Green Approach to Clean Marble Surfaces

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Research Article

Keywords: Iron Stains, Lactotransferrin, Ovotransferrin, XPS, Cultural Heritage, Restoration.

Posted Date: December 20th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1167704/v1>

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Abstract

In the field of cultural heritage restoration, the removal of iron corrosion stains is a difficult problem to deal with, especially in porous stone materials. Many studies in recent years have been aimed at finding simple and reliable methods using non-toxic chelating compounds. The search for natural compounds is therefore of great relevance, especially in the restoration of cultural heritage, where the use of toxic chemical compounds often involves risks for the environment and human health. Following this trend, the purpose of this preliminary work was to verify the use of two natural proteins, Lactotransferrin (Ltf) and Ovotransferrin (Ovt), for the removal of iron-based stains on marble surfaces. The two proteins, whose high affinity for iron "in vivo" has been widely documented, were extracted from their natural matrices. The protein extracts were then immobilized using a common cellulose pulp. The poultices obtained were spread on the surfaces of artificially stained marble specimens and, after a set time, were easily removed. The effectiveness of the removal, visually evident, was detected by spectrophotometry and image analysis. The surface analyses, before and after the treatment, carried out by X-ray photoelectron spectroscopy (XPS), confirmed that both proteins have a selective and effective complexing capacity for the ferric ions of rust stains.

Introduction

Monumental surfaces interact with the surrounding environment; the effects of these interactions proceed over time, depending on both the location and the characteristics of the constituting materials. The generation of stains, produced by iron corrosion phenomena, for example, can derive both from the oxidation of ferrous compounds constituting the stone, such as Pyrite and Siderite, and from the proximity of ferrous metals, which are oxidized for exposure to air, humidity or acid rains [1, 2, 3]. Therefore, atmospheric pollutants, humidity, suspended particulate, consisting also of biological components, are factors of great importance to the corrosion chemistry of metals and of calcareous stones. Examples of synergistic effects between chemical, physical, and biodeterioration factors are amply reported in the literature [4]. Specific chromatic alterations, occurring when metal parts, such as kingpins, brackets, nails, decorative elements, stabilization reinforcements, etc. are close to the limestones, are certainly related to some aspects of the corrosion chemistry [5, 6].

It is well known, in fact, how the combination of stone materials to some metals or alloys, like bronze statues on stone pedestals, promotes the formation of colored stains from corrosion products that affect, not only aesthetically, the surface of the monuments, favoring or inhibiting also specific biological activities [7]. XPS spectroscopic investigations on Roman monuments holding bronzes or other copper alloys artifacts have shown that, in outdoor conditions, the corrosion products of a copper-based alloy, directly exposed to rainwater, were drained off and migrated through the porous surfaces forming stains of different colors and intensities, causing the deterioration of limestone structures [8, 9]. In these XPS analyses, copper compounds and mixed calcium/copper carbonates, associated with the stains were identified, as well as the presence of other elements.

Similarly, the rust stains produced by corrosion of iron objects placed in proximity to marbles or carbonate stones were frequently observed on ancient and modern monuments [10, 11]. In these works, iron (III) compounds were easily identified by surface analysis [12–14]. Furthermore, attention was paid to understanding the formation and growth process of iron oxides on carbonate stones and the influence of environmental and biological factors contributing to the weathering process.

The detailed mechanism for rust formation is highly complex [11]; depending on the pH value, different species, all characterized by a brownish color, are formed. Typically, in the iron stains, Lepidocrocite has been generally found which tends to transform itself over time into better-defined forms, such as Goethite and Hematite [15].

Iron stains can be removed from stone surfaces by chemical treatments. The methods involve the application of different compounds with complexing and reducing action mixed on suitable supports. One of the most used complexing agents has been the citrate [5, 16, 17], although other salts of carboxylic acids, such as oxalic and tartaric acid, have also been tested. Other methods have involved the use of EDTA [18] or a hexadentate binder (TPEN) which, unlike EDTA, has a high affinity for iron and a low affinity for calcium [19]. The various complexing agents have been used singly or in combination with reducing compounds, such as thiosulfate and sodium dithionite [16, 20]. Thioglycolic acid and ammonium thioglycolate have been applied in various treatments on limestone [18]. Thioglycolate is presumably the most efficient binder for cleaning rust-stained marble [18, 21]. However, thioglycolic acid is a toxic chemical and is therefore difficult to acquire and handle by restorers.

More recently, attention has been paid to natural iron chelants (eg amino acids) and to compounds used in medical therapy, such as Deferoxamine and Deferiprone [22]. Amino acids containing sulfhydryl groups, such as cysteine, or thioether groups such as methionine, mixed with sodium dithionite, have been used with good results [10, 11].

The research described here was carried out as part of the "SMART CITIES" project n°SCN_00520, funded by the Italian Ministry of University and Research [23]. The first message that the research groups, making up the project team, wanted to address to the scientific community that deals with cultural heritage, is that preventing damage is better than restoring it; this means replacing the emergency approach with continuous and ordinary maintenance. The second message is of a technical nature and refers to the use of increasingly environmentally friendly materials for restoration. The goal is a sustainable restoration, respecting the environment and the health of the restorers, and the application of "green chemistry" to cultural heritage. Within this context, this work specifically regarded the development of a "green" cleaning procedure suitable for removing iron-based stains from architectural surfaces and walls of rupestrian churches of artistic-cultural interest, selected as case studies for the project, using non-toxic natural agents, both for respect for the environment and for human health.

Taking into account the historical and artistic cultural value of the study sites, the experimental search for the optimization of appropriate procedures was carried out preliminarily in the laboratory, using marble specimens properly stained with iron. It should be noted that the cleaning operation must ensure the

removal of unwanted alterations and maintain always the authenticity of the historical and cultural asset [24].

Specifically, the purpose of this work was to verify the action of two natural proteins, Lactotransferrin (Ltf) and Ovotransferrin (Ovt), as innovative products for the cleaning of the stone surfaces from rust patches. For this purpose, samples of Carrara marbles, exposed outdoor for one year in contact with metallic iron were used. Ltf (molecular weight 80-kDa) is a water-soluble multifunctional globular glycoprotein, which has two strong binding sites for Fe(III) ions. Ltf is present in bovine milk and colostrum at a concentrations ranging between 0.2 and 1.5 mg / ml respectively and is the second most abundant protein in milk after the casein [25]. Ovt is the second most abundant protein in egg white after ovalbumin, (~12-13% of the total egg proteins). The protein has a molecular weight of 76-kDa dalton and contains about 700 amino acids. It is a powerful natural antimicrobial agent and the major binder of ferric iron [26].

The two proteins both belonging to the transferrin's family, identified for their high affinity for ferric ions in vivo [27–30], have been tested, in replacing synthetic substances, to delete iron oxides, deposited on marble surfaces, through the formation of stable complexes.

The two proteins were first extracted from their natural matrices and then mixed with the common cellulose pulp, completely wet. The poultices obtained have been spread on the surface of iron-stained marbles and carefully removed when their action was completed. The ability to remove the stains for both immobilized proteins has been evaluated by a comparison of spectrophotometric and XPS data. The comparison proved to be very useful for associating the variations in color to the variation in the composition of the marble's surface before and after the treatment procedure. On the whole, the laboratory results have been very promising and made it possible to plan new actions to evaluate the products tested for the restoration of cultural heritage under study.

Materials Ad Methods

Extraction procedures of Lactotransferrin and Ovotransferrin

The extraction of Ltf was carried out from fresh commercial and whole milk, produced by the “*Centrale del Latte in Rome*”. The procedure adopted is that described by Parkar et al. [25]. Briefly, 40 ml of milk were centrifuged for 10 min at 4000 rpm and 4°C. After separation of the fats, 1 N HCl was added up to pH 4.6 to precipitate the casein. After centrifugation at 2000 rpm for 10 min, the supernatant, containing lactoferrin, was removed and stored at 4°C. The precipitation of the Lactoferrin was then effected at pH 8 in the presence of ammonium sulfate. After centrifugation at 4000 rpm for 10 minutes at 4°C, the precipitate obtained (about 80 mg) was resuspended in PBS (Phosphate Buffer Saline, pH 7.4) and stored at 4°C.

The separation of Ovt from chicken eggs (*Gold Feriali, category A*) was performed as described by Abeyrathne et al. [31]. 150 mL of egg white (4 commercial fresh eggs) were diluted with 300 mL of

distilled water and homogenized manually. The pH was adjusted to 4.6 with 3N HCl. The solution was centrifuged at 3400 rpm, for 30 minutes at 4°C. The supernatant was collected and treated with different levels of ammonium sulfate and acid combinations. The samples were kept overnight at 4°C and centrifuged at 3400 rpm for 20 min at 4°C. The precipitate was measured with weighing balance, dissolved with 2 volumes of DW, and then desalted using an ultrafiltration unit. After recovering the dialyzed solution, Ovotransferrin was precipitated from the solution by again adding various levels of ammonium sulfate and acid combinations. After storage overnight in the fridge at 4°C, the samples were centrifuged at 3400 rpm for 20 min at 4°C. The final precipitate, containing Ovt, approximately 4.3 g (1.075 g per egg), was resuspended in PBS buffer.

The final volume of the buffered solutions was adjusted according to the amount of precipitate to have approximately the same concentration of the two proteins, considering their molecular weight.

Cleaning procedures for remove iron-stained marble

To immobilize the extracts protein, cellulose pulp was used in that it belongs to the category of inert supports which act by the direct swelling of the cellulosic fibers (of different dimensions) due to imbibition of the pure solvents (water, alcohol, etc.) or their mixtures [32]. For the applications reported in this work, the extracted proteins (in aliquots of 20 mL, 1:1 m/V with PBS) were immobilized using the cellulose pulp white and de-resinated (Arbocel BWW40), composed of natural fibers, capable of retaining a quantity of water equal to 5 times its weight. The pH of the solutions was kept neutral (pH = 7.4) for both proteins with the use of the PBS buffer. The supported proteins were then applied over the surfaces of artificially stained Carrara marble specimens (100x100x20 mm). The stains were obtained by placing iron bars on the surface of the marble and promoting corrosion through exposure outdoor for one year (terrace, Department of Chemistry, University of Rome). This type of exposure was chosen to obtain an action mediated between the various conditions of temperature and rainfall over the four seasons.

Before the application of the cellulose pulp, any possible powdered fragment adhering to the surface of the marble has been removed. The pulp-covered samples were protected with cellophane to slow down the evaporation and thus prolong the cleaning action of the proteins. After 12 hours, the cellophane was removed, and the surfaces were cleaned with a cotton swab dipped in deionized water.

Carrara marble is mainly composed of CaCO_3 (~ 97%,) with lower percentages of other compounds ($\text{CaMg}(\text{CO}_3)_2$ 1.76%, MgO 1.32%, SiO_2 0.71%). Marble has a negligible total porosity (about 0.2%) [14]. The microscopic observation in reflected light of the section of an artificially stained marble specimen shows how the concentration of iron oxides progressively reduces from the surface of the specimen to the interior, down to a depth of about 1.37 mm %) [14]. With the oxidation of metallic iron, the iron compounds formed by the corrosion processes can diffuse through the inter-granular spaces of the marbles. On the surface of the sample, the corrosion process leads to the formation of iron (III) oxide, identified as Lepidocrocite, which tends to transform over time into Goethite and Hematite [15].

Spectrocolorimetric analysis and digital microscopy of iron stains

The instrument used for the colorimetric analysis was a Konica Minolta CM-2600d spectrocolorimeter set with the following measurement parameters: Spectral wavelength range between 360 nm and 740 nm, with a resolution of 10 nm; CIE Standard Illuminant D65; Observer at 10° [33].

The reflectance curves were used to evaluate the removal of iron oxides. In each treated area, 12 measurements were made in order to calculate the average trend. The color variation was also measured with a portable digital microscope, Dino-Lite AM4815ZT, and the acquired micro-photos, before and after the treatment, were processed with the software for image analysis "Image J" [34]. Using the RGB (Red/Green/Blue) profiler plug-in for the color processing, it was possible to obtain histograms of the average RGB values of the individual pixels, useful for evaluating chromatic variations relating to the removal of iron oxides [35].

X-ray Photoelectron Spectroscopy (XPS)

XPS spectra were acquired with a SPECS Phoibos 100- MCD5 spectrometer operating at 10 kV and 10 mA, in medium area (diameter = 2 mm) mode, using aMgK α (1253.6 eV) and AlK α (1486.6 eV) radiations. The pressure in the analysis chamber was less than 10⁻⁹ mbar during acquisition. Wide spectra were acquired in FAT (Fixed Analyser Transmission) or FRR (Fixed Retarding Ratio) modes with a constant pass energy of 20 eV and channel widths of 1.0 eV.

High-resolution spectra were acquired in FAT mode, with a constant pass energy of 9 eV and channel widths of 0.1 eV, and were curve-fitted using the program Googly, which allows estimating intrinsic and extrinsic features of XPS spectra [36, 37]. Peak areas and positions (Binding Energies, BE) as derived by curve-fitting were, respectively, normalized using proper sensitivity factors and referenced to the C1s aliphatic carbon set at 285.0 eV [38].

The assignments of the corresponding chemical group and the percentage compositions were derived from the analysis of standard compounds, acquired in our laboratory, and from the NIST X-ray Photoelectron Spectroscopy Database [39]. The sampling for XPS analysis of the marbles under study was performed by gently scraping their surface. The powder, collected from the marked zones, was firstly homogenized in an agate mortar and then pressed onto a double-sided copper tape, properly fixed on a steel sample holder, to be safely introduced in the analysis chamber.

Results And Discussion

Comparison between tested samples

Figure 1 shows the sequence adopted to remove iron stains, produced on two Carrara marble samples (C and S) subjected to exposure to the external environment for one year: On a part of their surface the

cellulose pulp, mixed with the two selected proteins, was applied, for 12 hours. To measure the extent of the color variations on marble surfaces, before and after the stains removal with the two protein extracts, spectrophotometric analysis together with digital microscopy with "Image Analysis" were used.

Reflectance spectra and Image Analysis

One of the most obvious characteristics of iron oxides, hydroxides, and oxy-hydroxides is the variety of their colors related to different types of electronic transitions. As a rule, iron oxides strongly absorb in the ultraviolet and blue spectral regions but are also strongly reflected in the red and infrared regions.

In these, in fact, significant differences can be observed between the individual oxides in the "warm" shades, ranging from the yellow of Goethite to the purplish-red of some Hematite. These absorption differences are the basis for a clear distinction among the different types of iron oxides.

As it can be seen in Fig. 2 (a) from the trends of the reflectance spectra, both for specimen C and S, there is a significant percentage increase in the post-treatment curve, especially in those areas of wavelengths characteristic of iron oxides (UV-Vis-near IR). The greater increase for specimen S, treated with the poultice containing Ovt, confirms the better cleanliness, also evident from an operational point of view, with a relative reduction of the distance from the blank curve that is to the color of the non-stained Carrara marble. The normal reflectance spectra obtained by spectrophotometric measurements can be parameterized according to the Kubelka-Munk model [40, 41], which provides one of the most useful transformations of the reflectance data. This model, of an empirical nature, relates the diffuse reflectance (R_d) of a homogeneously divided medium, not very absorbent and thick enough not to be crossed by light, to an absorption coefficient K and to a diffusion coefficient S of the medium. Since these spectra result from the overlap of the absorption bands at different wavelengths, their resolution is easier if the derivatives of the curves are obtained. The second-derivative of the curve usually provides more information than the first derivative, because a band in the original spectrum, even if overlapped on other bands and not producing a true absorption maximum (minimum reflectance in the curve of the first derivative), always produces a minimum in the second derivative curve.

The analysis, based on the second derivative curves of the original reflectance spectrum or of the remission function (K/S) shown in Fig. 2 (b), can be useful to get an indication about different iron oxides and their crystalline properties. The absorption bands shown by iron oxides come from electronic transitions within the five 3d shells of the Fe (III) ion. Second derivative spectra generally show four bands between 370 and 730 nm, as the result of the combined overlap of the different oxides bands listed in Table 1. Although their distinction can only be indicative, from the second derivatives of the spectra in Fig. 2 (b), it can be clearly individuated the presence of Hematite, of mixed phases of Lepidocrocite/Goethite and Maghemite, the latter discriminating for brown spots.

Table 1
Absorption bands based on 2nd derivatives of Fig. 2 (b)

Observed Colours	Wavelength (nm)						
	Ferrihydrite/ Lepidocrocite/ Goethite	Ferrihydrite/Maghemite/ Hematite/Goethite	Lepidocrocite	Hematite	—	Maghemite	
Brown	380	410	480	520	570	600	630
Orange	390	420	480	520	-		
Brown	390	420	480	520 (w)	560	600	630
Orange	380	410	480	540	590	620 (w)	650 (w)
Brown	-	410	490	540	580	620	660
Orange	390	420	490	550 (w)	-		
(w) weakpeaks							

Image analysis allowed the processing of images into fundamental components to extract meaningful information. The digital microphotographs of stained marbles in Fig. 3 have been processed by RGB color analysis, before and after the removal of iron stains using Ltf and Ovt proteins. RGB allowed us to represent the processed areas in histograms, Fig. 4 (a,b), from which are evident the good results obtained from the application of the two proteins, Ovt in particular. In both cases, in the post-treatment, the observed Red decrease and a Green and Blue increase, indicate the color changing toward the white of the marble.

XPS analysis of standard iron compounds

Based on reported XPS analysis of standard compounds, Fig. 5a effectively shows how different peak shapes are associated with different oxidation states of iron in various oxides [42]; Fig. 5b shows the Fe 2p region of standard iron (III) oxide (Sigma-Aldrich), curve-fitted with Googly program. The curve fitted Fe2p region shows the 2p_{3/2} and 2p_{1/2} positions (Binding Energies, eV), their broadening due to multiplet splitting (MS), and the presence of shake up (SU) satellites, all characteristic of the Fe³⁺ profile. From the combined analysis via curve-fitting of the Fe2p and O1s detailed regions, the obtained data were those corresponding stoichiometrically to hydrated Hematite, Fe₂O₃·H₂O. The acquisition of this reference spectrum proved to be the most useful in consideration of the subsequent analysis of the real samples consisting of powders gently scraped from the surface of the marbles, under study. In fact, all the analyses of rusted marbles showed the typical Fe2p shape of iron (III) oxides and were all curve-fitted by referring to Fig. 5b.

XPS analysis of marble surfaces

The surface areas of Carrara marbles, from which powders were collected for XPS analysis, are indicated in Fig. 6. The powders taken from lateral sections not treated (along the sample thickness) were first analyzed as blank marble C (BC) and blank marble S (BS). Comparing the wide spectra of the two blank samples, BC and BS in Fig. 7 (upper), no substantial differences were noted in their characterization, since the spectra were almost perfectly overlapped. Fig. 7 (lower) and Table 2 respectively show the C1s, Ca2p, O1s curve-fitted regions related to sample BS and the results for the whole set of detailed regions, obtained following a well-established curve-fitting procedure adopted for all samples in this study [36, 37].

This procedure allows estimating surface composition by assigning chemical states (corrected BEs) and relative intensities to each peak resolved by curve-fitting (normalized areas). The procedure also allows performing the mass balance, taking into account the stoichiometric coefficients of each chemical group in the given compound, in the limits of XPS technique accuracy ($\pm 10\%$) [38].

The data collected from both blank surfaces were then compared with those of the stained surfaces, before (BL and BO) and after (AL and AO) the cleaning treatment with Ltf and Ovt proteins supported by cellulose pulp. The XPS spectra of the powders taken from the various sampling areas outlined in Fig. 6 were processed by curve-fitting, and the results are summarized in the pie charts of Fig. 8. The curve fitting results of spectra from blank samples confirm their strongly comparable composition both for the main and minor constituents shown in the pie charts for BC and BS samples (Fig. 8). In fact, the percentages of calcium carbonate and carbonaceous constituents (C-C group) were for the two white marble samples: BC: CaCO_3 35%, C-C 48%; BS, CaCO_3 38%, C-C 46%. The non-negligible percentage of the carbonaceous components in the blank surfaces of non-rusted marbles certainly relates to the deposition of contaminants deriving by the city atmosphere during the one-year exposure outdoor.

As revealed by the number of peaks composing the carbon 1s signal, listed in Table 2, the blank surface of marbles is covered by carbonaceous contaminants, mainly consisting of aliphatic chains containing oxygen groups and some nitrogen and sulfur groups. Additional unspecified adsorbed compounds are grouped, in Table 2 assignments, as C organic belonging to the lower zone of Binding Energy typical of carbides, graphite, polycyclic and aromatic carbons [39, 43, 44]. Their co-presence, often found on monumental surfaces [45] long influenced by atmospheric pollutants, contributes to the formation of the characteristic 'patina'. Here the term "patina" indicates, without discrimination, all the sequential layers, of both biotic and abiotic origin, possibly deposited over the "noble patina", intrinsically formed over the underlying carbonate stone and eventually protective against the progressive erosion due to acidic rain and aggressive atmospheric components [46, 47].

By comparing the detailed spectra (here not shown) of the two samples sets (AL–BL and AO –BO), and the curve-fitting results reported in the corresponding pie charts of Fig. 8, the cleaning effects can be highlighted. After treatment with both proteins, the stoichiometry of CaCO_3 remains unaffected, while a sharp reduction of iron and sulfur content and a relative increase of the carbonaceous components, more evident after treatment with Ovt, are registered. The treatment with Ltf has not determined an appreciable

change in the C1s profile; however, the observed increase of nitrogen could be attributed to the eventual release of peptide chains from the protein extracts. It is important to note that the values of the At% of iron (III) oxide decreased from 3% to about 1% with Ltf treatment, and from 2.5% to under the detection limit with Ovt treatment, demonstrating that both proteins have shown a remarkable ability to reduce the percentage of iron. Due to the presence of iron compounds, the stained marbles were more contaminated than blank marble's surface, as evidenced by At% values in the relevant pie charts, where an increase of sulfur and nitrogen content were also observed. It is probable that during the corrosion phenomena, various contaminants (for example atmospheric particulate) can be incorporated into the rust stains [48], making it more difficult for the iron to bond with the chelating proteins and therefore its complete removal with single cleaning action. The overall cleaning efficacy, monitored after a single treatment, clearly depends on the conditions under which the rust deposits form on surfaces, on their amount and extension in-depth, and those of the associated contaminants.

It is worth to be noted that the third column of marble C (treated with Ltf) and the fourth column of marble S (treated with Ovt) in Fig. 6, were not considered for XPS analysis, appearing almost clean and representative of blank marbles.

Table 2
XPS curve-fitted data of the detailed regions of sample BS (Fig. 6).

Element/orbital	Corrected BE (eV $\pm$ 0.2 eV)	Normalized Area	Assignments (from NIST database, literature data and standard analyses)
C1s	282.8	3393	C organics
	284.3		
	285.0	7576	C aliphatics
	286.4	1454	C-O-C, C-N, C-OH, * Φ -OH
	288.5	1483	* Φ -CO-O, -CO-NH ₂
	289.7	9158	CO ₃ ²⁻
	293.1	42	aromatic/graphitic satellite
S2p	168.6	188	S linked to an aromatic ring/ SO ₄ ²⁻ / SO ₃ ²⁻
O1s	531.6	30157	●
N1s	399.5	204	-N<
Ca2p	345.1	462	Ca, CaO
	347.2	9137	CaCO ₃
(●) O1s: total area accounts for all the oxygenated species, taking into account the stoichiometric coefficients of each chemical group in the given compound, in the limits of XPS accuracy ($\pm$ 10%, [38]. Φ = aryl group			

The results obtained highlight the usefulness of the combined use of the two analytical techniques, which made it possible to correlate the color of the surface with its composition, providing information on the effectiveness of the treatment procedure for a single application. The two chelating proteins (Ltf and Ovt), supported in the cellulose pulp, have proved highly selective to solubilize iron in rusty marbles, without affecting the CaCO₃ substrate, whose integrity is confirmed by the At % calculated after curve-fitting of XPS regions as reported on the pie-charts (Fig. 8).

The results show that Ovt is more effective than Ltf; after application of the cellulose pulp, impurities of proteins, resulting from the extraction process, can be released on surfaces, thus reducing iron XPS signals within the sampled powder [49]. In this sense, further refinements of the method chosen for extracting the proteins from their raw material, (e.g.milk and eggs) will still be possible in order to minimize the release of additional residues on stone surfaces under treatment. Furthermore, the optimization of the laboratory procedures would allow reducing the extraction times and the costs that

would arise from the purchase of the two proteins. The results so far obtained also indicate how the surface patina induced by iron corrosion, contributes to the surface alteration of stone artifacts. It has been verified that the presence and the growth of iron oxides on carbonate stones are associated with the absorption of other components, as biological components and carbonaceous particulate, all contributing to the weathering process. Cleaning methods reported in the literature treat this complex problem with various strategies including the choice of chelants mixtures, the addition of redox components to control iron redox state, and removing other organic/inorganic pollutants [5, 11, 50].

Within the National Smart Cities project, further multidisciplinary researches are underway on innovative cleaning methodologies, in order to eliminate, at the same time, rust and the associated pollutants in bioactive forms, present in real samples exposed to the external environment. Iron chelating compounds, such as glutathione and deferiprone are also being evaluated [51, 52].

In this context, the choice of natural Ltf and Ovt transferrins for the chelation of iron and their solvation in the neutral aqueous cellulose pulp appears to be a promising way for cleaning artifacts of cultural interest.

Based on the obtained results, it is possible to foresee subsequent applications to remove rust patches still present on marble surfaces, after the first application. It is also important to underline the role of cellulose pulp also in the mechanical removal of surface contaminants associated with iron oxides, and the possible action of the two transferrins studied, given their antimicrobial activity, in the control of biodeterioration without affecting the calcareous substrate [53, 54]. All these premises support the proposed research as a “green method” with a lower environmental impact, which ensures safety, non-aggressiveness for the treated matrices and cleaning effectiveness.

Conclusions

The use of immobilized Ltf and Ovt has proved an effective technique for eliminating rust stains from marble artifacts, offering secure advantages in the field of Cultural Heritage.

Firstly, the advantage found in the use of these two proteins, as an alternative to traditional chemical cleaning methods, was that of obtaining highly selective and non-invasive chelation of ferric ions. In fact, these chelating proteins act only on target compounds, without attacking different compounds, such as calcium carbonate. Secondly, the immobilization system used was able to influence the degree of cleanliness obtained, through a good capacity of retaining water, good maintenance of the contact time and finally facilitating the mechanical removal of undifferentiated patina, which contribute to altering the stone surfaces. From these points of view, the innovative value of the developed methodology, which can be adapted according to the required needs, reflecting the criteria of minimum intervention, must be emphasized. Another further advantage is represented by the green aspect of the methodology, which proves not only safe for the artifacts but also for the environment and for operators in the cultural heritage sector.

Declarations

Availability of data and materials

All data analyzed during this study are included in this published article. Raw data (including spectra) are available from the corresponding authors on reasonable request.

Competing Interest

The authors declare that they have no competing interests

Funding

Not applicable

Author's contributions

All authors contributed to collect experimental data and their interpretation. All authors have read and approved the final manuscript.

Acknowledgment

The Authors acknowledge the financial support of the Smart Cities National (SCN 00520) project.

Thanks are due to Dr. Fausto Langerame for his valuable contribution to the XPS analyses.

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Figures

Figure 1

Iron stains produced on marbles and their removal by application of cellulose poultices of Ltf and Ovt.

Figure 2

(a) Reflectance spectra for samples C (left) and S (right), before and after the iron oxide removing, in relation to the blank curve; (b) remission function (K/S coefficients) and its second derivative, versus wavelengths (nm).

Figure 3

Images of magnified areas (75x) of samples C(Ltf) and S (Ovt), acquired by digital microscopy before and after the treatment with the two proteins.

Figure 4

(a) RGB averaged histograms of samples C (Ltf) and S (Ovt), before and after the treatment with the two proteins and (b) the relative difference (delta RGB).

Figure 5

a) Superimposed Fe2p spectral shape of iron standard from Graat et al. [42]; b) XPS Fe2p spectra of powdered Fe_2O_3 compound.

Figure 6

The sampling areas (dashed) of Carrara marbles used for XPS analysis; areas BC and BS: blank marble (along the sample thickness); areas BL and BO rusted before cleaning treatments; areas AL and AO treated with the supported proteins (Lft and Ovt respectively).

Figure 7

Upper, comparison of wide spectra: sample BC (orange) – sample BS (blue);

Lower, C1s, Ca2p and O1s curve-fitted regions for BS sample.

Figure 8

The graphic comparison of curve-fitting results for blank samples (BC and BS), rusted marbles before (BL and BO) and after (AL and AO) cleaning with Ltf and Ovt. Note: C-C includes both organic and aliphatic carbon.