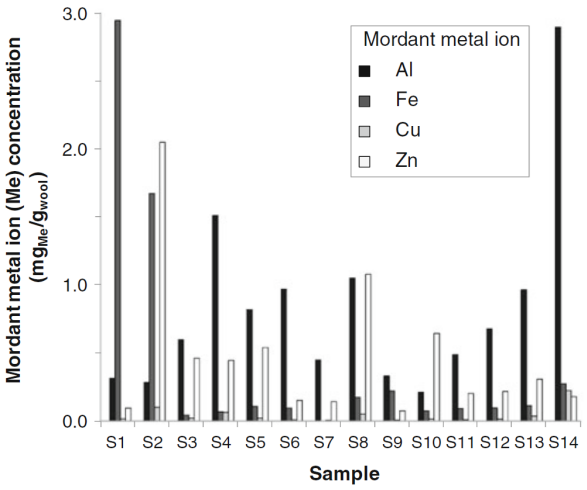

Fig. 6 ICP-MS mordant metal ion (Me) quantification (mg_{Me}/g_{wool}) in the Arraiolos carpet historical samples

Sample S6 is white, and no dye component could be extracted from it (Table 2) which led us, initially, to assume that natural sheep wool had been used for this embroidery area. However, mordant analysis detected a significant amount of Al in this sample (Fig. 6), an unusual finding considering the extremely lower amounts of Al obtained nowadays for untreated wool.

Whenever used, iron salts are known to substantially alter the resulting colours, with a shift towards dark shades [46]. In some regions of Europe, dyers learned early how to combine natural dyes with mud or silt rich in iron salts, which worked as mordants and darkened the fibre colours [53]. High amounts of iron were detected in black sample S1 and brown sample S2 (Fig. 6). The use of iron mordant partially explains the S1 fibre hue which would be unexpected based solely in the chromophore analysis. However, it is possible that the fibre black hue was also attained by the use of naturally dark coloured wool. Mordant analyses indicate that S2 sample presents high amounts of Fe and Zn (Fig. 6) despite the fact that no chromophore could be identified in it (Table 2). It is likely that dark brown wool was also used in this sample.

Conclusions

Natural dyes are chemically very different, making their effective extraction from textile fibres a complex and difficult task. Despite different extraction yields within the classes of natural dyes, the evaluation of eight extraction procedures available in the literature led us to conclude that the most adequate procedure to extract the tested natural dyes from dyed wool fibres is the use of a mixture of Na_2EDTA/DMF (EDTA-2 method) [22].

The Na_2EDTA/DMF procedure was used to extract samples collected from an eighteenth century Arraiolos carpet. The procedure proved to be very effective as several dye components could be recovered from most of the samples. The effectiveness of the extraction procedure was most evident in the extraction of up to nine different flavone derivatives from different samples, enabling the identification, with a high degree of certainty, of the usage of weld in the making of these carpets.

Despite being referred in the historical literature concerning Arraiolos carpets, the use of brazilwood dye was for the first time described, based on the extraction of a degradation product of the dye.

Mordant analysis also showed that apart from alum, which was referred in the historical literature, iron salts have also been used as mordants in the Arraiolos making. The identification of significant amounts Al in blue samples came as a surprise considering the nature of the indigo dye.

Acknowledgements This work was funded by the Portuguese Foundation for Science and Technology (FCT) (grant no. FCOMP-01-0124-FEDER-010482). A. Manhita also acknowledges FCT for a Ph.D. Fellowship (SFRH/BD/22411/2005). The authors acknowledge architect Teresa Pacheco Pereira from the National Museum of Ancient Arts, Institute of Museums and Conservation for the Arraiolos carpet selection and Mr. Luis Pedro from the Conservation and Restoration Department, Institute of Museums and Conservation for his help in collecting the historical samples. The authors also wish to thank Prof. Pat Sandra and Dr. Lieve Balcaen for the ICP-MS analyses.

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Submitted to the Journal of Chemical Education

Identification of onion dye chromophores in the dye bath and dyed wool by HPLC-DAD - an educational approach

Journal:	*Journal of Chemical Education*
Manuscript ID:	ed-2010-00668k.R1
Manuscript Type:	Laboratory Experiment
Date Submitted by the Author:	n/a
Complete List of Authors:	Barrocas Dias, Cristina; Universidade de Évora, Chemistry Miranda, Marco; Universidade de Évora, Chemistry Manhita, Ana; Universidade de Évora, Chemistry Candeias, António; Universidade de Évora, Chemistry Ferreira, Teresa; Universidade de Évora, Chemistry Teixeira, Dora; Universidade de Évora, Chemistry
Keywords:	Second-Year Undergraduate < Audience, Analytical Chemistry < Domain, Hands-On Learning / Manipulatives < Pedagogy, Dyes / Pigments < Topics, Natural Products < Topics, Chromatography < Topics

Identification of onion dye chromophores in the dye bath and dyed wool by HPLC-DAD - an educational approach

Cristina Barrocas Dias[1-3*], Marco Miranda[3], Ana Manhita[1,2], António Candeias[1-4], Teresa Ferreira[1-3], Dora Teixeira[1,3,5]

[1]HERCULES Laboratory, Largo do Marquês do Marialva, 8, 7000-554 Évora, Portugal

[2]Évora Chemistry Centre, Rua Romão Ramalho 59, 7000-671 Évora, Portugal.

[3]Chemistry Department, Rua Romão Ramalho 59, 7000-671 Évora, Portugal.

[4]José de Figueiredo Conservation and Restoration Laboratory, Institute of Museums and Conservation, Rua das Janelas Verdes 37, 1249-018 Lisboa, Portugal.

[5]ICAAM, Institute of Mediterranean Agricultural and Environmental Sciences, Núcleo da Mitra Apartado 94, 7002-774 Évora, Portugal

* Corresponding author: cmbd@uevora.pt

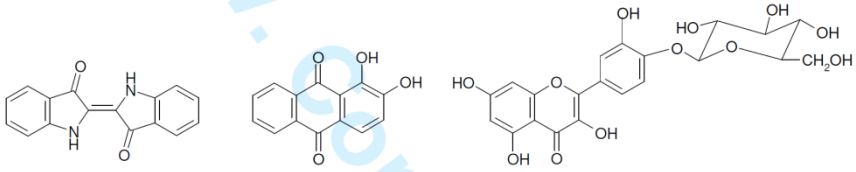
ABSTRACT:

Onion skins (*Allium cepa* L.) and hydrated potassium aluminum sulfate were used to dye wool samples. The main chromophores associated with this natural dye source, namely, quercetin and quercetin-4´-O-glucoside, were identified in the dye bath and in wool extracts by HPLC-DAD with the help of standards. Two procedures were used to extract the dye molecules from the dyed wool and the analytical methodology used was discussed in terms of the analysis of historical textile pieces dyed with natural sources.

KEYWORDS: natural dyes, onion, wool, flavonoids, HPLC-DAD

INTRODUCTION:

Natural dyes have been used since ancient times to dye vegetable and animal fibers and nowadays they are becoming more significant due to the realization that they are safer and more eco-friendly than the synthetic materials. Several reviews in the literature discuss in detail the natural sources of the different colors and the chemical nature of the dyes *(1-3)*. Although some dyes are of animal origin (ex: the red cochineal), the vast majority comes from plants, and all the dye molecules possess highly conjugated systems which absorb electromagnetic radiation between 400 to 800 nm therefore appearing colored (Figure 1).



Indigo (blue) Alizarin (red) Quercetin-4´-O-Glucoside (yellow)

Figure 1. Chemical structures of some natural dyes

Recent papers published in this journal describe the use of natural dyes to dye silk *(4)*, and the effect of different mordants on the color and hue obtained with the same dye *(5,6)* (for more information on mordants and mordant-wool bonds please see the Supplementary Material). A survey on interesting internet sites dealing with the use of natural dyes was also published in this Journal in 2008 *(7)*.

This laboratory experimental project brings students awareness to the use of natural dyes and to the importance of their study for purposes of cultural heritage and conservation of museum pieces. The chromatographic analysis of dyes extracted from

textiles of historical interest can give valuable information as to where, when and how the textiles were produced, and students will be using high pressure liquid chromatography (HPLC), the common analytical methodology to do it. This protocol was initially used in a workshop for the general public with interest in conservation science and later in an undergraduate 2nd year course on natural product chemistry.

A general introduction on the use of natural dyes was found to be essential because students were generally not aware of this subject. Information was given on the different classes and sources of natural dyes *(1-3)*, and also on the analytical methodology that can be used to extract and identify the natural dyes on historical textiles in the perspective of their importance in cultural heritage studies *(8-16)*.

LABORATORY SUMMARY

Student's directions and information for faculty are provided in the online documentation, but the experimental procedure is briefly summarized here.

To prepare the dye bath 2.0 g of yellow onion skins were boiled in 50.0 mL of water for 15 min, after which the solids were removed by filtration and the supernatant reserved. Hydrated potassium aluminum sulfate (alum), 0.50 g, was dissolved in 30.0 mL of water, and after addition of 1.0 g of white wool the mixture was heated at 90 ºC for about 15 min. The wool was removed, washed with distilled water and allowed to air dry.

The mordanted wool was then introduced in the dye bath, and the solution was heated to 90 °C for another 15 min. The dark yellow dyed wool was removed, washed with distilled water and allowed to air dry.

Two methods were used to extract the dyes from the wool namely the hydrochloric acid (HCl) method (*16*) and the ethylenediaminetetracetic acid disodium

salt/dimethylformamide (EDTA/DMF) method (*11*). Briefly, for both methods 20.0 mg sample of dyed wool were weighted and transferred in two test tubes with a small magnetic stirrer. Four mL of concentrated HCl/methanol/H_2O, 2:1:1 (*v/v/v*) and 10.0 mL of 0.1 % EDTA in H_2O/DMF 1:1 (*v/v*) were added to the test tubes respectively and the mixtures were heated at 100 ^oC for 10 min for the HCl method and 15 min for the EDTA/DMF method. After cooling to room temperature the solutions were filtered through a 0.45 µm filter and reserved for chromatographic analysis.

The HPLC-DAD analysis of the dye bath, the wool extracts and the standards of quercetin and quercetin-4´-O-glucose were accomplished with a 150×4.6 mm 3.5 µm C-18 column with DAD detection from 200 to 450 nm; chromatograms were recorded at 350 nm. A mobile phase consisting of two solvent systems (A: methanol, B: water with 0.1 % *v/v* formic acid) employed the following gradient program: 0-100 % A, 0-15 min; 100 % A, 15-20 min. The flow rate was 0.5 $mLmin^{-1}$ and the injection volume was 20 µL. Equilibration time of 5 min with 100 % B between sample injections.

Due to the length of the analyses and the use of manual injection students cannot perform all aspects of the analyses, and the UV spectra of quercetin and quercetin-4´-O-glucoside, used to identify both analytes in the chromatograms, are provided by the instructor. The waiting periods were used to read and review some literature reports dealing with the identification and use of natural dyes as well as their study in historical textile artifacts (see supplementary material for suggestions).

The wool final hue is strongly dependent on the dyeing conditions *(5)*. Instructors can introduce differences in the proposed protocol and encourage the students to look and discuss the HPLC data taking in consideration the wool final hue.

HAZARDS AND MATERIALS DISPOSAL

Formic acid and HCl solutions are corrosive to eyes, skin and mucous membranes. Methanol and dimethylformamide are toxic and the latter is highly flammable. Flavonoid compounds can be toxic if swallowed. EDTA may irritate the lungs if inhaled. Students should wear chemical safety goggles and compatible chemical-resistant gloves. The solvents should be handled in a well ventilated hood. Solution disposal should take into consideration the toxicity of the materials.

RESULTS AND DISCUSSION

The experimental procedure was used in an undergraduate course on natural product chemistry where the information on the sources of natural dyes provided very interesting discussions on the origins of the color and how color is perceived by humans and animals. The flavonoids, the chromophore molecules in the onion skins, are originated from two biosynthetic routes (shikimate and acetate pathways *(17)*) which makes them a very interesting subject for teaching purposes. Apart from the dye properties explored with this laboratory project, flavonoids exhibit an array of biological properties namely, antioxidant, anti-carcinogenic, anti-inflammatory among others *(18,19)*.

Figure 2 presents the HPLC chromatograms of the dye bath (A), the wool extracts obtained with HCl (B) and EDTA/DMF (C). Identification of the analytes was easily achieved by individually injecting the two standards phenolics solutions on the same conditions used to separate the extracts to record their retention time.

Identification of dyes in historical pieces requires the identification of the major chromophores obtained from a certain dye source; therefore the number of compounds and the amounts extracted are important factors when choosing an extraction procedure.

Acid hydrolysis with heated HCl is still a widely reported extraction method despite the fact these conditions lead to the hydrolysis of flavonoid glycosides common when studying yellow dyed textiles. HCl methodology is normally perceived as the most aggressive and therefore the most efficient methodology. With this project students easily understand that data was lost when the HCl method was applied as only the quercetin peak is observed in the chromatogram (Figure 2B). The EDTA/DMF procedure allowed the extraction of both flavonoids (Figure 2C) yielding therefore more information. Quercetin is a widely distributed flavonoid in plants and it can originate from different sources. If the dye source was unknown, it would have been difficult to attribute the onion skins as the dye source based solely on chromatogram 2B. Instructors with some experience on HPLC method development can easily perform this experiment using the same chromatographic system with small alterations in the gradient and other common dye sources, namely, weld (*Reseda luteola* L.) whose major chromophore is the flavonoid luteolin *(2,3)*, madder (*Rubia tinctorum* L.) which has the anthraquinones purpurine and alizarin as their major red colorants *(2,3)*, or cochineal which has the carminic acid derivatives as their red chromophores *(2,3)*. Indigo (*Indigofera tinctorum* L.) can also be used but the procedure to dye the textile fibers in blue is different because indigo is a vat dye and does not require mordants *(20,21)*.

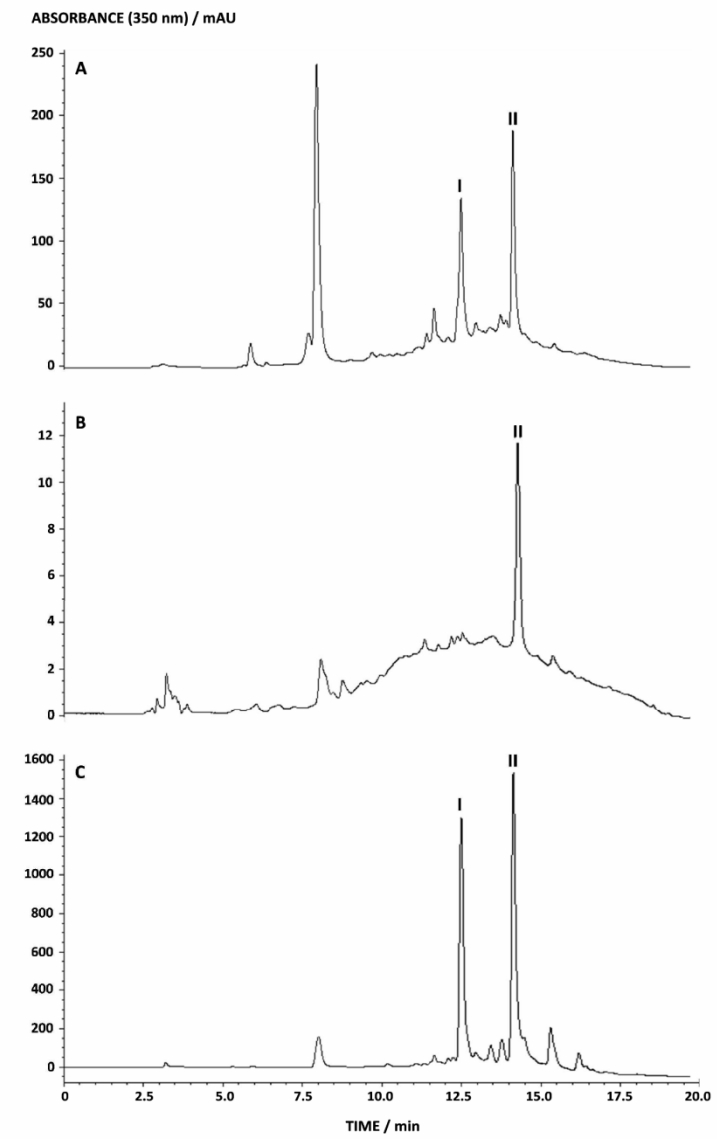


Figure 2. HPLC-DAD chromatograms recorded at 350 nm of (A)-onion skins dyed bath; (B)-HCl wool extract; (C)-EDTA/DMF wool extract. Identification of the peaks: I-quercetin-4´-O-glucoside; II-quercetin.

Mass spectrometry detection is a much more powerful technique than ultraviolet detection and, whenever available, instructors are encouraged to use it. A mass detector with an ESI (electrospray ionization) interface can be used to identify the major phenolic compounds in the onion skins *(22, 23)* without the need of standards.

CONCLUSIONS

This laboratory experiment was done with chemistry and biochemistry students in a natural product chemistry undergraduate course, bringing awareness to the use of plants as natural dyes.

Apart from the discussion on the role of the flavonoids as products of the secondary metabolism of the plants and their biosynthesis, this laboratory project enabled the students to perceive that worldwide plants were the main sources of color before the synthetic dyes became available in the second half of the XIX century.

Acknowledgements: The authors acknowledge the financial support of Project REMATAR, FCOMP-01-0124-FEDER-010482 (FCT PTDC/HAH/64045/2006) from the Portuguese Foundation for Science and Technology, FCT. A. Manhita also acknowledges FCT for the PhD fellowship (SFRH/BD/22411/2005).

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*Manuscript

TITLE: Ageing of brazilwood dye in wool – a chromatographic and spectrometric study

AUTHORS:

Ana Manhita[a-c], Vanda Santos[c], Helena Vargas[d], Isabel Ribeiro[d], Teresa Ferreira[a-c], António Candeias[a-d], Cristina Barrocas Dias[a-c*]

[a] HERCULES Laboratory, Largo Marquês de Marialva 8, 7000-554 Évora, Portugal

[b] Évora Chemistry Centre, Rua Romão Ramalho 59, 7000-671 Évora, Portugal

[c] University of Évora, Chemistry Department, Rua Romão Ramalho 59, 7000-671 Évora, Portugal

[d] José de Figueiredo Conservation and Restoration Laboratory, Institute of Museums and Conservation, Rua das Janelas Verdes 37, 1249-018 Lisboa, Portugal

*Corresponding author: cmbd@uevora.pt (C. B. Dias; Assistant Professor)

Phone: +351266745343; Fax: +351266745349

E-mail addresses and positions:

anaccm@uevora.pt (A. Manhita; Researcher)

vanda.1984@sapo.pt (V. Santos; Researcher)

lena_na_gabela@yahoo.com (H. Vargas; Researcher)

isarib@sapo.pt (I. Ribeiro; Researcher)

tasf@uevora.pt (T. Ferreira; Assistant Professor)

candeias@uevora.pt (A. Candeias; Assistant Professor)

Acknowledgements

The authors acknowledge the financial support of Fundação para a Ciência e a Tecnologia (FCT) through project FCT/HAH/64045/2006. A. Manhita also acknowledges FCT for the PhD fellowship (SFRH/BD/22411/2005).

ABSTRACT

Brazilwood was used in this work to dye wool mordanted with different amounts of copper (II) sulfate, alum and iron (II) sulfate. Two different dyeing methods were used: pre-mordanting (MD) and simultaneous mordanting (M+D) procedures. In order to evaluate the influence of the mordant ion in the brazilein chromophore photodegradation, samples were subjected to artificial light ageing. Colour measurements were made and, for the first time, LC-DAD-ESI-MS was used for chromophore analysis of the dyed fibres before and after light exposure. Mordant ion quantification was done after fibre acid digestion, by ICP-OES and FAAS.

Mordant metal ion, mordant bath concentration and dyeing procedure were found to have strong influence in the wool fibre hues. Colour variation was more pronounced in the alum dyed samples. Overall, mordant quantification showed that the amount of metal ions found in the fibres is very small when compared to the original concentrations of the dyeing bath, being Cu the ion with greater affinity for the wool fibres. MD dyeing procedure yielded fibres with larger amounts of metal ions and higher chromophore peak areas. Higher amounts of brazilein were extracted from wool mordanted with copper (II) sulfate. Finally, chromatographic analysis of the brazilwood dyed samples before and after light

exposure resulted in the detection of Type C compound as an outcome of the photodegradation process.

Keywords: **Brazilwood / Wool / Mordant / Photodegradation / LC-MS / ICP-OES / FAAS**

1. Introduction and research aims

The origins of dyeing are uncertain – the production of fabrics and their coloration precedes recorded history – but it is believed that several cultures had established dyeing technologies before 3000 BC [1,2]. It is likely that the ancient art of dyeing originally spread westwards from India, and it may well have been accidental staining from berries and fruit juices that initially stimulated its development [2-4]. Natural dyes were extracted from plant and animal sources with water, sometimes under conditions involving fermentation. Fabric was dyed by soaking it in the aqueous extract and drying [1].

Although water-dispersed colourants were used in paints 30 000 years ago, they easily washed off any material coloured with them. Dyes had only a limited range of dull colours and the dyeings invariably had poor fastness to washing and sunlight [1]. The fastness of a dye is a measure of its resistance to fading, or colour change, on exposure to a given agent or treatment. Wash-fastness can be improved if the fabric was first treated with a solution containing a salt of, for example, aluminium, copper or iron [1,5]. These metal salts are called mordants. When the pre-mordanted fabric is soaked in a bath of a suitable natural dye (or soaked in a bath containing the dye and the mordant), the dye penetrates into the

fibres and reacts with the metal ions present. This reaction decreases the water solubility of the dye, so the colour is less likely to bleed out on washing [1].

Soluble redwood dyes are extracted from various species of the genus *Caesalpinia*. The principal varieties are Brazilwood, Peachwood, Sappanwood, Limawood and Pernambuco wood, though they are frequently collectively known as Brazilwood [5-7]. The main colouring constituents of brazilwood are the homoisoflavonoids brazilin (*Fig. 1 (a)*) and brazilein (*Fig. 1 (b)*). Brazilin has been isolated, which by oxidation gives brazilein, the main chromophore in brazilwood [5,8,9]. The tree is indigenous to the East Indies, Central and South America and also to Africa. Brazilwood was known in Europe long before the discovery of South America because it was imported as a dyewood from the East Indies. Soon after the discovery of South America, large quantities of the valuable redwood were found in the forests along the Amazon and the newly discovered country was then given the name 'Brazil' [5,10,11].

The soluble brazilwood dyes wool and cotton with the assistance of a mordant. Different dye-mordant combinations can produce a wide range of colours. However, when exposed to light these colours easily fade or even change. Minimizing those fading changes has become crucial in conservation science [12]. Since lighting conditions carry the risk of causing irreversible colour changes, it is reasonable to seek a deeper understanding of the process of light fading. Few studies have been published concerning the influence of light exposure on brazilwood dyed textiles. Padfield and Landi [13] have studied the lightfastness of natural dyes in wool and cotton dyed fibres. Several mordants were used in the dyeing procedures and brazilwood was found to fade extremely fast when exposed to light. Zarkogianni *et al.* [14] also dyed cotton and wool fabrics with

several natural sources, including brazilwood, and used a variety of mordants. Wash, light and rub fastness were tested and colour measurements were made [14]. The authors concluded that, in general, pre-mordanting the fibres improves wash fastness for brazilwood wool dyed samples. Light fastness wasn't enhanced, except when using iron-based mordants. Rub fastness didn't improve after mordanting [14].

The aim of this work was to evaluate the influence of the mordant ion in the brazilein chromophore photodegradation. Brazilwood was used to dye wool mordanted with different amounts of copper, alum and iron salts. Two different dyeing methods were used: pre-mordanting and simultaneous mordanting procedures. Wool dyed samples were subjected to artificial light ageing in a solar box in order to simulate natural ageing. Samples were collected at different time intervals and differences in colour hues were measured by colourimetry, before and after light exposure. Mordant ion quantification was done, after fibre acid digestion, by inductively coupled plasma-optical emission spectroscopy (ICP-OES) and flame atomic absorption spectrometry (FAAS). After dye extraction from the wool samples, liquid chromatography coupled to diode-array and electrospray ionization mass spectrometry detectors (HPLC-DAD-ESI-MS) was used to evaluate chromophore behaviour throughout the ageing process.

2. Experimental section

2.1. Materials and reagents

The following reagents (analytical grade) were used: *N,N*-dimethylformamide and nitric acid (65 %) from Panreac (Barcelona, Spain),

EDTA dissodium salt from Sigma-Aldrich (Milwaukee, WI, USA), iron (II) sulfate pentahydrate (99 %) from Scharlau Chemie, S.A. (Barcelona, Spain) and copper sulfate pentahydrate (99 %) from Himedia Laboratories (Mumbai, India). Methanol and formic acid (HPLC gradient grade) from Merck (Darmstadt, Germany) were used in LC-MS analysis. For mordant quantification, aluminium and iron standards were inductively coupled plasma grade or equivalent, from Fluka (Buchs, Switzerland), and copper standard was spectroscopic grade, also from Fluka (Buchs, Switzerland). Alum and brazilwood (*Caesalpinia echinata* L.) were purchased from Kremer Pigmente (Aichstetten, Germany).Water from a Millipore Simplicity UV system (Billerica, MA, USA) was used throughout this work, including sample preparation. Undyed industrial Arraiolos sheep wool was acquired from Rosarios4 (Mira de Aire, Portugal).

2.2. Wool dyeing

Two methods were used for wool dyeing, which included a pre-mordanting procedure (MD procedure) and a simultaneous mordanting procedure (M+D procedure):

2.2.1. MD procedure

Sheep wool (1.0 g) was mordanted for 30 min in 50 mL of boiling water containing different amounts of mordant salts (concentration and sample notation in *Table 1*). Afterwards, the wool was removed and rinsed with cool water. The dye baths were prepared with 2.0 g of brazilwood immersed in 50.0 mL H_2O and heated at about 90 ºC for 30 min. Final volume was kept constant. The solution was allowed to cool and after simple filtration of the plant material, the previously

mordanted wool was added to the dye solution bath and reheated at 90 ˚C for 30 min.

2.2.2. M+D procedure

The dye bath was prepared as described for the MD procedure. After plant material filtration, the different amounts of mordant salts were added (concentration and sample notation in *Table 1*) to each solution. When the mordant salts were dissolved, 1.0 g of sheep wool were immersed in each solution that was kept at 90 ºC for 30 min. After the dyeing procedure, all wool samples were thoroughly rinsed with ultrapure water and left to dry in the open air protected from light.

2.3. Colourimetric studies

Datacolor International Mercury portable spectrophotometer used for colourimetric studies (L^*, a^* and b^*- CIELab space defined by Commission Internationale de l´Eclairage in 1976). The spectrometer was equipped with a Xenon lamp and a photodiode sensitive to the 360-750 nm spectral range. Black and white standards were used for calibration. Iluminant CIE D65; 10º of observation angle and specular component excluded. Analyses were performed in three different points of each wool sample, with the average value used for data interpretation.

2.4. Mordant quantification

2.4.1. ICP-OES analysis

Analyses were performed in an inductively coupled plasma-optical emission Horiba Jobin-Yvon spectrophotometer, model Ultima, equipped with an RF generator of 40.68 MHz and a Czerny-Turner monochromator with 1.00 m (sequential). Three analyses were done for each sample and the conditions were the following – potency: 1200 kW, argon flow: 12 dm^3/min, nebulisator: Mira Mist with 3 bar pressure, pump velocity: 15 rpm. Fe and Al calibration standards were prepared by diluting the respective stock solution to obtain a 5 point calibration curve (0.01 – 1.00 mg/dm^3; $R^2 = 0.9999$). For sample preparation, 1.5 mg of wool samples and 1.0 mL of concentrated HNO_3 were added to an *eppendorf* tube and digested in an ultrasound bath, without temperature control, until sample solubilisation (30 to 60 min). The solution was then transferred to a 25 mL flask and the volume was adjusted with ultrapure water. Replicate analyses were performed to evaluate analytical methodology reproducibility.

2.4.2. FAAS analysis

Analyses were performed on a Perkin Elmer 3100 flame atomic absorption spectrometer equipped with a Cu cathode lamp (resonance line at $\lambda = 324.8$ nm, 0.7 nm slit width and 15 mA lamp current intensity) and an air/acetylene flame. Six working solutions (0.50 – 5.00 mg/dm^3; $R^2 = 0.9999$) for calibration curve construction were obtained with adequate dilution of a 100 mg/dm^3 solution prepared from a Panreac 1.000 ± 0.002 g/dm^3 Cu stock solution. 50.0 mg of each wool sample were heated (without boiling) on a hot plate with 4.0 mL of concentrated nitric acid until the wool fibre was digested. The sample was then transferred to a 50 mL volumetric flask and the volume was adjusted with

ultrapure water. Replicate analyses were performed to evaluate analytical methodology reproducibility.

2.5. Accelerated ageing studies

A UV-Vis Solarbox 3000E equipped with a Xe lamp and a 310 nm filter was used. The ageing conditions were as follows – temperature: 55 ºC, irradiance: 400 W/m^2. Samples were collected after 48, 120, 360, 480, 600, 720, 840 and 960 h of light exposure.

2.6. Chromophore analysis

2.6.1. Chromophore extraction procedure

20 mg of dyed wool were placed in vials and 1 mL of 0.1 % EDTA in water/DMF (1:1, *v/v*) solution was added. The vials were capped and kept at 100 °C in liquid paraffin for 30 min. After it, they were cooled to room temperature and the samples were lyophilized. The dried residues were dissolved in 1.0 mL of MeOH/H_2O (1:1, *v/v*). All solutions were filtered through a 0.45 µm PTFE filter prior to analysis. Replicate analyses were performed to evaluate analytical methodology reproducibility.

2.6.2. LC-DAD-ESI-MS analysis

LC-DAD-ESI-MS analyses were carried out in a LCQ Fleet ThermoFinnigan mass spectrometer equipped with an ESI source, using an ion trap mass analyzer and a PDA detector (San Jose, CA, USA). The MS and PDA equipments were coupled to an HPLC system with autosampler (Surveyor ThermoFinnigan). The analytical column was a reversed phase Fortis-C18 (Fortis

Technologies; C_{18}, particle size 3.0 µm, 150 mm × 2.1 mm), used with a 0.2 mL/min flow and sample injection volumes of 10 µL. Column temperature was set at 30 ºC and tray temperature was set at 24 ºC.

For brazilein identification in brazilwood dyed samples before and after light exposure the following conditions were used: capillary temperature of 300 ºC, source voltage of 5.0 kV, source current of 100.0 µA and capillary voltage of -20.0 V, in negative full MS mode (*m/z* 100-600). PDA detector was set at 200-800 nm. The mobile phase consisted of acetonitrile (A) and water acidified with 0.1 % formic acid (B). The gradient used was 0-90 % A from 0-20 min, then 90 % A from 20-30 min. For brazilein MS quantification, the same capillary temperature, source voltage and source current were used. A capillary voltage of -25.0 V was used in negative ion mode. Brazilein was detected in SIM mode at *m/z* 283 and 100 % methanol was used as mobile phase.

3. Results and discussion

3.1. Colourimetry

Colour is a complex phenomenon. In the light–sample interaction different physical phenomena are observed: transmission, absorption, scattering, refraction, etc. One way of describing sample colour is to use numerical terms, which can be converted to CIE (Commission Internationale de l'Eclairage) colour specifications. The principal attributes of sample colours are lightness or value (L^*), chroma or saturation (C^*) and hue (h) [15,16]. Lightness considers colour as a source of reflected light ranging from black to white. Chroma is correlated to the degree of gray tone of the colour and is an indicator of colourfulness, so it

accounts for the effects of discolouration [17]. Hue is expressed in degrees and defines the tonality that we normally identify with the name of a colour (red, yellow, green or blue) [15,17].

Colour fading can be evaluated in terms of colour difference using the following formula of CIE Committee in 1976, $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$, where ΔL^* = lightness-darkness difference, Δa^* = redness-greeness difference and Δb^* = yellowness-blueness difference [17].

Table 2 presents the CIE*Lab*/*Ch* colour parameters for dyed samples preserved from light and after light exposure. As it can be seen, mordant metal ion, mordant bath concentration and dyeing procedure have strong influence in the wool fibre hues. For t = 0 h and using the MD procedure, red colours were observed when using Al as mordant. When using Cu and Fe as mordants, wool fibre hues ranged from red-brown to grey hues. In the case of the M+D methodology, at t = 0 h, purple, green or bluish hues can be observed when using Al, Cu and Fe as mordants, respectively. In general, the lightness of the samples increases during light exposure. As for the chroma value, the most systematic decrease observed during the ageing process is presented by the Al mordanted samples dyed by MD procedure.

For the results presented in *Fig. 2* it can be observed that, in general, fading is more pronounced in the first 400 h of light exposure and tends to stabilize beyond 600 h of light exposure. Colour variation was more pronounced for the alum dyed samples, as already observed by Crews [18], in wool dyed with natural yellow dyes, and by Manhita *et al*. [19], in madder dyed wool samples.

Apart from the mordant bath concentration, the dyeing technique had influence in the ΔE difference for the samples mordanted with Al and Cu.

Samples dyed by MD procedure are more affected by photofading. As for the Fe salt at a concentration of 0.0016 M, the colour variation is more accentuated in the first 48 h for the M+D procedure.

For Al mordanted samples, ΔE variation showed little dependence from the salt concentration. In the case of Fe mordanted samples, for the MD procedure, colour variation is independent from the concentration. Colour variation is also independent from the dyeing procedure for 0.0400 mol/dm^3 Fe salt bath concentration.

As for Cu mordanted samples, no stabilisation of the ΔE parameter was observed up to 960 h of light exposure for samples prepared by the M+D method.

3.2. Mordant evaluation

Brazilwood dyed wool samples were digested with nitric acid and analysed by ICP-OES (Al and Fe) and FAAS (Cu). These techniques were found to be highly reproducible with an RSD ≤ 5 % (data not shown).

Although larger mass values of metal ions were available in the dyeing baths (*Table 1*), the actual amounts of mordant metal ions found in the wool samples after HNO_3 digestion were, in general, much lower.

An increase in the mordant bath concentration results only in a small increase in the fibre metal ion content (*Fig. 3*). Nevertheless, the ratio between the metal ion content in the fibre and the mass of metal ion present in the dyeing bath is larger for the smaller bath concentrations of mordant salts. Cu ions seem to have greater affinity for the wool fibres and, in the case of CuMD16 samples, the mordant was almost fully bound to the fibre. Copper salts are well known for their ability to form complex amines [20]. Moreover, it has been previously established

that Cu ions react not only with the carboxylate groups in wool but also with the products of the cystine hydrolysis of wool keratin that occurs at high temperatures [21,22]. The high copper uptake may be attributed to the formation of copper sulfide and to the carboxylate reactions [21,22].

Overall, for equivalent mordant concentrations, the MD dyeing procedure results in samples with higher amounts of metal ions. Similar results were obtained in previous work [19] for madder dyed wool. This is probably explained by the differences between both dyeing methods. In the M+D method, metal ion and chromophore bind before the wool is added to the dyeing bath, making more difficult for the metal to bind to the wool protein structure. In the MD procedure, metal ion binds to wool beforehand, thus resulting in higher amounts of metal ions in the fibres.

3.3. Chromatographic studies

Brazilwood is well-known for its poor light fastness [10] but, as far as the authors are concerned, no study on the chromophore variation through light ageing has yet been published.

For the evaluation of brazilein in the wool before and during the light ageing process, LC-ESI-MS/MS technique was used. Samples were first subjected to a mild extraction procedure using Na_2EDTA/DMF [23,24]. *Fig. 4* represents the chromatographic peak areas of brazilein dyed wool before (t = 0 h) and after (t = 240, 600 and 960 h) light exposure.

For the same mordant, samples dyed by the MD procedure yielded higher brazilein peak areas (*Fig. 4*). In the MD dyeing procedure, higher amounts of

metal ions are bound to the fibres, probably resulting in a higher rate of metal-chromophore complexes attached to the wool fibres.

Brazilein degradation rate was not constant for both dyeing procedures, being overall more accentuated in the first 240 h for the MD dyeing method (*Fig. 5*), which is in agreement with the colour fading behaviour observed in the colourimetric studies.

For the same dyeing procedure, the use of copper sulfate as mordant results in an extraction of higher amounts of brazilein (*Fig. 4*). As already stated, this mordant seems to have greater affinity for the wool fibres (*Fig. 2*), probably allowing for a larger number of available sites for the formation of the brazilein-copper complexes.

The chromatographic profiles of brazilwood dyed wool AlMD1 before (t = 0 h) and after (t = 960 h) light exposure are shown in *Fig. 6*. An alum mordanted sample was chosen because of the historical importance of this mordant. Peak identification was done using UV-Vis and mass spectra (negative mode). As expected, at t = 0 h, the major compound identified in the brazilwood dyed wool sample was brazilein ($[M-H]^-$ *m/z* 283). After 960 h of light exposure, brazilein was still identified as the major compound in brazilwood dyed wool, but a new peak eluted at r_t = 18.8 min, yielding a deprotonated molecular ion $[M-H]^-$ at *m/z* 243 and UV-Vis characteristic bands at 257, 307 and 337 nm was detected. This compound, whose structure is not yet determined, is generally known as Type C compound and was first described by Nowik [9], after acid hydrolysis of several species of soluble redwoods, collectively known as brazilwood. Type C compound has also been described by Karapanagiotis *et al.* in samples collected from Cretan icons [25] and post-Byzantine textiles [26], but no explanation was

given for the nature of this compound. In the present work, no acidic extraction method was used for the chromophore extraction from dyed wool, thus the resulting Type C compound cannot be considered a product of an acid hydrolysis. Therefore, the authors believe that Type C compound can be considered a photodegradation product of brazilwood dye and that the detection of this analyte in historical textile samples can be used to identify brazilwood as dye source.

4. Conclusions

Fibre colour hue is highly dependent of mordant ion nature, mordant bath concentration and dyeing method, and this is probably a consequence of the different metal-chromophore complexes formed during the dyeing process.

Mordant quantification showed that the amount of metal ions found in fibres is very small when compared to the concentrations added to the dyeing bath. However, for the smaller concentrations of mordant salts, the ratio between the metal ion content in the fibre and the mass of metal ion present in the dyeing bath is higher. Cu ions seem to have greater affinity for the wool fibres than Al or Fe ions – for equivalent mordant bath concentrations, the uptake of Cu in the fibres is at least twice the amounts of Al or Fe. MD dyeing procedure yielded fibres with larger amounts of metal ions, which is likely due to the fact that metal ion binds to wool before complexing with the chromophore, thus resulting in higher amounts of metal ions in the fibres. This behaviour can also be used to explain the larger chromophore peak areas presented by MD dyed samples – if higher amounts of metal are bound to the fibre, it is likely that a higher number of wool-metal-brazilein structures are formed and then extracted for chromatographic analysis.

Brazilein degradation rate was not constant for both dyeing procedures, being overall more accentuated in the first hours for the MD dyeing method, which is in agreement with the colour fading behaviour observed in the colourimetric studies. Colour variation (ΔE) was more pronounced for the alum dyed samples. Chromatographic analysis on brazilwood dyed samples before and after light exposure detected the formation of Type C compound during the light ageing process. The latter had already been detected in historical samples but no hypothesis for its origin was clearly presented. The compound seems to be more a photodegradation product of brazilwood dye rather than an artefact caused by acid hydrolysis of the samples, as a mild EDTA-based extraction procedure was used in the present work. Therefore, the detection of this compound in historical textile samples can be used to identify brazilwood as dye source.

The chromatographic and spectrometric approach used in this work allowed a better understanding on the influence of mordant, dyeing technique and photodegradation of brazilwood dyed textiles, altogether, resulting in a useful contribute to the field of textile conservation.

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Table 1

Mordant salt bath concentration (mol/dm^3) and corresponding mass of metal ion available in the dyeing bath per gram of wool (mg/g). Sample notation presented was used throughout this manuscript.

Mordant metal ion	Mordant salt bath concentration (mol/dm^3)	Mass of metal ion available in the dyeing bath per gram of wool (mg/g)	Sample notation	
			M+D procedure	MD procedure
Al^{3+}	0.1000	135	AlM+D1	AlMD1
	0.0085	11	AlM+D85	AlMD85
	0.0030	4	AlM+D3	AlMD3
Cu^{2+}	0.0400	128	CuM+D4	CuMD4
	0.0016	5	CuM+D16	CuMD16
Fe^{2+}	0.0400	112	FeM+D4	FeMD4
	0.0016	5	FeM+D16	FeMD16

Table 2

Colourimetric data for dyed samples preserved from light (t=0 h) and after light exposure (t=48, 120, 240, 360, 480, 600, 720, 840 and 960 h).

		Al						Fe				Cu			
		M+D			MD			M+D		MD		M+D		MD	
Time in the solar box (h)	CIE*Lab*/*Ch* colour parameters	AlM+D1	AlM+D85	AlM+D3	AlMD1	AlMD85	AlMD3	FeM+D4	FeM+D16	FeMD4	FeMD16	CuM+D4	CuM+D16	CuMD4	CuMD16
0	*L**	37.47	34.29	34.00	34.80	38.76	39.51	35.40	53.62	22.96	25.54	20.58	24.62	23.42	21.50
	*a**	10.72	11.21	9.93	45.55	43.27	38.52	1.47	1.49	6.41	5.90	0.54	-0.55	17.37	12.13
	*b**	-9.61	-8.62	-7.48	21.33	19.69	18.40	-7.29	-8.19	0.10	1.15	-4.55	-3.73	5.72	1.86
	*C**	14.40	14.14	12.43	50.29	47.54	42.69	7.44	8.33	6.41	6.01	4.58	3.77	18.29	12.27
	h°	318.13	322.44	323.01	25.09	24.47	25.53	281.43	280.29	0.88	11.05	276.73	261.62	18.24	8.73
48	*L**	40.43	35.81	37.64	37.08	42.10	46.58	35.64	41.58	22.93	26.16	21.02	23.63	22.78	25.09
	*a**	9.80	8.91	8.13	38.49	31.80	26.06	0.82	0.62	5.78	5.09	-0.59	-0.61	17.42	12.59
	*b**	4.19	2.71	3.38	20.76	19.07	19.05	1.85	5.03	1.81	3.51	-2.46	-1.41	6.58	3.70
	*C**	10.65	9.31	8.81	43.73	37.08	32.28	2.02	5.07	6.05	6.18	2.53	1.54	18.62	13.12
	h°	23.15	16.91	22.56	28.34	30.95	36.17	65.95	82.93	17.40	34.58	256.51	246.71	20.69	16.36
120	*L**	43.31	38.29	39.45	43.25	50.84	54.64	37.11	42.33	25.97	31.52	20.58	27.05	26.70	27.69
	*a**	8.70	8.51	7.72	33.06	24.55	20.55	0.90	0.92	5.50	5.24	-0.95	-1.32	19.45	16.09
	*b**	9.08	5.09	6.14	22.01	20.07	20.96	4.30	6.98	3.64	4.95	-1.97	0.43	8.30	6.22
	*C**	12.58	9.91	9.86	39.71	31.71	29.35	4.40	7.04	6.59	7.21	2.18	1.39	21.14	17.25
	h°	46.21	30.89	38.52	33.65	39.26	45.57	78.21	82.46	33.49	43.33	244.19	161.94	23.12	21.14
240	*L**	51.05	44.50	48.20	53.08	58.20	62.46	42.97	47.92	30.70	36.79	22.56	30.81	32.23	36.22
	*a**	7.58	7.04	6.23	22.99	17.08	13.21	0.93	0.69	5.28	4.87	-1.01	-1.00	17.48	14.42
	*b**	13.87	8.03	9.78	22.44	19.95	21.26	7.66	9.36	6.71	8.79	-0.75	1.94	10.45	10.36
	*C**	15.80	10.68	11.60	32.13	26.26	25.03	7.72	9.39	8.54	10.05	1.26	2.18	20.36	17.76
	h°	61.36	48.76	57.49	44.30	49.43	58.14	83.07	85.81	51.79	60.98	216.58	117.33	30.88	35.70
360	*L**	53.25	49.91	53.44	58.15	61.41	67.26	45.20	48.07	34.78	38.81	21.66	31.77	34.01	39.19
	*a**	5.88	5.11	4.13	16.43	11.82	8.91	0.89	0.79	4.82	4.10	-1.87	-1.64	14.65	11.22
	*b**	15.95	9.14	11.61	22.27	18.90	20.67	9.49	10.18	9.66	9.95	0.35	5.11	12.18	12.02
	*C**	17.00	10.47	12.33	27.67	22.29	22.51	9.53	10.21	10.80	10.76	1.90	5.36	19.05	16.44
	h°	69.76	60.78	70.44	53.58	57.98	66.69	84.61	85.56	63.51	67.59	169.52	107.81	39.75	46.99
480	*L**	54.14	50.53	55.73	64.07	63.54	69.90	48.00	53.02	39.79	43.11	23.66	35.52	39.38	47.57
	*a**	6.25	4.29	2.89	12.57	9.16	6.53	1.01	0.78	4.70	3.98	-1.18	-1.61	12.93	9.24
	*b**	15.86	10.37	11.88	22.43	18.39	19.13	9.96	10.98	11.34	11.39	0.90	6.48	13.62	14.81
	*C**	17.05	11.22	12.23	25.71	20.54	20.21	10.01	11.01	12.27	12.06	1.48	6.48	18.78	17.45
	h°	68.51	67.53	76.35	60.74	63.52	71.15	84.19	85.93	67.47	70.75	142.86	103.93	46.49	58.03
600	*L**	58.61	53.43	61.39	66.26	65.83	68.38	49.80	52.87	41.03	43.29	25.05	36.73	42.59	49.78
	*a**	4.04	3.55	2.11	10.36	7.92	4.86	1.23	0.85	4.34	3.68	-1.69	-1.84	11.46	7.92
	*b**	17.36	10.50	12.65	22.82	18.67	17.72	11.21	11.43	12.71	12.72	2.00	6.90	15.09	15.92
	*C**	17.82	11.09	12.82	25.06	20.28	18.38	11.28	11.46	13.43	13.24	2.62	7.14	18.96	17.78
	h°	76.90	71.35	80.54	65.57	67.01	74.68	83.73	85.74	71.15	73.88	130.18	104.95	52.78	63.54
720	*L**	58.57	55.10	61.68	67.85	64.48	69.58	50.18	55.78	43.53	44.77	27.82	40.23	45.11	53.01
	*a**	3.26	2.94	1.56	8.72	5.50	4.29	0.86	0.41	4.15	3.34	-1.81	-1.54	10.40	5.82
	*b**	15.78	10.38	12.07	21.11	16.58	17.22	10.58	11.45	14.10	12.81	3.32	7.77	16.84	15.67
	*C**	16.12	10.79	12.17	22.84	17.47	17.74	10.61	11.46	14.70	13.24	3.78	7.92	19.79	16.72
	h°	78.34	74.20	82.63	67.55	71.65	76.02	85.35	87.95	73.58	75.40	118.65	101.18	58.29	69.61
840	*L**	59.25	58.72	62.08	69.12	67.17	68.74	46.58	54.39	39.06	45.05	25.45	43.14	43.95	52.25
	*a**	2.72	2.16	1.32	7.92	4.89	3.36	0.94	0.65	3.92	3.49	-1.40	-0.88	9.78	6.15
	*b**	14.67	10.85	11.76	21.06	15.55	15.10	10.06	11.29	12.09	13.22	4.26	9.43	15.99	16.32
	*C**	14.92	11.06	11.84	22.50	16.30	15.47	10.10	11.31	12.71	13.67	4.48	9.47	18.75	17.44
	h°	79.51	78.74	83.59	69.39	72.54	77.45	84.68	86.70	72.05	75.21	108.23	95.31	58.55	69.35
960	*L**	61.71	63.34	62.96	70.33	64.72	66.79	49.62	55.84	39.81	45.98	25.74	45.21	45.09	54.27
	*a**	2.47	1.66	1.00	7.01	3.34	2.46	1.30	0.62	3.97	3.39	-1.18	-0.53	9.22	5.48
	*b**	15.14	11.69	11.34	21.00	14.11	13.85	11.34	11.88	11.34	12.77	3.96	11.27	16.06	16.40
	*C**	15.34	11.81	11.38	22.14	14.50	14.07	11.41	11.90	12.01	13.21	4.13	11.29	18.52	17.29
	h°	80.75	81.90	84.95	71.53	76.67	79.91	83.48	87.01	70.68	75.14	106.63	92.70	60.15	71.52

Figure captions

Fig. 1. Chemical structures of brazilin and brazilein.

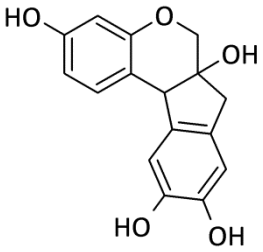
Fig. 2. Fading characteristics of wool dyed by the MD and M+D methods in different concentrations of alum (0.1000, 0.0085 and 0.0030 mol/dm^3), copper (II) sulfate and iron (II) sulfate (0.0400 and 0.0016 mol/dm^3 for both mordants) after t=48, 120, 240, 360, 480, 600, 720, 840 and 960 h of light exposure.

Fig. 3. Quantification of mordant metal ion (Al, Cu, Fe) in wool samples dyed with brazilwood at different concentrations of mordant bath: alum (A), copper (II) sulfate (B) and iron (II) sulfate (C), by the MD and M+D dyeing procedures.

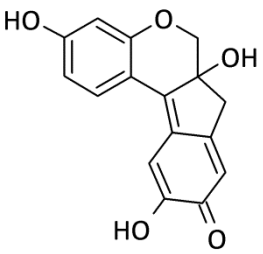
Fig. 4. LC-ESI-MS peak areas of brazilein in wool samples dyed at different concentrations of alum, copper (II) sulfate and iron (II) sulfate by the MD and M+D dyeing procedures before (t = 0 h) and after (t = 240, 600 and 960 h) light exposure.

Fig. 5. Relative LC-ESI-MS peak areas of brazilein in wool samples dyed with different concentrations of alum, copper (II) sulfate and iron (II) sulfate by the MD and M+D dyeing methods before (t = 0 h) and after (t = 240, 600 and 960 h) light exposure.

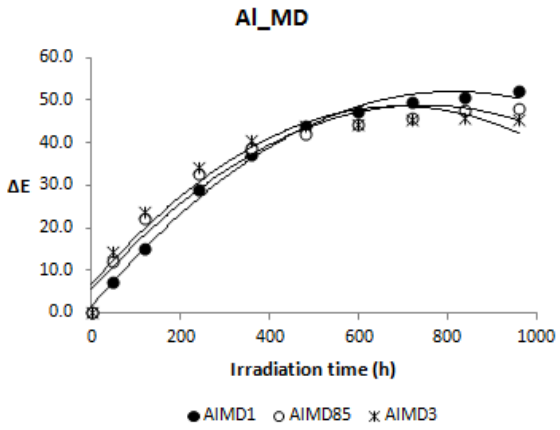
Fig. 6. Chromatographic profiles (DAD detection at 400 nm) of brazilwood dyed wool before (t = 0 h) and after (t = 960 h) light exposure. Brazilein (a) was identified in both samples and type C compound (b) was only detected after light ageing.

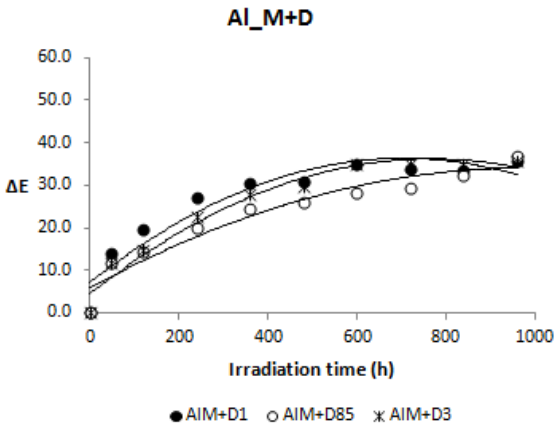


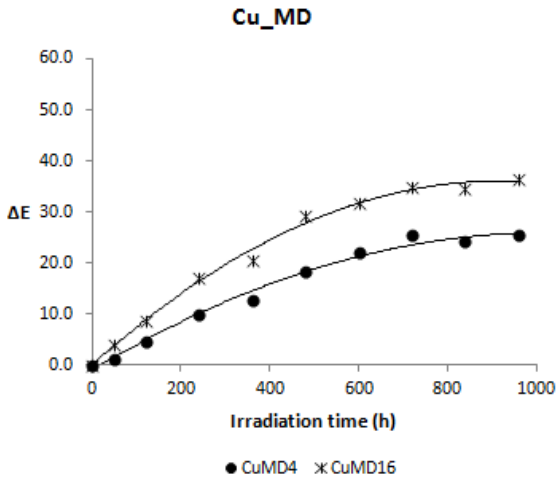
(a) Brazilin

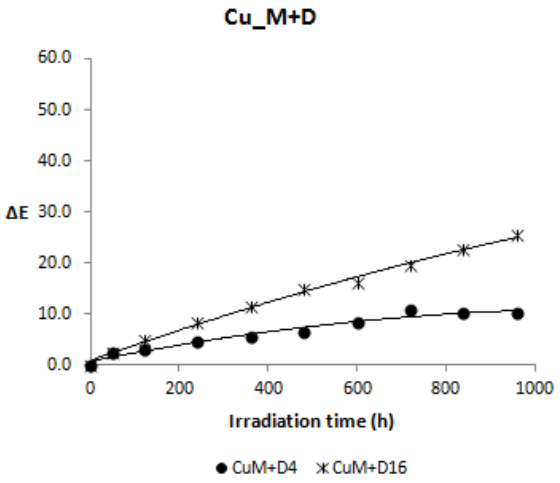


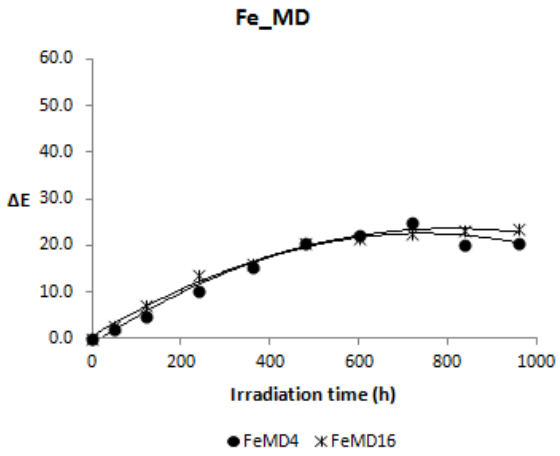
(b) Brazilein

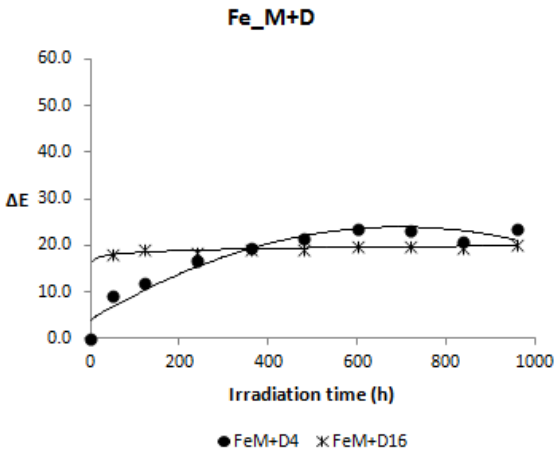

Al_MD
ΔE
Irradiation time (h)
AlMD1
AlMD85
AlMD3

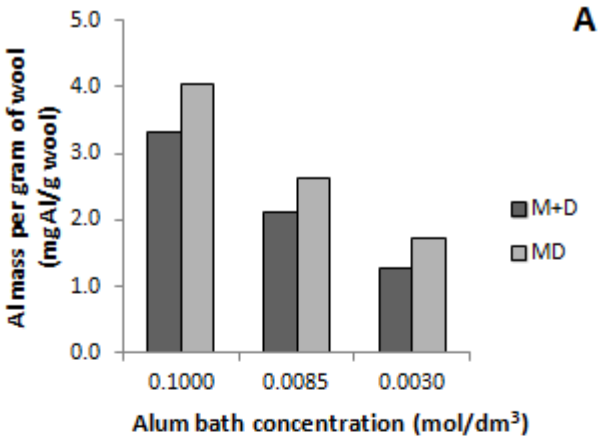

Al_M+D
ΔE
Irradiation time (h)
AlM+D1
AlM+D85
AlM+D3

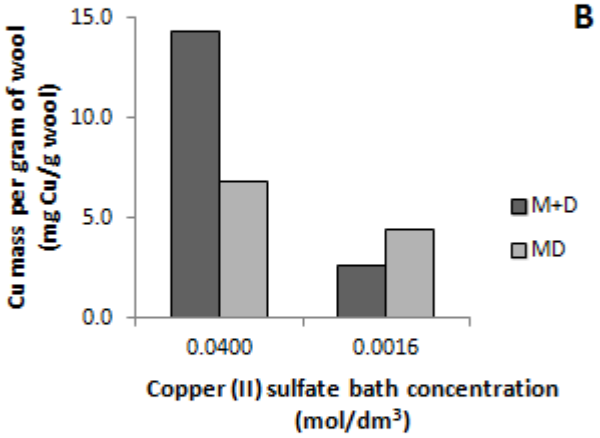

Cu_MD
ΔE
Irradiation time (h)
CuMD4
CuMD16

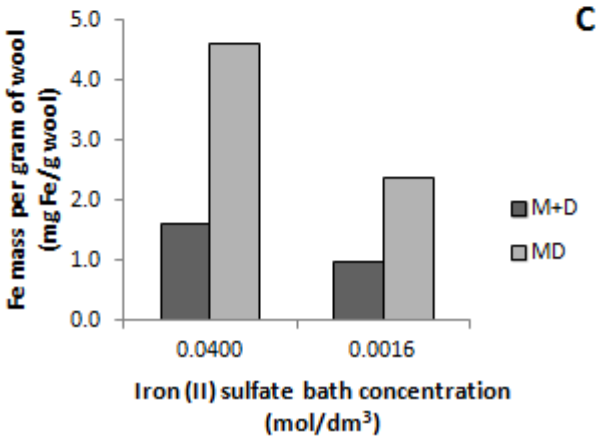

Cu_M+D
ΔE
Irradiation time (h)
CuM+D4
CuM+D16

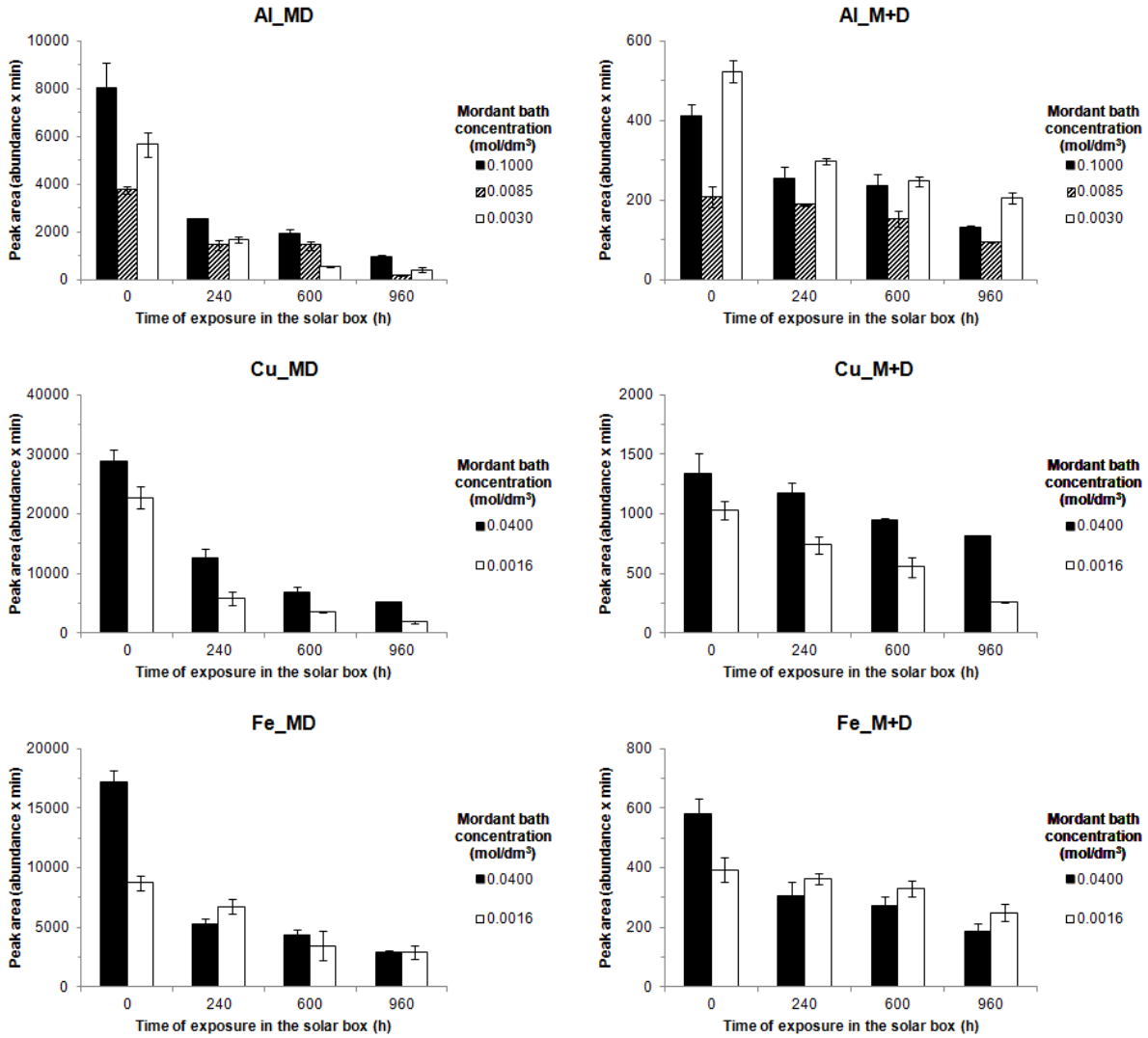

Fe_MD
ΔE
Irradiation time (h)
FeMD4
FeMD16

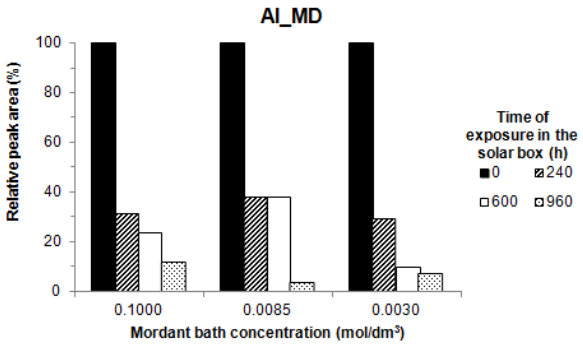

Fe_M+D
ΔE
Irradiation time (h)
FeM+D4
FeM+D16

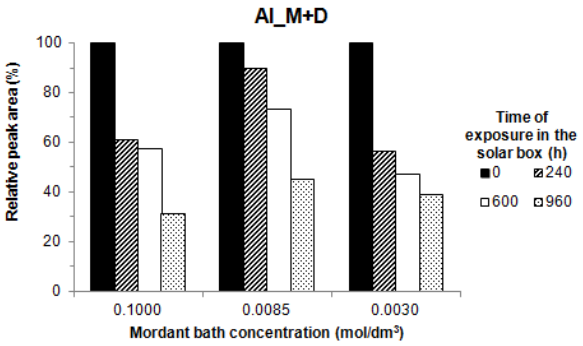

A
5.0
4.0
3.0
2.0
1.0
0.0
Al mass per gram of wool (mg Al/g wool)
M+D
MD
0.1000
0.0085
0.0030
Alum bath concentration (mol/dm³)

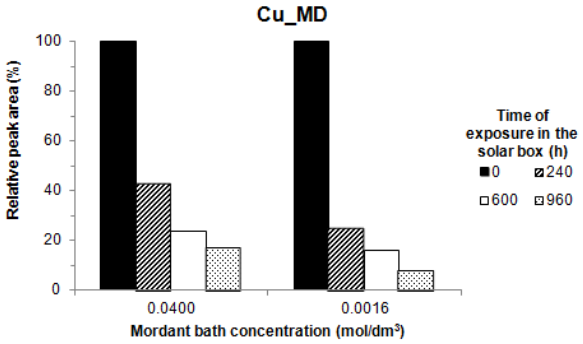

B
15.0
10.0
5.0
0.0
Cu mass per gram of wool (mg Cu/g wool)
M+D
MD
0.0400
0.0016
Copper (II) sulfate bath concentration (mol/dm³)

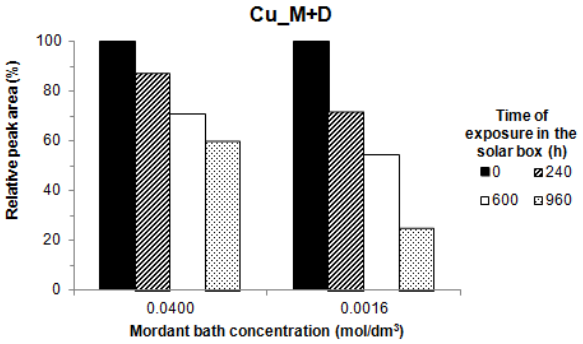

C
5.0
4.0
3.0
2.0
1.0
0.0
Fe mass per gram of wool (mg Fe/g wool)
M+D
MD
0.0400
0.0016
Iron (II) sulfate bath concentration (mol/dm³)

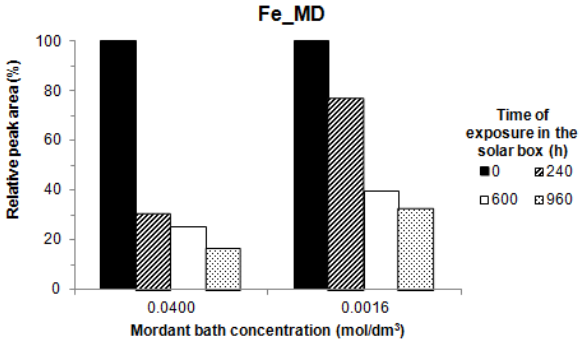

Al_MD
Peak area (abundance x min)
10000
8000
6000
4000
2000
0
0
240
600
960
Time of exposure in the solar box (h)
Mordant bath concentration (mol/dm³)
0.1000
0.0085
0.0030
Al_M+D
Peak area (abundance x min)
600
400
200
0
0
240
600
960
Time of exposure in the solar box (h)
Mordant bath concentration (mol/dm³)
0.1000
0.0085
0.0030
Cu_MD
Peak area (abundance x min)
40000
30000
20000
10000
0
0
240
600
960
Time of exposure in the solar box (h)
Mordant bath concentration (mol/dm³)
0.0400
0.0016
Cu_M+D
Peak area (abundance x min)
2000
1500
1000
500
0
0
240
600
960
Time of exposure in the solar box (h)
Mordant bath concentration (mol/dm³)
0.0400
0.0016
Fe_MD
Peak area (abundance x min)
20000
15000
10000
5000
0
0
240
600
960
Time of exposure in the solar box (h)
Mordant bath concentration (mol/dm³)
0.0400
0.0016
Fe_M+D
Peak area (abundance x min)
800
600
400
200
0
0
240
600
960
Time of exposure in the solar box (h)
Mordant bath concentration (mol/dm³)
0.0400
0.0016

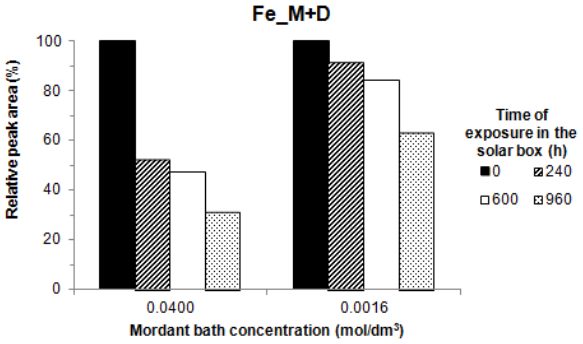

Al_MD
Relative peak area (%)
100
80
60
40
20
0
0.1000
0.0085
0.0030
Mordant bath concentration (mol/dm³)
Time of exposure in the solar box (h)
0
240
600
960

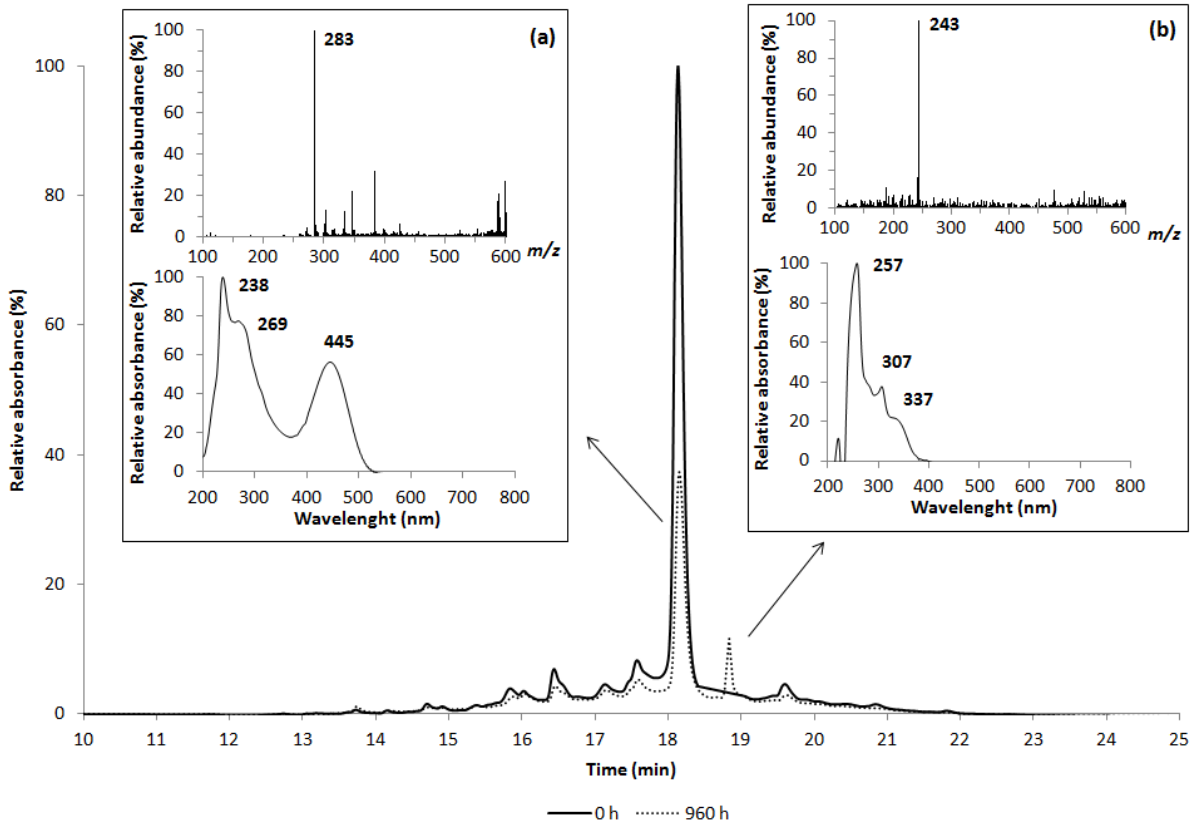

Al_M+D
Relative peak area (%)
100
80
60
40
20
0
0.1000
0.0085
0.0030
Mordant bath concentration (mol/dm³)
Time of exposure in the solar box (h)
0
240
600
960


Cu_MD
Relative peak area (%)
100
80
60
40
20
0
0.0400
0.0016
Mordant bath concentration (mol/dm³)
Time of exposure in the solar box (h)
0
240
600
960


Cu_M+D
Relative peak area (%)
100
80
60
40
20
0
0.0400
0.0016
Mordant bath concentration (mol/dm³)
Time of exposure in the solar box (h)
0
240
600
960


Fe_MD
Relative peak area (%)
100
80
60
40
20
0
0.0400
0.0016
Mordant bath concentration (mol/dm³)
Time of exposure in the solar box (h)
0
240
600
960


Fe_M+D
Relative peak area (%)
100
80
60
40
20
0
0.0400
0.0016
Mordant bath concentration (mol/dm³)
Time of exposure in the solar box (h)
0
240
600
960


(a)
283
Relative abundance (%)
m/z
238
269
445
Relative absorbance (%)
Wavelenght (nm)
(b)
243
257
307
337
Relative absorbance (%)
Wavelenght (nm)
Relative absorbance (%)
Time (min)
0 h
960 h