

## A DISCUSSION ON THE RED ANTHRAQUINONE DYES DETECTED IN HISTORIC TEXTILES FROM ROMANIAN COLLECTIONS

SCIENTIFIC PAPER

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**Biological sources containing anthraquinone dyes of vegetal and animal origin have been used for dyeing textiles from ancient times. Initially available only locally, later object of trade, different species were used in various areas of the world, in different historic periods. Moreover, the preference for certain biological sources also depended on the value, destination and manufacturing technique of the objects to be created. A large number of textiles from Romanian museum collections have been studied since 1997, in order to identify the natural dyes and their biological sources. Analysis were first performed by Liquid Chromatography with Diode Array Detection (LC-DAD) and more recently by Liquid Chromatography with Mass Spectrometric detection (LC-MS). Anthraquinones of vegetal origin, such as *Rubia tinctorum* L. (madder), as well as from scale insects: *Kerria lacca* Kerr (lac dye), *Dactylopiis coccus* Costa (Mexican cochineal), *Porphyrophora hamelii* Brandt (Armenian carmine dyeing scale insect), *Porphyrophora polonica* L. (Polish carmine dyeing scale insect), *Kermes vermilio* Planchon (kermes) were identified in various textiles dating from the 15th-20th century and in seal bound fibres, in 15th-16th century documents. This paper presents the procedures used in the identification of the above-mentioned biological sources, based on the use of an ion trap mass spectrometer as the LC detector. The preferences for certain biological sources are discussed, according to the textile manufacturing technique, period and provenance.**

### 1 Introduction

Anthraquinone-based dyes are among the oldest biological sources used for textile dyeing. *Rubia tinctorum* L. (madder) was used in ancient Egypt (not prior to 1350 BC), Mesopotamia, in the Greek and Roman times, India and surrounding countries, as well as in Middle East and Europe.<sup>1</sup> Other *Rubiaceae*, such as *Rubia peregrina* L. (wild madder), *Rubia cordifolia* L. (Indian madder or munjeet), *Galium verum* L. (lady's bedstraw), *Relbunium hypocarpium* L. (relbún) were used in various parts of the world.<sup>1,2</sup> The use of these plants in textile dyeing was also confirmed in scientific studies.<sup>2-8</sup> The widespread use of *Rubiaceae* plants may be explained by the variety of hues they may produce - from orange-red to pink, violet and brown - due to the high number of dye components contained in the same plant and the dye's ability to combine with several

metallic mordants.<sup>1</sup> Moreover, many of these plants are widely distributed around the world and were accessible as local, valuable dye sources to different civilizations.<sup>1</sup>

Although of less importance as compared to *Rubiaceae*, other anthraquinone based plants such as *Rheum*, *Rumex* and *Rhamnus* species have been also used in textile dyeing.<sup>1,2</sup>

Insect dyes have also played a prominent role in textile dyeing. Original to the Mediterranean basin where it may be found as a parasite on the kermes oak (*Quercus coccifera* L.), *Kermes vermilio* Planchon. (kermes) has been used by cultures on the shores of the Mediterranean Sea since prehistoric times. Later, it became an object of commerce, exchanged with various parts of the world.<sup>1</sup> Its appreciation as an expensive and precious dye culminated in 1464, when Pope Paul II decided that the cardinals' robes must be dyed with kermes. It declined rapidly in the second half of the 16<sup>th</sup> c., when cochineal became available from the New World.

*Kerria lacca* Kerr. (lac dye) has been known and used in India and China, mostly for dyeing silk, wool and cashmere.<sup>1</sup> Lac dye was identified in textiles from Palmyra<sup>9</sup> as well as in various textiles from Asia Minor.<sup>10,11</sup> It is rarely identified in European medieval silks, probably due to regulations restricting its use.<sup>1</sup>

There are two genres of carminic acid containing insects, both living as parasites on plants in different parts of the world: *Porhyrophora* species, that may be found in Europe and Asia and *Dactylopius*, in the Americas. *Porhyrophora polonica* L. (Polish cochineal, preferably called Polish carmine dyeing scale insect) and *Porhyrophora hamelii* Brandt (Armenian cochineal, preferably called Armenian carmine dyeing scale insect) are mostly discussed in relation to historic textiles. According to the current knowledge, other *Porhyrophora* species have also been known and used, sold as substitutes for the above and even mixed with them.<sup>1</sup> Identification of Polish and Armenian carmine dyeing scale insect dyes, and their distinction from Mexican cochineal (*Dactylopius coccus* Costa) in textiles of historic interest, became possible with the method developed by Wouters and Vercheken.<sup>12,13</sup> Based on this method, a mixture of Polish carmine dyeing scale insect with kermes could be identified in three Florentine borders dated 14<sup>th</sup>-15<sup>th</sup> c.<sup>14</sup> Polish carmine dyeing scale insect was also identified by thin layer chromatography in various textiles from Western European collections (1450-1600) as well as in a Hungarian embroidery from the 17<sup>th</sup>-18<sup>th</sup> c.<sup>8,15</sup> *Porhyrophora hamelii* Brandt (Armenian carmine dyeing scale insect) was identified in Coptic textiles (3<sup>rd</sup>-10<sup>th</sup> c.), in a cashmere cloth used in a Sassanid kaftan (6<sup>th</sup>-7<sup>th</sup> c.) as well as in Ottoman fabrics, such as velvets and lampas (15<sup>th</sup>-17<sup>th</sup> c.).<sup>16-18</sup> *Dactylopius coccus* Costa (Mexican cochineal), the main relevant representative of the *Dactylopius* genus, was known and used by Pre-Columbian civilizations in America. However, according to the analytical studies performed by Wouters and Rosario-Chirinos, it may not have been very widespread before the third century.<sup>3</sup> After the discovery of the New World, Mexican cochineal was very quickly imported and used in Europe and it soon prevailed over all the locally available insect dyes.<sup>2</sup> It

was identified in various textiles in European collections, starting with the third quarter of the 16<sup>th</sup> c.<sup>1,2,14,18-20</sup>

From the analytical point of view, useful analyses of anthraquinone containing dyes started with the application of thin-layer chromatography in the late 1960s. A systematic approach to the analysis of anthraquinone containing dyes and based on high-performance liquid chromatography and UV-VIS spectrometric detection and identification was published by Wouters in 1985.<sup>21</sup> Since then, the identification of such dyes in historic textiles based on a variety of liquid chromatographic separation methods kept attracting the attention of scientists in many laboratories and results obtained have been the subject of several publications.<sup>14-16,18,19,22-31</sup> Nowadays, liquid chromatographic separation is ideally combined with photodiode-array (PDA; or diode array detection, DAD) and mass spectrometric (MS) detectors in a hyphenated configuration.<sup>22-32</sup>

Since 1997, a collaborative project entitled "Dyes in textiles from Romanian collections" has been developed, between Romanian institutions and KIK/IRPA Brussels. The project aims to enrich the existing information on textiles in local collections, based on dye analysis. Several categories of textiles have been studied until now: religious embroideries (from the National Museum of Art of Romania, Putna and Sucevita Monasteries), brocaded velvets, knotted carpets, kilims and sumak from Minor Asia (National Museum of Art of Romania), Romanian ethnographical textiles (from "Bucovina" Museum, Suceava and the National Village Museum "Dimitrie Gusti", Bucharest), knotted carpets from the Black Church, Brasov. The categories of textiles should be considered as representative for the Romanian textile heritage. However, within each group of textiles studied, objects selection and sampling was made based on several criteria such as accessibility to collections, possibilities of sampling (in most cases sample withdrawal was possible for objects undergoing conservation interventions) and specific research interest (studies on dated objects to be used as references, objects of particular interest to art historians etc.) and did not follow any statistical sample selection.

This article is an overview of anthraquinone-based biological sources identified in textiles from Romanian collections, based on LC-PDA and LC-MS analysis,<sup>33-37</sup> with special emphasis on the progressive use of an ion-trap mass spectrometer as detector.<sup>38</sup> Rather than reporting the results themselves, the article intends to underline the variety of sources identified in textiles from local collections and the contribution this knowledge is bringing to better understanding the historic context in which the respective textiles have been created.

## 2 Experimental

### 2.1 Reference and Historic Samples

Samples from historic objects were provided by restorers, mostly from fragments, which could not be integrated in objects after conservation intervention. Small fibres, less than 0.5 cm long, were heated at 105 °C with 250 µL of hydrochloric acid

(37%)/methanol/water (2:1:1, v/v/v) mixture, for 10 min. The extract was evaporated to dryness in a nitrogen flow at 60 °C and the residue redissolved in 200 µL methanol/water mixture 1:1 (v/v), centrifuged and the supernatant was transferred into an injection vial. A library of references was used for data evaluation. This includes the UV-VIS, single stage and tandem MS data for the dye components discussed. These data were collected on references of dyes and standard dyed fibres - fibres dyed in the laboratory with known biological sources, following traditional recipes. A reference of *Dactylopius coccus* Costa dyed wool was prepared in the frame of the ARTECH and COST G8 projects, *Rubia tinctorum* L. dyed wool prepared in the MODHT project was kindly provided by Jo Kirby and *Rubia cordifolia* L. dyed wool was offered by Dr. Florica Zaharia, Metropolitan Museum of Art, New York, USA. Samples of *Rhamnus frangulae* L. and *Kerria lacca* Kerr. dyed wool were provided by Ana Roquero (Spain).

More details on the extraction procedure as well as on the single stage and tandem MS data of dyes in the database can be found in an earlier publication.<sup>38</sup>

## 2.2 Instrumentation

Experiments were performed using an Agilent 1100 LC equipped with an MS/MS ion trap detector using an ESI ion source, operated in negative mode. The Agilent ChemStation software LC incorporating the MSD trap control was used for data acquisition and processing. LC-MS separation was achieved on a Zorbax C18 column, 150 mm x 4.6 mm, 5 µm particle diameter. Gradient elution was applied to the mobile phase consisting of a mixture of aqueous 0.2% (v/v) formic acid (solvent A) and methanol/acetonitrile (1:1 v/v, as solvent B). The flow rate was set at 0.8 mL·min<sup>-1</sup>. An automated injector was used, 5 µL (if not otherwise specified) being injected from a total of about 200 µL solution. The DAD and the MS ion source were placed in series and in that order after the column. MS detection was made in the negative ion mode with the following ESI operational parameters: drying gas temperature 350 °C; drying gas flow rate 12 L·min<sup>-1</sup>; nebulising gas pressure 65 psi; capillary high voltage 2,484 V. The ion trap used a maximum accumulation time of 300 ms and a total charge accumulation (ICC) of 30,000. The multiplier voltage was set at 2,000 V and the dynode potential at 7 kV. When working in the MS/MS mode, the spectral width was 4 a.m.u. and the collisional induced dissociation amplitude 1.6 V. More details on the chromatographic and detection conditions can be found elsewhere.<sup>38</sup>

## 3 Results and Discussion

### 3.1 Anthraquinones of Vegetal Origin

Alizarin and purpurin, the main dye components in *Rubia tinctorum* L. (madder), were detected in several samples, based on the presence of their molecular ions and other ions produced in the ionization source (m/z 239 for alizarin, m/z 254 and m/z 227 for purpurin). This data was obtained from the chromatograms collected with the mass spectrometer working in Full Scan mode (FS) followed by extraction of chromatograms (IEC) corresponding to dyes in the

library of references. Other anthraquinones, such as rubiadin (m/z 239, [M-H]<sup>-</sup>), munjistin (m/z 283, [M-H]<sup>-</sup> and m/z 239, [M-H-44]<sup>-</sup>), xantopurpurin (m/z 239, [M-H]<sup>-</sup>) and anthragallol (m/z 255, [M-H]<sup>-</sup>), were also detected, based on the same procedure. *Rubia tinctorum* L. (madder) was detected in samples from Oriental carpets (15<sup>th</sup>-17<sup>th</sup> c.) preserved in Transylvania (Romania) as well as in local ethnographical textiles (19<sup>th</sup>-20<sup>th</sup> c.) and textiles (kilim, sumac, knotted carpets) from Asia Minor, also preserved in Romanian collections.

In three samples, all from the 19<sup>th</sup> c. kilim from Basarabia (Eastern European region), mainly purpurin and rubiadin and smaller amounts of other anthraquinones were detected in the absence of alizarin. This combination of dyes would suggest the use of either *Rubia peregrina* L. (wild madder) or *Rubia cordifolia* L. (idem *Rubia munjista*, munjeet).<sup>1,2</sup> According to literature, acid hydrolysates of yarns dyed with *Rubia peregrina* L. contain mainly rubiadin,<sup>39</sup> while for *Rubia cordifolia* L. a high percentage of munjistin should be expected,<sup>21</sup> both assumptions based on calculations of relative ratios resulting from integration values obtained at 254 nm. The analysis performed on a standard of *Rubia cordifolia* L. dyed wool showed that the ratio between munjistin and rubiadin in the respective ion extracted chromatograms is very different when compared with the sample from the 19<sup>th</sup> c. kilim (Figure 1).

Although component ratios may differ considerably when calculated either from UV integration values or from mass spectrometric data, it is suggested here that, very probably, *Rubia peregrina* L. was used in the 19<sup>th</sup> c. kilim and not *Rubia cordifolia* L. Unfortunately, at the time of analysis, a sample of *Rubia peregrina* L. dyed wool was not available for confirmation.

Emodine, the main dye component in *Rhamnus frangula* L. (alder buckthorn bark), *Rheum* sp. (rhubarb), *Rumex* sp. (yellow dock) and other biological sources was also detected in some samples.<sup>1,2</sup> The detection of emodine was suggested by the presence of its molecular ion (m/z 269) in the chromatogram obtained with the mass spectrometer working in FS mode, followed by data processing by IEC.

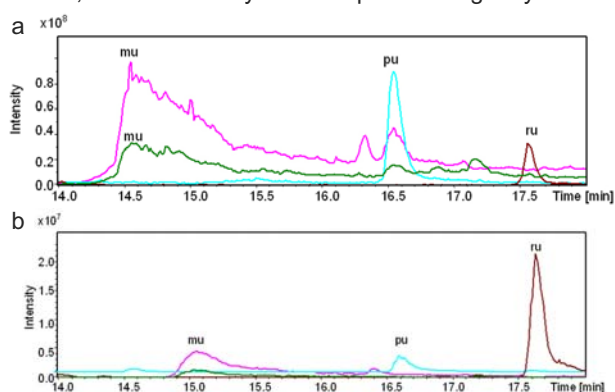


Figure 1. Image supporting the identification of *Rubia peregrina* L. in a sample belonging to a 19<sup>th</sup> c. kilim from Basarabia. Images obtained by extracting the chromatograms corresponding to the molecular ions (and the ion produced by its decarboxylation in the ionization source) of munjistin (mu), purpurin (pu) and rubiadin (ru) for a *Rubia cordifolia* L. standard dyed wool (a) and a sample from the kilim (b).

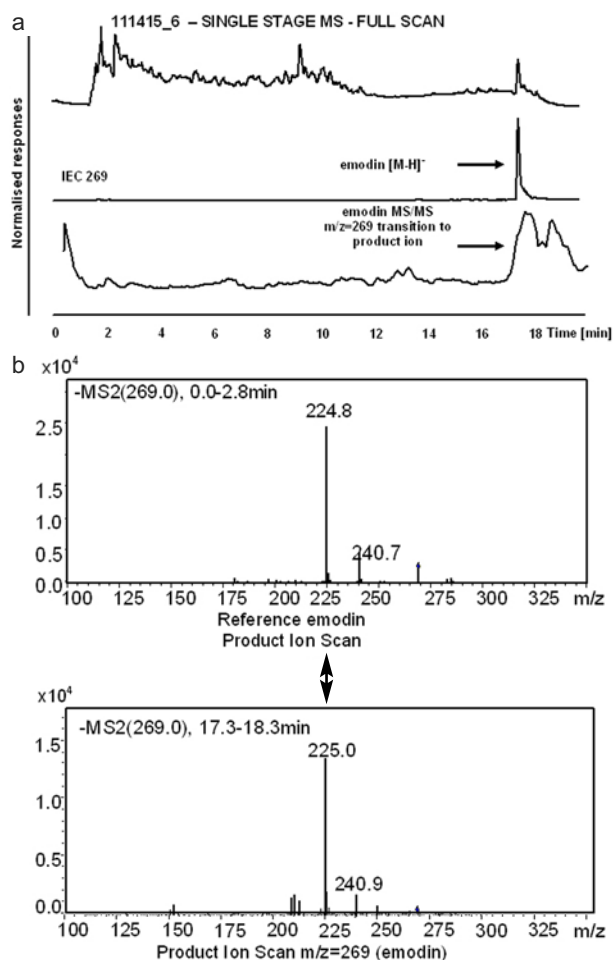


Figure 2. Image supporting the identification of emodine in a sample from a kilim in Minor Asia (19<sup>th</sup> c.). From top to bottom: (a) chromatogram in FS mode; ion extracted chromatogram of ion m/z 269 corresponding to the molecular ion of emodine; (b) chromatogram collected in Multiple Reaction Monitoring (MRM) corresponding to the transition to product ion in emodine; product ion scan of ion m/z 269, as compared to that obtained on a standard of emodine.

Considering that this was the only dye component detected, its presence was always confirmed by the product ion scan, as compared with data collected using a standard of emodine (Figure 2). Even if according to literature, in many biological sources, emodine is present together with chrysophanic acid (m/z 253, [M-H]<sup>-</sup>), the latter was never detected in the analysed samples. A dedicated study, including analysis of a pure standard of chrysophanic acid (unavailable in the research described here) is needed in order to be able to identify it in historic samples, based on the LC-MS analytical protocol developed and to eventually suggest more refined identifications of relevant plant species.

### 3.2 Anthraquinones of Animal Origin

Kermesic and flavokermesic acids were the main dye components detected in some samples, suggesting the use of *Kermes vermilio* Planchon (kermes). Their presence was indicated by the molecular ions in the FS - ion extracted chromatograms (m/z 329 for the former and m/z 313 for the latter) and confirmed by the ions produced by their decarboxylation in the ion-

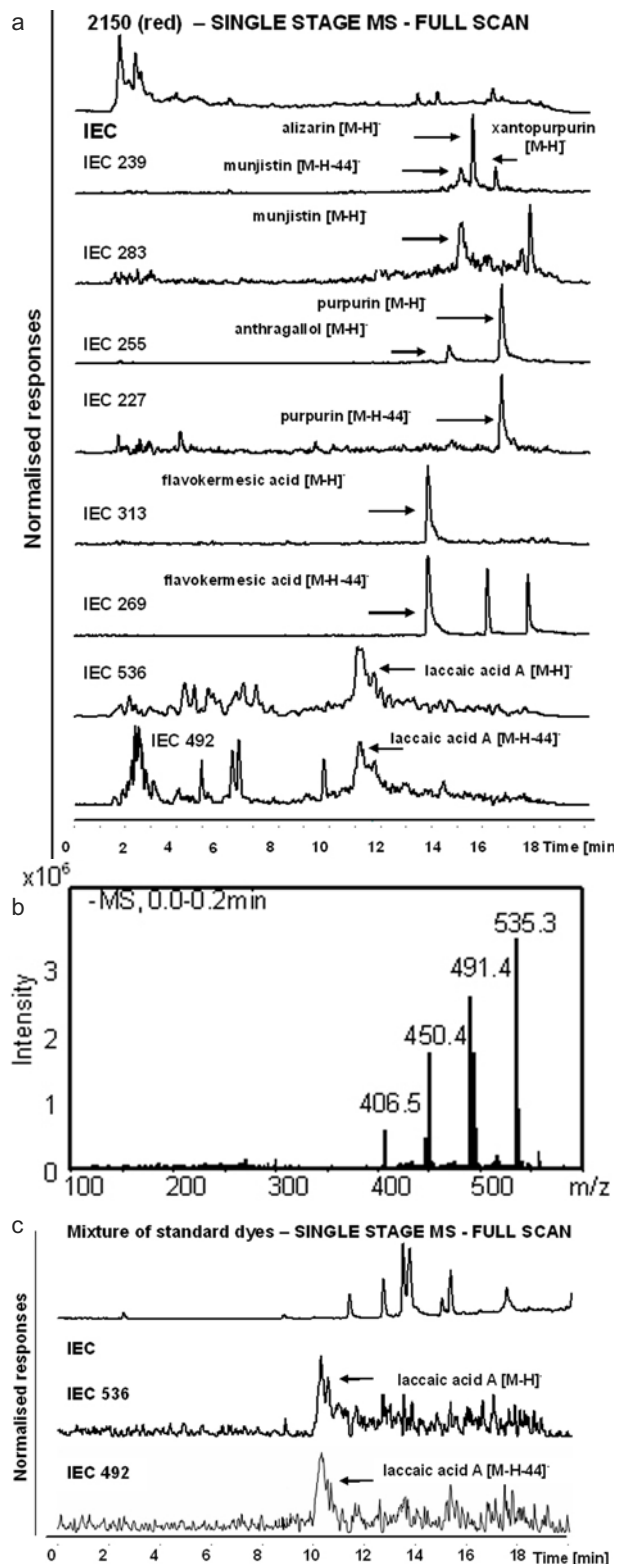


Figure 3. Image supporting the identification of a mixture of *Rubia tinctorum* L. (madder) and *Kerria lacca* (lac dye), in a sample belonging to a 15<sup>th</sup> c. seal thread. (a) from top to bottom: chromatogram in FS mode; ion extracted chromatograms according to molecular ions (and other ions in the ionization source) of anthraquinones in madder, flavokermesic acid and laccic acid A; (b) top: TIC and ion extracted chromatograms according to the molecular ion of laccic acid A (m/z 536) and the ion produced by its decarboxylation in the ionization source (m/z 492); bottom: mass spectrometry spectra of a standard of laccic acid A to illustrate fragmentation of the molecular ion in the ionisation source.



ization source ( $m/z$  285 for kermesic acid and  $m/z$  269 for flavokermesic acid). This valuable biological source was detected in three 15<sup>th</sup> c. religious embroideries preserved at Putna and Sucevita Monasteries.<sup>35,37</sup>

In some samples from document seal threads, flavokermesic acid was detected in the absence of carminic and kermesic acids (Figure 3a). Flavokermesic acid is one of the minor compounds in cochineal insects where the major compound is carminic acid, in kermes – major compound is kermesic acid and in *Kerria lacca* Kerr. (lac dye) where the main dye component is laccaic acid A. However, flavokermesic acid is the major component in a rarely cited kermes species, *Kermes ballotae* Signoret.<sup>13</sup> Hence, whether the detection of flavokermesic acid, in the absence of carminic and kermesic acids, indicates the use of lac dye or of the rare kermes species, the presence of the diagnostic laccaic acid A must be confirmed. With a PDA detector as the only detector, retention time and the UV-VIS spectrum of laccaic acid A will allow unequivocal identification of the lac. Likely, the sensitivity may be improved by having access to MS detection. However, in the case of laccaic acid A, detection suffers from the fragmentation of its molecular ion in the ion source (observed on a laccaic acid A standard), so that amounts larger than expected will have to be injected (Figure 3, b). This example illustrates the excellent compatibility of PDA and mass detectors for the analysis of natural organic dyes. This procedure allowed confirming the presence of laccaic acid A with both detectors in historic samples that contained both madder and lac.

Carminic acid, the main dye component in *Dactylopius coccus* Costa (Mexican Cochineal), *Porphyrophora hamelii* Brandt (Armenian carmine dyeing scale insect) and *Porphyrophora polonica* L. (Polish carmine dyeing scale insect) was detected in several samples from various textiles, based on detection of the molecular ion of carminic acid ( $m/z$  491) and the ion produced by its decarboxylation in the ionization source ( $m/z$  447). Minor compounds, such as dcll (C-glycoside of flavokermesic acid) ( $m/z$  475, [M-H]<sup>-</sup>), kermesic and flavokermesic acids ( $m/z$  329, [M-H]<sup>-</sup>;  $m/z$  285, [M-H-44]<sup>-</sup> and  $m/z$  313, [M-H]<sup>-</sup>;  $m/z$  269, [M-H-44]<sup>-</sup>, respectively) were also detected in most cases.

Identification of carminic acid containing insects down to the species level could be only performed based on LC-PDA analysis.<sup>12,13</sup> According to this method, Mexican cochineal was detected in religious embroideries and brocaded velvets dated later than 16<sup>th</sup> c. as well as in 19<sup>th</sup>-20<sup>th</sup> c. Romanian ethnographical textiles<sup>33,35</sup> while Polish carmine dyeing scale insect was detected in a brocaded velvet from 1502.<sup>34</sup> Armenian carmine dyeing scale insect was only identified in 19<sup>th</sup>-20<sup>th</sup> c. Romanian ethnographical textiles.<sup>33</sup>

### 3.3 Anthraquinone Dye Sources Detected According to the Technique and Period

All the results obtained for anthraquinone dyes by LC-DAD and LC-MS in textiles from Romanian collections, are presented in Table 1. It illustrates the number of detections for each biological source according to the group of textile (religious embroideries, brocaded velvets, sumak, kilim, knot carpets, ethnographical) and period. Even if the number of samples analysed from each group depends solely on the objects undergoing conservation intervention at the time of the research as well as the possibility of sampling, some important features may be observed. The combination of *Kerria lacca* Kerr. (lac dye) and *Rubia tinctorum* L. (madder) was mostly preferred for 15<sup>th</sup>-16<sup>th</sup> c. (mainstream) religious embroideries while *Kermes vermilio* Planchon (kermes) was used for more valuable textiles. It should be mentioned that the mixture of lac dye and madder is the only combination of anthraquinone dye sources detected in the textiles under discussion. *Dactylopius coccus* Costa (Mexican cochineal) was identified in all groups of textiles, starting with the second half of the 16<sup>th</sup> c. *Rubia tinctorum* L. (madder) was used in 15<sup>th</sup>-17<sup>th</sup> c. Oriental knotted carpets preserved in Transylvania as well as in later (19<sup>th</sup>-20<sup>th</sup> c.) textiles from Minor Asia. *Rubia peregrina* L. (wild madder) was detected in a kilim from Basarabia. Carminic acid containing dyes (*Dactylopius coccus* Costa and *Porphyrophora hamelii* Brandt) were used to obtain red-violet hues in Romanian ethnographical textiles (19<sup>th</sup>-20<sup>th</sup> c.) and *Porphyrophora polonica* L. in a brocaded velvet dated 1502.

Table 1. Anthraquinone based biological sources detected in textiles from Romanian collections. Number of detections according to textile (manufacturing) techniques and period.

\* identified in 16th c. pieces

	Documents, 15th-16th c.	Religious embroideries, 15th-16th c.	Religious embroideries, 17th-18th c.	Religious embroideries, 19th c.	Brocaded velvets, 15th-16th c.	Brocaded velvets, 18th c.	Oriental carpets (knots), 15th-17th c.	Sumak, Asia Minor, 19th c.	Kilim, Asia Minor, 19th c.	Knots carpets, Asia Minor, 19th c.	Kilim, Basarabia, 19th c.	Romanian ethnographic textiles, 19th-20th c.
Carminic acid containing insects (all)	-	6	4	1	2	1	-	-	4	2	-	23
<i>Porphyrophora hamelii</i> Brandt (Armenian carmine dyeing scale insect)	-	-	-	-	-	-	-	-	-	-	-	(2)
<i>Dactylopius coccus</i> Costa (Mexican Cochineal)	-	(3)*	(2)	-	-	(1)	-	-	-	-	-	(13)
<i>Porphyrophora polonica</i> L. (Polish carmine dyeing scale insect)	-	-	-	-	1	-	-	-	-	-	-	-
<i>Kermes vermilio</i> Planchon (kermes)	-	4	-	-	1	-	-	-	-	-	-	-
<i>Kerria lacca</i> Kerr. (lac dye)	4	21	-	-	-	-	-	-	-	-	-	-
<i>Rubia tinctorum</i> L. (madder)	4	29	4	-	-	1	15	3	9	3	-	3
<i>Rubia peregrina</i> L. (wild madder)	-	-	-	-	-	-	-	-	-	-	3	-
<i>Rhamnus / Rheum / Rumes</i> sp. (emodine containing plant)	-	2	4	1	-	-	-	-	1	-	-	-

*Rhamnus frangula* L. (alder buckthorn bark), or another emodine containing plant, was only detected in religious embroideries and once in a kilim from Minor Asia. Considering that this/these source/s, as well as *Kerria lacca* Kerr., were not identified in textiles from Western Europe but were detected in Oriental textiles,<sup>10,11,18</sup> it could be proposed that (at least) part of the fibres used in the manufacture of the religious embroideries have an Oriental origin.

#### 4 Conclusions

A large number of anthraquinone dye sources were successfully detected in textiles from Romanian collections and a preference for certain biological sources according to the (manufacturing) technique, period and provenance can be suggested. Detection in religious embroideries of sources never or rarely identified in textiles from Western Europe but frequently detected in Oriental textiles suggests that at least some of the materials used in the manufacture of these pieces are of an Oriental origin. The high value of some embroideries was certified by the identification of *Kermes vermilio* Planchon (kermes), a very expensive dye. The analytical protocol developed, based on the use of the ion trap mass spectrometer as a detector in liquid chromatography, was successful in the identification of various dye components and biological sources. The results obtained were in perfect agreement with those previously generated with the well established LC-PDA methodology.

The LC-MS procedure for the identification of anthraquinone-containing biological sources reported in this paper is based on data collected from standard dyed fibres (fibres dyed in the laboratory with known biological sources, following traditional recipes) and has shown its strength as a dedicated research tool. However, as exemplified by the discussion of the detection of laccaic acid A, as well as when complex mixtures of dye sources in one and the same sample are involved, or when ratios of components need to be calculated for exact source identifications, preference should be given to LC-PDA-MS.

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