Supplementary materials

Experimental details

Reagents, reference products and MB-like specimens preparation. Synthetic indigo (Fluka), isatin, (Aldrich), Indirubin (RG Chromadex), lapachol (Extrasynthèse), were used as dye blanks. Methanol, dimethylsulfoxide (Carlo Erba), hexamethyldisilazane (HMDS) (Sigma-Aldrich) (purity 99%). Indigo (Fluka), isatin, (Aldrich) and indirubin (RG Chromadex) and commercial indigo pigment from *Isatis tinctoria* (Kremer 36002) were used as reference indigoid compounds. Dehydroindigo was prepared, following reported procedures,[1-4] by oxidation of indigo with $KMnO_4$ in the presence of acetic acid and subsequent alkaline treatment of the intermediate diacetate. Palygorskite, collected from the *Sak lu'um* classical site in Yucatan and sepiolite (obtained from the repository of the Clay Mineral Society) were used to prepare MB-type specimens. These were prepared by finely grinding and mixing 1.0 % (w/w) of indigo (IN) or lapachol (LA) with palygorskite in an agate mortar and pestle during 60 min. The resulting specimens were separated in different portions (labeled here as LA@$clay_t$ and IN@$clay_t$) and submitted to heating at temperatures between 100 and 180 ºC in furnace during 24 h and subsequently were repeatedly rinsed with DMSO and acetone to remove indigo which remains unattached to the clay. In order to ensure minimal excess of non-associated indigo, the initial proportion of indigo to palygorskite (1% w/w) in such samples was taken below the theoretical maximum of ca. 4 % w/w. Blank experiments were also performed with palygorskite and sepiolite clays and dye samples with no heating (IN@PL_{25}, IN@SP_{25}, LA@PL_{25}, LA@SP_{25}).

Extraction procedures. Aliquots of dye@clay samples were subjected to extraction with DMSO, 90:10 (% v/v) H_2O:DMSO and 50:50 (% v/v) MeOH:DMSO mixtures in closed vials. Prior to the extraction the suspensions were maintained 24 h under magnetic stirring. The resulting extracts were studied conjointly with solutions of indigo and lapachol in the same solvents.

Instrumentation and methods. The extracts were injected into a LC-DAD equipment consisted of an Agilent 1200 Series HPLC system equipped with a UV diode array detector set at 286 nm (Agilent Technologies, Palo Alto, CA, USA). The column was a Agilent Zorbax XDB C18 150x4,6 mm 5 µm particle size (Agilent, Palo Alto, USA)

preceded by a Agilent C18 12 mm, 4.6 µm guard cartridge. Signals were processed by Agilent ChemStation software Ver. 10.02 [1757]. Analysis was performed in the gradient mode. The mobile phase used was a mixture of two solvents (solvent A: water - 0.1% formic acid and solvent B: acetonitrile). Gradient conditions were initiated by holding the mobile phase composition for 0.1 min with 7% B, after that it was changed linearly to 75% B during 12 min. The composition was then changed to 98% B in 3 min and maintained for 4.5 min as a cleaning step in order to improve the results. After cleaning, the eluent composition was returned to the initial 7% B. The flow rate of the mobile phase was 1.2 mL/min and injection volume was 10 µL. The column oven was operated at 35 ºC.

UPLC-MS analyses of the extracts obtained from samples from the different reference materials and specimens were performed in an ACQUITY UPLC system (Waters Corp.) with a conditioned autosampler at 4 °C. Liquid samples were prepared by diluting 0.1 mL of the extracts in 1.0 mL of acetonitrile. Typically, 20 µL of the prepared sample was injected into the UPLC system equipped with Phenomenex Kinetex XB-C18 column (100 x 4.6 mm i.d.; 2.6 µm particle size). The column temperature was maintained at 40 °C. The mobile phase, pumped at 1.0 $ml \cdot min^{-1}$, consisted of 0.1% formic acid in water (A) and acetonitrile (B). The gradient applied was the following: 7% B isocratic to 0.1 min, to 75% B (linear) at 12 min, to 98% B (linear) at 15 min, to 98% B isocratic to 19.5 min, to 7% B (linear) at 20.5 min and 7% B isocratic until 25 min. Separated components of sample mixture were detected by means of a Waters ACQUITY™ XevoQToF Spectrometer (Waters Corp.) connected to the UPLC system via an electro-spray ionization (ESI) interface. The ESI source was operated in positive ionization mode with the capillary voltage at 1.5 kV. The temperature of the source and desolvation was set at 100 °C and 400 °C, respectively. The cone and desolvation gas flows were 100 $L \cdot h^{-1}$ and 800 $L \cdot h^{-1}$, respectively. All data collected in Centroid mode were acquired using Masslynx™ software (Waters Corp.). Leucine-enkephalin was used as the lock mass generating an $[M+H]^+$ ion (m/z = 556.2771) at a concentration of 2 $ng \cdot mL^{-1}$ and flow rate of 50 $\mu L \cdot min^{-1}$ to ensure accuracy during the MS analysis.

Pyrolysis-silylation gas chromatography-mass spectrometry (Py-GC-MS) experiments were carried out with an integrated system composed of a CDS Pyroprobe 1000 heated

filament pyrolyser (Analytical Inc., New York, USA), and a gas chromatograph Agilent 6890N (Agilent Technologies, Palo Alto, CA, USA) coupled to an Agilent 5973N mass spectrometer (Agilent Technologies) and equipped with pyrolysis injection system. A capillary column HP-5MS (5% phenyl–95% methylpolysiloxane, 30 m, 0.25 mm i.d., 0.25 μm film thickness, Agilent Technologies) was used in order to provide the adequate separation of components. Pyrolysis was performed at 600 ºC for 10 s using a precalibrated Pt coil type pyrolyzer (CDS pyroprobe). The pyrolyser interface and the inlet were set at 250 ºC. The samples were injected in split mode (split ratio 1:40).

The chromatographic conditions were as follows: initial temperature of 50 ºC held for 10 min. and then increased at 5 ºC min^{-1} up to 300 ºC held for 8 min. Helium gas flow was set at 1.5 ml min^{-1}. The inlet pressure of the carrier gas was 89.1 kPa. The electronic pressure control was set to constant flow mode with vacuum compensation. Ions were generated by electron ionisation (70 eV). The mass spectrometer was scanned from m/z 20 to m/z 800, with a cycle time of one second. An Agilent Chemstation software G1701CA MSD was used for GC–MS control, peak integration and mass spectra evaluation. Tuning of the mass spectrometer was checked using perfluoro-tributylamine. EI mass spectra were acquired by total ion monitoring mode. The temperatures of the interface and the source were 280 and 150 ºC, respectively. Wiley Library of Mass Spectra and NIST were used for identifying compounds. Samples were placed in a micro quartz pyrolysis tube and then two small portions of quartz wool were introduced in both sides of the quartz tube in order to avoid undesirable displacements of the sample and, after this, 5–10 μL of HMDS were added. Afterwards, the sample was placed in the pyrolysis coil and introduced in the pyrolysis interface. At ca. 1 μg of solid sample of each reference material and specimens was introduced in a quartz tube with a small plug of quartz wool and 1 μL of HMDS was afterwards added.

Voltammetry of microparticles experiments were performed at sample-modified paraffin-impregnated graphite electrodes (PIGEs) using a CH I660 equipment. A standard three-electrode arrangement was used with a platinum auxiliary electrode and a Ag/AgCl (3M NaCl) reference electrode in a cell at 298 K. Experiments in aqueous media were performed with 0.25 M acetic acid/sodium acetate solutions at pH 4.75. For modified electrode preparation, ca. 0.5 mg of the samples were thoroughly powdered in an agate mortar and pestle and extended forming a spot of finely distributed material. The lower

end of the graphite electrode was pressed over that spot of sample to obtain a sample-modified surface, as previously described. EIS measurements were performed in the 0.01 to 100000 Hz frequency range with amplitude of 10 mV at different potentials between +0.65 and -0.65 V at sample-modified graphite electrodes immersed into 2.5 mM $K_4Fe(CN)_6$ + 2.5 mM $K_3Fe(CN)_6$ solution in 0.25 M HAc/NaAc, pH 4.75.

SECM experiments were performed with a CH 920c equipment. The bipotentiostat mode was used to apply potentials to the tip (E_T) and the electrode substrate (E_S). A microdisk platinum electrode tip CH 49 (diameter 20 μm) was used. The substrate electrode was a graphite bar (geometrical area 0.785 cm^2) and the reference electrode was again AgCl (3M NaCl)/Ag. Microparticulate deposits of selected samples (with disposal of 1-2 mg) were mechanically transferred to the surface of the graphite substrate by placing it on agate mortar and pressing the graphite bar on the pellets. The distance between the tip and the substrate (d) was determined from the observed tip current ratio, based on theoretical curves, as described in literature.[19a] 2.0 mM $K_4Fe(CN)_6$ as was used as a redox probe in in 0.25 M HAc/NaAc, pH 4.75 with various combinations of E_T and E_S based on redox competition mode schemes.[19c]

ATR-FTIR spectra of sample-modified electrodes were obtained with a Bruker Vertex 70 Fourier-transform infrared spectrometer with an FR-DTGS (fast recovery deuterated triglicine sulphate) temperature-stabilised coated detector and an MKII Golden Gate Attenuated Total Reflectance (ATR) accessory. A total of 32 scans were collected at a resolution of 4 cm^{-1} and the spectra were processed using the OPUS/IR software. UV-Vis absorption spectra of the liquid extracts and diffuse reflectance spectra of powdered samples were obtained with a Perkin-Elmer lambda35 spectrometer, slit width 1 nm, scan speed 480 nm/min. ^1H and ^{13}C NMR measurements were acquired on a Bruker Advance 400 spectrometer operating at 399.91 MHz in deuterated DMSO.

XRD measurements were made using a Bruker-AXS D8-Discover diffractometer equipped with parallel incident beam (Göbel mirror), vertical θ-θ goniometer, XYZ motorized stage and with a GADDS (General Area Diffraction System). Samples were placed directly on a low background sample holder (Si(510)) and the area of interest was selected with the aid of a video-laser focusing system. An X-ray collimator system allows to analyze areas of 500 μm. The X-ray diffractometer was operated at 40 kV and

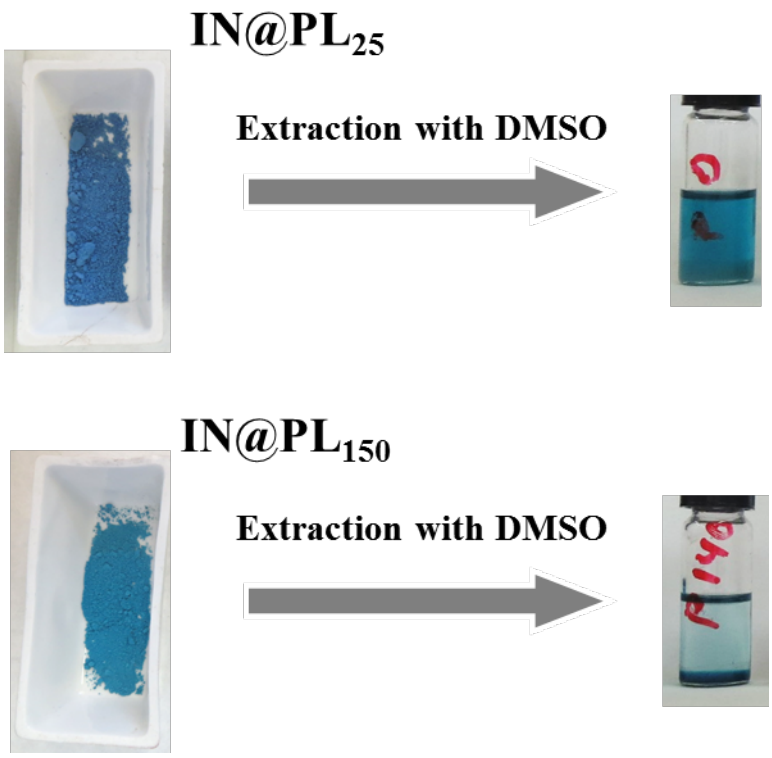
40 mA to generate CuKα radiation. The GADDS detector was a HI-STAR (multiwire proportional counter of 30x30 cm with a 1024x1024 pixel).

TEM images were obtained with a Philips CM10 transmission electron microscope equipped with Keen view camera: Soft imaging system was used operating voltage 100 kV. Samples were prepared by grinding a few micrograms in an agate mortar and then dispersing them by the help of an ultrasons bath in dichloroethane. A drop of the dispersion was poured on TEM grids pretreated with a polymer film layer with holes to improve the quality of the images.

References

(1) Kalb, L. *Ber. Dtsch. Chem. Ges* **1909**, *42*, 3642-3652.
(2) Klessinger, M.; Luettke, W. *Tetrahedron* **1963**, *19* (Suppl. 2), 315.
(3) Luettke, W.; Klessinger, M. *Chem. Ber.* **1964**, *97*, 2342.
(4) Hein, M.; Phuong, N. T. B.; Michalik, D.; Görls, H.; Lalk, M.; Langer, P. *Tetrahedr. Lett*. **2006**, *47*, 5741-5745.

Figure S.1. Images of the extracts from indigo plus palygorskite specimens prepared at room temperature ($IN@PL_{25}$) and treated at 150 ºC during 24 h ($IN@PL_{150}$), using DMSO such as extraction solvent.



The extracts contained indigo, isatin, dehydroindigo and often other minority organic components.

Table S.1. NMR data from extracts in deuterated DMSO:

Compound	**^{1}H NMR**	**^{13}C NMR**
Indigo	7.04-7.63 (m, 8H), 10.7 (N-H)	191.1 (C-3), 153.3 (C-2), 137.8 , 125.1, 124.1, 122.2, 120.6, 113.3
Dehydroindigo	7.76-7.64 (m,8H)	189.48 (C-3), 159.50 (C-2), 155.21 (C-3a), 139.10 (C-7a), 136.81 (C-6), 130.37 (C-4), 125.04 (C-5), 124.40 (C-7)
Isatin	4.30 (N-H), 6.86 (d), 7.00 (t), 7.47 (d) and 7.53 (t)	184.6 (C-3), 159.5 (C-2), 151.0 (C-7a), 138.8 (C-6), 124.6 (C-4), 123.2 (C-5), 118.0 (C-3a), 112.3 (C-7)

Figure S.2. UV-Vis spectra of the compounds eluted in the HPLC-DAD system from the DMSO extract of indigo plus palygorskite specimen treated at 150 ºC during 24 h: indigotin, t_r: 9.6 min; dehydroindigo, t_r: 9.4 min; isatin, t_r: 3.9 min.

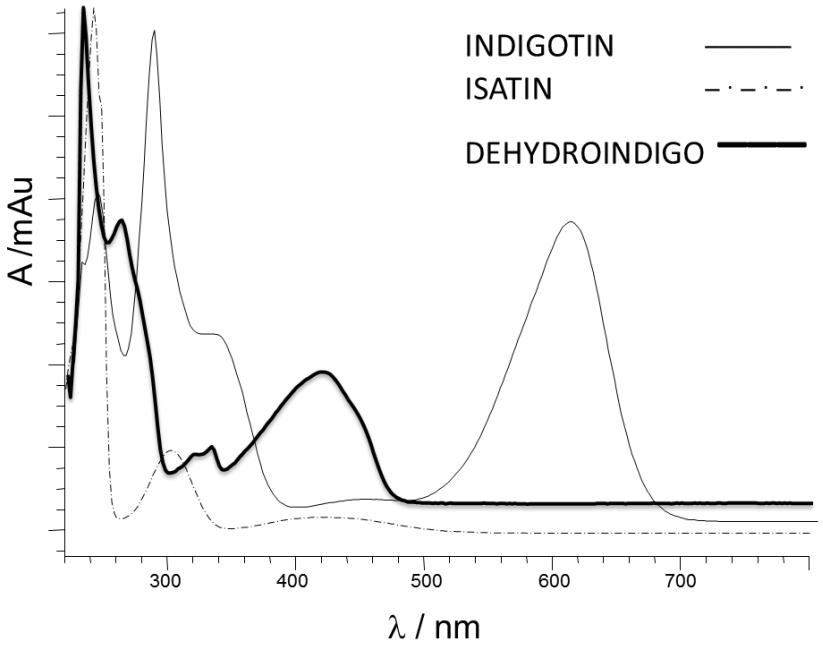


Figure S.3. Pyrogram corresponding to Py-GC-MS with UPLC-MS of palygorskite specimen treated at 150 ºC for 24 h (IN@PL_{150}). See Table S.2. for marker compound identification.

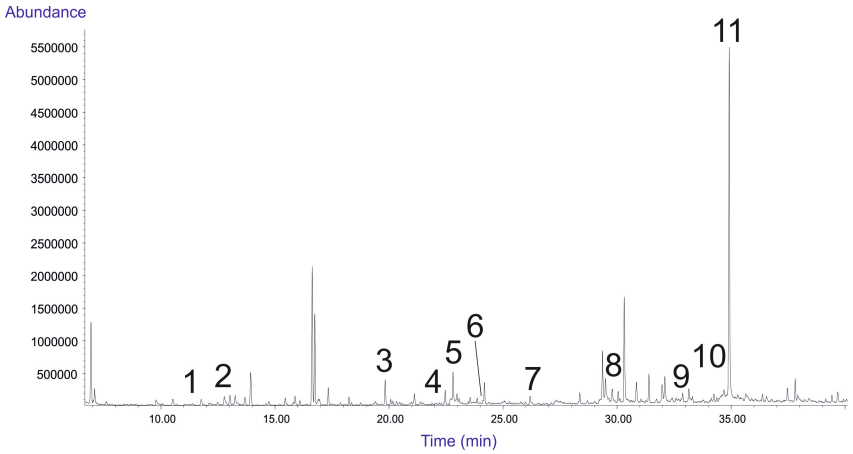


Table S.2. Main compounds identified by Py-GC-MS in IN@P_{150} specimen together with molecular weight, retention time and main fragment ions present in their mass spectra (relative abundance in brackets). Marker compounds for dehydroindigo (*) and indigo (**) are indicated.

Ref.	Compound	t_r (min)	M_w	*m/z* ion fragments
1	Benzaldehyde	11.38	106	51(50), 77(100), 105(86), 106(86)
2	Aniline	12.39	93	66(32), 93(100)
3	Benzil alcohol, TMS ether*	19.92	180	91(100), 165(82), 135(70)
4	N-Trimethylsilylaniline	21.36	165	73(7), 150(100), 165(62)
5	Benzoic acid, TMS ester	22.79	194	77(46), 105(75), 135(36, (179)100
6	1 H-Indole**	24.16	117	63(16), 90(40), 117(100)
7	4H-3,1-Benzoxazin-4-one, 2-methyl	27.13	161	90(24), 119(28), 146(68), 161(100)
8	2-Aminobenzoic acid, TMS ester	29.44	209	92(56), 120(100), 194(94), 209(70)
9	Benzoic acid, 2-(trimethylsilyl)amino-trimethylsilyl ester	32.59	281	73(71), 266(100), 281(6)
10	1 H-indole-2,3-dione	34.54	147	64(34), 92(75), 119(100), 147(65)
11	N-Acetil-2-aminobenzoic acid, TMS ester	35.04	251	119(72), 194(100), 209(72), 251(26)

Figure S.4. UPLC-MS chromatogram for indigo + palygorskite specimen treated at 150 ºC during 24 h (IN@PL_{150}) shown chromatographic peaks for indigo and dehydroindigo at 11.86 and 10.64 min.

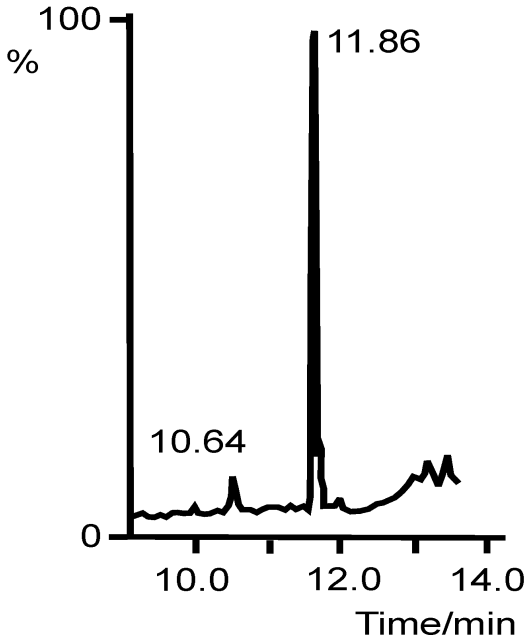
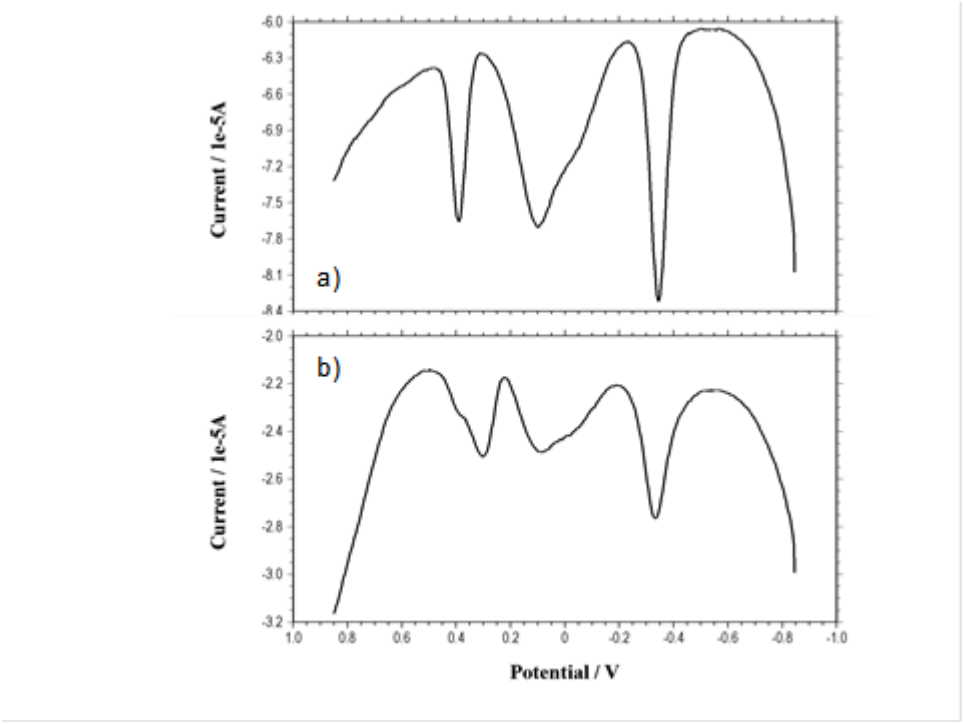


Figure S.5. Square wave voltammograms for paraffin-impregnated graphite electrodes modified with samples: a) IN@PL_{25} and b) IN@PL_{150} in contact with aqueous 0.25 M HAc/NaAc, pH 4.75. Potential scan initiated at -0.85 V in the positive direction. Potential step increment 4 mV; square wave amplitude 25 mV; frequency 50 Hz).



Solid indigo displays two well-defined peaks at +0.45 and -0.30 V corresponding, respectively to the proton-assisted oxidation of indigo ($C_{16}H_{10}O_2N_2$) to dehydroindigo ($C_{16}H_8O_2N_2$), and the proton-assisted reduction of indigo to leucoindigo ($C_{16}H_{12}O_2N_2$) (see Chart). For IN@PL and IN@SP specimens, such processes can be represented by means of the equations: [4]

$$\{C_{16}H_{10}O_2N_2\} \rightarrow \{C_{16}H_8O_2N_2\} + 2H^+(aq) + 2e^- \quad (1)$$

$$\{C_{16}H_{10}O_2N_2\}\ 2H^+(aq) + 2e^- \rightarrow \{C_{16}H_{12}O_2N_2\} \quad (2)$$

These equation, where { } denotes indigo-clay complex in the hybrid materials. For IN@PL_{25}, tall indigo signals are recorded, the ratio between the peak intensities being close to that recorded in voltammograms for indigo blanks. For IN@PL_{150}, the peaks become wider and even peak splitting appears and the peak current ratio varies significantly relative to that of indigo. These features denote that strongly clay-attached indigo accompanied by dehydroindigo appear.

Dehydroindigo and isatin are products of aerobic indigo oxidation:

$$\{C_{16}H_{10}O_2N_2\} + (1/2)O_2\ (g) \rightarrow \{C_{16}H_8O_2N_2\} + H_2O$$

$$\{C_{16}H_{10}O_2N_2\} + O_2\ (g) \rightarrow 2\{C_8H_5O_2N\}$$

The formation of dehydroindigo from indigo, relative to the indigo to isatin oxidation, will be favored by water abstraction at temperatures above 100 ºC.

Chart S.1. Schemes for the aerobic opxidation of indigo to dehydroindigo and isatin.

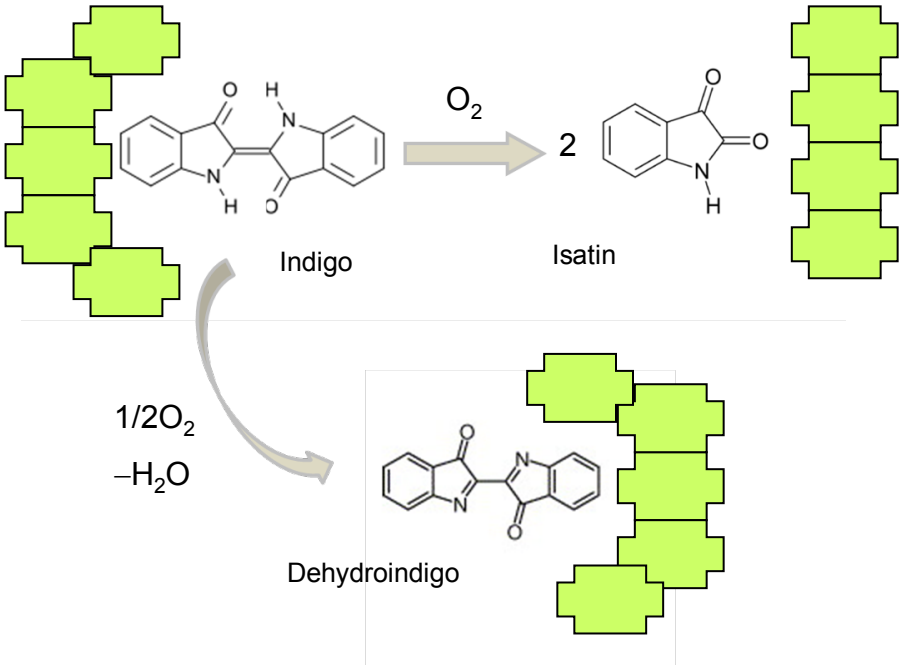
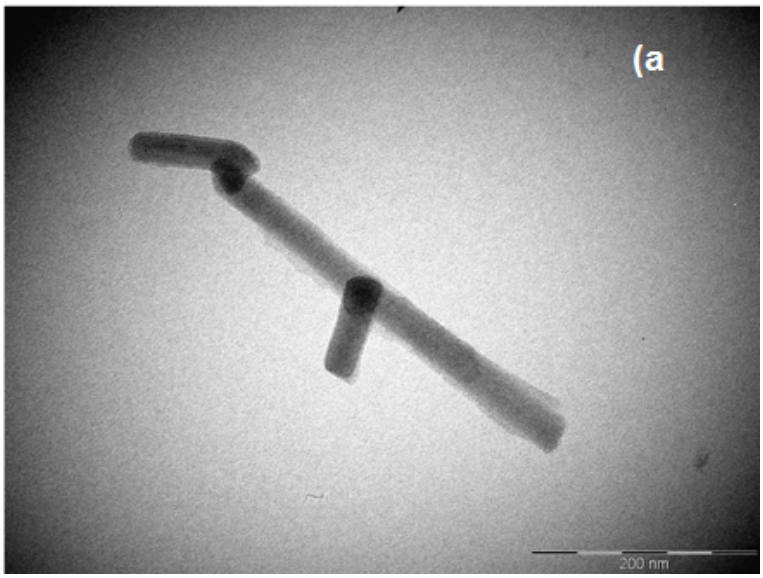


Figure S.6. TEM images of palygorskite crystals of: a) LA@PL_{25}; b) LA@PL_{150} specimens. In agreement with previous observations for Maya Blue samples,[4] acicular palygorskite crystals exhibit textural changes with appearance of pores due to the evacuation of zeolitic water after thermal treatment. Equivalent images for IN@PL and IN@SP can be found in refs.[5-7]



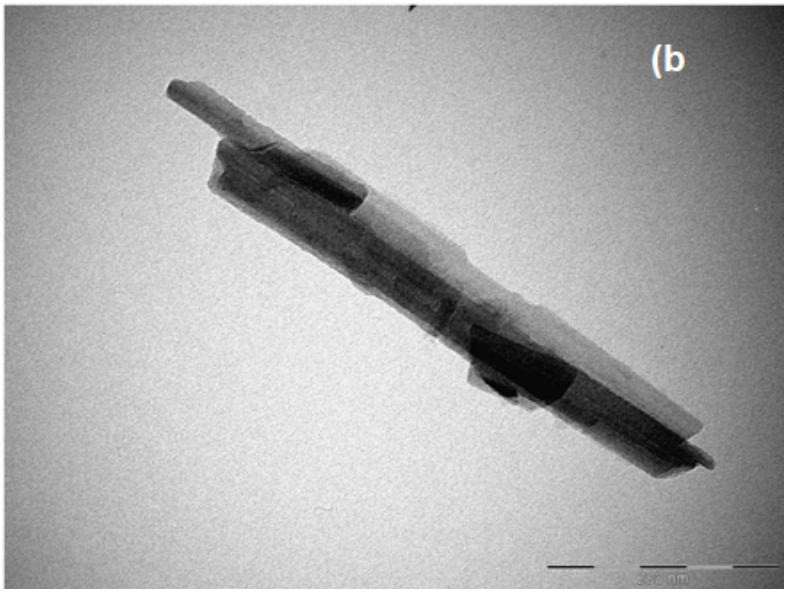
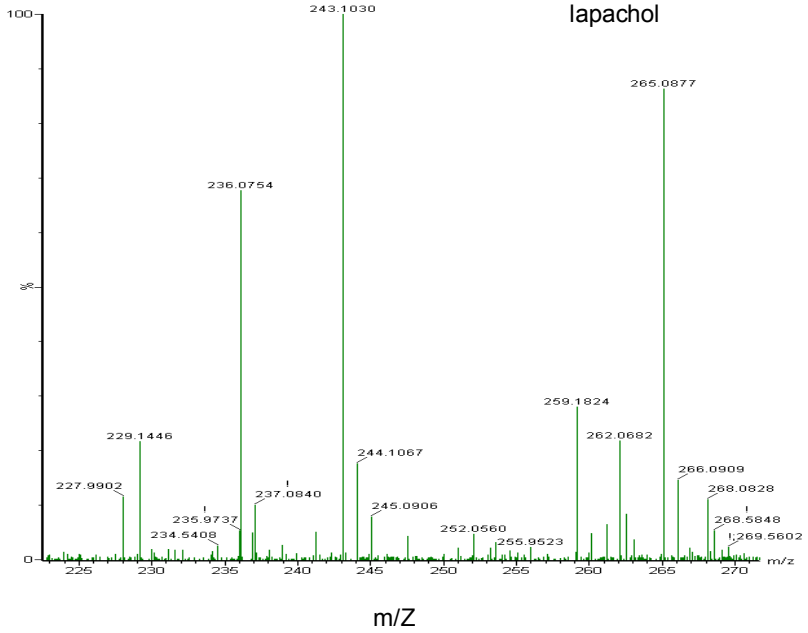


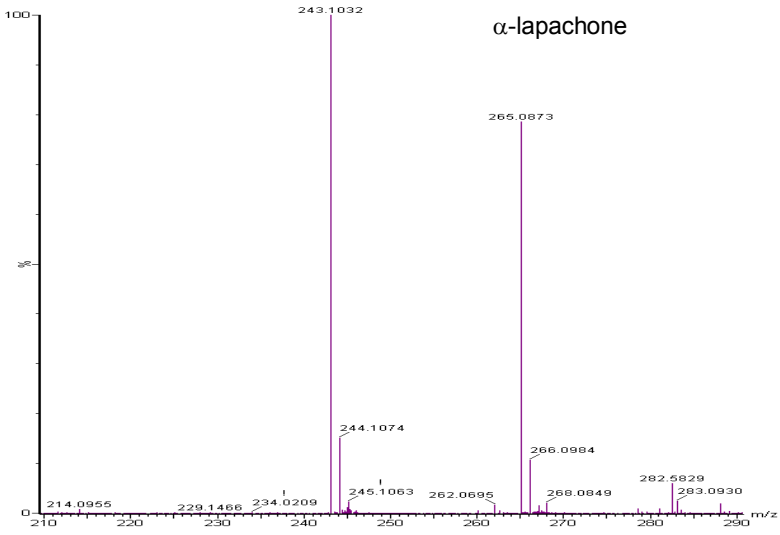
Table S.2. Majority components identified using HPLC-DAD, UPLC-MS and Py-GC-MS analysis of MeOH extracts in dye plus palygorskite specimens in this study.

Sample	Compounds identified in the extracts	$[M+H]^+$ or $[M+Na]^+$ (m/z)
LA@PL_{25}	Lapachol	243.1030
LA@PL_{150}	α-Lapachone	243.1032
	Lapachone isomer 1	265.0831
	Lapachone isomer 2	243.1022
	Lapachone isomer 3	243.1027
	4-Hydroxy-α-lapachone	281.0490
	Dihydro-4-hydroxy-α-lapachone	261.1117
	Dehydro-α-lapachone	263.0694

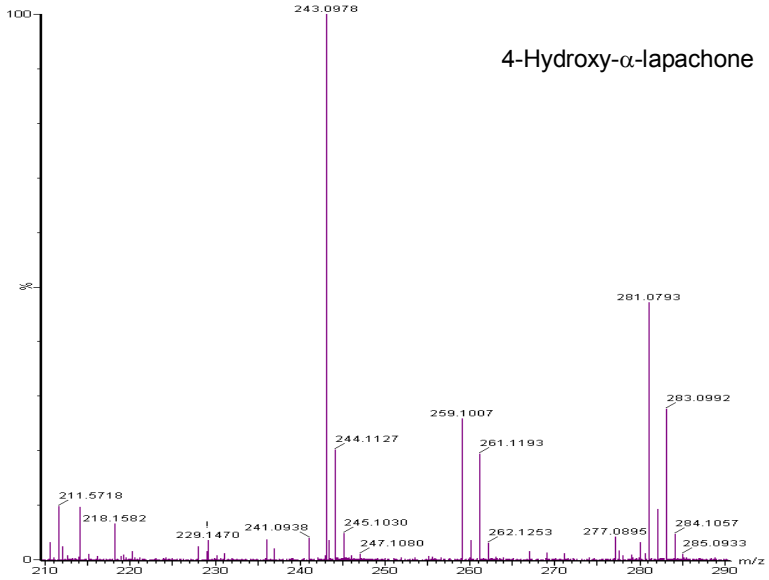
Figure S.7. MS spectra of compounds in compounds in MeOH extracts from lapachol plus palygorskite specimens treated at 150 ºC during 24 h.



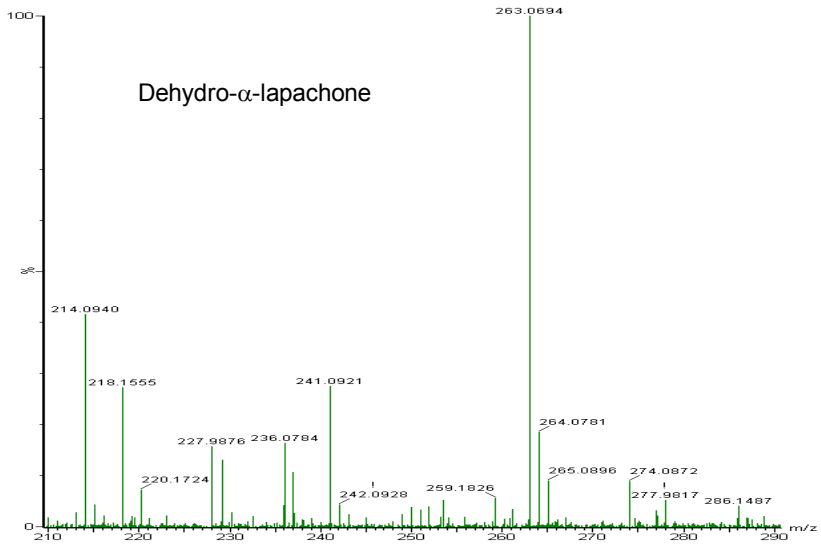
m/Z



m/Z



m/Z



m/Z