

# The purple *Codex Rossanensis*: spectroscopic characterisation and first evidence of the use of the elderberry lake in a sixth century manuscript

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**Abstract** This paper presents the results obtained during the measurements campaign started in June 2012 and ended in November 2013 on the invaluable purple *Codex Rossanensis*, sixth century, one of the oldest surviving illuminated manuscripts of the New Testament. The tasks of the chemistry laboratory were to answer a variety of questions posed both by historians and restorers, concerning the materials used in a previous restoration, the composition of the pictorial palette and the different inks and to determine which colouring material had been applied to dye the parchment support. It was also requested to determine the state of preservation of the manuscript, as a result of its interactions with the environment in which the manuscript had been stored and the vicissitudes experienced during its life (fire, previous restoration, exhibition). The spectroscopic analyses performed by micro-Raman, micro-Fourier transform infrared and X-ray fluorescence allowed to fill a gap in the knowledge of the pictorial materials used in the Early Middle Ages. The pictorial palette, the inks, the dye applied to obtain the purple parchments, the support and the materials used in the previous restoration treatment executed in 1917–19 were fully characterised. Moreover, to the author's knowledge, the article shows the first experimental evidence of the use of the elderberry lake in a sixth century-illuminated manuscript. The lake was characterised by Raman spectroscopy.

**Keywords** Elderberry lake · Pigments · Micro-Raman · Micro-FTIR · XRF · Rossano Codex

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## Introduction

The *Codex Rossanensis* is a sixth century Byzantine-illuminated manuscript written on purple parchment, conserved at the Museo Diocesano in Rossano Calabro (Cosenza, Italy)<sup>1</sup>. It was found in 1879 in the sacristy of the Cathedral of Maria Santissima by Adolf von Harnack and published soon after by Oscar von Gebhardt (von Gebhardt 1883). It is a Gospel in Greek consisting of 188 parchment sheets (31 cm×26 cm, with a posterior numbering of each page from 1 to 376). It contains the Gospel of Matthew and the Gospel according to Mark (the latter with one lacuna, Mark 16:14–20), as well as part of the letter from Eusebius to Carpianum on the concordance of the Gospels. Originally, it seems that it should have contained all four canonical Gospels, as shown in the miniature with the symbols of the four Evangelists, and in particular by the presence of the Eusebian concordances. The written part is laid in biblical uncial capital in two columns.

The manuscript is famous for its prefatory cycle of 13 miniatures of subjects from the Life of Christ, arranged in two tiers on the page; the miniature of the four Evangelists; the golden decoration of the letter to Carpianum; the magnificent illumination of Mark inspired by the Sophia and for the use of the precious purple dye as background for all the parchments and gold and silver for the text.

In 1917–19, the codex was subjected to a restoration treatment, carried out by Nestore Leoni, a famous miniaturist, active from the end of nineteenth century to mid-twentieth century. The manuscript was actually in a worse state of conservation: stains, carbonised parts, holes and tears, folds and undulations were evident, due both to microbiological

<sup>1</sup> Information on the history of the *Codex Rossanensis* as well as images of the miniatures can be seen at the dedicated site: <http://www.codexrossanensis.it/en/>

attacks and to a fire occurred in the Cathedral of Rossano Calabro in the seventeenth century. Scholars suppose that the two lacking Gospels were destroyed during that fire.

Unfortunately, Leoni's intervention irreversibly modified the aspect of the illuminated sheets. Nestore Leoni never wrote which materials he used for the restoration. Another cause of deterioration of the manuscript is related to the almost continuous display of the illuminated pages in the museum. The light—solar and artificial—has induced oxidative processes in the parchment support and in the dye used to obtain the purple pages, changing the original colour and the mechanical resistance of the support. These effects are more evident and intense in the restored part of the codex.

In June 2012, the codex arrived at the Istituto Centrale Restauro e Conservazione Patrimonio Archivistico e Librario (Icrcpal) of Rome, for a complete characterisation of the pigments, the support and the materials used by Nestore Leoni and the state of conservation and for the restoration.

The laboratory of chemistry of Icrcpal performed spectroscopic analyses, by micro-Raman (378 spectra), micro-Fourier transform infrared (80 spectra) and X-ray fluorescence (35 spectra), on the whole volume, both on the pigments and on the support.

The challenge of the analysis of the *Codex Rossanensis* lies in the lack of analytical information on the pictorial media used in Early Middle Ages (fourth–ninth centuries). Even though old mediaeval-illuminated manuscripts have been deeply studied from the historical standpoint, they have been rarely described in their material composition (Aceto et al. 2012). Moreover, a recently discovered mediaeval Arab manuscript containing recipes on inks and pigments manufacture (Zaki 2011) and the careful translation work carried out during a Ph.D. thesis not yet published (Sara Fani, *Studi sul Vicino Oriente e Maghreb. Specificità culturali e relazioni interculturali*, Università degli Studi di Napoli “L'Orientale”) on another mediaeval Arab manuscript (al-Qalalūsī 2007), never translated before, offered precious tools for the interpretation of the experimental data.

In this paper, I present the results obtained during the measurement campaign on the *codex* executed between June 2012 and November 2013.

For a better understanding of some spectra acquired on red lakes, laboratory samples were prepared, using historical lake samples either belonging to the collection of the Icrcpal chemistry laboratory or newly synthesised.

## Materials and methods

### Instrumentation

Measurements were performed by means of a Renishaw inVia Reflex Raman microscope equipped with a Renishaw diode

laser at 785 nm (nominal output power 300 mW) and a 1,200 line/mm grating to disperse the backscattered light. The Raman signal is detected by a Peltier-cooled ( $-70\text{ }^{\circ}\text{C}$ ) deep-depletion charge-coupled device (CCD RD-VIU,  $578\times 384$  pixel) optimised for near-infrared and ultraviolet. The nominal spectral resolution obtained for the measurements is about  $3\text{ cm}^{-1}$ . The system, equipped with a Leica DM LM microscope to focus the laser on the sample and a colour video camera, allows for the positioning of the sample and the selection of a specific region for the investigation. Spectral acquisitions (one–ten accumulations, 50 s each) were performed with a  $\times 50$  objective (N.A. 0.75). Under these conditions, the laser spot measures about  $20\text{ }\mu\text{m}^2$ . Depending on the sample investigated, the laser power has been reduced with neutral density filters up to 0.03 mW.

Micro-FTIR measurements were performed using a Nexus Nicolet interferometer and a Continuum microscope, equipped with a KBr beam splitter, a liquid nitrogen-cooled MCT/A detector and an Infinity Refflachromat™ 15X  $\infty/V$  objective with N.A.=0.58. Measurements on a surface of  $100\times 100\text{ }\mu\text{m}^2$  were performed in the  $4,000\text{--}650\text{ cm}^{-1}$  range at a resolution of  $8\text{ cm}^{-1}$ , averaging 200–400 acquisitions per sample. No ATR spectra were collected in order to avoid any direct contact with the manuscript during the analyses.

X-ray fluorescence (XRF) spectra were recorded by means of an Assing Lithos 3000 portable spectrometer, equipped with a Mo X-ray tube. With such an instrument, the radiation can be collimated at different beam diameters (from 0.5 to 4 mm), depending on the area of interest. In this experiment, the 2-mm collimator was used together with a Zr filter. A red laser (695 nm) and a camera (both integrated into the system and controlled by the instrument software) were used to choose the area to be sampled. Measurements were performed with the tube operating at 25 kV, 0.300 mA, in the 0–25 keV range with a resolution of 160 eV at 5.9 keV, lasting 10–60 min for each acquisition. Long-time acquisitions were performed in order to obtain information on the elements present in minor amount.

### Laboratory samples

Laboratory samples were prepared as follows:

- Historical samples of lakes, belonging to the collection of the Icrcpal chemistry laboratory (gift of Lorilleux (Milano), 1938) obtained from *Rubia tinctorum* (red) and *Porphyrophora hamelii* (Armenian cochineal, red), were prepared following the indication of Bisulca et al. (2008), by using eggs: yolks and egg whites were separated and vigorously whisked. They were then combined by mixing 2:1:1 yolk/egg white/water and added to the pulverised lake, until the correct viscosity of the lake was

obtained. The pigment was then applied on the surface of a parchment.

- Aluminium lake pigments from *Crocus sativus* (red) and *Sambucus nigra* (pink-mauve) were prepared following the ancient recipes reported in Carriera (2005) and in Caffaro (2004). Each pigment was then mixed with egg white as reported in Caffaro (2004) and Anonimous (1976) and applied on the parchment.
- Two different kinds of purple-dyed parchment samples were prepared, by treating the parchment with (a) aqueous solution of *Rocella tinctoria* prepared as described in recipe 131 of the Stockholm Papyrus (Caley 2008) and (b) aqueous solution of *Rocella tinctoria* and sodium carbonate, as described in the recipe 123 of the same manuscript (Caley 2008).

## Results and discussion

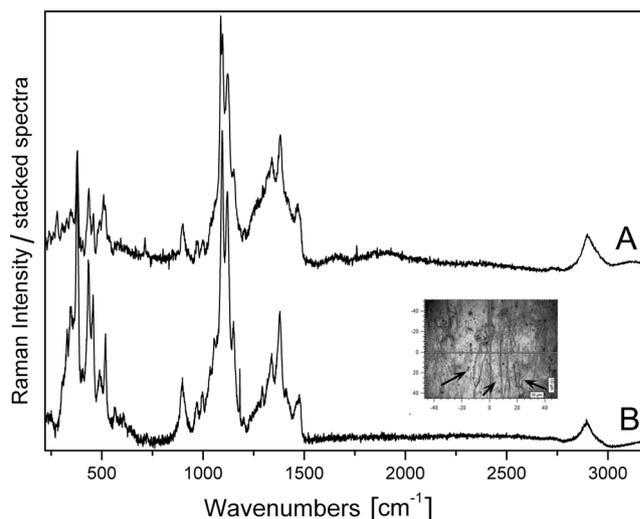
Analysis of the materials used in the previous restoration (1917–19)

On the first 20 pages of the codex, a layer of an unknown material was applied during the restoration performed in 1917–19 by Nestore Leoni, who never wrote any detail on the compounds used in the restoration, not even in the technical reports he had to present to the ministry. The unpublished reports are conserved at the Archivio Centrale dello Stato, Roma (Italy).

The materials used to reinforce the parchment sheets deeply penetrated into the bulk of the membranaceous support, modifying its optical characteristics: the sheets restored in this way are now completely transparent and their colour appears more brown than purple. On some pages, the applied layer was partially or completely detached and it was possible to analyse a fragment of the layer of about 2 cm<sup>2</sup>.

By observing the detached layer under the Raman microscope, there was an evidence of the presence of some fibres, from which the spectra of cellulose had then been collected (Fig. 1). In the remaining part of the pellicle, both Raman (Fig. 2) and infrared analyses gave the unmistakable spectrum of collagen, but the analyses carried out directly on other pages showed also the presence of cellulose nitrate, in some restricted regions (Fig. 3). The mentioned figures report, for comparison, the spectra collected from pure standard samples.

Three products were basically used between the end of the nineteenth century and the beginning of the twentieth century for the reinforcement of damaged parchments: pure, high-quality gelatine mixed with formaldehyde, directly applied on the parchment or reinforced with Japanese paper, Archiv-Zapon (cellulose nitrate dissolved in amyl acetate with

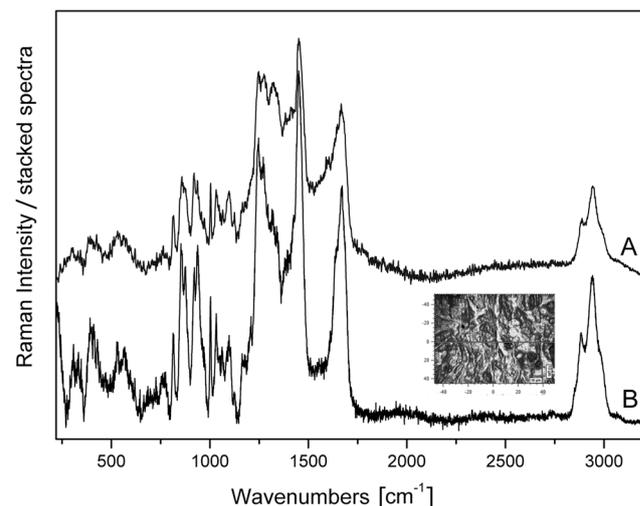


**Fig. 1** Raman spectra collected from *A* fibres included in the restoration layer (marked with arrows in the inset) and, for comparison, *B* spectrum of standard microcrystalline cellulose

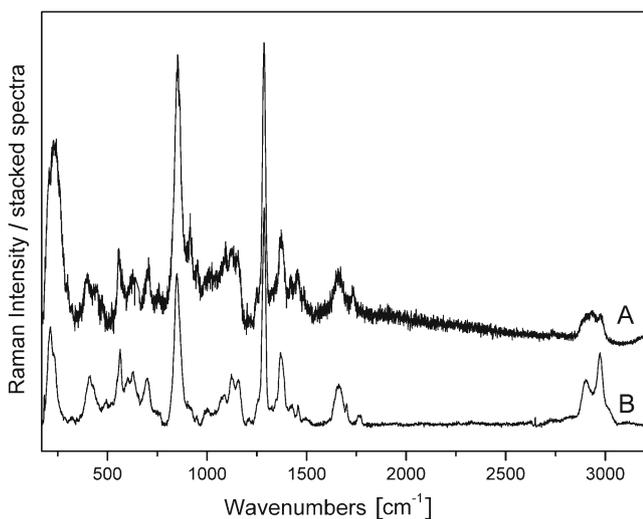
addition of camphor or formaldehyde) and Cellit (cellulose acetate mixed with acetic ether, ethanol, acetic acid and camphor) (Casanova 1928; Schill 1899; Sello 1902; Perl 1904; Frederking 1910; Posse 1911; Ramos Rubert 2000; Riccardi 2006; Spadaccini 2012). No traces of cellulose acetate were found in the codex.

At that time, the mentioned methods were supposed to be safe and reversible. On the contrary, we know now that the three products irreversibly penetrate into the support that becomes transparent and brittle, by ageing (Posse 1899; Tanasi et al. 1985; Spadaccini 2012).

On the last 15 pages of the codex, where insects' attack was evident, as well as an extensive corrosion caused by the silver



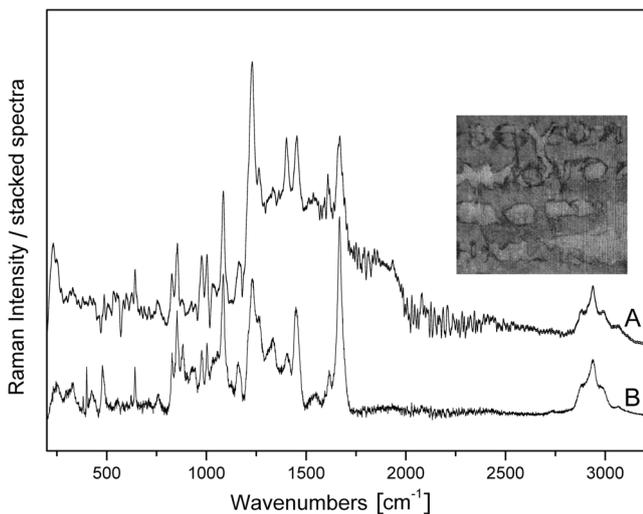
**Fig. 2** Raman spectra of *A* restoration layer made of gelatine compared to *B* standard sample of collagen. The inset shows the appearance of the layer under the Raman microscope



**Fig. 3** *A* Raman spectrum collected in restricted areas (page 3 in this case) of the restoration layer that gives the signals of cellulose nitrate and *B* spectrum of a standard sample of cellulose nitrate reported for comparison

ink, the parchment sheets were particularly fragile. Leoni used a different restoring technique: he applied a fabric with a loose weave, the so-called crêpeline (Anonymous 1900–1901), made of silk as confirmed by Raman (Fig. 4).

The analyses on the material used in the previous restoration were carried out to document the work of Nestore Leoni and to verify whether other compound could have been used in a potential—but not documented—later restoration that could have been performed before the exhibit “Mostra bibliografica per la storia della Chiesa in Campania e in Calabria. Anno Santo 1950” (AA.VV. 1950). No other materials, except those previously mentioned, were found.



**Fig. 4** *A* Raman spectra collected from the crêpeline layer and *B* spectrum of pure silk fabric reported for comparison

### Analysis of the graphic media

Many colours were used in the precious manuscript: white, pink, red, orange, green, light and dark blue, grey, black and gold in the illuminations; gold and silver in the text of the Gospels and black inks in the title, in the explanations of the miniatures and in posterior notes. Some parts of the no longer readable text in silver had been, at some time, rewritten using black ink.

The pigments in the manuscript had not been finely ground by the miniaturist and this allowed to collect individual Raman spectra from each pigment applied in the mixtures of colours, facilitating the identification of the colouring matters. In some cases, XRF spectra were recorded to confirm the Raman attribution. Only for the organic dyes, lakes and purple infrared technique was employed, trying to elucidate the Raman results. The black inks were analysed by using the three techniques. The whole palette, the inks and the techniques for their detection in the codex are reported in Table 1. It is important to stress that no preparatory layer for the pigments (Armenian bole or lead white or gypsum) was found. The direct painting on the parchment support is typical of the Byzantine area in which the codex was written and decorated.

A typical XRF spectrum of the purple parchment is reported in Fig. 5 for comparison with the spectra collected from the pigments and to explain the apparent high concentration of lead, visible in all the measurements.

The Mo cathode, used in the present work, excites the lines of lead with high efficiency. This is supported by the instrumental minimum detection limit (MDL) value for lead ( $1.5 \times 10^{-3}$  % in weight), that is, a factor of 10 lower than the MDL of calcium ( $1.3 \times 10^{-2}$  % in weight), element that is predominantly present in the parchment. Taking into account the limits of the application of quantitative analysis to a support as thin as parchment, the amount in weight percent of Ca and Pb calculated for the support are Ca=0.88 % and Pb=0.02 %. When the measures were performed on gold, the layer of this metal acted as an absorber to the lead signal arising from the support.

### White colours

Raman measurements detected the presence of white lead as the unique source of white. It was confirmed by the presence of lead peaks in XRF. Moreover, white lead was used as pure pigment or mixed with other colours, as a brightener. In some miniatures, the white heightening had darkened; nevertheless, spectra collected from those regions only evidenced the presence of white lead. No indication of the possible presence of either lead dioxide or of lead sulphide was obtained with Raman nor with XRF, which did not detect any sulphur. The

**Table 1** Palette of the *Codex Rossanensis* with attribution to specific pigments/dye and applied technique

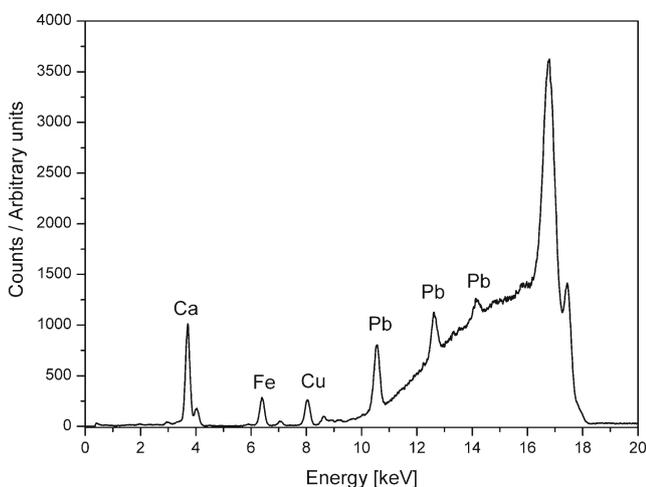
Colour	Compound	Raman	Infrared	XRF
White	Lead white	X		X
Red	Red lead Cinnabar	X		
Pink	Red lead mixed with white lead Pink-mauve lake from <i>Sambucus nigra</i>	X X	X	X
Orange	Red lead+goethite	X		X
Yellow	Goethite Orpiment, only two occurrences on pages 3 and 241	X X		X X
Green	Goethite+lapis lazuli; goethite+indigo; orpiment+indigo (only on pages 3 and 241)	X		
Blue	Lapis lazuli	X		X
Indigo	Indigo	X		
Violet	Inorganic red+lapis lazuli Pink-mauve lake+lapis lazuli	X X	X	X
Black	Carbon black	X		X
Gold	Gold, traces of iron	X		X
Original black inks	Carbon black	X	X	X
Black inks added on top of silver inks	Carbon black	X	X	X
Posterior inks	Iron gall inks	X	X	X
Silvery inks	Silver with a high amount of copper	X		X
Golden inks	Pure gold	X		X
Purple support	Purple lake from orchil	X	X	X

darkening is probably due to biological attack or to a chemical degradation occurred at some time.

### Black colours and inks

To obtain black colours, carbon black was applied alone or as a darkener for brown, violet and grey hues. Black inks were originally used for the title and in the explanations of the

miniatures. Their Raman spectra gave the characteristic signals of carbon black (1,315 and 1,590  $\text{cm}^{-1}$ ), which was also used in the more recent numbering of each page of the codex and to rewrite—at unknown time—some faded parts of the original text drawn in silver. The posterior annotation were realised in iron gall ink, detected by Raman (main peak at 1,478  $\text{cm}^{-1}$ ) and infrared and confirmed by the intense iron peak in XRF. Two examples of the collected Raman spectra, more discriminating than infrared, insensitive to the presence of carbon, are shown in Fig. 6.



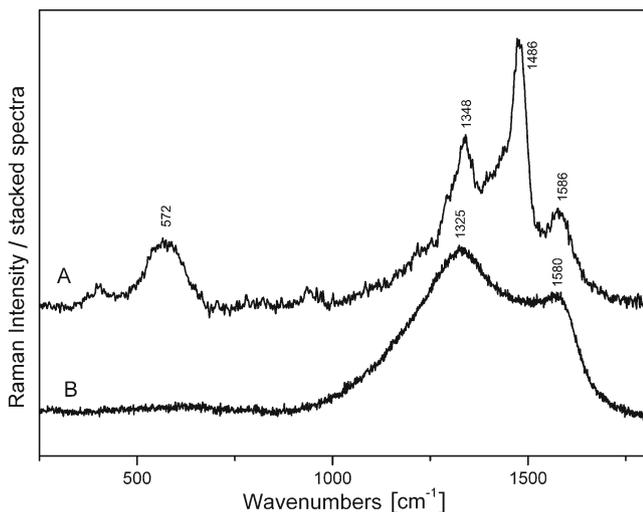
**Fig. 5** XRF spectrum of the parchment on a purple page (page 19)

### Yellow, green, gold and silver colours and inks

Along the whole manuscript, goethite was used as yellow pigment or mixed with lapis lazuli or indigo to obtain different green tones. The only exceptions are present at pages 3 (The cleansing of the Temple) and 241 (Mark the Evangelist inspired by the Sophia), where a particular dark and brilliant green was obtained by mixing orpiment and indigo. The presence of arsenic in the pigment was confirmed by XRF.

No real green pigments, such as malachite or *verdigris*, were found in the manuscript.

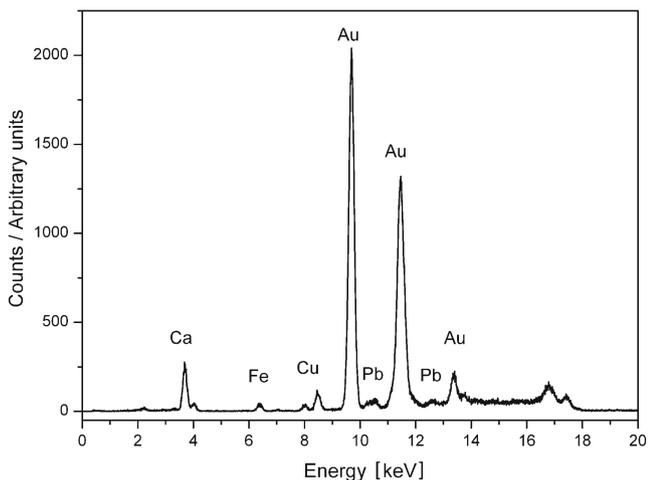
As concerning the golden areas, Raman analyses were performed in order to ascertain if mosaic gold could have been used. No traces of this pigment were found, and XRF data confirmed the presence of pure gold containing very



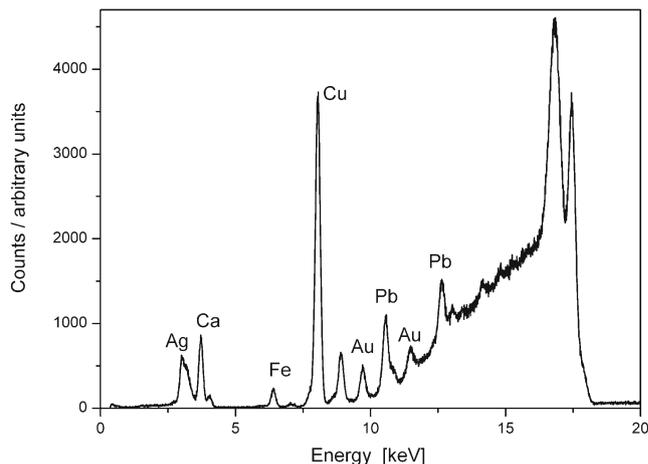
**Fig. 6** Raman spectra of the two kinds of black inks found in the codex. *A* iron gall ink, found in all the posterior notes at page 21; *B* carbon black found in the page’s numbering of page 21, in the title and in the explanations of the illumination and used to rewrite some faded areas of the silver ink. The wavelength of the characteristic peaks of each ink are reported in the figure

small amount of iron and copper (Fig. 7). There are no substantial differences in the composition between the gold applied in the decorations and that used as ink. The lead peak belongs to the substrate.

Silver has been applied as metallic ink in the text but never in the illuminations. XRF spectra (Fig. 8) show that silver was mixed with copper, the latter being responsible for the degradation of some written areas, with a strong corrosion that modified the aspect of the silver, by its darkening, and sometimes caused corrosion of the parchment substrate (van der Most et al. 2010), in particular in the last 15 pages that were then reinforced with crêpeline.



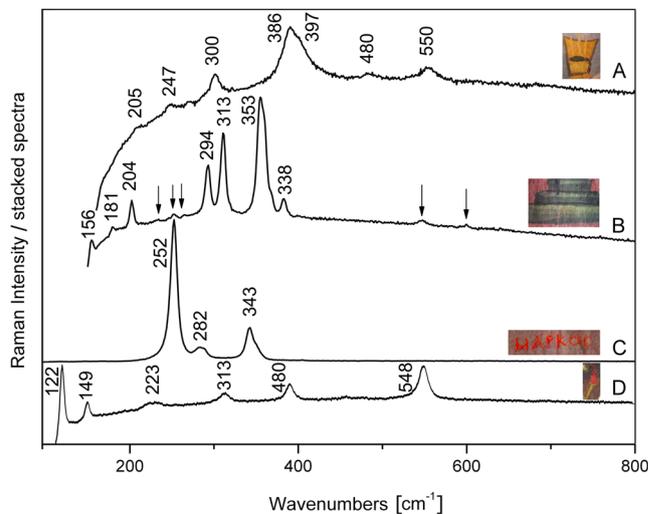
**Fig. 7** XRF spectrum of the golden ink (page 19). The gold used along the codex, both for inks and illuminations, is quite pure. Only small traces of copper and iron were found. Lead signals are related to its presence in the parchment support



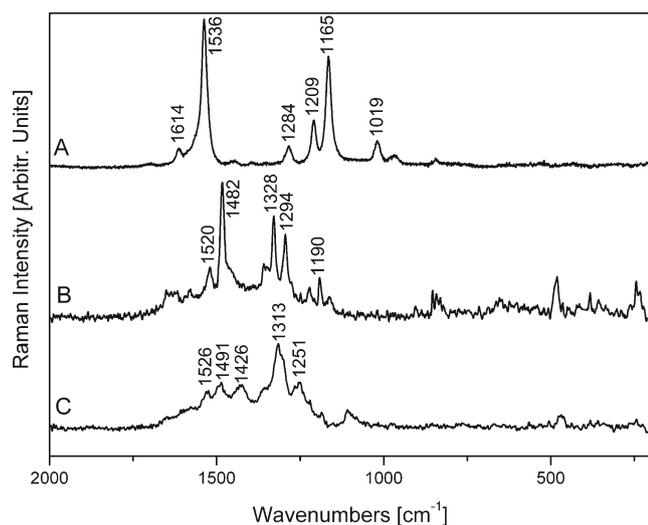
**Fig. 8** XRF spectrum of the silver ink. All L-lines are present, but in the ink there is a noticeable amount of copper. Lead signals are related to its presence in the parchment support

Red, orange, brown, violet and pink inorganic colours

Red hues were obtained with lead oxide; there is only one evidence for the use of the most expensive cinnabar, at page 241 (Mark the Evangelist inspired by the Sophia), where this pigment was employed to write the name of the Evangelist “Markos”. Red lead was also found in mixture with goethite, to obtain orange tones, with white lead for some pink colours and with lapis lazuli—sometime mixed with carbon black—for the violet shade. A different brown-violet hue was obtained by mixing goethite, carbon black and lapis lazuli.



**Fig. 9** Raman spectra collected from the different yellow and red pigments. All the yellow areas were realised by using goethite (spectrum *A*). In two miniatures (page 3 and page 241) orpiment was applied mixed with indigo to obtain a particular green hue (spectrum *B*). In the *B* spectrum, orpiment is dominating, but some peaks of the indigo of the mixture are also visible. They are marked with arrows and are located at 233, 250, 263, 544, 598  $\text{cm}^{-1}$ . Cinnabar (spectrum *C*) was found only in the red applied to write the name “Markos” at page 241, whereas minium (spectrum *D*) was used in the remaining red areas of the codex



**Fig. 10** Raman spectra of three laboratory samples of red lakes. *A* saffron, *B* madder and *C* cochineal. The wavelength of the characteristic peaks of each lake are reported in the figure

The Raman spectra obtained from the different red and yellow pigments are shown in Fig. 9.

#### Pink and violet organic colours

In all the illuminations where no red lead was found, Raman spectra of the same organic compound were collected, hence evidencing the usage of a single organic lake throughout the whole manuscript.

A lake is a pigment manufactured by precipitating a dye on an inert binder; usually the mordant, a metallic salt, was added. In ancient times, the organic dyes were extracted from plants (bark, leaves, fruits, seeds), and the most used mordant was alum. In some recipes, sodium carbonate was also employed, as well as vinegar or lime, depending on the pH

**Table 2** Assignments for the most important Raman bands in saffron lake

Saffron	Approximate assignments
523, 846, 953 w	Skeletal C-C vibrations
1,019 m	$\rho$ (CH <sub>3</sub> )/ $\nu$ (CC)
1,165 s	$\rho$ (CH <sub>3</sub> )/ $\nu$ (CC)
1,209 s	$\nu$ C-O-C
1,284 s	$\nu$ (CO) $\nu$ (CC)/ $\delta$ (CCC)
1,356 w	$\nu$ (CC)/ $\delta$ (COH)
1,390 w	$\delta$ (CH <sub>3</sub> )
1,445 w	$\delta$ (CH <sub>3</sub> ) asym
1,536 vs	$\rho$ (CH <sub>3</sub> )/ $\nu$ (CC)
1,614 m	$\nu$ (C=C)
1,698 w	$\nu$ (C=O)

$\nu$  stretching,  $\delta$  deformation,  $\rho$  rocking

**Table 3** Assignments for the most important Raman bands in madder lake

Madder	Approximate assignments
239, 382, 481 m	Skeletal C-C vibrations
853 w	$\delta$ (C=O)/ $\delta$ (CCC)
1,161 w	$\nu$ (CC)/ $\delta$ (CH)
1,190 m	$\nu$ (CC)/ $\delta$ (CH)/ $\delta$ (CCC)
1,221 m	$\delta$ (CH)/ $\delta$ (CCC)
1,294 s	$\nu$ (CO)/ $\nu$ (CC)/ $\delta$ (CCC)
1,328 s	$\nu$ (CC)
1,358 ms	$\nu$ (CC)/ $\delta$ (COH)
1,482 vs	$\nu$ (CO)/ $\nu$ (CC)/ $\delta$ (CH)
1,520 ms	$\nu$ (CC)
1,570 w	$\nu$ (CC)
1,624 br	$\nu$ (C=O)

$\nu$  stretching,  $\delta$  deformation

necessary to develop a specific colour (Caley 2008; Carrier 2005; Caffaro 2004).

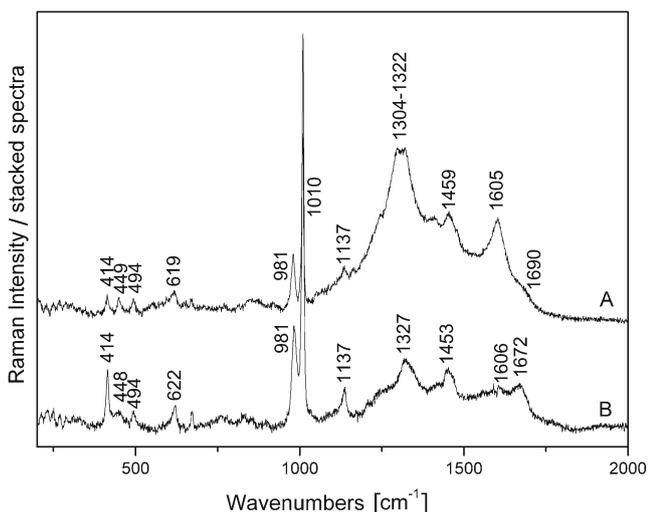
The Raman analysis of dyes and lakes is particularly difficult, because these colourants are generally poor Raman scatterers and because the concentration needed to achieve an intense colour is very low, thus rendering Raman spectroscopy often not sensitive enough to acquire significant spectra. Sometimes, better results are obtained by using surface-enhanced Raman spectroscopy (SERS), but the policy of the Italian Ministry of Cultural Heritage does not allow the direct intervention on original books or documents and the application of a destructive technique, even if micro-invasive as SERS.

For this reason, I decided to try to obtain information by comparison of the spectra collected from the original miniatures with some red and red-violet lakes prepared in the laboratory and to apply Raman and infrared techniques.

**Table 4** Assignments for the most important Raman bands in cochineal lake

Cochineal	Approximate assignments
245, 359, 468, 551 w	Skeletal
1,016 w	$\rho$ (CH <sub>3</sub> )
1,103, 1,188 m sh	Ring breathing/ $\nu$ (CO)
1,222 m sh	$\delta$ ring
1,251 s	$\delta$ (CH)
1,313 vs, 1,360 sh	$\delta$ (COH) carboxylic
1,426 s	$\nu$ (CC) ring/ $\delta$ (COH)
1,491 s	$\nu$ (CO)/ $\nu$ (CC)/ $\delta$ (CH)
1,526 s	$\nu$ ring
1,608 m sh	$\nu$ (C=C)
1,649 w sh	$\nu$ (C=O)

$\nu$  stretching,  $\delta$  deformation,  $\rho$  rocking

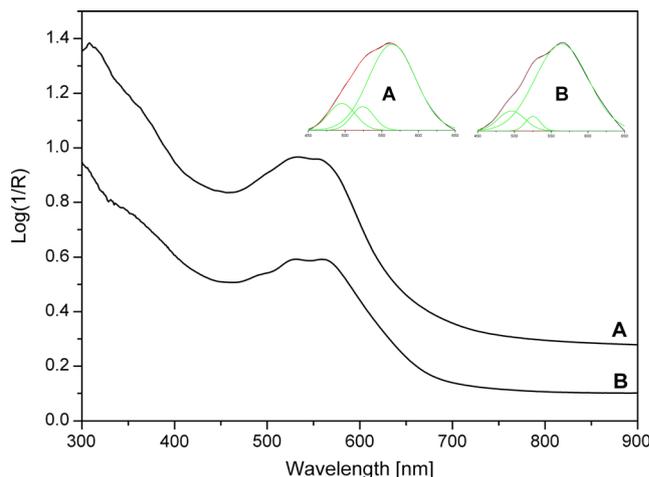


**Fig. 11** Raman spectra of *A* pink-mauve lake in the *Codex Rossanensis* and *B* elderberry lake prepared in laboratory. The wavelength of the characteristic peaks of each sample are reported in the figure

The chosen lakes represent four different classes of dyes. Madder, obtained from *R. tinctorum*, is an anthraquinonic dye containing mostly alizarin; cochineal-carminium from *P. hamelii* is essentially constituted by carminic acid with an anthraquinonic structure linked to a glucose sugar unit; saffron is a carotenoid dye containing crocetin and the dye obtained from elderberries contains anthocyanins.

The comparison with the spectra reported in the scientific literature is not so easy, due to the fact that the most used technique for their detection is SERS and not normal Raman. This can induce modifications in the spectra, as well as the use of different excitation lines of lasers. Moreover, very often, the spectra reported in the literature were obtained from pure or purified compounds or from a single dye extracted from the originals and not directly from the lakes applied on a writing support.

The Raman spectra of the laboratory samples are in good agreement with the most recent publications on this subject (Schmidt and Trentelman 2009; Bruni et al. 2011; Cañamares



**Fig. 12** FORS spectra of *A* original mauve lake and *B* elderberry lake. The insets show the deconvolution of the peaks in the 450–650-nm region

et al. 2004; Whitney et al. 2006), taking into account the different techniques and the different excitation lines used in the literature spectra.

Raman spectra (Fig. 10) are very useful for a possible assignment of the vibrational frequencies that are reported in Tables 2, 3 and 4 for saffron, madder and cochineal lakes, respectively.

The spectra of the lakes presented in Fig. 10 do not match those recorded from the original manuscript. The better correlation between the literature data, the measurements on the laboratory samples and the spectra collected from the *Codex Rossanensis* is obtained for the elderberry lake, as shown in Fig. 11.

The small differences that can be noted in the two spectra (enlargement in the 1,200–1,600-cm<sup>-1</sup> region) are related to the presence of minor amount of carbon black, always found in the original lakes, and from the peculiar composition of the elderberry. *S. nigra* contains non acetylated cyanidin-based anthocyanins as major pigments (mainly cyanidin 3-glucoside and cyanidin 3-sambubioside) (Gebhardt et al. 2002; European Medicines Agency 2013). Other compounds in the

**Table 5** Assignments for the most important Raman bands in elderberry lake and comparison with the original pink-mauve lake in the codex

Elderberry	Approximate assignments	Original pink-mauve lake
233, 304, 414, 448, 494 w	Skeletal	414, 449, 494
622, 673, ca. 778 w	$\nu$ S-S	619, 673
834, 860, 901 w	$\nu$ glycosidic C-O-C linkage	856, 876 broad, 928
981 vs	$\nu$ (S=O) (lake)	981
1,010 vs	$\nu$ (SO <sub>4</sub> ) out of phase (lake)	1,010
1,137 ms	$\nu$ (C-O-C)	1,137
1,258 m sh	$\nu$ (C-O-C)	1,255
1,327 s	$\nu$ (CN), $\delta$ (CH) ring	1,304–1,322
1,453 s	$\nu$ (C=O) amide, $\delta$ (CH <sub>2</sub> )	1,459
ca. 1,606 m	Amide I/ $\nu$ (C=C)	1,605
ca. 1,672 m	Amide I/ $\nu$ (C=O)	ca. 1,690 (sh)

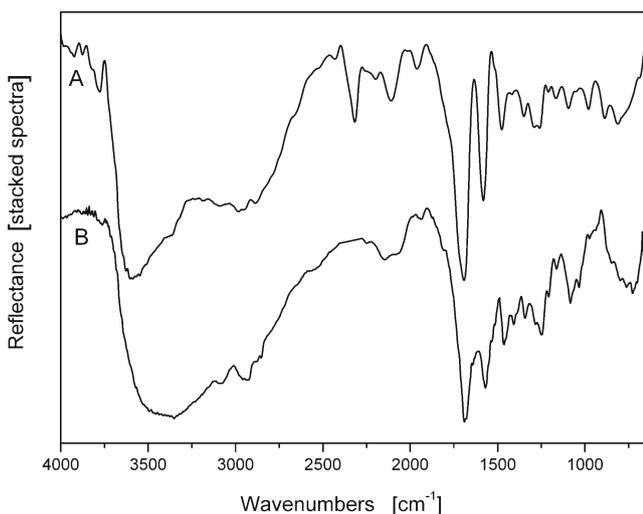
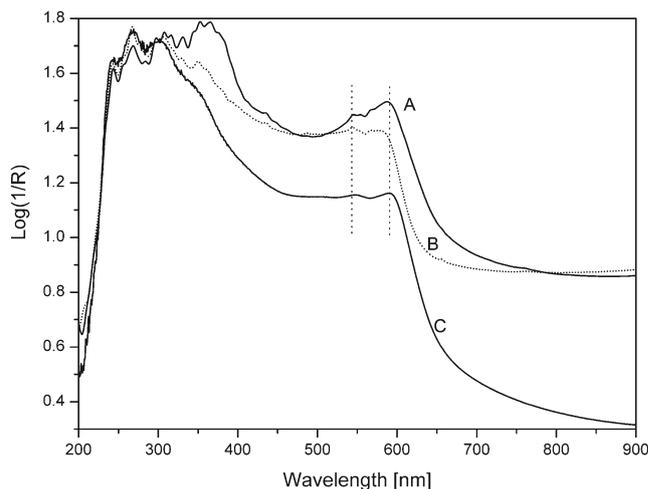
**Table 6** Peak position (nm), height and area for the original pink-mauve lake and the elderberry lake

	Original mauve colour	Laboratory elderberry lake
$x_1$	496±1	496±1
$w_1$	37±1	37±1
$A_1$	4.6±0.3	2.5±0.1
$x_2$	524.1±0.8	525.1±0.7
$w_2$	30.3±0.9	20±1
$A_2$	3.3±0.4	1.0±0.1
$x_3$	564.0±0.2	564.2±0.3
$w_3$	63.7±0.3	71.9±0.4
$A_3$	25.6±0.2	21.1±0.1
	$\chi^2/\text{DoF}=3.3 \times 10^{-6}$	$\chi^2/\text{DoF}=9.5 \times 10^{-6}$
	$R^2=0.999$	$R^2=0.998$

$x$  wavelength of the maximum,  $w$  peak height,  $A$  peak area,  $\text{DoF}$  degrees of freedom

seeds are flavonols, amino acids, essential oils, carbohydrates such as pectin, glucose and fructose, vitamins and minerals in small amounts (Jungmin and Chad 2007). The presence of proteins and carbohydrates (Schulz and Baranska 2007; Buchweitz et al. 2012; Gamsjaeger et al. 2011) is quite well visible in the collected spectra. Jungmin and Chad (2007) reported how specific *Sambucus* cultivars can be recognised by their different chemical composition.

Few Raman spectra of the pure compounds found in elderberry—usually dispersed in water solution—are reported in the literature (Merlin et al. 1994). The comparison between the spectra obtained from a single pure component and those collected from a lake, containing all the chemical products of elderberries, is difficult and it is almost impossible to attribute the recorded peaks to a specific compound. Moreover, the Raman spectra of the anthocyanins and anthocyanidins

**Fig. 13** Micro-FTIR spectra of *A* purple parchment in the *Codex Rossanensis* (page 208) and *B* STD not dyed parchment sample**Fig. 14** FORS spectra of *A* orchil mixed with sodium carbonate, *B* orchil and *C* purple parchment, page 241 of the *Codex Rossanensis*

contained in different vegetal sources or cultivars are obviously quite similar and, depending on the species of origin, they present small shifts in the frequency of the peaks.

Table 5 presents the tentative assignment for the elderberry lake and the position of the peaks for the original pink-mauve lake.

In the analysis of the *Codex Rossanensis*, there is a perfect correspondence between the spectrum collected from the laboratory elderberry-aluminium lake and the spectra obtained from all the red-mauve or violet areas present in the original. The two intense peaks of the lake at 981 and 1,009  $\text{cm}^{-1}$  are related to the presence of aluminium sulphate in the lake.

Figure 12 reports the fibre optics reflectance spectra (FORS) obtained from red-mauve areas in the original manuscript and from the elderberry lake prepared in laboratory. Table 6 contains the results of the deconvolution of the superimposed peaks in the 450–650-nm region for both samples.

#### Analysis of the purple dye

Scholars and art historian supposed that Tyrian purple (6,6'-dibromoindigo extracted from *Murex*) should have been used to dye the parchment sheets of a so precious manuscript.

To confirm this hypothesis, many purple pages were analysed by XRF, looking for bromine, which presence was not detected, even after prolonged acquisition time of 1 h. Only some sheets gave a possible bromine peak, not discriminating because of about the same intensity of the noise. This result was a first indication for the use of a dye different from the Tyrian purple.

All the XRF spectra collected from the parchment substrate showed, see Fig. 5, the presence of lead, presumably related to the tools used in the dye preparation, historically made of lead.

**Table 7** Band position (nm) for orchil, orchil+sodium carbonate and original purple-dyed page

Sample	Peak 1	Peak 2	Peak 3	Correlation
Orchil+sodium carbonate	536±6	545.1±0.8	592±2	$R^2=0.995$ $\chi^2=7\times 10^{-5}$
Orchil	526±1	555±2	589.6±0.5	$R^2=0.998$ $\chi^2=2\times 10^{-5}$
Original parchment (page 241)	508.0±0.9	547.6±0.4	594.6±0.2	$R^2=0.999$ $\chi^2=3\times 10^{-6}$

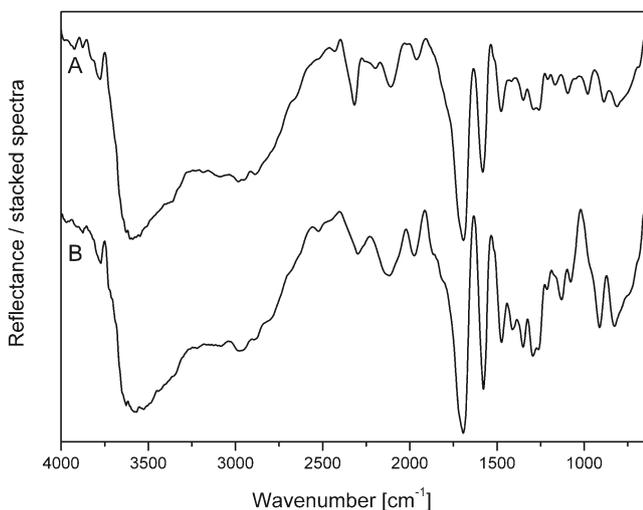
The purple parchments were then analysed by Raman, but it was impossible to collect good-quality spectra due to the intense fluorescent band that masked all Raman signals.

On the other side, the infrared spectra were dominated by the signals of the collagen substrate and, even if some differences between the spectra of not dyed and dyed parchment are visible, they are not discriminating enough to recognise the used dye as can be seen in Fig. 13, where the spectrum of a purple page in the codex is plotted together with the spectrum of a standard not dyed parchment.

Trying to understand the nature of the purple dye, the Ircrcal physics laboratory, during the measurement campaign on the *Codex Rossanensis*, collected fibre optics reflectance spectra (FORS with a Zeiss MCS 600 spectrometer) from many purple pages of the codex. They were then compared with those obtained from red lakes mostly used in antiquity as purple dye. No matches were found neither with madder, litmus or sapanwood.

On the contrary, an excellent match was found with the parchment samples dyed with orchil, prepared as explained in the “Materials and methods” section.

The spectra shown in Fig. 14 are plotted as log (1/reflectance) vs wavelength, in order to be compared with those reported in the scientific literature (Aceto et al. 2014b).



**Fig. 15** Micro-FTIR spectra of A purple parchment of *Codex Rossanensis* and B purple parchment of *Sarezzano Codex*

The regression statistically considered acceptable in the region 500–700 nm was achieved with the least number of peaks and of peaks whose assignment is known (Aceto et al. 2014b). The deconvolution (Origin software, Gaussian multi-peak fit) allowed to find the exact position of the bands for the analysed samples of parchment dyed with orchil, with orchil and sodium carbonate and original parchment from the *Codex Rossanensis*. The results are reported in Table 7.

The band position reported in the literature for orchil (Aceto et al. 2014c) are located at 549 and 595 nm. They are in good agreement with the position calculated for the measurements on original purple-dyed parchment and on the parchment dyed with orchil prepared in mixture with sodium carbonate. Pure orchil has a different band position in respect to the original parchment.

In a previous work carried out at the Ircrcal chemistry laboratory during the restoration of some pages of another purple codex, the *Sarezzano Codex* (ff. 72—Tortona, Curia vescovile, fifth–sixth century), XRF and micro-FTIR spectra were collected from the purple pages.

In that case, XRF showed a noticeable presence of bromine in the dyed parchment, but the infrared spectra did not show the typical features of 6-6' dibromoindigo.

Micro-FTIR spectra obtained from *Rossanensis* and *Sarezzano Codices* show identical spectral features for the purple parchments, as can be seen in Fig. 15. Let us suppose that also the *Sarezzano Codex* was not prepared using Tyrian purple as principal dye, as confirmed by the FORS analyses performed by Aceto Aceto et al. (2014c).

**Conclusions**

The long work executed on the *Codex Rossanensis* allowed for its complete characterisation: three different materials used in the previous restoration (gelatine, cellulose nitrate and silk) were detected; the whole palette, compatible with the period of its realisation, and the compound used to obtain the purple-dyed parchments were characterised.

It is moreover the first time, to the author's knowledge, that experimental evidence on the use of the elderberry lake in such an ancient document is shown.

The characterisation of the precious illumination at page 241 with Mark the Evangelist inspired by the Sophia is also of a paramount historical importance. Some scholars, in fact, supposed that the illumination did not belong to the original manuscript, but could be dated back to the twelfth century and realised with pigments different from those applied in the remaining manuscript (Kresten and Prato 1985). What instead differentiates such a miniature from the others present in the codex is that as it was not subjected to any previous invasive restoration and it maintains the freshness of the original colours.

All the experimental results show that indeed the same palette was used throughout the entire codex. In particular, our results show the peculiar use of the elderberry lake and of orpiment mixed with indigo to obtain in the supposed posterior miniature the precise shade of green, already found at page 3, that was considered as original.

The absence in the whole manuscript of any kind of preparation layer for the illuminations underlines and confirms the Byzantine origin of the codex.

It seems also very important, from the historical point of view, to extend the analyses on purple codices, in order to elucidate if or not a real Tyrian purple could have been used.

Until now, in fact, there are no evidences of its use for writing purposes.

The scientific data collected from the manuscript underlined the importance and the authenticity of the codex that is now under evaluation for being declared UNESCO World Heritage.

Moreover, all the data collected from the different laboratories of my institution demonstrated the noxious effect of the continuous manipulation and display of the codex (I cite as example the shift to brown of the parchment purple colour and the presence of spots caused by bacteria), stimulating the construction of a new controlled display case and the definition of the maximum allowable time of exhibit per year.

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