

Green Chitosan:Thiourea Dioxide Cleaning Gel for Manganese Stains on Granite and Glass Substrates

Bruno Campos

Universidade Católica Portuguesa: Universidade Catolica Portuguesa

Alexandra Marco

Universidade Católica Portuguesa: Universidade Catolica Portuguesa

Guilhermina Cadeco

Instituto Politecnico do Porto Instituto Superior de Engenharia do Porto

David M. Freire-Lista

Universidade de Trás-os-Montes e Alto Douro: Universidade de Tras-os-Montes e Alto Douro

Joaquin Silvestre-Albero

Universidad de Alicante: Universitat d'Alacant

Manuel Algarra

Universidad de Málaga: Universidad de Malaga

Eduarda Vieira

Universidade Católica Portuguesa: Universidade Catolica Portuguesa

Manuela Pintado

Universidade Católica Portuguesa: Universidade Catolica Portuguesa

Patrícia Moreira (✉ prmoreira@ucp.pt)

Universidade Católica Portuguesa: Universidade Catolica Portuguesa

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Abstract

The cleaning or removal of manganese stains on Cultural Heritage has not been much tested or successful so far. The aim of this article was to assess a new green cleaning gel for Mn-rich black-blue stains on different substrates. The black-blue stains were characterized at optical and chemical level through RGB data, colorimetry, optical microscope, FTIR, XRF and XPS.

Mn-stained granite found on historical churches at Vila Real (North of Portugal) and glass jars of Leclanché cells, belonging to the ISEP's Museum (Portugal) collection, were the ideal case studies to test the efficiency of Chitosan:Thiourea Dioxide (TD) cleaning gel. TD proved to be the best candidate to reduce insoluble manganese oxides, over Hydroxylamine Hydrochloride and Hydroxymethanesulfinic Acid. Cleaning assays performed on stained granite samples collected at a historical quarry and *in situ* application on stained granite churches showed the complete removal of the stains. Similar results were obtained on stained glass jars.

This article presents an innovative, feasible and green Chitosan gel embedded with TD and phosphoric acid, applied to the cleaning of Mn-stained granite and glass substrates.

Introduction

Manganese staining involving built heritage and cultural artefacts is a rare occurrence that has been recently reported, mainly in underground sites, like caves and historical buildings. For instance, the cases of Neang Khmau temple-Cambodia (laterite) [1], Freiburger Münster-Germany (sandstone) [2], Smithsonian Institute-Washington D.C. (red seneca sandstone) [3], Paranhos spring water tunnel-Portugal (granite) [4] or the caves of Lauscaux-France (sandstone) [5] and Pautler-Southwest Illinois (limestone) [6]. Some articles describe this type of staining as a rock varnish defined by a thin dark Mn-enriched coating on rock surfaces. The thickness of this coating could range from 10 µm to 1 mm and the Mn percentage from 2 to 45 wt%, approximately [7].

The phenomenon associated to this type of stains is still unclear. Notwithstanding, two hypotheses are associated to them. One, indicating the leaching and redeposition of Mn aerosol at the surface, from airborne dust grains, followed by autocatalytic oxidation [8], [9]. A second, indicating the efflorescence and precipitation phenomenon, where the Mn source is assigned to the diffusion of soluble Mn through the rocks, originated from enriched Mn rocks, soils or groundwater [10], [11]. The formation of Mn-rich coatings occurs through the oxidation of soluble $Mn^{2+/3+}$ to insoluble Mn^{4+} , like MnO_2 , and inherent to this process, it can be ensued by autocatalysis and/or biogenic oxidation [12]–[19].

Regarding cultural artefacts, a type of Mn-staining in Leclanché cells is reported on glass cell jar. Currently, the solutions to remove this type of stains is scarce. Nevertheless, an unfeasible solution can be found to remove manganese oxides from laterite rock samples, immersing them into an oxalic acid solution. This strategy discloses some problems inherent to the toxicity of oxalic acid, the high diffusion of aqueous solvents into porous stones and the destructive behaviour of this method [10].

This article presents an innovative, feasible and green chitosan gel embedded with thiourea dioxide and phosphoric acid, applied in the cleaning of Mn-stained granite and glass found on historical churches, placed at Vila Real (North of Portugal), and inside the glass jar of Leclanché cells.

The main goal of this work was to evaluate the potential and effectiveness of cleaning gel solutions on two manganese-stained substrates in order to propose conservation-restoration procedures in Cultural Heritage context. To evaluate the results of the three different gels, several Romanesque granite churches in Vila Real district were chosen due to their type

of Mn-rich black-blue stains on granite blocks and even on *frescoes*, as well as a stained-glass jar of Leclanché cells, property of the ISEP Museum-Porto (Portugal).

Methods

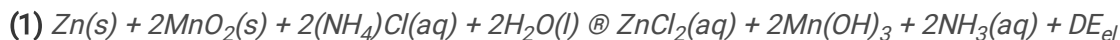
Case studies

Vila Marim Church (**Fig. A1 and A2**) was built on the 12th century and the inner walls of the chancel and nave are partially decorated with *frescoes*, showing different campaigns. The oldest campaign from the 15th century is located on the nave, on the gospel side, observed between the *lacunae* of the second, 16th century, campaign. On the chancel, there are also two campaigns but from different guilds, the first of the 15th century and the latter from the 16th century with a cartouche bearing the date 1549 and attributed to the painter Arnaus F. [20].

Folhadela Church (**Fig. B1 and B2**) is a temple from the 13th century with profuse alterations during the 18th century. The remaining mural paintings survived by being concealed behind baroque altars. They are distributed by the lateral (left and right) walls and top of the triumphal arch as well as the main chapel overall bottom wall and fragments at its lateral walls. The fragments on the upper part of the triumphal arch's lateral walls and the lower left wall area have similarities to Arnaus F. craftsmanship operating in this region between 1535 and 1549 and also, the painting on top of the triumphal arch, although afterwards repainted. The bottom of the triumphal right wall is from a later hand, around the second half of the 16th century. The bottom wall on the main chapel presents at least three different campaigns from 15 and 16th centuries [21].

Mouçós Church (**Fig. C1 and C2**) was built at the beginning of the 16th century along with its remaining mural painting at the main chapel and altar table, dated from 1529 as a legend conveys [22].

Leclanché cell (**Fig. 2**) was invented by the french scientist, Georges Leclanché in 1868. It is considered a single-fluid cell because it is composed by a square shaped glass jar with a saturated aqueous solution of ammonium chloride (electrolyte), a zinc bar (anode) and a porous pot (cathode), dipping into it but not touching. The porous pot is filled with a mixture of pulverized carbon and black oxide of manganese and a carbon bar inside [23], [24]. The electric current of this cell is generated by a chemical reaction in which Mn (IV) is reduced to Mn (III) at the cathode and Zn (0) is oxidized to Zn (II) at the anode [25], producing a net cell potential (DE_{el}) of 1.4 V, roughly **(1)**.



Analytical methodology

RGB data

Cleaning effectiveness of the gel on the stained-glass cell jar was assessed through the analysis of photos taken before and after gel application. A Canon 5D Mark II camera was used to register the process with the following settings: ISO 640; 50 mm lens; F/2,8; 1/100s; White Balance of 3650°K and a TINT 28+. Two points were chosen in each glass jar to verify the before and after RGB data. It was used a white sheet inside the glass jar to isolate the face behind. The glass itself is not totally transparent but when comparing the results of the same dots of the paper sheet is clear the differences between before and after cleaning process.

Optical microscope (OM)

Microscopic analysis of the stained granite was performed and recorded with the portable OM Dino-lite Pro2 AD 4000 series, using magnifications of 50x and 430x.

Colorimetry

The colorimetric data of black-blue stains were measured using the Chroma Meter CR-400, made by Konica Minolta, equipped with a standard colorimetric observer (2°) and a standard illuminant (C&D65) inside. The size of measurement aperture is about Ø8 mm and illumination area is Ø11 mm. After a soft cleaning with a brush, three measurements were performed at each sampling site, pressing the equipment against the selected area of analysis, avoiding the interference of external light. The results were evaluated by CIELAB colour system, defined by the International Commission on Illumination (CIE) in 1976. The measured chromatic coordinates were L^* , a^* and b^* , corresponding to the lightness (0-100), red/green axes and yellow/blue axes (0-100), respectively.

Fourier-transform infrared spectroscopy (FTIR)

FTIR was used to determine the functional groups corresponding to each vibrational frequency. The infrared spectra in transmission mode were recorded in the $4000\text{--}400\text{ cm}^{-1}$ frequency region using a Bruker Tensor 27 spectrometer. The measurements of the transmission bands were made using OPUS software supplied by the Bruker instrument. The pellet disks of 1.5 cm diameter were prepared by mixing 1 mg sample with 200 mg KBr (Aldrich) and pressing at 10 kg/cm^2 .

Portable X-ray fluorescence (pXRF)

Elemental analyses of the patina were performed using a pXRF device conducted with a Thermo Scientific Niton XL3t-900 GOLDD (Geometrically Optimized large Drift Detector) analyser equipped with a silver (Ag) target and a 25 mm^2 Silicon Drift Detector (SDD) with a resolution of 145 eV. The areas of interest were pinpointed using the integrated colour CCD camera and the integrated 3 mm small-spot collimation. The analyses were performed in triplicate using the mining mode with a total time of analysis settled at 180 seconds, putting the pXRF nozzle directly on the surface of the stain. The semi-quantification of the elements was obtained through deconvolution of X-ray spectra using the WinAxil software.

X-ray Photoelectron Spectroscopy (XPS)

XPS enables the determination of the surface chemistry until a near-surface region ($\sim 10\text{ nm}$ in depth). Providing the chemical bonding at surfaces/interfaces, a quantitative information and the electronic state of these elements allows the identification of their oxidation condition.

The spectra were recorded using a Physical Electronics PHI 5701 spectrometer with a non-monochromatic Al K α radiation (300 W, 15 kV, $h\nu = 1486.6\text{ eV}$) as the excitation source. Spectra were recorded at 45° take-off angle by a concentric hemispherical analyser operating in the constant pass energy mode at 25.9 eV, using a 720 mm diameter analysis area. Under these conditions the Au $4f_{7/2}$ line was recorded with 1.16 eV FWHM at a binding energy of 84.0 eV. The spectrometer energy scale was calibrated using Cu $2p_{3/2}$, Ag $3d_{5/2}$ and Au $4f_{7/2}$ photoelectron lines at 932.7, 368.3 and 84.0 eV, respectively. Charge referencing was done against adventitious carbon (C 1s 284.8 eV). Samples were mounted on a sample holder without adhesive tape and kept overnight in high vacuum in the preparation chamber before being transferred to the analysis chamber of the spectrometer. Each region was scanned with several sweeps until a good signal to noise ratio was observed. The pressure in the analysis chamber was maintained lower than 10^{-9} Torr. A PHI ACCESS ESCA-V6.0 F software package was used for acquisition and data analysis. A Shirley-type background was subtracted from the signals. Recorded spectra were always fitted using Gauss–Lorentz curves in order to determine more accurately the binding energy of the different element core levels. The accuracy of binding energy (BE's) values was within $\pm 0.1\text{ eV}$.

Atomic Absorption Spectroscopy (AAS)

The determination of soluble manganese was achieved by AAS with a Manganese (Mn) Lumina Hollow Cathode Lamp. 15 mL of HNO₃ (0.1M) was diluted and acidified in 500 µL of each sample. The manganese stock solutions were prepared by diluting different aliquots of a 1000 µg/mL manganese standard stock solution. The measurements were performed in a concentration range of 0.5-4 mg/L for the calibration curve with a linear regression of 0.9962.

Cleaning assays

The formulation of a gel able to clean unaesthetically Mn-rich stains was based on four main requirements: (i) a renewable biopolymer able to impart a gel like-behaviour to delay the evaporation of embedded solvents, prolonging their action, improving the contact between the solution gel and the substrate, reducing, concomitantly, the penetration of the solvent inside the substrate; (ii) the acidity of the media. The solubility of insoluble oxide metals, like MnO₂, is facilitated in pH under 7.0, at 25°C (as can be proved by Pourbaix diagrams); (iii) the mandatory use of a reductant to promote the reduction of insoluble Mn (IV) to soluble Mn (II/III); (iv) all the previous parameters should respect one of the Green Chemistry principles, using safer, natural and eco-friendly materials mainly based on renewable feedstocks, generating minimal or innocuous waste whenever possible.

The products chosen to fulfil the previous requisites are 1% (w/v) of Chitosan (dissolved in 1% (v/v) of glacial acetic acid) and phosphoric acid, being these constant parameters. The reductants chosen to promote the reduction of insoluble Mn (IV) to soluble Mn (II/III) were Thiourea Dioxide (TD), Hydroxylamine Hydrochloride (HH) and Hydroxymethanesulfinic Acid (HSA), in function of the time and at room temperature. Aqueous solutions of TD, HH and HSA, were prepared separately, with a concentration of 40 mM, in a volume of 30 mL of deionised water, containing 10 mg of dispersed MnO₂, under stirring. Within a period of 75 minutes, 500 µL of each solution was removed every 15 minutes, in three replicas. It was then centrifuged to separate the supernatant from the insoluble MnO₂, diluted to 15 mL with a nitric acid solution (0.1 M) and, finally, the soluble manganese was quantified by AAS. These procedures were replicated three times.

The green gel used to clean Mn-rich stains on granite and glass substrates was prepared weighing 20 g of Chitosan (6% w/v, high molecular weight) solution in Acetic Acid (1% v/v), adding later 5 mL of deionised water and 5 mL of Phosphoric Acid (85 wt. % in H₂O), manually stirring until obtaining a homogeneous gel. After the confection of Chitosan gel, a Thiourea Dioxide aqueous solution was prepared dissolving 1 g of TD in 20 mL of deionised water at 50°C, under stirring, enabling the dissolution process. Finally, Chitosan gel and TD solution were manually mixed and stirred until obtaining a uniform gel.

The cleaning gel used to test on different stained substrates was freshly prepared and the assays respected the following order: (i) granite samples collected from historical quarries; (ii) *in situ* application on manganese-stained granite churches; and (iii) manganese-stained glass of Leclanché cells.

Mn-stained granite samples were collected at an historical granite quarry placed at Vila Real district, in which, a stained area was selected for surface application of the cleaning gel and left to act for 2 minutes. It was then gently wiped with cotton swabs, performing circular movements for 1 minute. Finally, the gel was removed, spraying the surface with deionised water. Residues were rinsed with absorbent kraft paper. Tests performed *in situ* on stained granite ashlar at Sta. Marinha, S. Tiago and N.S. Guadalupe churches followed identical procedures as described on granite samples.

Cleaning tests performed on Mn-stained Leclanché cells, followed the same procedures described above.

Results And Discussion

The identification of the stains on the Leclanché glass jar was successfully achieved by Guilhermina Cadeco *et al.*, where the presence of manganese dioxide on the stained glass was verified. XRF analyses demonstrated that manganese was the most abundant element (~25%) and XPS confirmed the presence of MnO₂ [26].

The chemical characterization of black-blue stains on granite at Santa Marinha, São Tiago and N. Sra. of Guadalupe churches were attained through XRF, FTIR and XPS analysis, demonstrated by the results reported hereinafter.

Characterization of black-blue stains in Vila Real churches (north of Portugal)

Unusual Mn-rich black-blue stains have been causing aesthetical changes on granite walls of Vila Real churches. These black-blue stains are mainly visible on granite support at all churches and in some areas of their *frescos*. After an exhaustive searching of churches with Mn-rich stains, were found a typology of black-blue stains at Sta. Marinha (Vila Marim), St. Tiago (Folhadela) and Nossa Senhora de Guadalupe (Mouços) churches, all located in Vila Real district (Fig. SI1).

Table 1. Iron and Manganese percentage of black-blue stains on granite and CIELab color coordinates. Un = unstained; St = stained; L* = lightness; a* = red/green value; b* = yellow/blue value

		Elemental Analysis				CIELab coordinates		
Churches	Granite	Fe (%)	Fe _{st} /Fe _{un}	Mn (%)	Mn _{st} /Mn _{un}	L*	a*	b*
Sta. Marinha	Un	1.01±0.19	0.96	0.08±0.02	18.73			
	St	0.97±0.11		1.50±0.77		23.86±1.03	3.36±0.34	3.90±1.20
St. Tiago	Un	0.72±0.21	0.85	0.08±0.04	17.25			
	St	0.61±0.30		1.38±0.53		21.16±1.39	3.48±0.47	3.07±0.37
N.S. Guadalupe	Un	0.79±0.31	1.31	0.05±0.03	23.35			
	St	1.04±0.20		1.17±0.11		24.04±2.40	3.59±0.74	4.15±1.98

Table 1 shows the similarity between Mn-rich stains found in three churches of Vila Real. These black-blue stains are also characterized by the presence of iron because its chemical similarity with manganese. In terms of elemental percentage, was uncovered that stained granite presents a manganese percentage 17.25 to 23.35 times higher than the unstained granite and the levels of iron are almost identical, resulting in Mn/Fe ratios of 0.08±0.02 and 1.64±0.57 for unstained and stained granite, respectively. Concerning to colorimetric characterization, the lightness of these stains is 23.02±1.61 being concordant with the visual evaluation that revealed an apparent brightness (common in biofilms). The parameters a* and b* revealed values of 3.48±0.12 and 3.70±0.57 being also plausible with stains ranging around black colorations.

Were performed analytical techniques at Sta. Marinha, St. Tiago and N.S. Guadalupe churches to unveil the chemical composition of these type of stains on granite. Black-blue stains were analysed by FTIR (Fig. SI2) and is observed the presence of symmetric and asymmetric stretching vibrations of νCH₃ and νCH₂ between 2920 and 2850 cm⁻¹, and the stretching vibration of νOH at 3433±5 cm⁻¹. The bands at 2360, 2337 and 1385 cm⁻¹ are due to the absorption of atmospheric CO₂. The range of bands between 1100 and 670 cm⁻¹ may be ascribed to silicate vibrations (e.g. quartz, typically present on granite). The assignments between 528 and 420 cm⁻¹ suggest the presence of Mn oxides, equivalent to Mn oxide coatings found on Lascaux cave (520, 470 and 420 cm⁻¹) [5] and Paranhos spring water tunnel (529, 468 and 425 cm⁻¹) [4].

These stains were also analysed with XPS on samples of the three churches (**Fig. SI3** and **Table SI1**). The binding energies at 532.40 (O_{1s}) (**Fig. SI3e**), 102.92 (Si_{2p3}) (**Fig. SI3g**) and 74.38 eV (Al_{2p3}) (**Fig. SI3h**) indicate the presence of silicates and aluminosilicates, concordant assignments with the granite substrate. The presence of organic elements is also shown at 284.55 (C_{1s}) (**Fig. SI3d**) and 406.90 eV (N_{1s}) (**Fig. SI3f**), corresponding to adventitious carbon and nitrates, respectively. The presence of halite (NaCl) at 1071.35 eV (Na_{1s}) (**Fig. SI3j**) might be an evidence of the migration of some ions from groundwater, passing through the porous granite, and precipitating at the surface. The existence of manganese oxides in black-blue stains could be proved at 641.24 and 643.88 eV (**Fig. SI3b**), both assigned to Mn(II/III) (*e.g.* Mn₃O₄) and Mn (IV) (*e.g.* MnO₂), respectively. The same occurs for iron oxides at 709.52 and 712.45 eV (**Fig. SI3c**) attributed to Fe (II/III) (*e.g.* Fe₃O₄) and Fe (III) (*e.g.* Fe₂O₃), respectively. The spectral interpretation for samples collected at S. Tiago and N.S. Guadalupe is similar and is summarized in **Table SI1**. It is also observed the presence of calcite, confirmed by the spin-dublet of Ca 2p region at 347.13 (Ca 2p_{1/2}) and 350.65 eV (Ca 2p_{3/2}), giving a chemical shift of Δ Ca 2p $\sim$ 3.5 eV and an intensity peak ratio approximately 2:1, respectively (**Fig. SI3i**) [27]. The presence of calcite might indicate that some granite ashlar were whitewashed with lime, probably covered with *frescoes*, a common practice at that time.

Cleaning assays

Determination of soluble Mn by AAS

This study intended to ascertain the suitable reactant able to reduce insoluble Mn oxides (Mn⁴⁺) to soluble manganese oxidation states (Mn^{2+/3+}). Thiourea Dioxide (TD), Hydroxylamine Hydrochloride (HH) and Hydroxymethanesulfonic Acid (HSA) were assayed. HSA has been proposed as a reductive bleaching for textile, paper and wood pulp industries; HH is commonly used to extract selectively manganese and iron from soils and ores [28]; TD has been widely used as a green and affordable reducing agent in industries like paper, photography, leather processing and textile printing because of its outstanding safety and nontoxic properties [29], [30].

The results obtained by AAS showed that TD and HH are the most effective Mn⁴⁺ reducing agents (**Fig. SI4**). At the first 15 minutes, $\sim$ 80% of Mn⁴⁺ was reduced by TD and $\sim$ 90% by HH. Mn⁴⁺ was almost completely reduced at 75 and 60 minutes by TD and HH, respectively, being the best candidates to be used in the cleaning of Mn-rich stains, inversely to HSA that showed a weak reductive capacity, reaching $\sim$ 10% after 75 minutes.

Application of Chitosan: TD gel on different Mn-stained substrates

The previous study showed that TD and HH are effective in the reduction of Mn⁴⁺ to soluble Mn^{2+/3+}. Nevertheless, HH is known by its acute toxicity and carcinogenicity, inversely to TD that is considered a green reducing agent.

The optimal gel preparation is described in the Section 2.3 and the main premise for its formulation is the use of green products, namely, the chitosan, acetic acid, phosphoric acid and thiourea dioxide. Chitosan is a cationic natural polymer and its remarkable and unique properties such as biodegradability, biocompatibility and antimicrobial activity has been proved [31]. Nonetheless, chitosan needs an acid solution to be solubilized and acetic acid is commonly used for this effect. Aqueous solutions of acetic acid are ranked high as relatively green solvents [32]. Phosphoric acid was added to chitosan gel solution because it acts as co-solvent, aiding the dissolution of insoluble heavy metals (*e.g.* Fe and Mn) [33]. Phosphoric acid is considered a green product because it respects the energy efficiency principle, concerning to the 12 principles of Greener Chemistry. The proposed green gel was applied on different Mn-stained substrates to test its cleaning efficiency.

Application on stained granite samples

Cleaning efficiency of green gel was assayed, applying it on three different granite samples (**Fig. 3** and **Fig. SI5**). The black stains labelled inside yellow marks showed Mn and Fe percentages ranging from 1.87 to 2.97% and 0.86 to 2.23%, respectively. These percentages originated a Mn/Fe ratio of 1.22 ± 0.56 , decreasing 35% in comparison with 1.64 ± 0.57 from **Table 1**. This Mn/Fe ratio seems to influence the changing of CIELab coordinates because it also led to different L*, a* and b* parameters (**Table 2**). Moreover, granite samples collected at historical quarry appears to present higher Mn percentages ($2.53\pm0.58\%$) when compared with stained granite at the indicated churches ($1.35\pm0.17\%$), being perfectly suitable to the proposed cleaning study.

Table 2. Iron and Manganese percentage of stained granite samples and CIELab color coordinates. L* = lightness; a* = red/green value; b* = yellow/blue value

Elemental analysis				CIELab coordinates		
Granite Sample	Fe (%)	Mn (%)	Mn/Fe	L*	a*	b*
I	1.68±0.09	2.97±0.24	1.77	27.29±0.40	3.42±0.18	9.74±0.32
II	2.23±0.02	2.76±0.09	1.24	38.69±0.03	3.50±0.01	6.74±0.01
III	0.86±0.04	1.87±0.31	0.65	38.27±0.30	3.28±0.19	9.55±0.40

As can be seen in the granite samples I, II and III (**Fig. 3** and **Fig. SI5**), the cleaning gel effectively removed the black stains within the yellow circles. The images captured by Dino-lite before and after the cleaning procedure, substantiate that these Mn and Fe oxides are overlapped at the granite surface, similar to a patina, and the cleaning cycles repetition, where the gel is applied and after removed, is directly associated to the thickness of these superimposed Mn and Fe oxides. Due to the abrasive effect of cotton swabs, is also observed some remaining cotton fibres on the cleaned area (**Fig. 3B2**).

In situ application on stained granite churches

Mn-stains characterized in Section 4.1 were also submitted to cleaning assays. The areas where the gel was applied are labelled in **Fig. SI1** with dashed yellow circles. As expected, the green gel showed its cleaning efficiency by removing effectively black-blue stains on granite, within the yellow marks, at the three churches (**Fig. 4** and **Fig. SI6**). Following the same procedure adapted from granite samples, the cleaning cycles were repeated until full stain removal, with reference to the thickness of the patinas that seem to differ. The patina porosity and gel viscosity are factors that influence the penetration depth of the gel. The dissolution/reduction processes promoted by phosphoric acid and thiourea dioxide are enabled on thin layers of manganese oxide. Thus, it is estimated that the number of cleaning cycles is proportional to the porosity and thickness of the patina.

Application on stained Leclanché cells

Nowadays, Leclanché cells are being used as museum artifacts due to its relevance in the electrochemistry history. Were provided two Mn-stained glass jar cells by ISEP’s Museum (**Fig. 5**) to test the green gel efficiency, intending to recover the materials originality.

Photos were taken before and after gel application on a stained face and two points were selected to get RGB data (1-8 points) using a Photoshop software. The same procedure was done with a white paper sheet and its RGB parameters (I-

IV points) were setting as a cleaned material.

RGB parameters taken at 1-8 and I-IV points are represented in the **Table 3**. The colorimetric methodology to reveal the cleaning gel efficiency is based on a formula used to calculate the magnitude of a vector ($|u|$) with two points (paper sheet coordinates as reference), exemplified by the following formula (2):

$$(2) \; |u|_1 = ((R_1-R_1)^2+(G_1-G_1)^2+(B_1-B_1)^2)^{1/2}$$

Table 3. RGB data before (1, 2, 5 and 6) and after (3, 4, 7 and 8) the cleaning assay. R = red, G = green, B = blue, $|u|_{ac}$ = magnitude of a vector after cleaning and $|u|_{bc}$ = magnitude of a vector before cleaning.

Points	R	G	B	$ u $	$ u _{ac}/ u _{bc}$
1	150	89	26	292	0.40
3	209	185	159	117	
I	249	249	249		
2	209	173	124	146	0.42
4	229	216	194	62	
II	246	246	245		
5	195	162	110	171	0.26
7	233	227	212	44	
III	248	248	248		
6	133	99	70	256	0.28
8	211	209	195	72	
IV	246	247	245		

The points 1, 3; 2, 4; 5, 7 and 6, 8 are compared with paper sheet RGB coordinates in which the points are I, II, III and IV, respectively. A high $|u|$ indicates a more stained glass, in reverse, a low $|u|$ suggests a more cleaned glass. The same logic is followed by the ratio $|u|_{ac}/|u|_{bc}$, where a low ratio value is indicative of a high cleaning efficiency. Considering the thickness of the glass and the inclination of the light, the results show, in general, an effective cleaning activity of the Chitosan:TD gel on glass substrates stained with manganese oxide.

Conclusions

More sustainable pathways aiming at green products extended to the conservation of Cultural Heritage is a trend followed by conservator-restorers. This article presents a safer and innovative cleaning product applied to Mn-rich black-blue stains. Conservation and Restoration interventions should be more sustainable and greener, as much as possible, benefiting not only the artworks, but also the operator health and the environment as well.

The application of a cleaning product in gel mode offers advantages regarding the evaporation rate and the capillary product penetration. The proposed Chitosan:TD cleaning gel showed a good cleaning efficiency on Mn-stained granite and glass. Even more, fulfils the previous assumptions and some of the principles inherent to Green Chemistry, being considered a safer and green gel able to clean Mn-rich stains on cultural artifacts.

Declarations

Authors' contributions

BC and GC designed this study and performed the laboratory work. DL collected granite samples. AM, JA and MA accomplished analytical techniques. BC and AM drafted this manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

References

1. Casanova Municchia A, Bartoli A, Bernardini F, Caneva S, Della Ventura G, Ricci G, Boun Suy M, T. & Sodo, "Characterization of an unusual black patina on the Neang Khmau temple (archaeological Khmer area, Cambodia): a multidisciplinary approach.," *J. Raman Spectrosc.*, vol. 47, no. 12, pp. 1467–1472, 2016, [Online]. Available: <https://doi.org/10.1002/jrs.4969>.
2. Macholdt DS, et al. Black manganese-rich crusts on a Gothic cathedral. *Atmos Environ.* Dec. 2017;171:205–20. doi:10.1016/j.atmosenv.2017.10.022.
3. Vicenzi Z, Grissom E, Livingston C, R. & Weldon-Yochim, "Rock varnish on architectural stone: microscopy and analysis of nanoscale manganese oxide deposits on the Smithsonian Castle, Washington DC," *Herit. Sci.*, vol. 4, no. 1, p. 26, 2016, [Online]. Available: <https://doi.org/10.1186/s40494-016-0093-2>.
4. Miller AZ, et al., "Biogenic Mn oxide minerals coating in a subsurface granite environment," *Chem Geol*, vol. 322–3, pp. 181–191, Sep. 2012, doi:10.1016/j.chemgeo.2012.07.005.
5. Saiz-Jimenez M, Miller C, Martin-Sanchez A, P. & Hernandez-Marine, "Uncovering the origin of the black stains in Lascaux Cave in France," *Environ. Microbiol.*, vol. 14, no. 12, pp. 3220–3231, 2012, [Online]. Available: <https://doi.org/10.1111/1462-2920.12008>.
6. Frierdich AJ, Hasenmueller EA, Catalano JG. Composition and structure of nanocrystalline Fe and Mn oxide cave deposits: Implications for trace element mobility in karst systems. *Chem Geol.* May 2011;284:no. 1–2.

doi:10.1016/j.chemgeo.2011.02.009., “”, , pp. 82–96.

7. Macholdt DS, et al. Characterization and differentiation of rock varnish types from different environments by microanalytical techniques. *Chem Geol.* May 2017;459:91–118. doi:10.1016/j.chemgeo.2017.04.009.
8. Dorn RI, Krinsley DH, Langworthy KA, Ditto J, Thompson TJ, “The influence of mineral detritus on rock varnish formation,” *Aeolian Res.*, vol. 10, pp. 61–76, Sep. 2013, doi: 10.1016/j.aeolia.2013.04.005.
9. Goldsmith Y, Stein M, Enzel Y. From dust to varnish: Geochemical constraints on rock varnish formation in the Negev Desert, Israel. *Geochim Cosmochim Acta.* Feb. 2014;126:97–111. doi:10.1016/j.gca.2013.10.040.
10. Uchida S, Watanabe E, R. & Osawa. “Precipitation of manganese oxides on the surface of construction materials in the Khmer temples, Cambodia,” *Herit. Sci.*, vol. 4, no. 1, p. 16, 2016, [Online]. Available: <https://doi.org/10.1186/s40494-016-0086-1>.
11. Huang H, J. & Zhang. “Redox reactions of iron and manganese oxides in complex systems,” *Front. Environ. Sci. Eng.*, vol. 14, p. 76, 2020, [Online]. Available: <https://doi.org/10.1007/s11783-020-1255-8>.
12. Tebo S, Bargar B, Clement J, Dick B, Murray G, Parker K, Verity D, R. and Webb, “BIOGENIC MANGANESE OXIDES: Properties and Mechanisms of Formation,” *Annu. Rev. Earth Planet. Sci.*, vol. 32, no. 1, pp. 287–328, 2004, [Online]. Available: <https://doi.org/10.1146/annurev.earth.32.101802.120213>.
13. Sunda D, W. & Kieber. “Oxidation of humic substances by manganese oxides yields low-molecular-weight organic substrates,” *Nature*, vol. 367, no. 6458, pp. 62–64, 1994, [Online]. Available: <https://doi.org/10.1038/367062a0>.
14. Spiro BM, Bargar TG, Sposito JR, G. & Tebo, “Bacteriogenic manganese oxides,” *Acc. Chem. Res.*, vol. 43, no. 1, pp. 2–9, 2009, [Online]. Available: <https://doi.org/10.1021/ar800232a>.
15. Piazza N, Ciancio Casalini A, Pacini L, Sanguinetti VA, Ottado G, J. & Gottig, “Environmental Bacteria Involved in Manganese(II) Oxidation and Removal From Groundwater,” *Front. Microbiol.*, vol. 10, pp. 1–13, 2019, [Online]. Available: <https://doi.org/10.3389/fmicb.2019.00119>.
16. Martínez-Ruiz EB, Cooper M, Fastner J, Szewzyk U. Manganese-oxidizing bacteria isolated from natural and technical systems remove cylindrospermopsin. *Chemosphere.* Jan. 2020;238:124625. doi:10.1016/j.chemosphere.2019.124625.
17. Dakal S, T. & Cameotra, “Microbially induced deterioration of architectural heritages: routes and mechanisms involved,” *Environ. Sci. Eur.*, vol. 24, no. 1, p. 36, 2012, [Online]. Available: <https://doi.org/10.1186/2190-4715-24-36>.
18. Zhou C, H. & Fu, “Manganese-oxidizing microbes and biogenic manganese oxides: characterization, Mn(II) oxidation mechanism and environmental relevance,” *Rev. Environ. Sci. Bio/Technology*, vol. 19, no. 3, pp. 489–507, 2020, [Online]. Available: <https://doi.org/10.1007/s11157-020-09541-1>.
19. Tebo BM, Johnson HA, McCarthy JK, Templeton AS, “Geomicrobiology of manganese(II) oxidation,” *Trends in Microbiology*, vol. 13, no. 9. Elsevier Current Trends, pp. 421–428, Sep. 01, 2005, doi: 10.1016/j.tim.2005.07.009.
20. Caetano J. O Marão E As Oficinas De Pintura Mural Nos Séculos XV E XVI. Lisbon: Aparição; 2001.
21. Cavaleiro E. A arte dos frescos em Vila Real. João Azevedo Editor; 2004.
22. Bessa P. “Pintura mural na Igreja de Nossa Senhora de Guadalupe. In: ” in Estudos em Homenagem ao Professor Doutor José Marques. Faculdade de Letras da Universidade do Porto, Ed.; 2006. pp. 193–215.
23. Jenson A, Singh G, Bhama G, J. K., & Ratner. Hydrogel Leclanché Cell: Construction and Characterization. *Energies.* 2020;13(3):594. doi:<https://doi.org/10.3390/en13030594>.
24. Larcin F, Maskell J, W., & Tye, “Leclanché cell investigations. Part II: Zinc potential as a tool for studying intermittent discharge,” *Electrochim. Acta*, vol. 44, no. 2–3, pp. 191–199, 1998, [Online]. Available: [https://doi.org/10.1016/s0013-4686\(98\)00172-8](https://doi.org/10.1016/s0013-4686(98)00172-8).
25. Jensen WB, “The Leclanché Cell Notes from the Oesper Collections,” Cincinnati, 2014. [Online]. Available: <http://hdl.handle.net/2374.UC/731246>.

26. Cadeco E, Costa G, Campos P, Algarra BC, Martínez de Yuso M, Moreira MV, Silva PR, Vieira P. The scientific collection of ISEP Museum: Grenet and Leclanché cells. *Conserv Património*. 2021. doi:10.14568/cp2020067.
27. Sahoo J, Kausar P, Lee F, J. & Han, "Facile fabrication of silver nanoparticle embedded CaCO₃ microspheres via microalgae-templated CO₂ biomineralization: application in antimicrobial paint development," *RSC Adv.*, vol. 4, no. 61, pp. 32562–32569, 2014, [Online]. Available: <https://doi.org/10.1039/C4RA03623A>.
28. Suda T, Makino A, T. and Higashi. Extractability of manganese and iron oxides in typical Japanese soils by 0.5 mol L⁻¹ hydroxylamine hydrochloride (pH 1.5). *Soil Sci Plant Nutr*. 2012;58(6):684–95.
29. Dong Y, Ding L, Huo Y, Zhang W, Lu W, Jin J, Zhao L, Wu Y, G. and Zhang. A green and facile synthesis for rGO/Ag nanocomposites using one-step chemical co-reduction route at ambient temperature and combined first principles theoretical analyze. *Ultrason Sonochem*. 2019;53:152–63.
30. Mendonça J, Ribeiro P, Abreu J, Guliashvili C, Serra T, A. and Coelho. Thiourea Dioxide As a Green and Affordable Reducing Agent for the ARGET ATRP of Acrylates, Methacrylates, Styrene, Acrylonitrile, and Vinyl Chloride. *ACS Macro Lett*. 2019;8(3):315–9.
31. Yaneva M, Ivanova Z, Nikolova D, N., & Tzanova, "The 21st century revival of chitosan in service to bio-organic chemistry," *Biotechnol. Biotechnol. Equip.*, vol. 34, no. 1, pp. 221–237, 2020, [Online]. Available: <https://doi.org/10.1080/13102818.2020.1731333>.
32. Tobiszewski F, Namieśnik M, J., & Pena-Pereira, "Environmental risk-based ranking of solvents using the combination of a multimedia model and multi-criteria decision analysis," *Green Chem.*, vol. 19, no. 4, pp. 1034–1042, 2017, [Online]. Available: <https://doi.org/10.1039/c6gc03424a>.
33. YM et al. Taha, Masoud MH, Khawassek AM, "Cadmium and iron removal from phosphoric acid using commercial resins for purification purpose," *Env. Sci Pollut Res*, vol. 27, pp. 31278–31288, 2020, [Online]. Available: <https://doi.org/10.1007/s11356-020-09342-7>.

Figures



Figure 1

Outside overview and respective black-blue stains at Sta. Marinha (A1 and A2), St. Tiago (B1 and B2) and N.S. Guadalupe churches (C1 and C2).



Figure 2

Current conservation state of a Leclanché cell, belonging to ISEP Museum-Porto (Portugal).

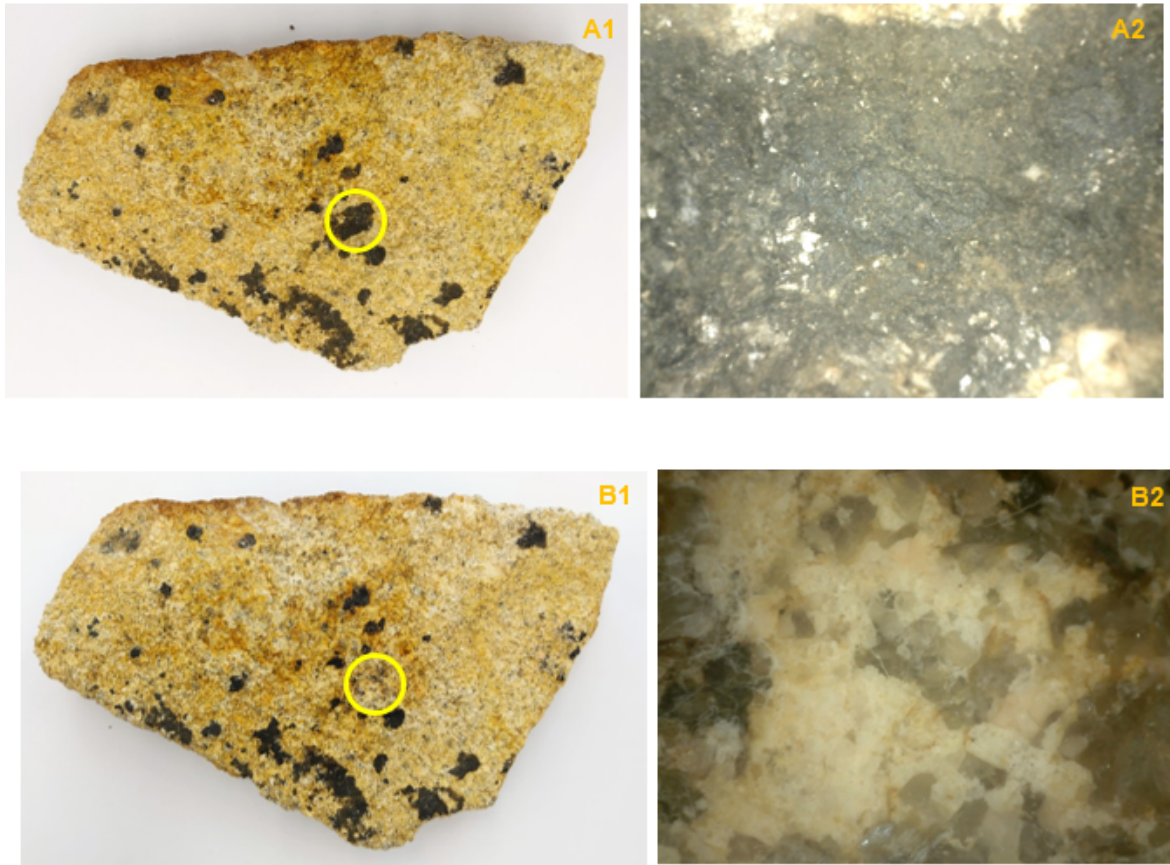


Figure 3

Cleaning assay performed on stained granite sample I. Assays were done inside the labelled yellow marks; A1 and A2 are images of stained granite; B1 and B2 are images of removed stain. A2 and B2 images were taken by Dino-lite (435x magnification).

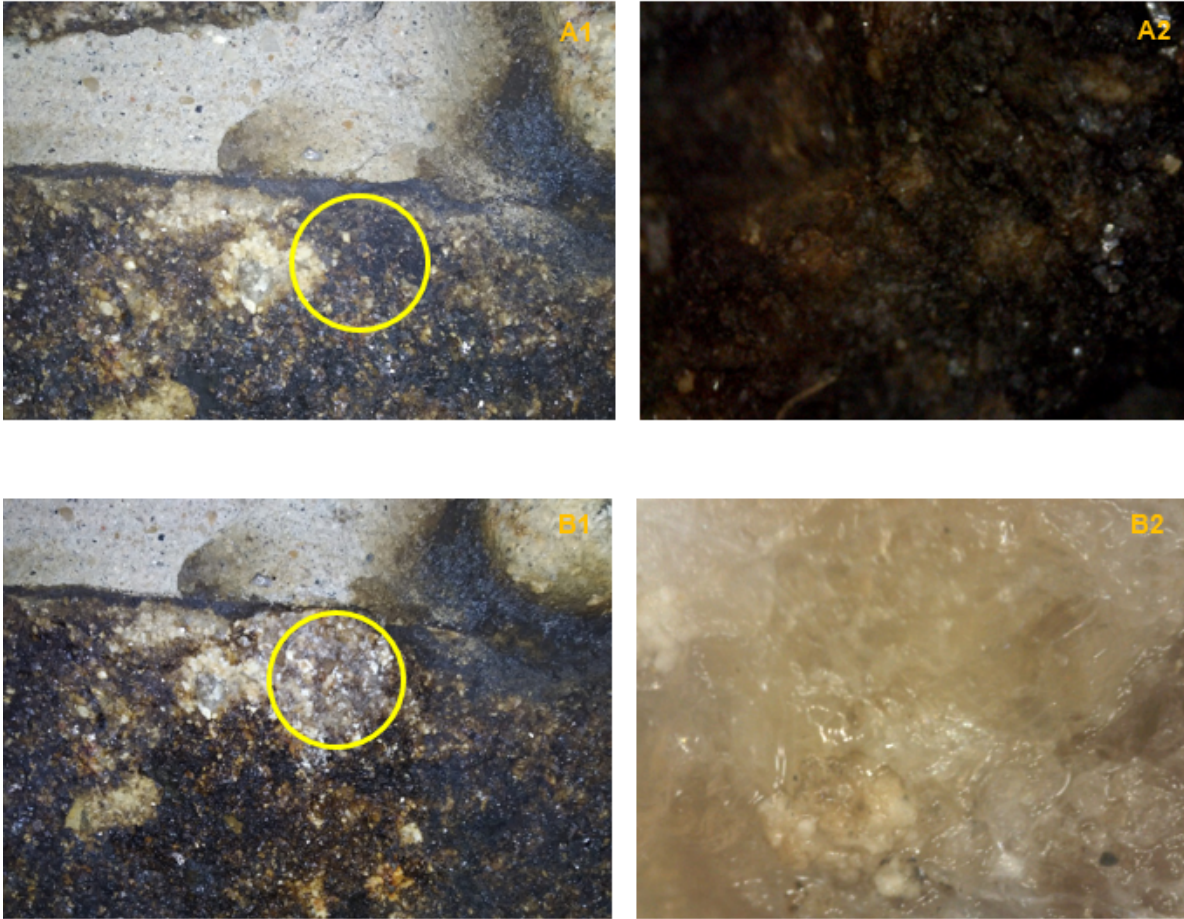


Figure 4

Cleaning assay performed on stained granite at St. Tiago church. Assays were done inside the labelled yellow marks; A1 and A2 are images of stained granite; B1 and B2 are images of removed stain. A2 and B2 images were taken by Dino-lite (435x magnification).

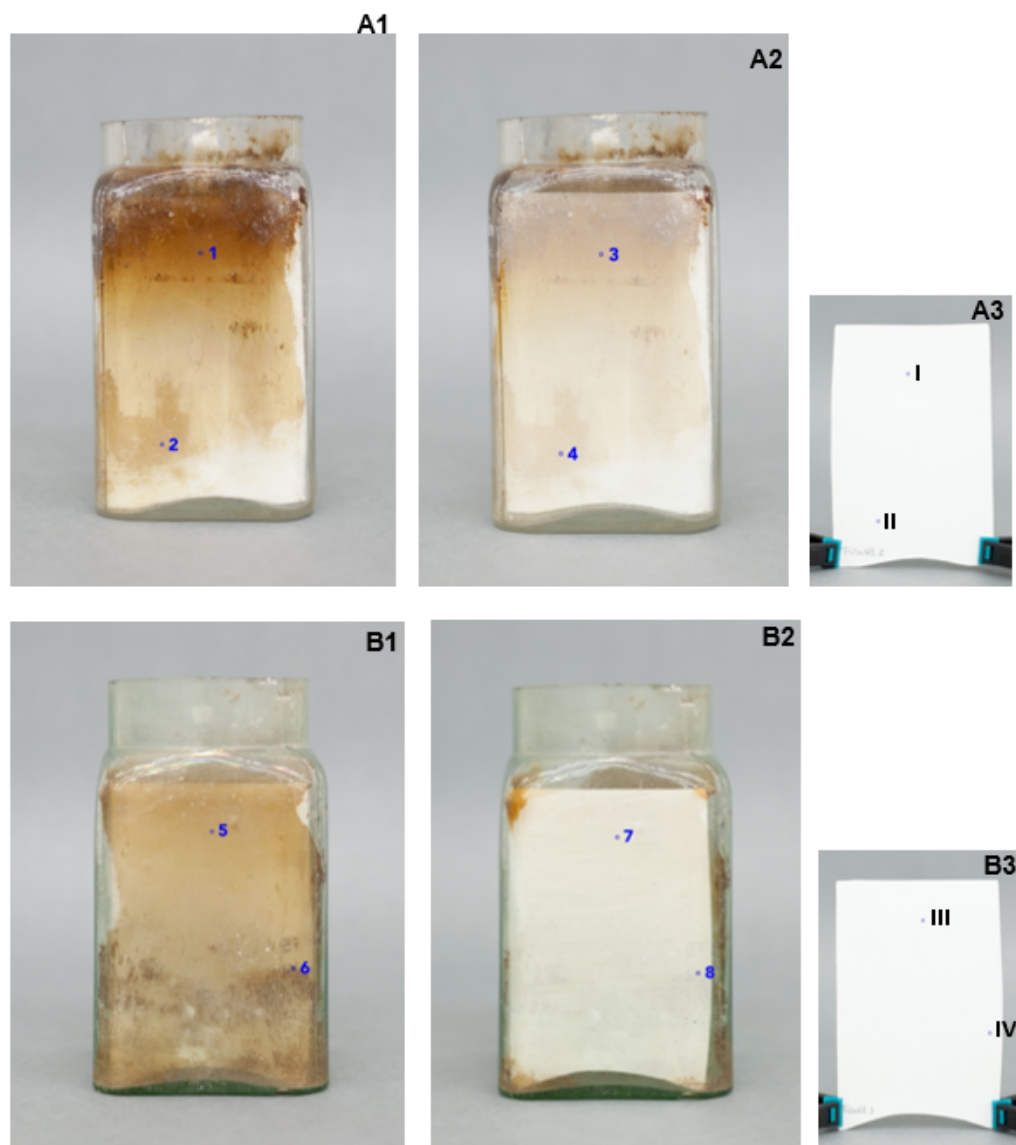


Figure 5

Two Leclanché cells where the left image is before cleaning (A1 and B1) and the right image is after cleaning (A2 and B2). A3 and B3 are the paper sheets used inside the glass jar.

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