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The NYMPHA Algae Extract as a New Consolidant for the Restoration of Cultural Heritage: Studies and Considerations on Its Effectiveness on Painted Marble

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Abstract: This work aimed to assess the potential efficacy of a novel polysaccharides-based extract as a green consolidant for the pictorial layer on marble substrates. Understanding its properties could lead to determining whether it can ensure the correct conservation and transmission of the cultural heritage. Four different types of marble specimens were prepared with paint based on malachite (egg, *tempera grassa*, rabbit glue, and linseed oil as binders). Colorimetric analyses, peeling tests, SEM-EDS, and FTIR spectroscopy were used to test the properties of the consolidant. Results reveal that no aesthetic changes occur when using the extract on painted surfaces and that the cohesive properties of most pictorial films increase after its application, confirming the consolidating effect. In conclusion, the use of an innovative green product for the restoration of marble-painted surfaces can be considered a possible good solution for consolidating treatment. Through this initial explorative research, we suggest an innovative approach to the protection of cultural heritage that doesn't cause harm to both the restorer's health and the environment.

Keywords: green materials; microalgae; natural consolidant; painted marbles; SEM-EDS; FTIR spectroscopy

1. Introduction

Cultural heritage has always been regarded as an essential means of transmitting to future generations the rich cultural legacy of ancient populations and cultures.

However, considering that artefacts are subject to the inevitable course of time that can cause their degradation [1–3], conservation and restoration practices are necessary in order to fulfill this purpose.

As far as pictorial films are concerned, damage triggers can have both anthropogenic and natural origins. Among the latter, the most common are seasonal climatic changes and weathering, catastrophes, or biological agents, while other factors such as pollution, wars, and errors in handling or restoring the artifact arise from human influence [4–6]. The selection of the optimal product for the preservation and protection of the pictorial film depends upon the substrate under examination and the extent of the damage found. After careful consideration, conservation scientists and restorers can select the most suitable option from a range of organic and inorganic substances [7–10]. Those are usually divided into three main categories, namely protectants, adhesives, and consolidants [11–16].

Specifically, consolidants are defined as substances whose aim is to re-establish the physical and mechanical conditions of an artefact, thereby restoring a sufficient degree of cohesion between the pictorial film and the substrate to which it was applied [17–19].

To consolidate the pictorial layer, organic consolidants such as animal glue, animal waxes, and vegetable gums have historically been widely used [20–23], while in more recent times, thermoplastic, vinyl, or acrylic resins such as polyvinyl acetate, have also been employed [14,24,25].

In addition to some commercially available consolidants, there are ongoing studies to continuously test new alternatives that are more effective and safer for both the environment and the operators [26–29]. Most researches are, however, often focused on the area of consolidation of panel paintings or frescoes [24,30,31], while products to consolidate the paint layer on stone substrates such as marble are, generally, less investigated. Although very rare examples have come down to the present day on which the color is visible, historically speaking, many marble works, both statuary and buildings, were actually painted [32,33]. Unfortunately, in most cases, the original pictorial film has not survived, making it challenging to determine the materials used. Some techniques, such as XRF [34], allow the analysis of trace elements, which are useful in identifying and characterizing the inorganic part, which comprises most of the pigments. The organic material originally present as certain pigments and the binders, however, deteriorate almost completely over time, and even in the very rare cases when the scarce remains are present on the artwork, they can't be detected. Recently, there has been an increase in the use of sophisticated alternatives such as gas chromatography coupled with mass spectrometry and pyrolysis [35], which have made it possible to identify, for example, proteinaceous substances, such as egg and glue, and lipidic substances, such as vegetable oils [36], whose use as binders is also confirmed by historical literary sources [37]. However, due to difficulties mainly related to the interference of other species and conservation conditions [36], their unambiguous identification still represents a challenge, in particular as it concerns the composition of the pictorial films on the statues, which to a greater extent present the problem of the scarcity of available and collectable samples.

Heritage objects of this kind can be considered complex systems because of the different nature and, therefore, the different characteristics of the two parts involved that must be taken into account. The marble substrate is inorganic, crystalline, and not breathable, whereas the surface paint layer may contain organic fractions represented by binders or any dyes, in addition to inorganic pigments [14,38,39].

A suitable consolidating agent for this type of artifact should eliminate the discontinuity between substrate and pictorial film. In addition to ensuring good adhesion, it should not cause any aesthetic changes to the item to preserve its original appearance [40–43]. Furthermore, the long-lasting effects of a consolidant and, above all, its stability over time are essential to ensure that the restoration does not cause more long-term problems than immediate benefits.

Previous treatments of marble surfaces have been demonstrated to result in the formation of certain degradation products subsequently identified, such in the case of silica compounds, which are by-products arising from the decomposition of fluorosilicate used as consolidant [44], or orange-brown patinas originated from an artificial coating improperly applied for aesthetic reasons [45].

In this early stage study, the authors wanted to specifically test the effects of applying on marble with pictorial films a natural algae extract called NYMPHA, which was created to facilitate the restoration of polymateric works and, therefore, might be potentially effective on different types of substrates [46]. NYMPHA is a new product based on polysaccharides extracted from the *Chlamydomonas reinhardtii*, a unicellular algae with a very fast rate of reproduction and ease of cultivation [47–49]. Following extraction with hot water, precipitation in ethanol, and a freeze-drying step (carried out by the spectroscopy and microalgae-based biosensors laboratory of the Institute of Crystallography—CNR), this extract was turned into a dusty, easily usable solid.

The use of polysaccharide extracts from natural sources in the field of cultural heritage is not a complete novelty, as some South American populations already applied the mucilage obtained from *Opuntia ficus-indica*, consisting of complex carbohydrates and commonly called *Nopal*, since ancient times as a consolidating agent for mortar mixtures [50–52]; some products such as *agar-agar*, a polysaccharide extracted from several species of red seaweeds [53] and *funori*, a polysaccharide derived from the red algae genus *Gloiopeltis* [54], are beginning to catch on as protective or adhesive agents [28,55]. The latter is used for the conservation of several artefacts, generally employed as a mild consolidant for paper, painting layers, and gold or silver leaves, where a weak binding is required [54]. Their versatility in terms of both properties and substrates they are compatible with, combined with their green, natural, and environmentally safe characteristics, as well as their non-toxicity, make polysaccharide extracts a class of substances worthy of testing and further investigation.

2. Materials and Methods

2.1. Sample Preparation

A series of marble bricks were cut from a white marble block from the Gioia basin (Colonnata, Province of Massa-Carrara, central Italy), in the Apuan Alps. It is a white meta-limestone widely used as an ornamental stone since the Etruscan time [56–60].

Four different types of *tempera* were manually prepared according to Cennini [37] and Hebborn [61] to test the behavior of the extract on different binders: egg yolk, *tempera grassa*, rabbit glue, and linseed oil. Copper (II) carbonate, basic from Merk (product number 207896), was used as a pigment for all binders, and the final mixture was applied to the marble samples using a synthetic brush.

Within the four sets of samples, each prepared with one of the chosen binders, two subgroups were formed: half of the specimens were painted with a mix consisting of pigment and binder in a 1:1 ratio, as in common practice, whereas the remaining half of the specimens were painted using a *tempera* mixed with a 2:1 ratio, in which the amount of binder was therefore halved compared to conventional practice; the aim was simulating the loss of binder over time.

Once prepared and set, all the specimens underwent cycles of temperature (T) and relative humidity (RH) for the artificial aging. The samples were exposed in the climatic chamber ACS Challenge CH250 (distributed by Angelantoni Industrie S.p.A, Massa Martana, Italy) for 38 days, following this regimen:

- 12 h at T = 10 °C and RH = 95%
- 12 h at T = 50 °C and RH = 40%

Subsequently, the samples were treated with UVA radiation (λ = 340 nm) in the QUV/se UV test Chamber (distributed by Q-LAB for 7 days) at T = 50 °C and RH = 20%.

Once completed, one sample of each kind of *tempera* was kept for comparison analysis while NYMPHA was applied to the remaining samples, forming three more sets containing the extract dissolved at 0.5%, 1%, and 2% (w/w) in water.

Application of the NYMPHA solution was done by synthetic brush by laying an equal number of brush strokes on all samples until the entire surface was wet.

2.2. Methods

2.2.1. Fourier Transform Infrared Spectroscopy

FTIR spectroscopy was chosen to characterize the paint film, consisting of the pigment mixed with different binders, as well as to identify the presence of NYMPHA extract on the surface. An FTIR Nicolet iS50 instrument (Thermo Scientific, Waltham, Massachusetts, USA) coupled with the IR microscope Continuum and equipped with a liquid nitrogen-cooled MCT-A (mercury cadmium telluride) detector was used to acquire the spectra.

Spectra were acquired in µ-transmission mode in the 4000–650 cm^{-1} range, with a spectral resolution of 8 cm^{-1}. Fourier transform was applied to the sum of 40 scans. Finally, the spectra were processed using the Omnic Spectra software ver. 9.12.993.

2.2.2. Peeling Test

The peeling test, also known as Scotch test, was performed to test the effective consolidating power provided by the NYMPHA solution, which is one of the most important parameter that had to be evaluated in this experimental phase [62,63].

The aim was to determine the capacity of the paint layer applied on the marble to resist interaction with external agents and, therefore, to maintain a good level of adhesion to the substrate. For each sample, whether consolidated by NYMPHA or not, the test was carried out by applying ten strips of double-sided adhesive tape, 3.5 cm long and 2 cm wide. During the test, light pressure was applied to the double-sided tape for approximately 10 s, after which the adhesive was removed by seizing the free end and pulling it off steadily (without jerking and at an angle of 90°) with the force applied in a direction perpendicular to the surface under examination. This procedure was repeated ten times on each sample, placing each new piece of tape on the same spot every time. To verify the degree of adhesion of the film to the substrate, it was necessary to determine the amount in weight of material removed: each adhesive strip was therefore weighed before and after the process.

2.2.3. Colorimetry

Colorimetric analysis was carried out to assess the presence of any chromatic change between the samples on which NYMPHA was applied and the unconsolidated ones, using the portable spectrophotometer CM-2600d (distributed by Konica Minolta, Tokyo, Japan). The measurements were performed in the color space CIE L*a*b* with the following features: illuminant D65, 10° observer, 100% UV included, 3 mm spot, in both SCE (Specular Component Excluded) and SCI (Specular Component Included) mode. The software Spectra Magic (NX version) was used for data processing. In this research, the color difference (ΔE*) between untreated and treated samples was used to assess the visual impact of NYMPHA.

2.2.4. Scanning Electron Microscopy

SEM-EDS investigation was conducted to observe the morphologies and structures of the analyzed surface in detail. A FEI-Quanta 400 instrument (FEI company, Hillsboro, Oregon), operating at 20 kV, equipped with X-ray energy-dispersive spectroscopy (EDAX Genesis Microanalysis system by FEI company) was used.

3. Results and Discussion

3.1. Fourier Transform Infrared Spectroscopy

µ-FTIR analysis confirmed the nature of the pigment used for the pictorial layer, which is a basic copper carbonate corresponding to the pigment malachite [64]. The bands at 873 cm^{-1} and around 1434 cm^{-1} could be attributed to the out-of-plane bending vibration of the carbonate groups [65], while the one at 710 cm^{-1} to the in-plane bending vibration. The antisymmetric stretching bands of CO_3^{2-} are visible at 1506 cm^{-1} and 1395 cm^{-1} [66]. The peak at 815 cm^{-1} could be caused by the C-O deformation vibrations, and the O-H stretching vibrations in the crystalline water are visible at 3310 cm^{-1} and 3400 cm^{-1} [64] (Figure S1). The four raw binders underwent µ-FTIR analysis to serve as a benchmark for both unconsolidated and consolidated specimens (Figure S2).

Next, the µ-FTIR spectra of the samples were compared with those of samples with the same binder consolidated with NYMPHA.

No differences emerged between the two samples, from which it can be claimed that under the experimental conditions in which the NYMPHA product was used, in terms of the amount and type of substrate, the NYMPHA product is not detectable with the FTIR technique (Figure 1).

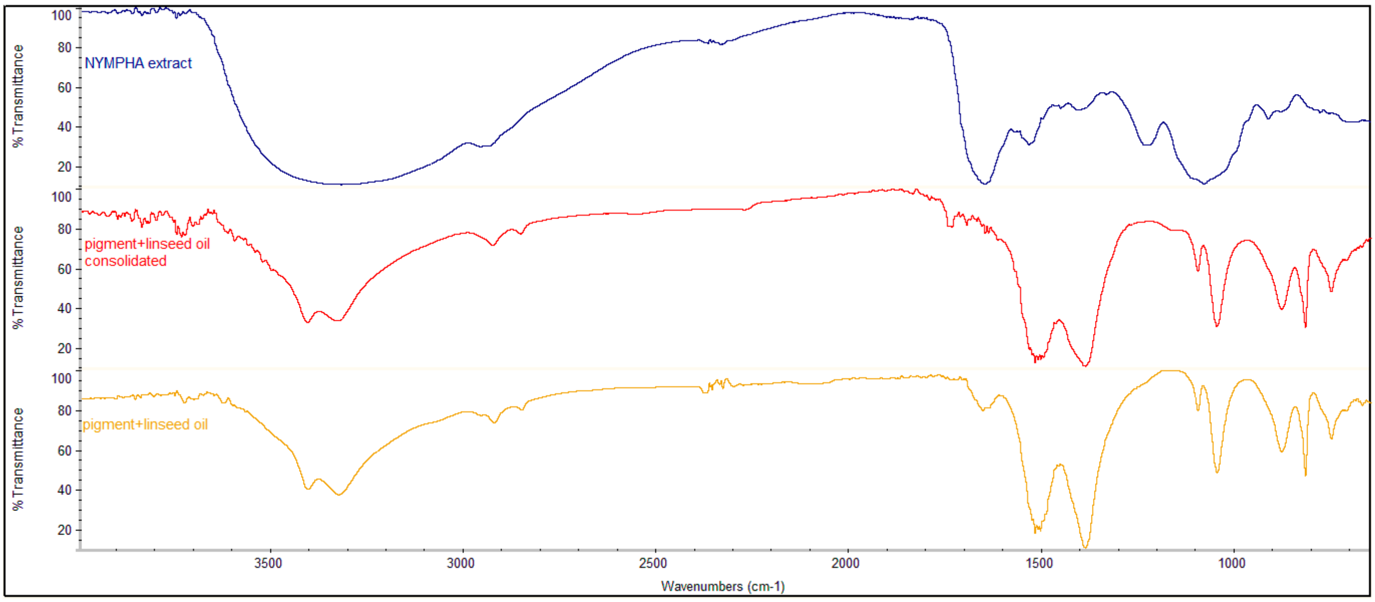

Figure 1. Comparison between μ-FTIR spectra in transmission of the NYMPHA extract in powder and an example of pictorial layer (pigment and linseed oil) untreated and consolidated.

No differences could be observed between spectra, except a slight variation in the intensity of peaks. The same difference was notable when comparing the samples with different pigment binder ratios (Figure S3). In both cases, the differences were attributed to the heterogeneity of the sample, given their manual preparation.

3.2. Peeling Test

The peeling test showed overall interesting results.

In all sets of samples, the amount of material removed by tearing from NYMPHA-treated samples was lower on average than that removed from untreated samples. The only exception was observed in samples in which the binder used was rabbit glue (Figure 2a).

Both on samples containing *tempera grassa* and oil as a binder in a 1:1 ratio with pigment, good efficacy of the product was recorded, specifically when the concentration of the applied solution was 0.5% and 1% (Figure 2b).

Sets with the same two binders, applied in a 1:2 ratio with the pigment, maintain fairly good efficacy, which is higher in cases of 0.5% NYMPHA application (Figure 2c).

Lastly, from all samples in which the binder was egg yolk, the amount of material removed was very low, particularly close to zero for samples with a 1:1 ratio pigment:binder to which NYMPHA was applied at 0.5% (Figure 2d).

Overall, despite the invalid efficacy recorded on samples containing rabbit glue as binder, the results could be considered satisfactory as a low amount of material removed with the tape is indicative of good adhesion. In most cases, this amount was much less than the material removed from the untreated specimens, indicating a good consolidation ability of the NYMPHA product when applied on egg, oil, and *tempera grassa* binders, especially in 0.5% concentration.

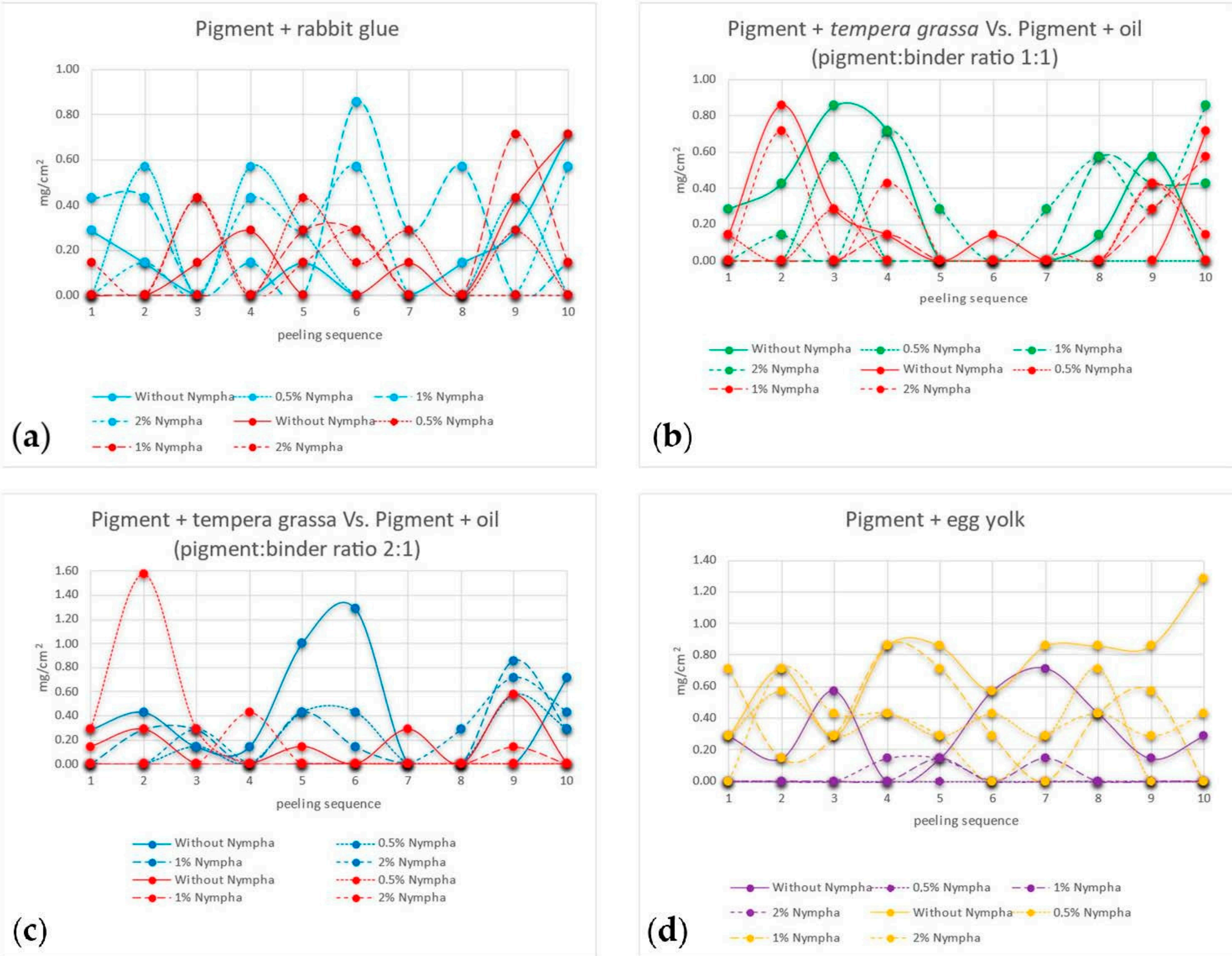

Figure 2. Scheme of peeling test results: (**a**) Test on pigment and rabbit glue; ratio 1:1 in blue, ratio 1:2 in red; (**b**) Comparison of the tests on pigment and *tempera grassa* (green) versus pigment and linseed oil (red), for the 1:1 ratio; (**c**) Comparison of the tests on pigment and *tempera grassa* (blue) versus pigment and linseed oil (red), for the 1:2 ratio; (**d**) Test on pigment and egg yolk; ratio 1:1 in purple, ratio 1:2 in yellow.

3.3. Colorimetry

In the interpretation of the data presented here, it should be kept in mind that this is a punctual analysis, and the surface small under investigation is, in most cases, potentially unrepresentative of the entire statuary cultural heritage.

The results are shown in Table 1, in which one can note that most of the values lie below 3, which can be considered to be the threshold value below which, in most cases, human eyes do not detect a color change [67,68].

The ΔE* value obtained from the sample "*tempera grassa* (1:1)" treated with the NYMPHA solution at 1% and 2% is higher than 7.

This type of test could also be influenced by the thickness of the pictorial layer, which is not uniform due to the application method.

In addition, it's worth mentioning that on the NYMPHA-treated samples, for technical and control reasons, a small, marked area on which the product was not applied was always left on the margin. This area has no clear demarcation given by the color variation compared to the area on which the product was applied.

Keeping in mind all the variables related to sample preparation, it may not be excluded that unexpected values of ΔE* could be due to the points on which the analyses were carried out and, rather than to a real optical difference, probably because of different punctual composition in terms of the amount of binder, pigment, and thickness of the pictorial layer.

Therefore, it might be concluded that the application of NYMPHA could result in no significant color alterations in the short term.

Table 1. Colorimetric ΔE values obtained by comparing, for each sample, the values measured on untreated specimens and those treated with NYMPHA. ΔE values > 3 are in bold.

Binder (Pigment:Binder Ratio)	NYMPHA%	ΔE
Egg yolk (1:1)	0.5%	2.54
Egg yolk (1:1)	1%	**4.46**
Egg yolk (1:1)	2%	2.67
Egg yolk (1:2)	0.5%	0.86
Egg yolk (1:2)	1%	1.72
Egg yolk (1:2)	2%	1.60
Tempera grassa (1:1)	0.5%	**5.61**
Tempera grassa (1:1)	1%	**7.01**
Tempera grassa (1:1)	2%	**7.46**
Tempera grassa (1:2)	0.5%	1.28
Tempera grassa (1:2)	1%	1.17
Tempera grassa (1:2)	2%	2.22
Rabbit glue (1:1)	0.5%	1.41
Rabbit glue (1:1)	1%	2.00
Rabbit glue (1:1)	2%	**3.26**
Rabbit glue (1:2)	0.5%	**5.32**
Rabbit glue (1:2)	1%	**4.37**
Rabbit glue (1:2)	2%	1.96
Linseed oil (1:1)	0.5%	**3.83**
Linseed oil (1:1)	1%	2.37
Linseed oil (1:1)	2%	**4.11**
Linseed oil (1:2)	0.5%	2.81
Linseed oil (1:2)	1%	0.83
Linseed oil (1:2)	2%	**3.68**

3.4. Scanning Electron Microscopy

SEM analyses were performed to detect any changes in the morphology of the pictorial layer following the application of NYMPHA. To investigate the appearance that the extract would have on the marble substrate, images of a specimen to which NYMPHA was directly applied without the pigment layer were acquired (Figure 3). As shown in Figure 3, the application of the extract resulted in an irregular and fractured surface. The collected EDS spectrum showed high contents of magnesium and calcium, depending on calcite and dolomite as the main components of marble, and phosphorus. The presence of the latter was expected such as algae like *C. reinhardtii* can absorb it from the outside environment while growing [69].

On the SEM images collected on the *tempera grassa* samples, the pigment and binder structures that make up the paint film were visible (Figure 4); moreover, this layer was clearly distinguishable from the part of the pure marble sample, on which no brush strokes were applied (Figure 4a).

In the samples containing rabbit glue as binder (Figure 5), the latter appeared as a series of irregular, spongy-looking particles, at times distinguishable from the spherical malachite particles. It was also possible, at large magnifications, to notice the action of the glue, which incorporates the pigment (Figure 5b).

A comparison of the samples containing different ratios of binder and pigment revealed, as shown in Figure 5, that in the case of the pictorial film with higher binder concentration, the structure appears more porous. This phenomenon could be attributed to the fact that air bubbles are formed during the glue preparation or after it is applied to the substrate, which, upon bursting, then cause the presence of voids between the film particles themselves.

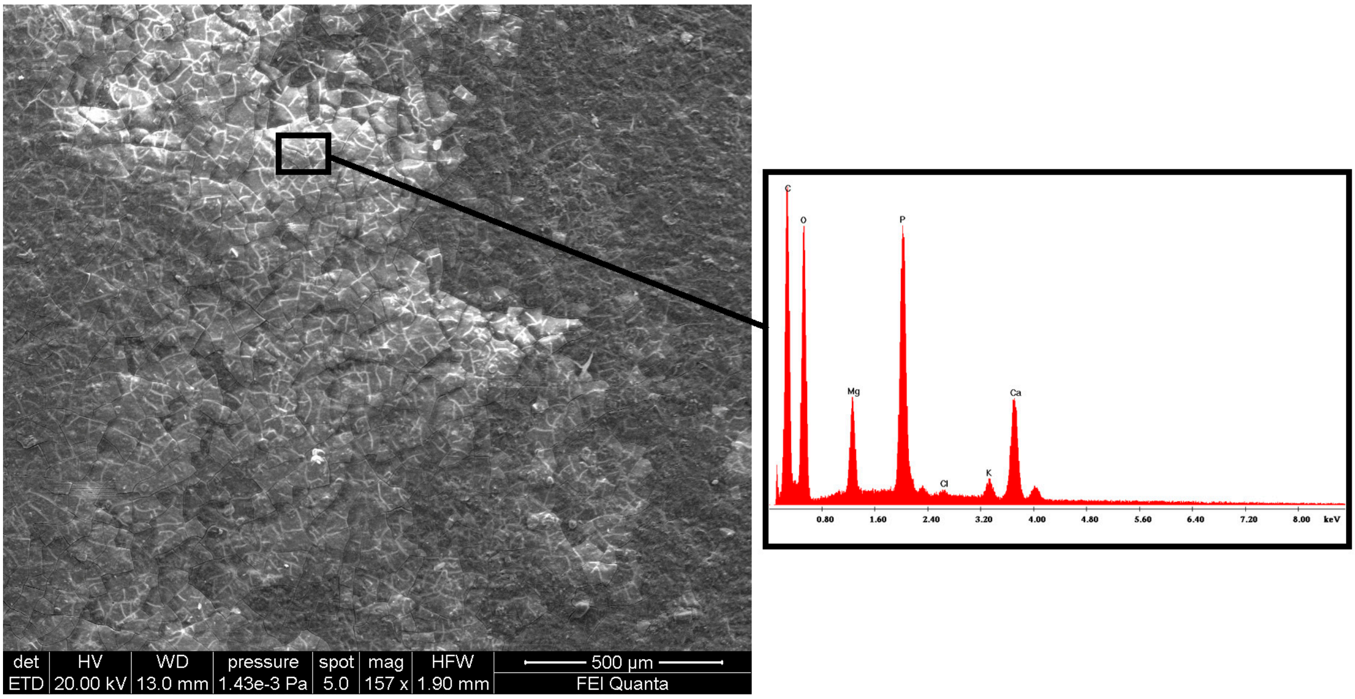

Figure 3. SEM image and EDS analysis of NYMPHA solution dried on the marble surface, without the presence of any pictorial layer.

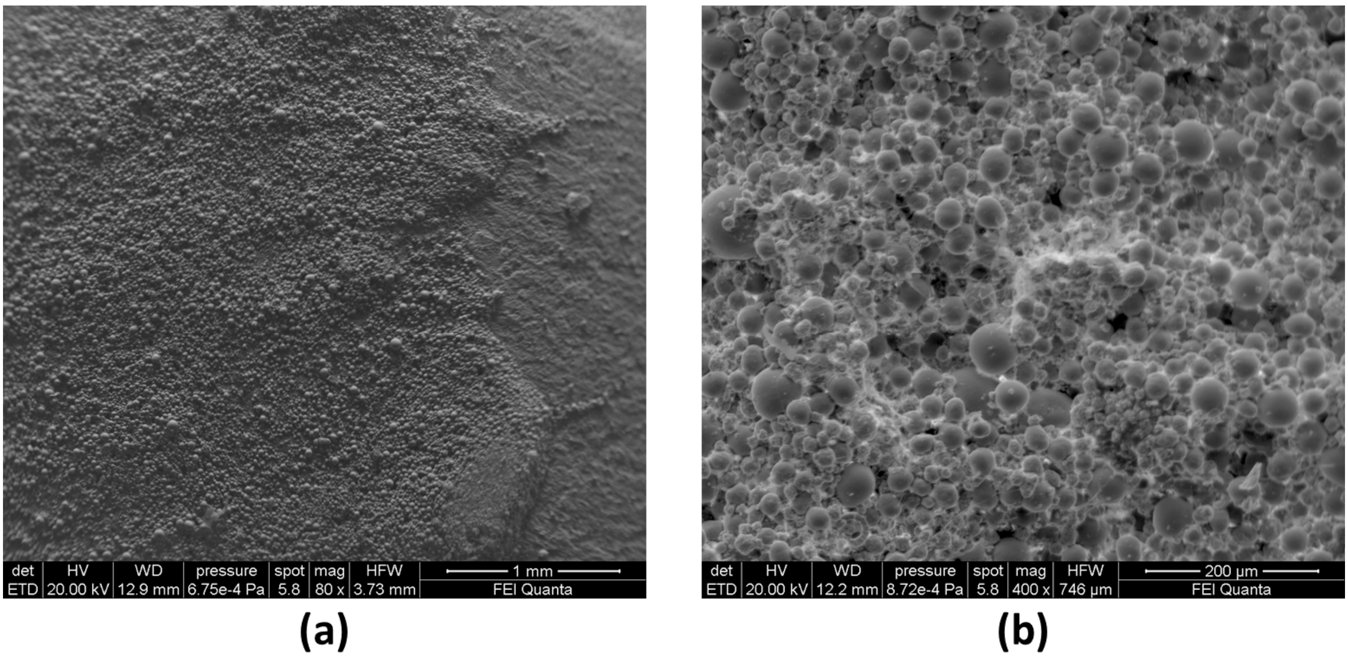

Figure 4. SEM images of the sample painted with *tempera grassa* treated with NYMPHA solution: (**a**) detail of the edge of the pictorial layer and the marble substrate, on the right; (**b**) high magnification of the pictorial layer.

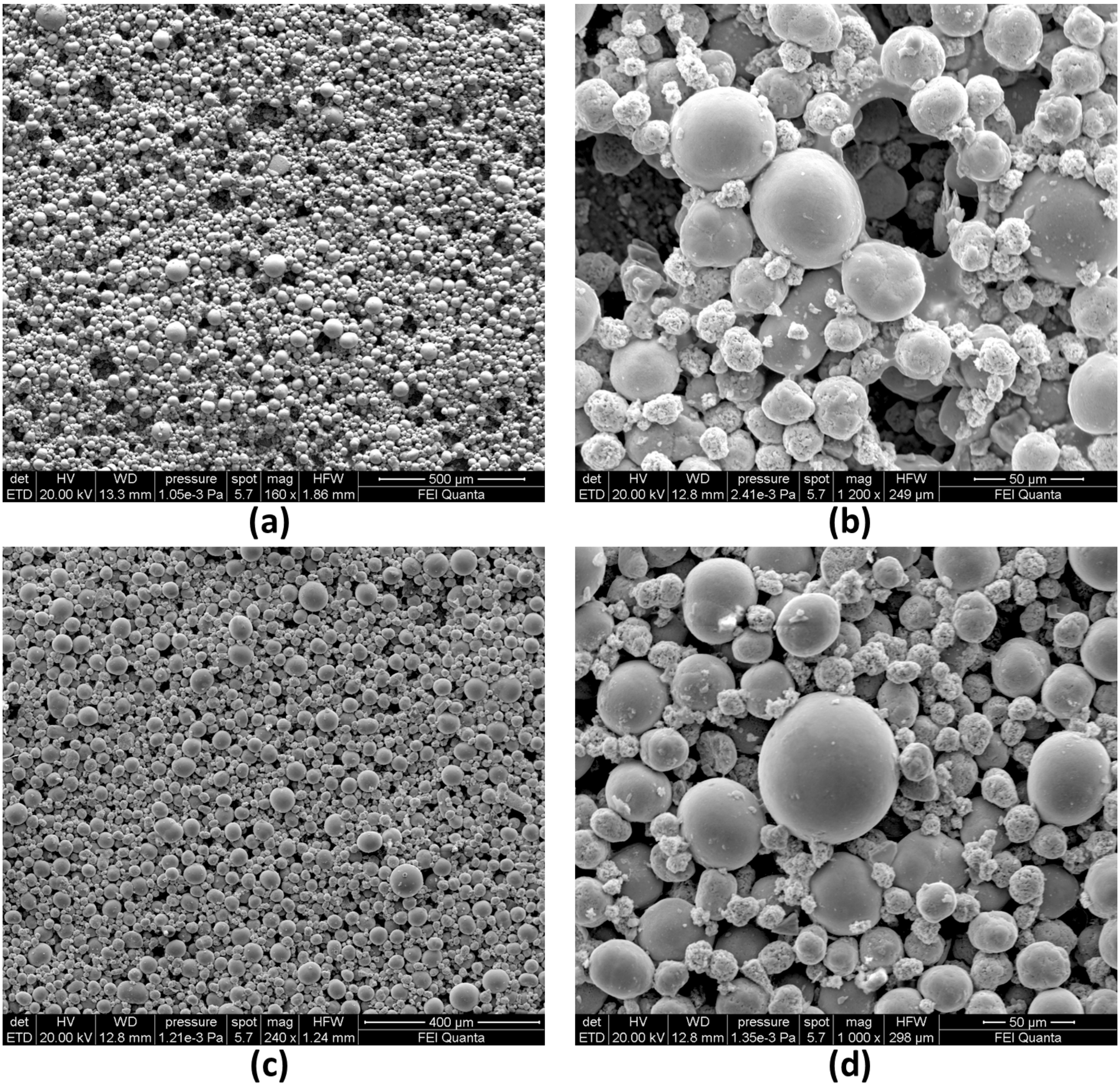

Figure 5. Comparison of SEM images of sample painted with rabbit glue and pigment treated with NYMPHA solution: (**a**) overview of the 1:1 binder-pigment ratio film, with a certain degree of porosity; (**b**) detail of the same pictorial film; (**c**) overview of the 1:2 binder-pigment ratio film, with a less degree of porosity; (**d**) detail of the same pictorial film.

The lower amount of glue in the samples with the halved binder ratio may explain the less porous appearance (Figure 5c,d). Also, in those, the liquid glue layer is much less than the solid pigment's particles.

Likewise, for the samples in which linseed oil and egg yolk were used, SEM analysis exhibit the pigment particles distinguishable by morphology from those of the respective binders (Figure 6a,b). Indeed, also in these samples, the spherical malachite particles were clearly visible, and the binders showed different characteristics: linseed oil (Figure 6a) appears in the form of lumpy and small particles, separated from the pigment, whereas the egg yolk forms a more compact film that surrounds the malachite spheres.

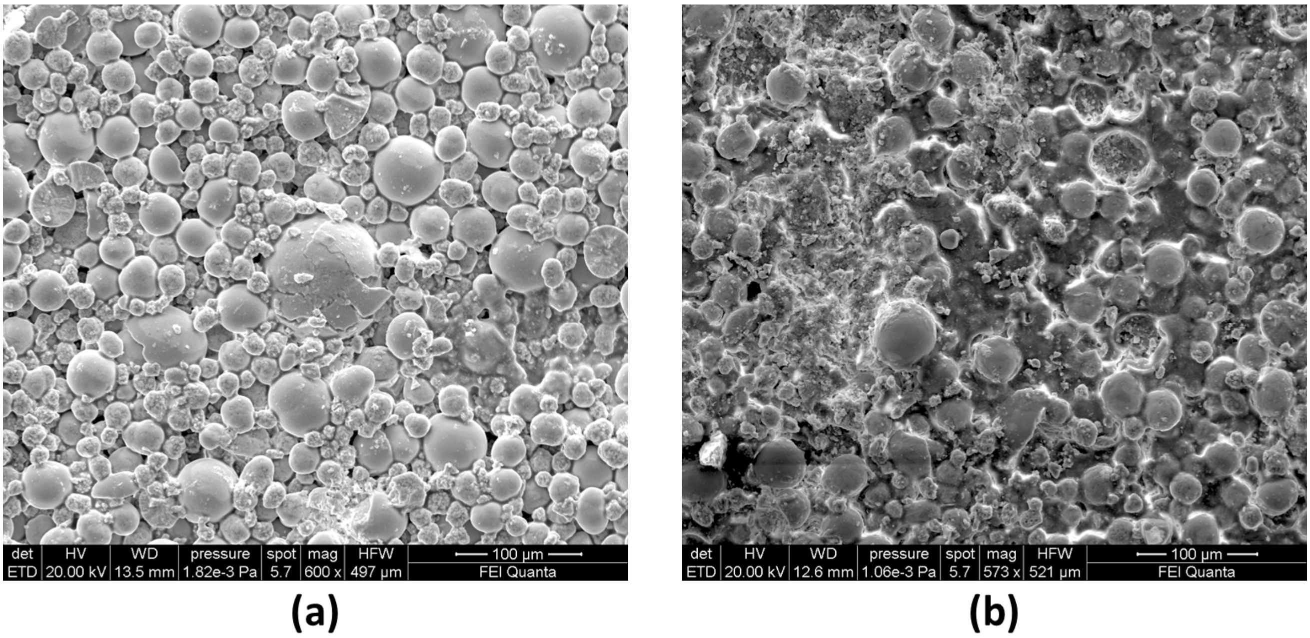

Figure 6. SEM images of sample painted surfaces treated with NYMPHA solution: (**a**) linseed oil; (**b**) egg yolk.

However, comparing the SEM image of the dried NYMPHA solution (Figure 3) with the SEM images of the painted treated surfaces, in none of the specimens there were visible structures that could be traced back to the presence of NYMPHA. No differences can be noted between surfaces treated with NYMPHA and the untreated ones (Figure S4).

The main hypothesis that might explain this kind of behavior is related to the possibility that the solution, once spread on the films, does not remain on the surface of the painting film but penetrates deeper into the layers, probably reaching the interface with the marble.

4. Conclusions

The results presented and discussed here lead to several key conclusions:

- The interpretation of FTIR spectra might suggest that the application of the NYMPHA product, independently from the concentration of the applied solution, does not alter the composition of the pictorial layers. The absence of characteristic peaks identifying the polysaccharides of NYMPHA in all collected spectra may be attributed to the low concentrations at which the solution was diluted; the absorption bands of NYMPHA may have been overshadowed by the pigment and binder bands.
- The results from the peeling test indicate a good consolidation treatment for most painted layers, except for those with rabbit glue as binder. Particularly, the observed increase in cohesive properties appears to be most pronounced for the 0.5% NYMPHA solution, suggesting that the extract might be more effective at lower concentrations.
- According to the colorimetric results, the application of the NYMPHA solution on the pictorial film would not induce any distinguishable changes in color, preserving the aesthetics of the specimens.
- SEM imaging does not detect the presence of the NYMPHA solution, at least on the surface. These results suggest the idea that the product may have penetrated under the external layer of the samples at the pictorial-marble interface.

From the above-mentioned considerations, the NYMPHA extract could be considered a potential efficient green product for the consolidation of paint films on marble substrates. Indeed, the results here may be regarded as an encouraging preliminary indication of the potential for a satisfactory product to be deployed in the context of cultural heritage.

However, the inability to detect the solution on the surface through FTIR and SEM analyses, alongside the proposed hypothesis of its penetration, currently hinders a comprehensive understanding of the consolidation mechanisms of NYMPHA.

Further studies involving different analytical techniques are planned to investigate how the product interacts with the paint layer and the stone substrate and to elucidate these mechanisms. In addition, analyses will be carried out to investigate the performance of NYMPHA after aging, to confirm that the extract does not cause long-term chemical and visual changes to the substrate to which it is applied and to confidently assess the viability of NYMPHA as a green restoration product for cultural heritage artefacts.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su16166868/s1, Figure S1: μ-FTIR spectrum of Copper (II) carbonate; Figure S2: μ-FTIR spectra of the four raw binders; Figure S3: Comparison between μ-FTIR spectra of the pictorial film realized with different pigment-binder ratio); Figure S4: Comparison of SEM images of untreated sample.

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References

1. Baglioni, P.; Chelazzi, D.; Giorgi, R. *Nanotechnologies in the Conservation of Cultural Heritage: A Compendium of Materials and Techniques*; Springer: Dordrecht, The Netherlands, 2015; ISBN 978-94-017-9302-5.
2. Sesana, E.; Gagnon, A.S.; Ciantelli, C.; Cassar, J.; Hughes, J.J. Climate Change Impacts on Cultural Heritage: A Literature Review. *WIREs Clim. Chang.* **2021**, *12*, e710. [CrossRef]
3. Patil, S.M.; Kasthurba, A.K.; Patil, M.V. Characterization and Assessment of Stone Deterioration on Heritage Buildings. *Case Stud. Constr. Mater.* **2021**, *15*, e00696. [CrossRef]
4. Coccato, A.; Moens, L.; Vandenabeele, P. On the Stability of Mediaeval Inorganic Pigments: A Literature Review of the Effect of Climate, Material Selection, Biological Activity, Analysis and Conservation Treatments. *Herit. Sci.* **2017**, *5*, 12. [CrossRef]
5. Li, Z.; Wang, L.; Chen, H.; Ma, Q. Degradation of Emerald Green: Scientific Studies on Multi-Polychrome Vairocana Statue in Dazu Rock Carvings, Chongqing, China. *Herit. Sci.* **2020**, *8*, 64. [CrossRef]
6. Gil, M.; Costa, M.; Cvetkovic, M.; Bottaini, C.; Cardoso, A.M.; Manhita, A.; Barrocas Dias, C.; Candeias, A. Unveiling the Mural Painting Art of Almada Negreiros at the Maritime Stations of Alcântara (Lisbon): Diagnosis Research of Paint Layers as a Guide for Its Future Conservation. *GEC* **2021**, *20*, 105–117. [CrossRef]
7. Geiger, T.; Michel, F. Studies on the Polysaccharide JunFunori Used to Consolidate Matt Paint. *Stud. Conserv.* **2005**, *50*, 193–204. [CrossRef]
8. Sadeghifar, H.; Venditti, R.; Jur, J.; Gorga, R.E.; Pawlak, J.J. Cellulose-Lignin Biodegradable and Flexible UV Protection Film. *ACS Sustain. Chem. Eng.* **2017**, *5*, 625–631. [CrossRef]
9. Sassoni, E.; Franzoni, E.; Stefanova, M.; Kamenarov, Z.; Scopece, P.; Verga Falzacappa, E. Comparative Study Between Ammonium Phosphate and Ethyl Silicate Towards Conservation of Prehistoric Paintings in the Magura Cave (Bulgaria). *Coatings* **2020**, *10*, 250. [CrossRef]

10. Tsakalof, A.; Manoudis, P.; Karapanagiotis, I.; Chryssoulakis, I.; Panayiotou, C. Assessment of Synthetic Polymeric Coatings for the Protection and Preservation of Stone Monuments. *J. Cult. Herit.* **2007**, *8*, 69–72. [CrossRef]
11. Alonso-Villar, E.M.; Rivas, T.; Pozo-Antonio, J.S. Adhesives Applied to Granite Cultural Heritage: Effectiveness, Harmful Effects and Reversibility. *Constr. Build. Mater.* **2019**, *223*, 951–964. [CrossRef]
12. Serafini, I.; Ciccola, A. Chapter 14—Nanotechnologies and Nanomaterials: An Overview for Cultural Heritage. In *Nanotechnologies and Nanomaterials for Diagnostic, Conservation and Restoration of Cultural Heritage*; Lazzara, G., Fakhrullin, R., Eds.; Elsevier: Amsterdam, Netherlands, 2019; pp. 325–380, ISBN 978-0-12-813910-3.
13. Artesani, A.; Di Turo, F.; Zucchelli, M.; Traviglia, A. Recent Advances in Protective Coatings for Cultural Heritage—An Overview. *Coatings* **2020**, *10*, 217. [CrossRef]
14. Negri, A.; Nervo, M.; Di Marcello, S.; Castelli, D. Consolidation and Adhesion of Pictorial Layers on a Stone Substrate. The Study Case of the Virgin with the Child from Palazzo Madama, in Turin. *Coatings* **2021**, *11*, 624. [CrossRef]
15. Weththimuni, M.L.; Licchelli, M. Heritage Conservation and Restoration: Surface Characterization, Cleaning and Treatments. *Coatings* **2023**, *13*, 457. [CrossRef]
16. Horie, C.V. 10—Polymers Derived from Cellulose. In *Materials for Conservation*; Horie, C.V., Ed.; Butterworth-Heinemann: Oxford, UK, 1987; pp. 124–134, ISBN 978-0-408-01531-8.
17. Matteini, M.; Rescic, S.; Fratini, F.; Botticelli, G. Ammonium Phosphates as Consolidating Agents for Carbonatic Stone Materials Used in Architecture and Cultural Heritage: Preliminary Research. *Int. J. Archit. Herit.* **2011**, *5*, 717–736. [CrossRef]
18. David, M.E.; Ion, R.-M.; Grigorescu, R.M.; Iancu, L.; Andrei, E.R. Nanomaterials Used in Conservation and Restoration of Cultural Heritage: An Up-to-Date Overview. *Materials* **2020**, *13*, 2064. [CrossRef] [PubMed]
19. Baglioni, M.; Poggi, G.; Chelazzi, D.; Baglioni, P. Advanced Materials in Cultural Heritage Conservation. *Molecules* **2021**, *26*, 3967. [CrossRef] [PubMed]
20. Ntasi, G.; Sbriglia, S.; Pitocchi, R.; Vinciguerra, R.; Melchiorre, C.; Dello Ioio, L.; Fatigati, G.; Crisci, E.; Bonaduce, I.; Carpentieri, A.; et al. Proteomic Characterization of Collagen-Based Animal Glues for Restoration. *J. Proteome Res.* **2022**, *21*, 2173–2184. [CrossRef] [PubMed]
21. Berger, G.A. Testing Adhesives for the Consolidation of Paintings. *Stud. Conserv.* **1972**, *17*, 173–194. [CrossRef]
22. Andreotti, A.; Izzo, F.C.; Bonaduce, I. Archaeometric Study of the Mural Paintings by Saturnino Gatti and Workshop in the Church of San Panfilo, Tornimparte (AQ): The Study of Organic Materials in Original and Restored Areas. *Appl. Sci.* **2023**, *13*, 7153. [CrossRef]
23. Aggelakopoulou, E.; Sotiropoulou, S.; Karagiannis, G. Architectural Polychromy on the Athenian Acropolis: An In Situ Non-Invasive Analytical Investigation of the Colour Remains. *Heritage* **2022**, *5*, 756–787. [CrossRef]
24. Zhang, Y.; Li, X.; Zhu, J.; Wang, S.; Wei, B. Hybrids of CNTs and Acrylic Emulsion for the Consolidation of Wall Paintings. *Prog. Org. Coat.* **2018**, *124*, 185–192. [CrossRef]
25. Normand, L.; Duchêne, S.; Vergès-Belmin, V.; Dandrel, C.; Giovannacci, D.; Nowik, W. Comparative in Situ Study of Nanolime, Ethyl Silicate and Acrylic Resin for Consolidation of Wall Paintings with High Water and Salt Contents at the Chapter Hall of Chartres Cathedral. *Int. J. Archit. Herit.* **2020**, *14*, 1120–1133. [CrossRef]
26. Stagno, V.; Ciccola, A.; Curini, R.; Postorino, P.; Favero, G.; Capuani, S. Non-Invasive Assessment of PVA-Borax Hydrogel Effectiveness in Removing Metal Corrosion Products on Stones by Portable NMR. *Gels* **2021**, *7*, 265. [CrossRef]
27. Di Turo, F.; Medeghini, L. How Green Possibilities Can Help in a Future Sustainable Conservation of Cultural Heritage in Europe. *Sustainability* **2021**, *13*, 3609. [CrossRef]
28. Palma Prieto, M.; Santos Gómez, S.; Pérez-Estébanez, M.; De La Roja, J.M. Evaluation of Chia and Flax Mucilages as Consolidants of Paint Films and as Hydrogels Used in the Cleaning of Canvases Reverses: First Results. *Eur. Phys. J. Plus* **2023**, *138*, 555. [CrossRef]
29. Mariani, A.; Malucelli, G. Consolidation of Stone Materials by Organic and Hybrid Polymers: An Overview. *Macro Chem. Phys.* **2023**, *224*, 2300053. [CrossRef]
30. Giorgi, R.; Dei, L.; Baglioni, P. A New Method for Consolidating Wall Paintings Based on Dispersions of Lime in Alcohol. *Stud. Conserv.* **2000**, *45*, 154–161. [CrossRef]
31. Pondelak, A.; Kramar, S.; Kikelj, M.L.; Sever Škapin, A. In-Situ Study of the Consolidation of Wall Paintings Using Commercial and Newly Developed Consolidants. *J. Cult. Herit.* **2017**, *28*, 1–8. [CrossRef]
32. Karydas, A.G.; Brecoulaki, H.; Bourgeois, B.; Zarkadas, C. In-Situ XRF Analysis of Raw Pigments and Traces of Polychromy on Marble Sculpture Surfaces. Possibilities and Limitations. In Proceedings of the 28th International Symposium on the Conservation and Restoration of Cultural Property, Tokyo, Japan, 1–3 December 2004.
33. Saint, A.-C.; Cheilakou, E.; Dritsa, V.; Koui, M.; Kostanti, K.; Christopoulou, A.; Zezza, F. The Combined Use of Non-Invasive Methods for the Identification of Pigments and the Weathering Damage on Marble Figurines and Statues. In *10th International Symposium on the Conservation of Monuments in the Mediterranean Basin: Natural and Anthropogenic Hazards and Sustainable Preservation*; Koui, M., Zezza, F., Kouis, D., Eds.; Springer International Publishing: Cham, Switzerland, 2018; pp. 233–242, ISBN 978-3-319-78093-1.
34. Calza, C.; Oliveira, D.F.; Freitas, R.P.; Rocha, H.S.; Nascimento, J.R.; Lopes, R.T. Analysis of Sculptures Using XRF and X-Ray Radiography. *Radiat. Phys. Chem.* **2015**, *116*, 326–331. [CrossRef]

35. Calvano, C.D.; Van Der Werf, I.D.; Palmisano, F.; Sabbatini, L. Revealing the Composition of Organic Materials in Polychrome Works of Art: The Role of Mass Spectrometry-Based Techniques. *Anal. Bioanal. Chem.* **2016**, *408*, 6957–6981. [CrossRef]
36. Geddes Da Filicaia, E.; Evershed, R.P.; Peggie, D.A. Review of Recent Advances on the Use of Mass Spectrometry Techniques for the Study of Organic Materials in Painted Artworks. *Anal. Chim. Acta* **2023**, *1246*, 340575. [CrossRef] [PubMed]
37. Cennini, C. *Il Libro Dell'arte di Cennino Cennini*; Frezzato, F., Ed.; I colibrì; Neri Pozza: Vicenza, Italy, 2009; ISBN 978-88-7305-910-3.
38. Colombini, M.P.; Andreotti, A.; Bonaduce, I.; Modugno, F.; Ribechini, E. Analytical Strategies for Characterizing Organic Paint Media Using Gas Chromatography/Mass Spectrometry. *Acc. Chem. Res.* **2010**, *43*, 715–727. [CrossRef] [PubMed]
39. Brøns, C.; Rasmussen, K.L.; Di Crescenzo, M.M.; Stacey, R.; Lluveras-Tenorio, A. Painting the Palace of Apries I: Ancient Binding Media and Coatings of the Reliefs from the Palace of Apries, Lower Egypt. *Herit. Sci.* **2018**, *6*, 6. [CrossRef]
40. Pascoal, P.; Borsoi, G.; Veiga, R.; Faria, P.; Santos Silva, A. Consolidation and Chromatic Reintegration of Historical Renders with Lime-Based Pozzolanic Products. *Stud. Conserv.* **2015**, *60*, 321–332. [CrossRef]
41. Becherini, F.; Durante, C.; Bourguignon, E.; Li Vigni, M.; Detalle, V.; Bernardi, A.; Tomasin, P. Aesthetic Compatibility Assessment of Consolidants for Wall Paintings by Means of Multivariate Analysis of Colorimetric Data. *Chem. Cent. J.* **2018**, *12*, 98. [CrossRef] [PubMed]
42. Baiza, B.; Gil, M.; Galacho, C.; Candeias, A.; Girginova, P.I. Preliminary Studies of the Effects of Nanoconsolidants on Mural Paint Layers with a Lack of Cohesion. *Heritage* **2021**, *4*, 3288–3306. [CrossRef]
43. Collado-Montero, F.J.; Calero-Castillo, A.I.; Melgosa, M.; Medina, V.J. Colorimetric Evaluation of Pictorial Coatings in Conservation of Plasterworks from the Islamic Tradition. *Stud. Conserv.* **2019**, *64*, 90–100. [CrossRef]
44. Fassina, V. New Findings on Past Treatments Carried out on Stone and Marble Monuments' Surfaces. *Sci. Total Environ.* **1995**, *167*, 185–203. [CrossRef]
45. Polikreti, K.; Maniatis, Y. Micromorphology, Composition and Origin of the Orange Patina on the Marble Surfaces of Propylaea (Acropolis, Athens). *Sci. Total Environ.* **2003**, *308*, 111–119. [CrossRef]
46. Masi, A.; Medeghini, L.; Masi, A.; Sadori, L.; Leonelli, F.; Favero, G.; Antonacci, A. Natural Polysaccharides from Microalgae for the Protection of Cultural Heritage. *SEL* **2021**, *16*, 24. [CrossRef]
47. Schulze, T.; Prager, K.; Dathe, H.; Kelm, J.; Kießling, P.; Mittag, M. How the Green Alga Chlamydomonas Reinhardtii Keeps Time. *Protoplasma* **2010**, *244*, 3–14. [CrossRef] [PubMed]
48. Bellido-Pedraza, C.M.; Calatrava, V.; Sanz-Luque, E.; Tejada-Jiménez, M.; Llamas, Á.; Plouviez, M.; Guieysse, B.; Fernández, E.; Galván, A. Chlamydomonas Reinhardtii, an Algal Model in the Nitrogen Cycle. *Plants* **2020**, *9*, 903. [CrossRef] [PubMed]
49. Masi, A.; Leonelli, F.; Scognamiglio, V.; Gasperuzzo, G.; Antonacci, A.; Terzidis, M.A. Chlamydomonas Reinhardtii: A Factory of Nutraceutical and Food Supplements for Human Health. *Molecules* **2023**, *28*, 1185. [CrossRef] [PubMed]
50. Chandra, S.; Eklund, L.; Villarreal, R.R. Use of Cactus in Mortars and Concrete. *Cem. Concr. Res.* **1998**, *28*, 41–51. [CrossRef]
51. León-Martínez, F.M.; Cano-Barrita, P.F. de J.; Castellanos, F.; Luna-Vicente, K.B.; Ramírez-Arellanes, S.; Gómez-Yáñez, C. Carbonation of High-Calcium Lime Mortars Containing Cactus Mucilage as Additive: A Spectroscopic Approach. *J. Mater. Sci.* **2021**, *56*, 3778–3789. [CrossRef]
52. Alisi, C.; Bacchetta, L.; Bojorquez, E.; Falconieri, M.; Gagliardi, S.; Insaurralde, M.; Martinez, M.F.; Orozco, A.M.; Persia, F.; Sprocati, A.R.; et al. Mucilages from Different Plant Species Affect the Characteristics of Bio-Mortars for Restoration. *Coatings* **2021**, *11*, 75. [CrossRef]
53. Sansonetti, A.; Bertasa, M.; Canevali, C.; Rabbolini, A.; Anzani, M.; Scalarone, D. A Review in Using Agar Gels for Cleaning Art Surfaces. *J. Cult. Herit.* **2020**, *44*, 285–296. [CrossRef]
54. Caruso, M.R.; D'Agostino, G.; Milioto, S.; Cavallaro, G.; Lazzara, G. A Review on Biopolymer-Based Treatments for Consolidation and Surface Protection of Cultural Heritage Materials. *J. Mater. Sci.* **2023**, *58*, 12954–12975. [CrossRef]
55. Rubio, L.B.; Cabezas, M.J.; Cortés, M.G.; Morelli, P.K. Evaluation of Funori as Adhesive for Stone Polychromy. The XV Century Ramon Llull's Sepulchre as Case Study. *Estud. Conserv. Restauro* **2019**, *10*, 44–55. [CrossRef]
56. Dean, N.E. Geochemistry and Archaeological Geology of the Carrara Marble, Carrara, Italy. In *Classical Marble: Geochemistry, Technology, Trade*; Herz, N., Waelkens, M., Eds.; Springer: Dordrecht, The Netherlands, 1988; pp. 315–323, ISBN 978-94-015-7795-3.
57. Bruschi, G.; Criscuolo, A.; Paribeni, E.; Zanchetta, G. 14C-Dating from an Old Quarry Waste Dump of Carrara Marble (Italy): Evidence of Pre-Roman Exploitation. *J. Cult. Herit.* **2004**, *5*, 3–6. [CrossRef]
58. Oesterling, N.; Heilbronner, R.; Stünitz, H.; Barnhoorn, A.; Molli, G. Strain Dependent Variation of Microstructure and Texture in Naturally Deformed Carrara Marble. *J. Struct. Geol.* **2007**, *29*, 681–696. [CrossRef]
59. Massa, G.; Pieruccioni, D. Geological Characterization of the Marble Commercial Varieties Outcropping in the Frigido Valley (Apuan Alps, Italy). *Geoheritage* **2020**, *12*, 60. [CrossRef]
60. Gentili, R.; Alderighi, L.; Errico, A.; Salvatore, M.C.; Citterio, S.; Preti, F.; Baroni, C. Human-Induced Changes and Phyto-Geomorphological Relationships in the Historical Ravaneti Landscape of the Carrara Marble Basin (Tuscany, Italy). *Int. J. Min. Reclam. Environ.* **2023**, *37*, 297–318. [CrossRef]
61. Hebborn, E. *Il Manuale Del Falsario*; Neri Pozza: Vicenza, Italy, 1995; ISBN 88-7305-519-2.
62. Drdácký, M.; Slížková, Z. In Situ Peeling Tests for Assessing the Cohesion and Consolidation Characteristics of Historic Plaster and Render Surfaces. *Stud. Conserv.* **2015**, *60*, 121–130. [CrossRef]
63. Drdácký, M.; Lesák, J.; Rescic, S.; Slížková, Z.; Tiano, P.; Valach, J. Standardization of Peeling Tests for Assessing the Cohesion and Consolidation Characteristics of Historic Stone Surfaces. *Mater. Struct.* **2012**, *45*, 505–520. [CrossRef]

64. Zou, W.; Yeo, S.Y. New Methods for the Identification of Malachite Pigments with Varying Particle Sizes Used in Ancient Chinese Murals by Spectroscopic Techniques. *Dye. Pigment.* **2024**, *226*, 112111. [CrossRef]
65. Franquelo, M.L.; Duran, A.; Herrera, L.K.; Jimenez De Haro, M.C.; Perez-Rodriguez, J.L. Comparison between Micro-Raman and Micro-FTIR Spectroscopy Techniques for the Characterization of Pigments from Southern Spain Cultural Heritage. *J. Mol. Struct.* **2009**, *924–926*, 404–412. [CrossRef]
66. Buti, D.; Rosi, F.; Brunetti, B.G.; Miliani, C. In-Situ Identification of Copper-Based Green Pigments on Paintings and Manuscripts by Reflection FTIR. *Anal. Bioanal. Chem.* **2013**, *405*, 2699–2711. [CrossRef]
67. Mokrzycki, W.S.; Tatol, M. Colour Difference ΔE—A Survey. *Mach. Graph. Vis.* **2011**, *20*, 383–411.
68. Khimchenko, S.V.; Eksperiandova, L.P. Colorimetric and Stochastic Assessment of the Visual Limit of Color Perception for Visual Colorimetric Analysis. *J. Anal. Chem.* **2014**, *69*, 322–326. [CrossRef]
69. Dean, A.P.; Nicholson, J.M.; Sigee, D.C. Impact of Phosphorus Quota and Growth Phase on Carbon Allocation in Chlamydomonas Reinhardtii: An FTIR Microspectroscopy Study. *Eur. J. Phycol.* **2008**, *43*, 345–354. [CrossRef]