

Inside the Scriptorium: Non-Invasive In Situ Identification of Dyes in Illuminated Manuscripts by Microspectrofluorimetry and Multivariate Analysis: The Crescenzago Choir Books (End 15th Century) as a Case Study

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Supplementary Material

Table S1. Summary of the recipes followed to prepare the historically accurate mock-up samples of the different lakes examined in the present work.

Dye	Lake	Recipe
Carmine	CA	Carminic acid dissolved in lye (K_2CO_3 in distilled water, pH=9) with gentle heating. Saturated alum solution added dropwise until a red suspension is formed. Washed and filtered.
	C5	Crashed cochineals dispersed in lye. Heated and, when boiling, added alum. Filtered.
	C6	Crashed cochineals dispersed in lye, heated and left to boil. Cooled to 50°C and added alum solution up to pH of 6-7. Filtered.
Brazilwood	L1	Brazilwood extract dissolved in Milli-Q water. Added alum and $CaCO_3$. Filtered.
	L2	The extract is dissolved in ammonia solution.
	L1(M1)	Lye added to <i>Caesalpinia sappan</i> fragments. Heated and added alum and $CaCO_3$. Filtered.
	L1(M3)	Same as L1(M1), but with more alum.
	B1	Redwood fragments boiled in distilled water and concentrated. Filtered and added ground alum; everything is boiled and, once cooled, a K_2CO_3 solution is added dropwise. Filtered.
	E1	Like B1, but with extract powder.
	B3	Redwood fragments in ammonia solution, heated without boiling. Filtered. Alum and $CaCO_3$ added to the liquid. Filtered.
	R1(RA)	Redwood fragments and synthetic urine added to ground alum and white lead. Place everything under extractor fan stirring several times a day. Filtered. The liquid part is put in a chalk bowl. The bowl is put under extractor fan until the inside is completely dry and then the powder is scraped away from the sides.

	LB1(RA)	Same as R1(RA) with <i>Caesalpina sappan</i> fragments and ammonia solution instead of synthetic urine.
Madder	GA	Ground madder roots in water for one day at room temperature. Saturated alum solution added and heated at about 70° C. Filtered and lake precipitated by Na ₂ CO ₃ . Rinsed with cold water.
	LG1	Ground madder roots put in distilled water and boiled. Filtered and added alum and K ₂ CO ₃ . Again filtered.
	LG2	Same as LG1, but reversing the order of additions and decreasing the amount of K ₂ CO ₃ and alum.
	LG3	Same as LG2, but maintaining the quantities of LG1.
	LG4	Same as LG3, but the ground madder roots soaked overnight in water.
Lac dye	B131	Ground shellac added with <i>zucarino</i> alum" and gum arabic to synthetic urine. The filtered solution is decanted until a deposit is formed, which must be filtered again
	B140	Ground shellac added with alum solution to very strong boiling lye. The solution is left to stand for 2 days and then filtered to retain the solute.
	P90	Finely ground resin added to a boiling lye solution to extract the dye. Once filtered to remove impurities, heated until it becomes more viscous and spread out on a shelf to dry.
	Mont	Two containers of synthetic urine: added ground shellac and <i>zaccurino</i> alum to the first. Once the resulting solution was filtered, more <i>zuccarino</i> alum added and unheated urine to the solution. Filter and keep the solute.
	MC	Finely ground shellac put in synthetic urine and boiled to a third of its volume. Filtered- Added alum to the eluted solution to promote precipitation. Filter the solution and keep the precipitate.
Kermes	K1	Grains of kermes dispersed in lye. Boiled and filtered. Added alum solution. Filtered.
	K2a	Same as K1, but more alum.
	K2b	Saturated solution of alum added to the mother liquor of K2a, resulting from the final filtration, until the end of the precipitation. Filtered.
	K3	Wool dyed with kermes put in lye (K ₂ CO ₃ in distilled water). Boiled and filtered. Added alum. Filtered.
	OR	Orcein extract directly diluted in the binder and spread on parchment.
Folium	FO-Aq	Folium dye extracted from a commercial cloth soaked in <i>chrozofora tinctoria</i> juice with distilled water and spread on parchment as such.
	FO-A	Same as FO-Aq, but spread on parchment with egg white as a binder.
	FO+BU	Same as FO-Aq, then the extract was mixed with burnt umber and spread with egg white as a binder.

FO@AZ	Folium lake FO-A spread as a glaze over a layer of azurite.
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FO@UM	Folium lake FO-A spread as a glaze over a layer of ultramarine.
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Table S2. L*a*b* color coordinates and chroma (C*) values for the paint mock-ups examined in the present work.

Dye		L*	a*	b*	C*
Carmine	CA_A	51.3	32.4	-2.5	32.5
	CA_G	58.4	35.9	-1.6	35.9
	C5_G	47.0	28.7	-5.5	29.2
	C6_A	46.7	37.6	4.8	37.9
	C6_G	44.4	41.7	0.6	41.7
Kermes	K1_A	64.6	24.7	8.4	26.0
	K1_G	67.2	23.7	9.2	25.4
	K2A_A	79.2	22.0	15.4	26.9
	K2B_A	70.3	22.7	9.6	24.7
	K2B_G	78.4	16.6	8.9	18.9
	K3_G	76.4	16.4	4.3	16.9
Madder	GA_A	57.8	45.0	20.6	49.5
	LG1_A	62.2	33.2	17.4	37.5
	LG1_G	63.4	35.3	17.2	39.3
	LG2_A	68.6	30.8	15.9	34.6
	LG2_G	48.1	43.0	21.7	48.1
	LG3_A	50.7	35.5	13.7	38.1
	LG4_A	59.3	39.1	21.8	44.7
Brazilwood	E1B_G	80.4	20.1	9.1	22.1
	E1S_G	43.5	22.6	5.2	23.2
	E1C_G	66.5	27.1	11.4	29.4
	LDB1_A	53.2	29.5	13.8	32.6
	LDB3_A	51.5	24.5	9.4	25.2
	L1_G	73.2	25.8	10.0	27.7
	L2_G	74.5	23.5	9.0	25.1
	L1M3_A	81.3	7.1	-1.7	7.3
	L1M3_G	91.4	1.9	-2.9	3.5
	L1M1_A	84.1	12.8	4.4	13.6
	L1M1_G	90.2	6.9	-0.5	1.2
	LB3E_G	68.3	26.2	14.7	30.0
	R1R_A	79.6	1.1	-0.5	1.2
	R2R1_G	74.5	23.5	9.0	25.1
	LB1R_A	61.1	28.7	-1.1	28.7
	LB1R_G	60.7	30.8	2.7	31.0
Lac dye	B131_A	49,2	16,7	1,5	16,8
	B131_G	34,5	23,3	2,4	23,4
	B140_A	47,4	26,0	3,0	26,2
	B140_G	50,6	29,1	4,0	29,4
	MC_A	34,8	15,7	-0,3	15,7
	MC_G	39,8	33,73	3,0	33,89
	MP_A	46,5	29,2	7,2	30,1
	MP_G	54,4	43,2	-8,1	44,0

	P90_G	46,4	26,4	2,2	26,5
Folium	FO@AZ	31,3	-7,8	-23,5	24,7
	FO+BU	62,6	7,4	-4,6	8,8
	FO_Aq	66,3	2,0	-16,1	16,3
	FO_A	54,6	6,9	-16,5	17,8
	FO@UM	29,7	-2,7	-29,9	30,0
Orcein	O_G	66,8	19,4	4,5	19,9
	O_A	72,9	11,0	-19,2	22,1

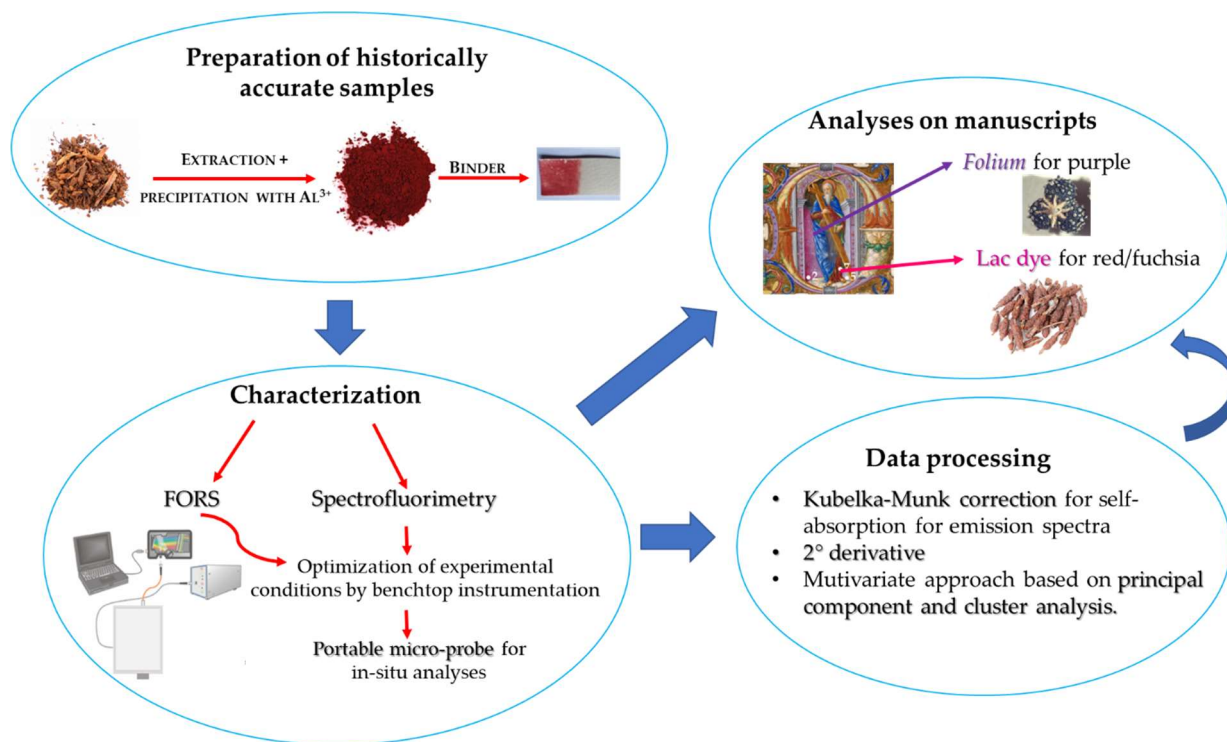


Figure S1. Flowchart of the experimental protocol followed in the present work.

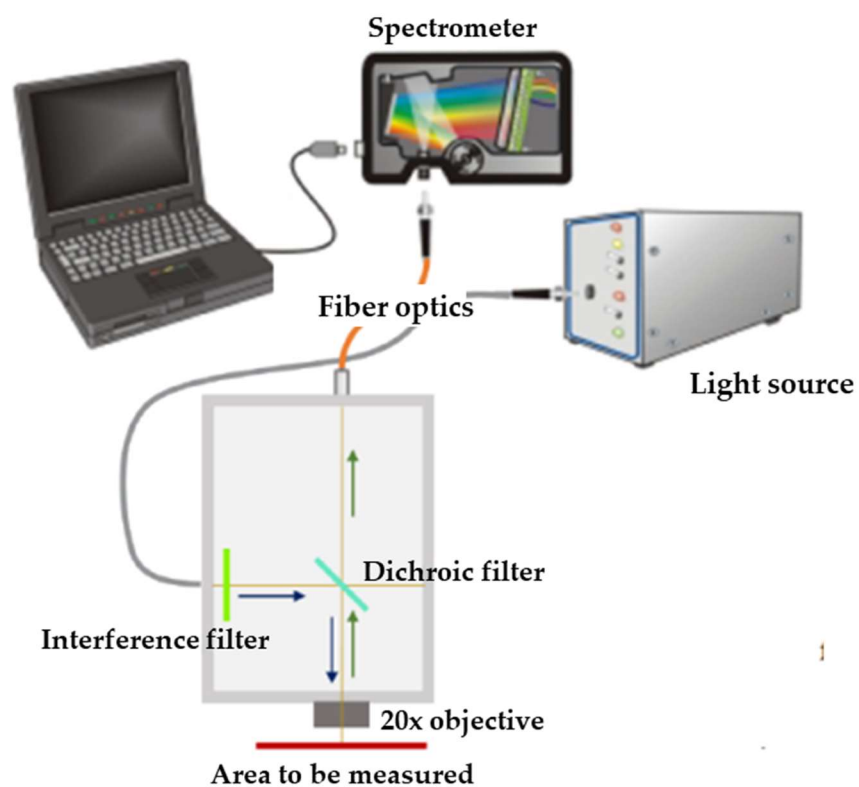


Figure S2. Scheme of the instrumental setup used for in situ fluorescence measurements.

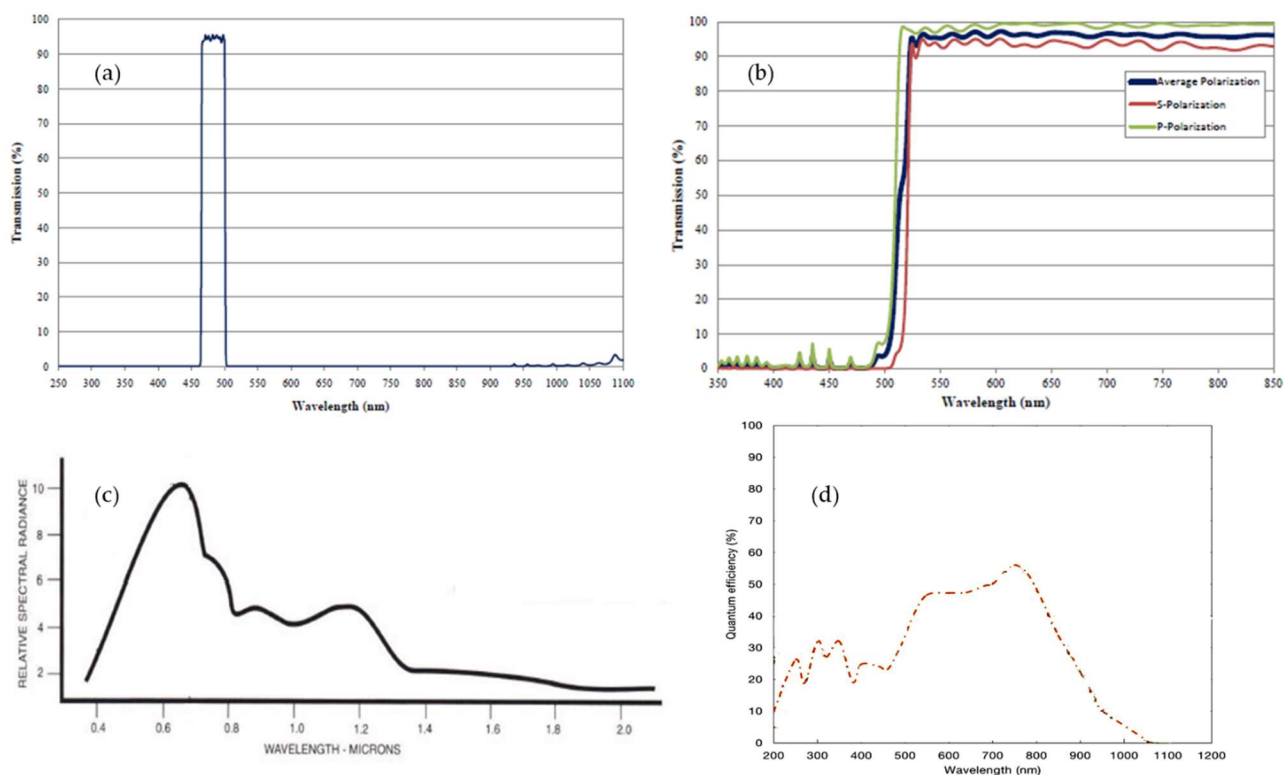


Figure S3. (a) Transmission curve of the interference filter; (b) transmission curve of the dichroic filter; (c) spectral radiance of the halogen source; (d) quantum efficiency of the CCD detector. For the optical components see the scheme of the microprobe shown in Figure S1.

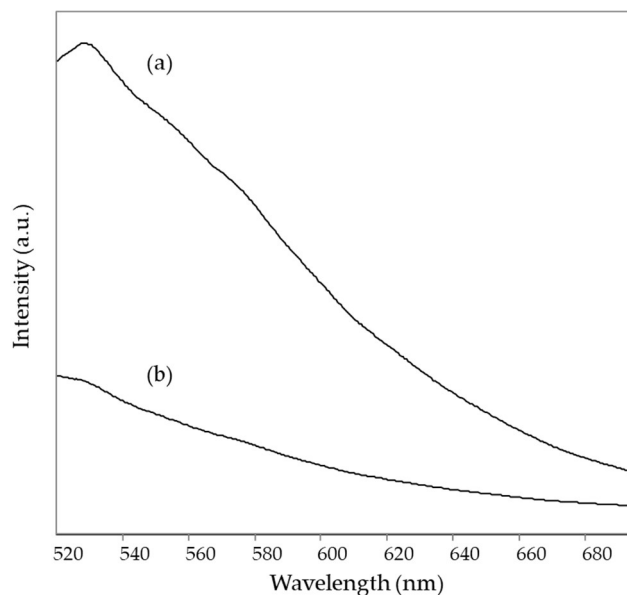
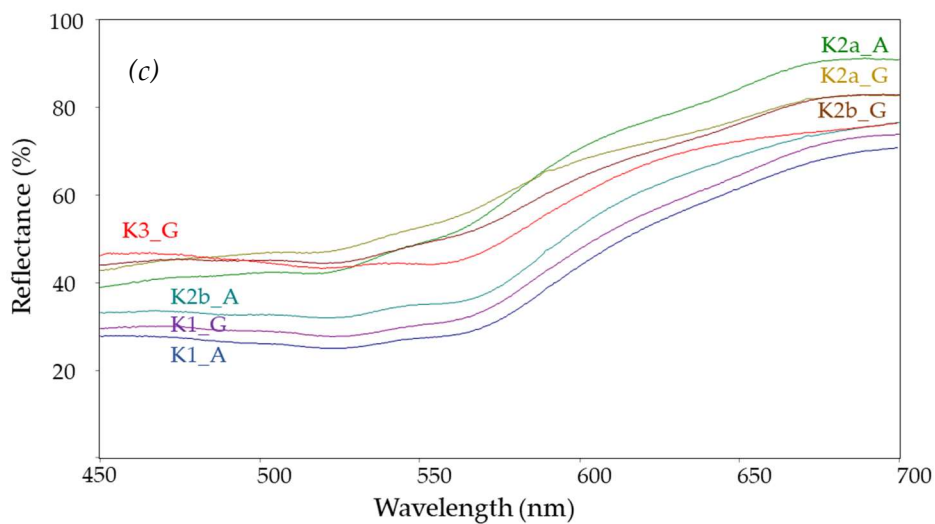
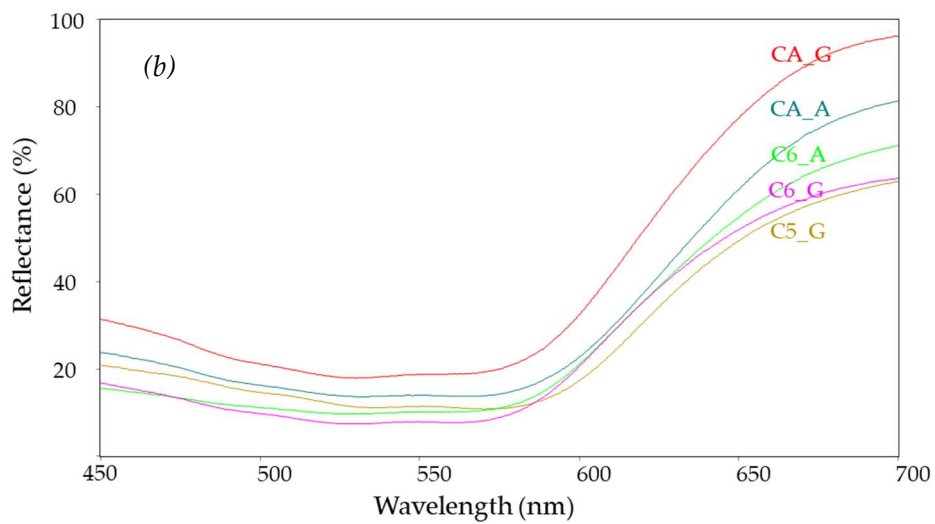
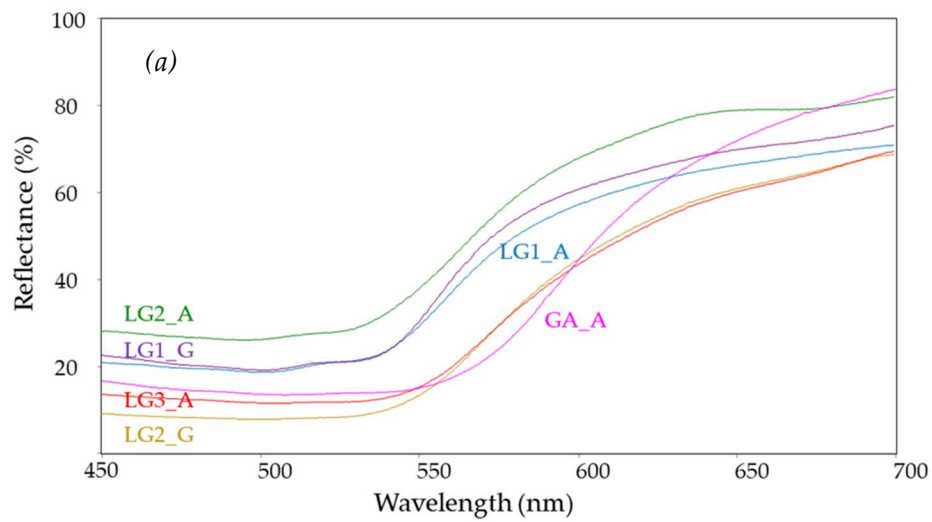
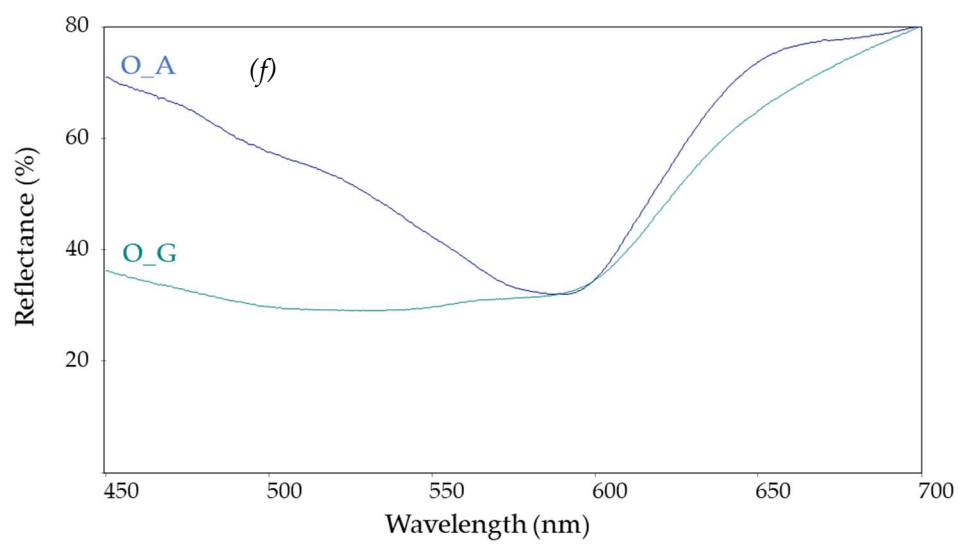
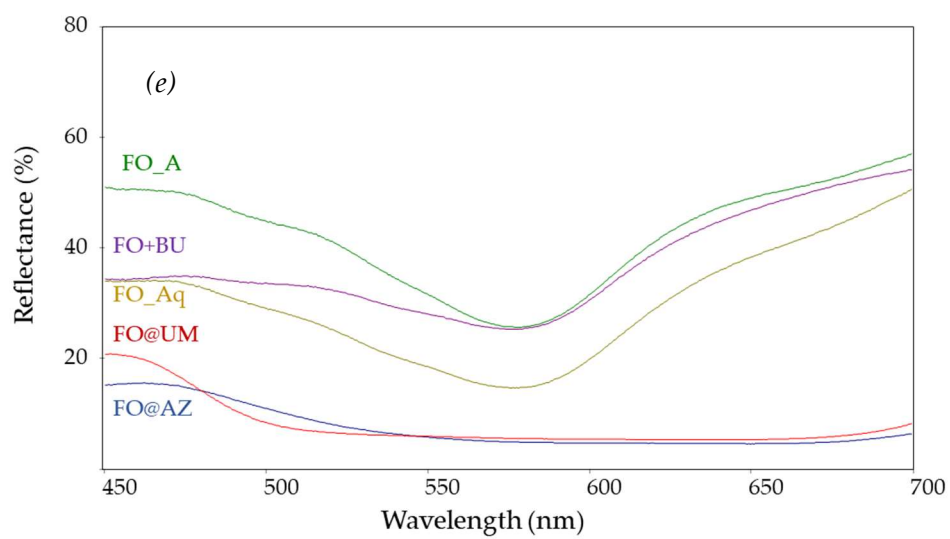
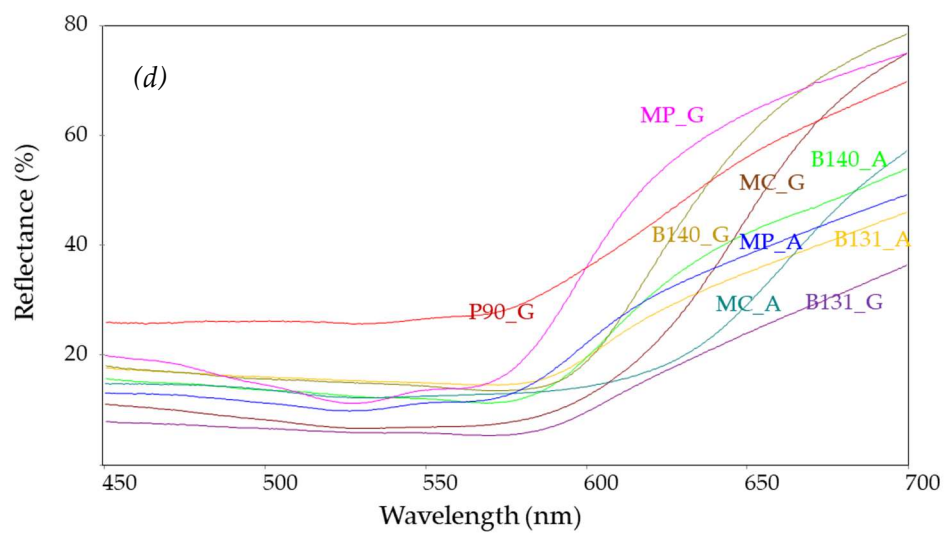


Figure S4. Emission spectra acquired from (a) bare parchment used to prepare the paint mock-ups; (b) a BaSO₄ target analysed as non-fluorescent material. The contribution to the support is evident in the first case. The apparent maximum at around 530 nm is due to the cut on of the dichroic filter used to reject the component associated with the exciting radiation.





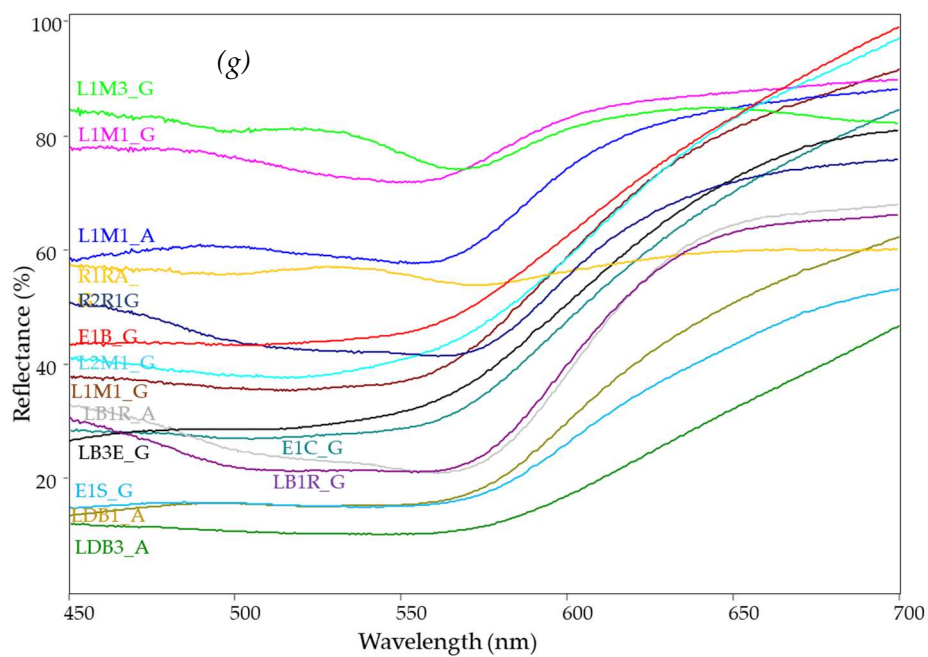


Figure S5. Visible reflectance spectra of the mock-up samples of red and purple lakes analysed: a) carmine; b) madder; c) kermes); d) lac dye; e) folium; f) orcein and (g) brazilwood.

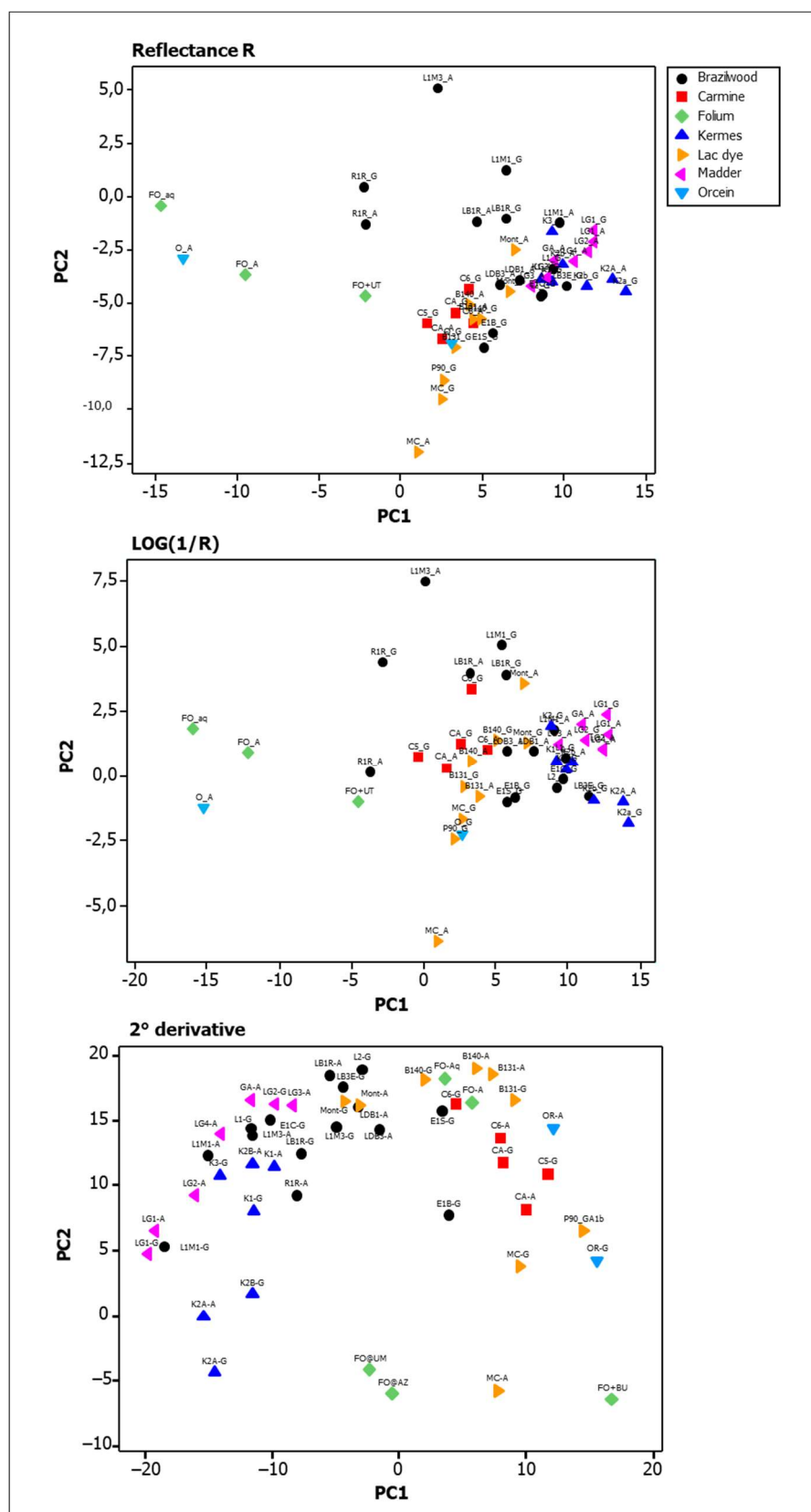


Figure S6. Score plots of the first two principal components of the reflectance spectra of the reference lake samples: (top) reflectance (R) spectra (explained variance 89.4%); (middle) spectra transformed as $\log(1/R)$ (explained variance 87.9%); (bottom) second derivative of the reflectance spectra (explained variance 69.6%).

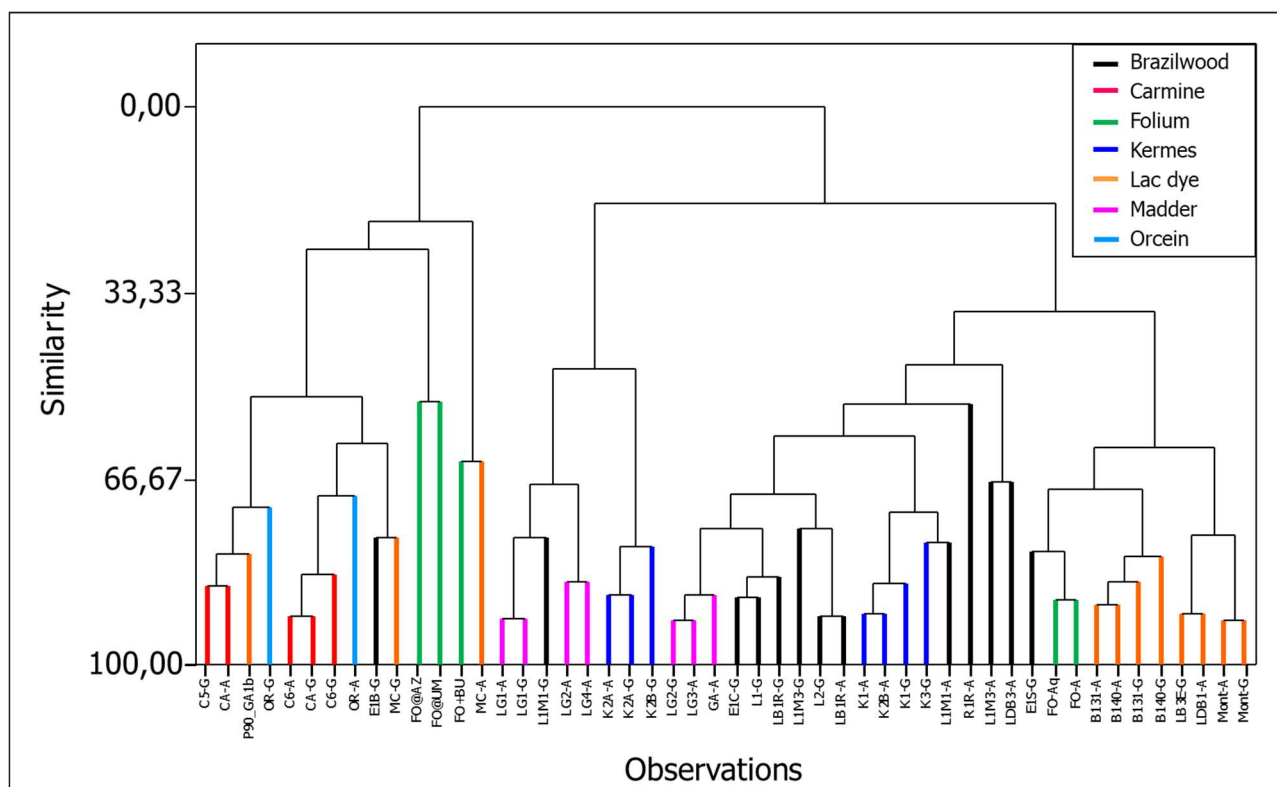
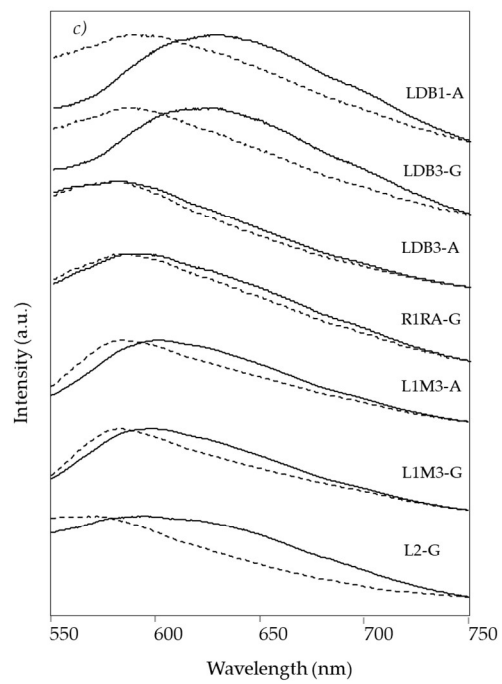
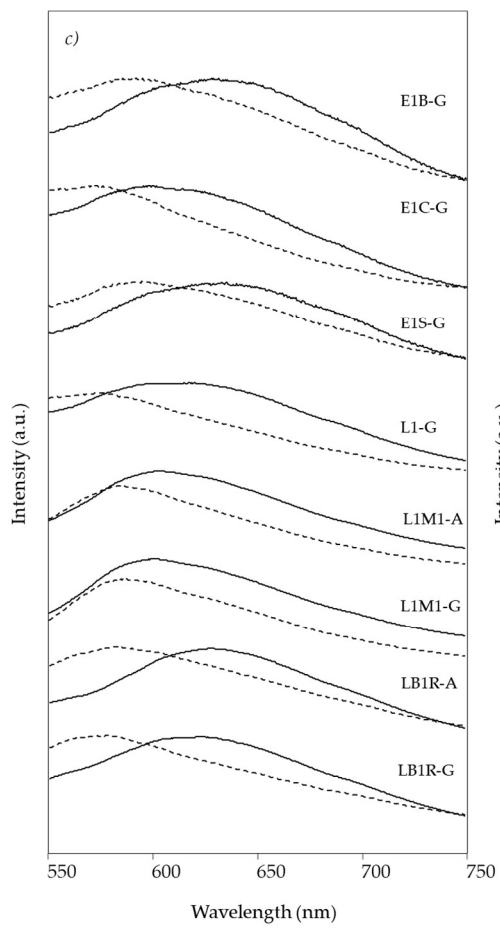
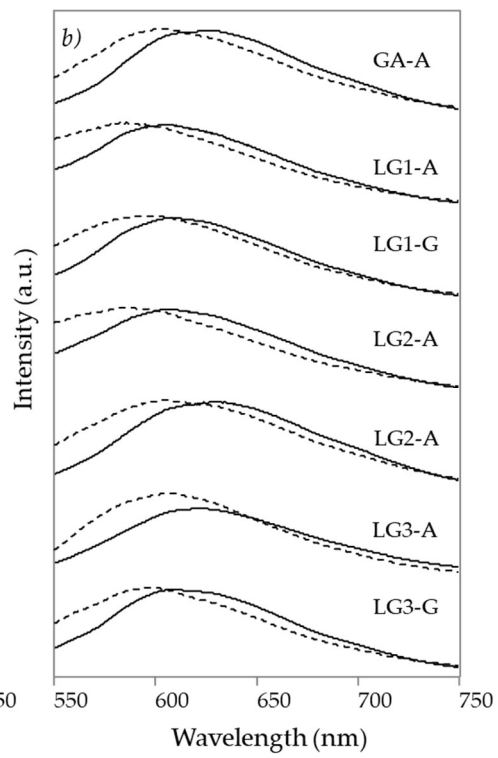
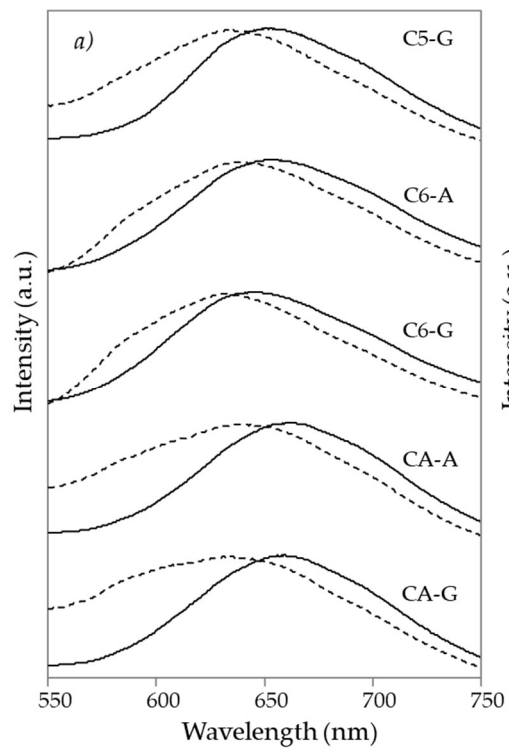


Figure S7. Dendrogram generated by CA applied to the second-derivative reflectance spectra of the reference lake samples.



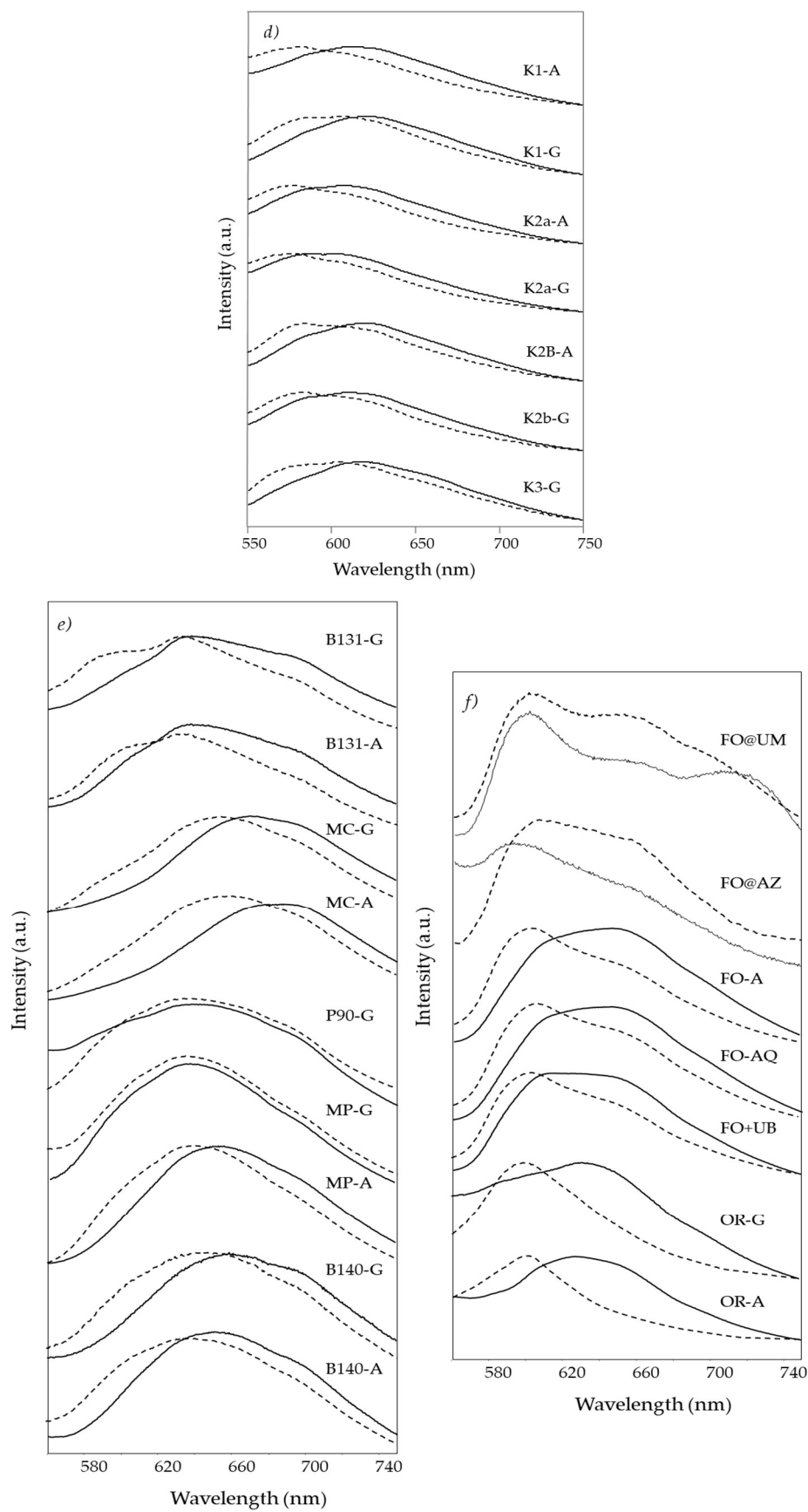


Figure S8. Emission spectra (λ_{exc} 482.5 nm) of the lake mock-up samples analyzed in the present work before (solid line) and after (dotted line) the Kubelka-Munk correction. a) carmine lakes; b) madder lakes; c) brazilwood lakes; d) kermes lakes; e) lac dye lakes and f) purple lakes (folium and orcein).

Table S3. Emission maxima (λ_{exc} 482.5 nm) of the mock-ups of lakes on parchment. The values in parentheses refer to the spectra corrected according to the Kubelka-Munk theory.

	Sample	λ_{em} (nm)
Carmine	C5-G	658 (636)
	C6-A	653 (639)
	C6-G	645 (632)
	CA-A	662 (641)
	CA-G	658 (636)
Brazilwood	E1B-G	621 (581)
	E1S-G	634 (599)
	L1-G	617 (576)
	L1M1-A	615 (585)
	L1M1-G	615 (587)
	L1M3-A	615 (584)
	L1M3-G	618 (583)
	L2-G	614 (573)
	LB1R-A	629 (586)
	LB1R-G	624 (579)
	LB2G_CH	578 (570)
	LDB1-A	628 (595)
	LDB3-A	622 (587)
	R1R-A	585 (580)
	R1R-G	590 (584)
Madder	GA-A	625 (602)
	LG1-A	605 (585)
	LG1-G	611 (597)
	LG2-A	610 (586)
	LG2-G	628 (608)
	LG3-A	621 (606)
	LG4-A	618 (598)
Kermes	K1-A	615 (612+586)
	K1-G	585+602 (585+609)
	K2A-A	577+603 (585+607)
	K2a-G	586+601 (585+609)
	K2b-A	619+586 (585+609)
	K2b-G	588+612 (587+609)
	K3-G	617 (604+585)
Lac dye	B131-A	637+691 (606+633+691)
	B131-G	597+632+696 (638+690)
	B140-A	652+689 (638)
	B140-G	652 (628)
	P90-G	662+603 (603+630)
	Mont-G	630 (604+636)
	Mont-A	649 (640)
	MC-A	675+695 (655+692)
	MC-G	662+692 (650+691)

Orcein	OR-G	635 (601)
	OR-A	630 (601+626)
Folium	FO-A	646 (606+646)
	FO-Aq	646 (606+646)
	FO+BU	612+646 (604+646)
	FO@AZ	595+696 (606+653)
	FO@UL	603+711 (603+658+694)

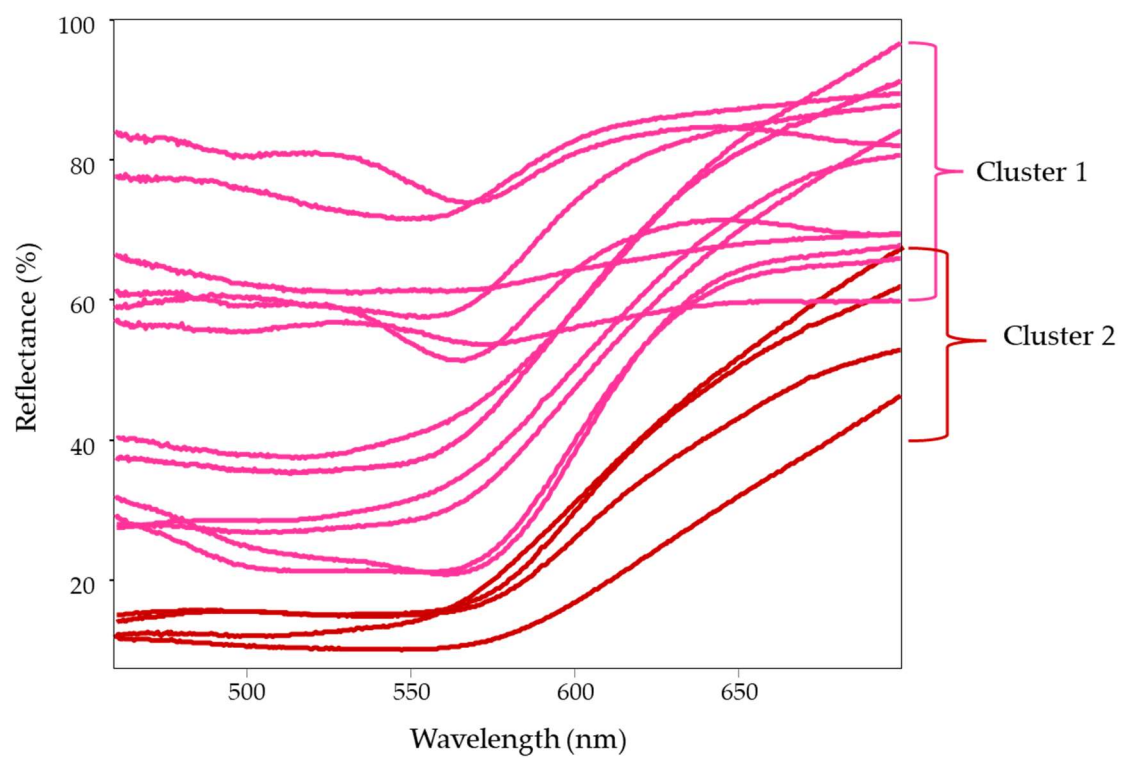
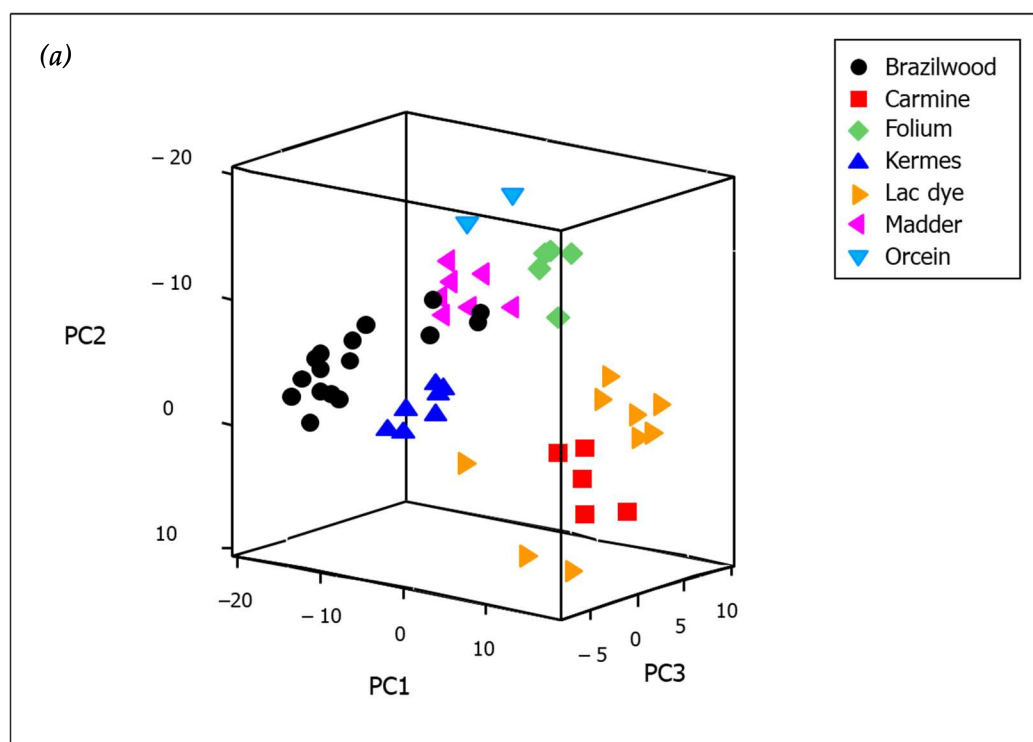


Figure S9. Reflectance spectra of brazilwood lake mock-up samples. The spectra corresponding to the lightest colored lakes (pink line) form cluster 1, those corresponding to the darkest ones form cluster 2.



(b)

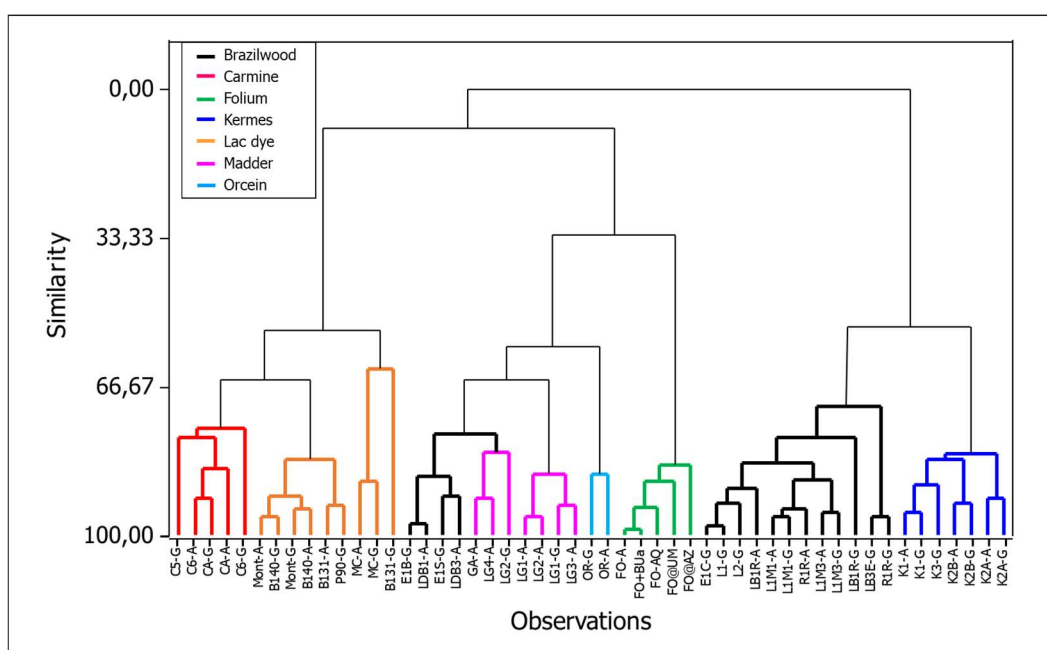


Figure S10. (a) Score plot (obtained from Minitab software) of the first three principal components of the second-derivative emission spectra of the reference lake samples (explained variance 88,8%); (b) dendrogram obtained from the cluster analysis of the first three principal components.

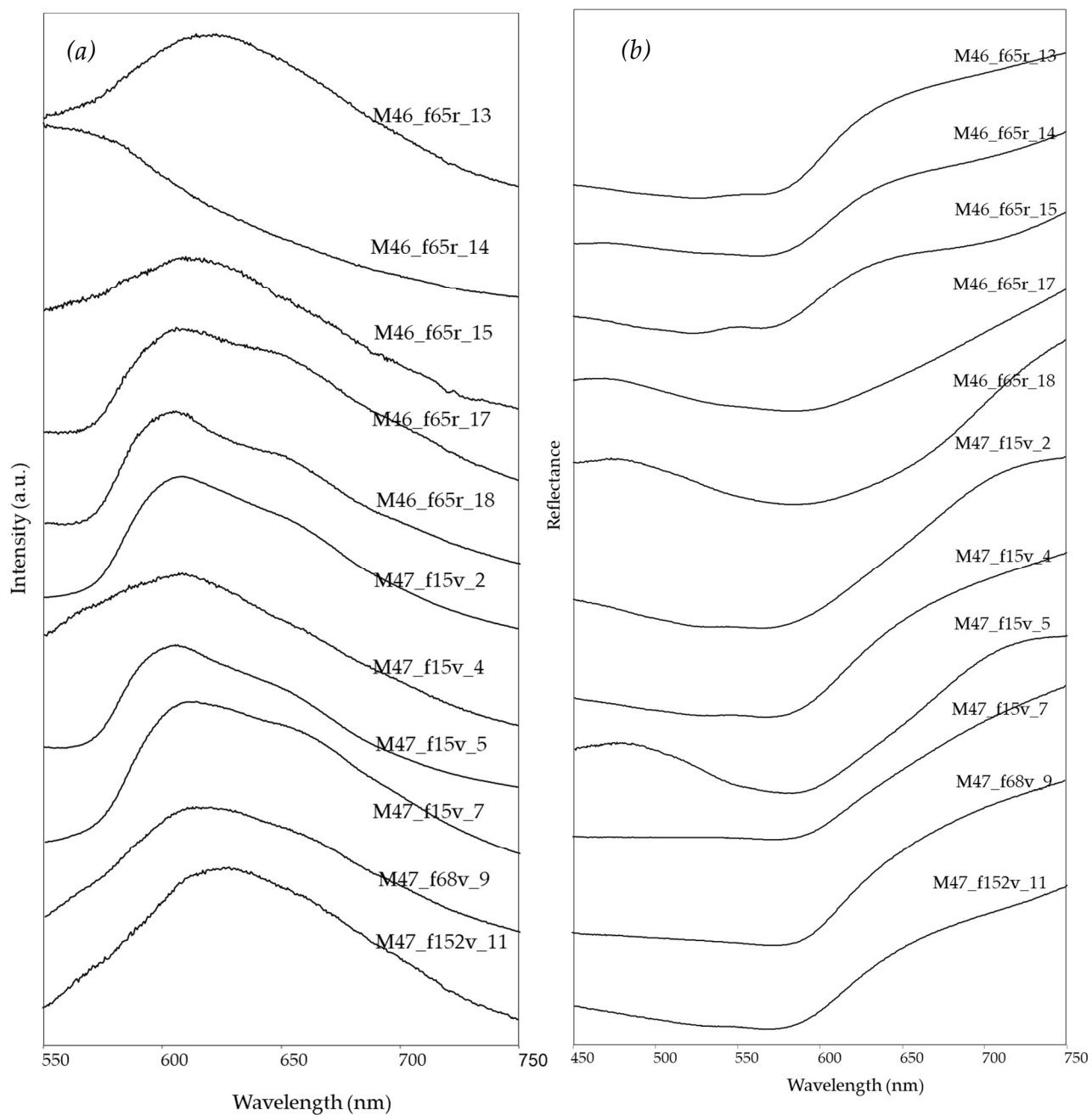


Figure S11. (a) Emission spectra (corrected for self-absorption) and (b) visible reflectance spectra acquired in situ from illuminated details of the Crescenzo choir books.

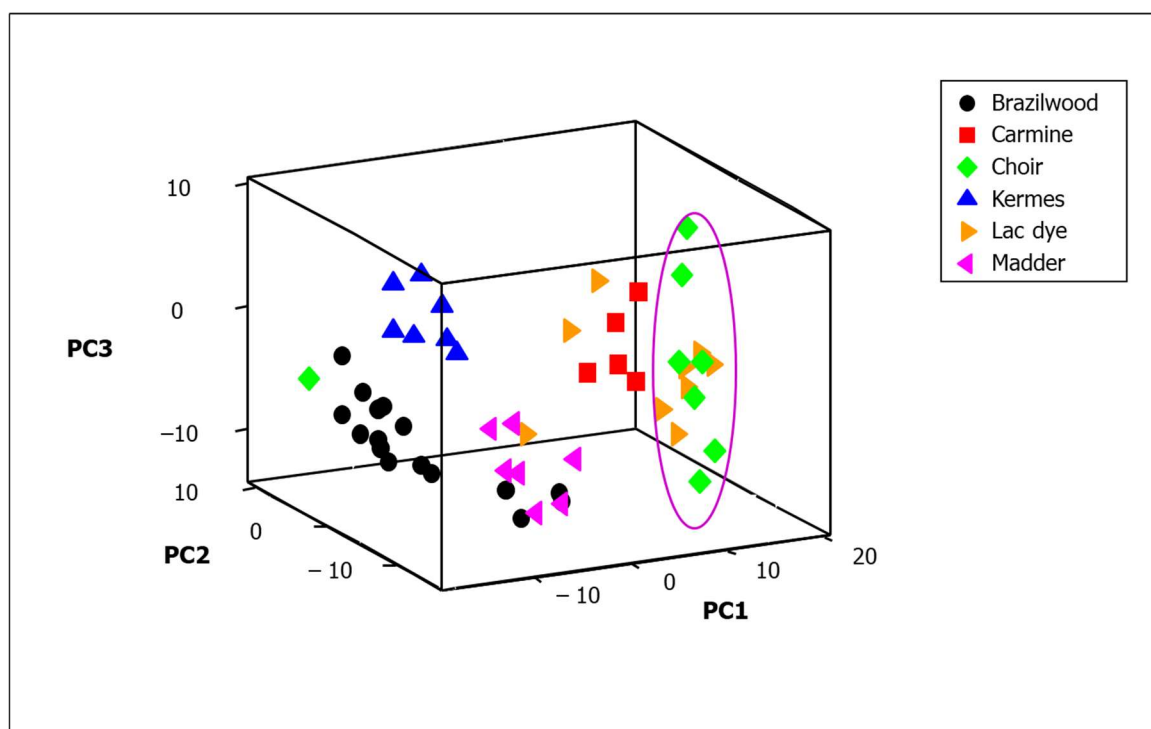


Figure S12. Score plot of the first three principal components of the second-derivative emission spectra of the reference lake samples and those obtained from red and fuchsia areas of the Crescenzo choir books (explained variance 90,2%).

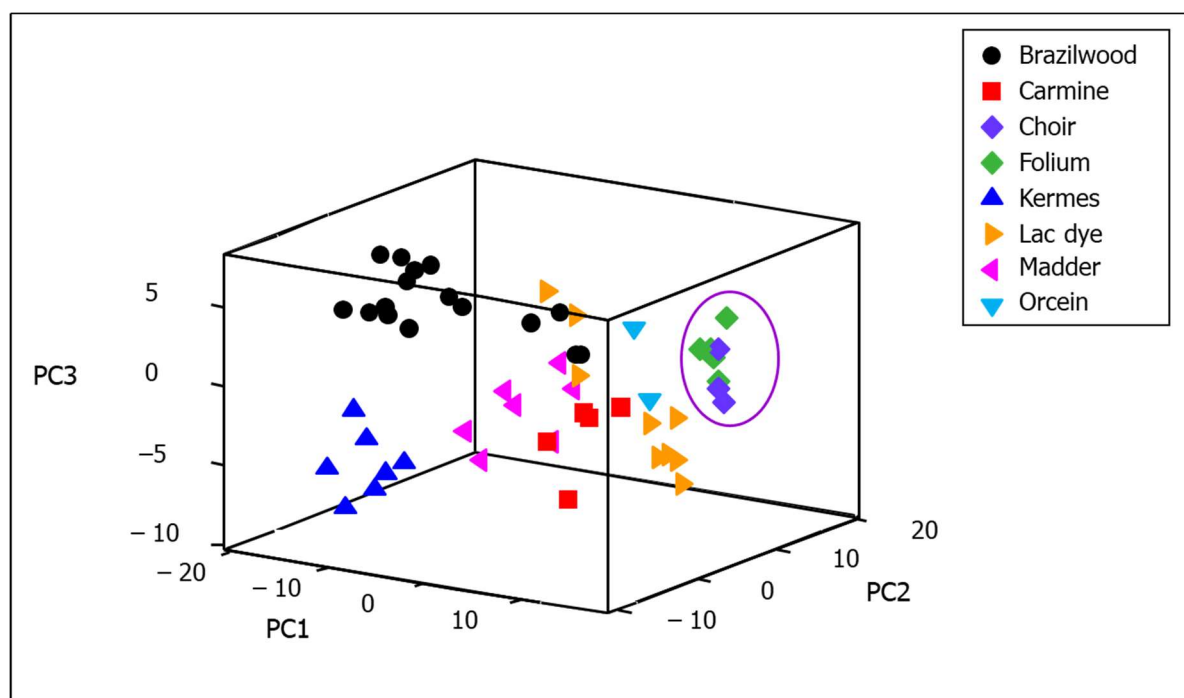


Figure S13. Score plot of the first three principal components of the second-derivative emission spectra of the reference lake samples and those obtained from purple areas of the Crescenzo choir books (explained variance (explained variance 89,1%).