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NYMPHA, a natural product for the conservation of ancient wood

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ABSTRACT
In recent years, there has been a significant interest on sustainability and health-safety across various domains. Notably, many traditional chemicals employed in the preservation of Cultural Heritage pose environmental and human health risks. The NYMPHA project aimed to develop an eco-friendly solution using microalgae-derived polysaccharides to remove biological patinas from cultural heritage wooden materials, addressing sustainability and health concerns. To validate its efficacy, the product underwent testing on diverse woods such as silver fir, beech, and sessile oak, selected for their distinct anatomical characteristics. The analytical methodology involves three key steps: 1) determining the optimal extraction and application method through spectro-colorimetric measures and UV imaging; 2) evaluating surface color stability; and 3) assessing the product's effectiveness before and after exposure to a biological attack using spectro-colorimetry.
Results indicate that the NYMPHA product can induce a color variation on some wood surfaces. Moreover, although it is reported that algae can have biocidal effects, in this experiment, this action is not observed probably due to the absence of sulphates in the polysaccharide molecule extracted from this specific strain. This emphasizes the necessity for further research and to explore new solutions beyond controlled laboratory conditions, specifically on naturally degraded materials.

Section: RESEARCH PAPER

Keywords: microalgae; antimycotic; biocide; cultural heritage; green material

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1. INTRODUCTION

All materials undergo decay over time, including those in cultural heritage. Regardless of their chemical composition or physical properties, materials are susceptible to deterioration from thermo-hygrometric conditions, mechanical stresses, and electromagnetic radiations. Biodeterioration, defined as "any undesirable change in the properties of a material caused by the vital activity of organisms", [1], [2] is a significant factor. This issue becomes more critical with culturally valuable items, where microorganisms can adhere to surfaces through various vectors, leading to deterioration.

Wood, an organic material in cultural heritage, is durable but prone to deterioration when exposed to water, fostering the growth of fungi and bacteria. Biodeterioration goes beyond aesthetics, causing structural damage to wooden artifacts. Effects include stains, embrittlement, bending, warping, and deformation, altering the historical narrative.

Biological patinas result from the interplay of moisture, microorganisms, and wood, posing challenges to conservation [3], [4]. Restoration procedures easily expose operators to hazardous substances for extended periods and guaranteeing a safe environment is not always straightforward since security systems installation could be challenging in historical or unique locations. Also, chemical waste disposal can be expensive and environmentally impactful if not handled properly.

In recent years, there's a growing preference for "greener" chemistry, seeking less toxic alternatives with lower environmental impact [5], [6]. Algae, with proven applications in restoration, offer alternatives to harmful solvents. Algae already have attested applications in the restoration field. Agar-agar is, for example, a polysaccharide extracted from the cell walls of some species of red algae, used by restorers as gelling agent. *Gloiopeltis* extract (Funori) is also used due to its adhesive and consolidating property on paper [7], [8]. Antimycotic and antibacterial effects of different extracted algal polysaccharides are already reported in literature such as in the clinical and agronomical field [9], [10], [11] and encouraged to test these properties also on cultural heritage. The NYMPHA project aimed to test a new product (following called NYMPHA) based on microalgae polysaccharides, addressing biological degradation on polymaterial surfaces while being compatible with heritage materials and safe for both human health and the environment. The project, funded by the Lazio Region (POR-FESR), involves collaboration with Sapienza University of Rome, CNR Crystallography Institute, and Central Institute of Restoration.

2. MATERIAL AND METHODS

2.1. Polysaccharides extraction and their application on wooden samples

The freshwater microalgae *Chlamydomonas reinhardtii*, (CC125 strain), a model system widely used in laboratory, was carefully chosen and cultivated to induce a natural accumulation of carbohydrates. This strain, the basic "137c" wild type kindly provided by Prof. K.K. Niyogi of UC Berkeley, was grown on Tris acetate phosphate (TAP) medium according to Harris E., 2009 [12] under continuous light and temperature controlled conditions (50 μmol photons/m^2/s and 25 °C, respectively). In order to promote the photobiological processes of our microalgae, particularly powerful fluorescent lamps at the blue and red ends of the spectrum (OSRAM Fluora) were chosen. For the conservation of the strains, Petri dishes and the previously mentioned medium solidified with 1.5 % noble agar (Difco) were used. Additionally, three experimental approaches (below defined as P1 [13], P2 [14] and P3 [15]), according to the scientific literature, were tested to obtain polysaccharide mixtures from algae biomass. In particular, the polysaccharides were extracted according to P1 from *C. reinhardtii* cells (collected to plateau phase of growth curve) by hot water treatment (3 h) and 80 % ethanol precipitation at -20 °C over night. The chemical composition of polysaccharides is still under investigation by FTIR, GC-MS antioxidant assays. We tested three NYMPHA solutions, applying them at three concentrations (0.3 % w/v, 0.6 % w/v, 1.2 % w/v) in distilled water using three different application methods (brush, spray, drop) on ten wooden samples (silver fir). To determine the optimal extraction product and application method while considering potential staining effects, we measured colour with a Konica Minolta CM 700d, employing a D65 light source, 10° observation angle, and a 2 mm spot. The measurements were taken after 24 hours and 7 days of natural irradiation following treatment, and results were compared to an untreated sample as a reference.

As the NYMPHA extract exhibits fluorescence under UV light, we utilized UV imaging to preliminarily assess the presence of surface residues of the product. A Wood-lamp served as the light source for this examination.

2.2. Selection of the wooden samples

A set of modern wood belonging to three different species: silver fir (*Abies alba*), beech (*Fagus sylvatica*), and sessile oak (*Quercus petraea*) have been selected due to their microstructure, showcasing distinct levels of porosity and homogeneity. Silver Fir exhibits softwood with a fine and regular texture and fine porosity. Beech and Sessile oak features hardwood. The first one with large, diffuse vessels evenly distributed across the transversal surface, the latter with larger vessels arranged in concentric circles. The samples fashioned into a 3 cm-sided cube to expose the typical diagnostic surfaces: transversal, radial longitudinal section, and tangential longitudinal section. The samples have been identified through morphological analysis of the diagnostic features under a transmitted light microscope at magnifications of 5x, 10x, and 40x.

2.3. Aging, biological patina and biocidal effects

Different amount of samples have been used to assess the effect of the application. The polysaccharide extracted using protocol P1 was chosen due to its minimal coloration, and it was subsequently diluted in distilled water at a concentration of 2 % w/v.

To establish the aging, two samples were taken for each wood type, and NYMPHA was applied with a brush (following the results reported in paragraph 3.1) onto transversal and tangential surfaces. Measurements were taken again after 24 hours and 1 week.

To establish the biocidal effects, two groups of nine samples (comprising three silver firs, three beeches, and three sessile oaks) underwent biological colonization through natural processes for a month. One group was exposed to a hypogeum environment, specifically an ancient Etruscan tomb [16]. This unique selection served two key purposes: firstly, the environment's remarkable stability, maintaining constant microclimatic conditions day and night, with minimal fluctuations around 15 °C and 98 % humidity throughout the year. Secondly, aligning with NYMPHA's primary objective, this environment simulates the type of biological attack on Cultural Heritage that the product aims to counter. The second sample group was directly buried in the earth for a month to induce significant biological colonization. For each set, six samples (two silver firs, two beeches, and two oaks) were treated with NYMPHA before the colonization process, another six were treated after, and the remaining ones served as references. Colorimetric measurements were once again recorded for all samples in each of the aforementioned conditions.

The analyses have been carried out using visible light reflectance spectroscopy. Reflectance spectra were recorded using a BWtek Exemplar® LS FORS spectrometer (200-850 nm spectral range; 1.5 nm spectral resolution), employing a D50 illuminant and an optical fibre with a 6.4 mm spot probe and a 45° holder. Colorimetric parameters in the CIELab colour space were extracted using the BWSpec® 4 software. Due to the highly heterogeneous nature of wood surfaces, six measurement points were taken for each surface (using a mask), and acquisitions were repeated five times. Chromatic variation ΔE was calculated for each of the six points using the 1967 formula:

$$\Delta E = \sqrt{(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2}\,, \qquad (1)$$

where L^*: lightness value, a^*: red/green value, b^*: blue/yellow value.

The application of the NYMPHA product took place on the transversal and tangential sections of two silver fir samples, two beech samples, and two oak samples for both the buried and underground sets. The impact of the biocide was assessed through colorimetric analyses conducted at 24 hours and three weeks after treatment on all samples within the two sets.

The colorimetric data, derived from averaging measurements across each analysed surface over six repetitions, are graphically presented in the CIE 1931 chromaticity diagram using OriginLab 2023b software.

3. RESULTS AND DISCUSSION

3.1. Polysaccharides application on wooden samples: effect upon aging and biological patina

When silver fir wood samples underwent UV imaging after being treated with P1, P2, and P3 protocols using various application methods, there was no fluorescence observed compared to the untreated sample. This lack of fluorescence can be attributed to the treated samples absorbing the solution effectively, rendering any product residues invisible on the surface for all the tested solutions. Therefore, there is no difference between the three application methods. The brush is the most suitable in terms of application and it was chosen for the following phases of the experimentation.

The colorimetric measurements on both the transversal and tangential surfaces of the samples using the CIELab colour space have been provided in order to assess the chromatic variation induced by the product. The colorimetric values revealed a significant colour change before and after treatment (Figure 1). The values exceed 3 CIELab units, the chromatic variation perceived by human eye [17]. The results displayed notable variability, likely influenced by the non-uniformity and distinct porosity of the surface.

On less porous surfaces like the tangential surface, where trachea and tracheid walls are pretty continuous with only small pores, penetration is considerably lower compared to the transversal section. In contrast, the wider openings in the transversal section favour higher penetration. In fact, a greater colour difference on the tangential section is recorded with a less noticeable shift where the product is readily absorbed.

In some cases, the decrease in ΔE values after a week could be attributed to higher absorption zones. However, in all instances, the application led to a discernible chromatic variation exceeding 3 CIELab units, making it visible to the naked eye ($\Delta E > 3$) [18], [19]. Across all samples, there was a shift towards yellow, potentially influencing the chromatic characteristics of an artifact upon application. This underscores the need for a careful pre-use assessment of the product. In most scenarios, there was an observable colour variation that seems to intensify over time. This change was particularly noticeable in silver fir samples (Figure 1a, b, c, d), the transversal surface of beech (Figure 1g), and the tangential section of oak (Figure 1l). The yellowing observed is a common phenomenon in aging polymer materials due to oxidation reactions that escalate with time [20], [21].

Figure 2 presents the average a^* and b^* parameter values across surfaces at three analysis stages (untreated, after 24 hours, and after 1 week). The distinctive characteristics of the two surfaces under investigation are evident: a) the transversal surface exhibits a gradual shift towards higher b^* values, indicating a progressively yellow hue over time; b) on the tangential surface, the values trend towards lower a^* values, implying a more green/blue hue (Figure 2).

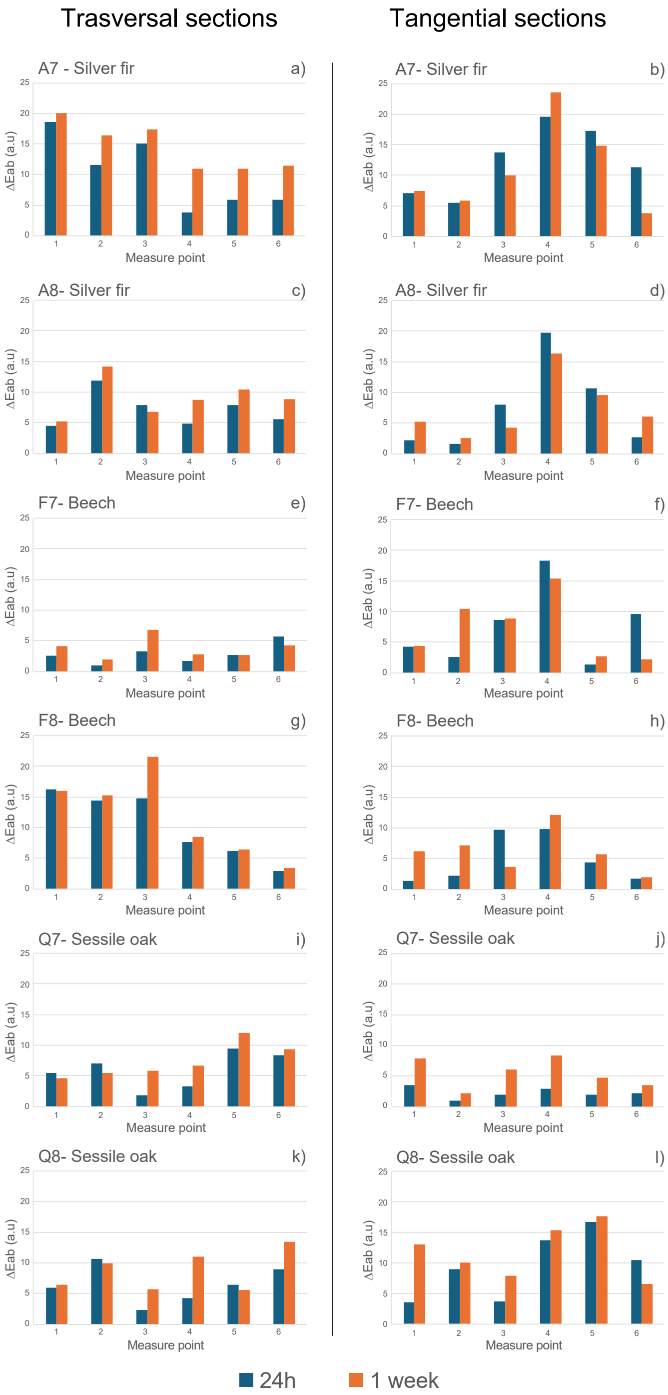

Figure 1. The graph shows ΔE values (1) of the 6 measure points compared to the relative untreated sample on transversal and tangential surfaces of two samples of silver fir (a; b; c; d), two of beech (e; f; g; h) and two of sessile oak (i; j; k; l) for both the repetitions of the experiment: 24 hours and a week from treatment. A= silver fir; F= beech; Q= sessile oak.

Interestingly, on tangential surfaces, the values consistently stay below the perceptibility threshold ($\Delta a^* < 3$; $\Delta b^* < 3$). In contrast, on transversal surfaces, colour changes are imperceptible within the initial 24 hours. However, after one week, a noticeable yellowing becomes evident ($\Delta a^* > 3$; $\Delta b^* > 3$), except for the transversal surface of one beech sample (F7).

It is important to highlight that across all samples, a marked difference in behaviour was observed between the two surfaces under examination, with the transversal surface being more prone to yellowing.

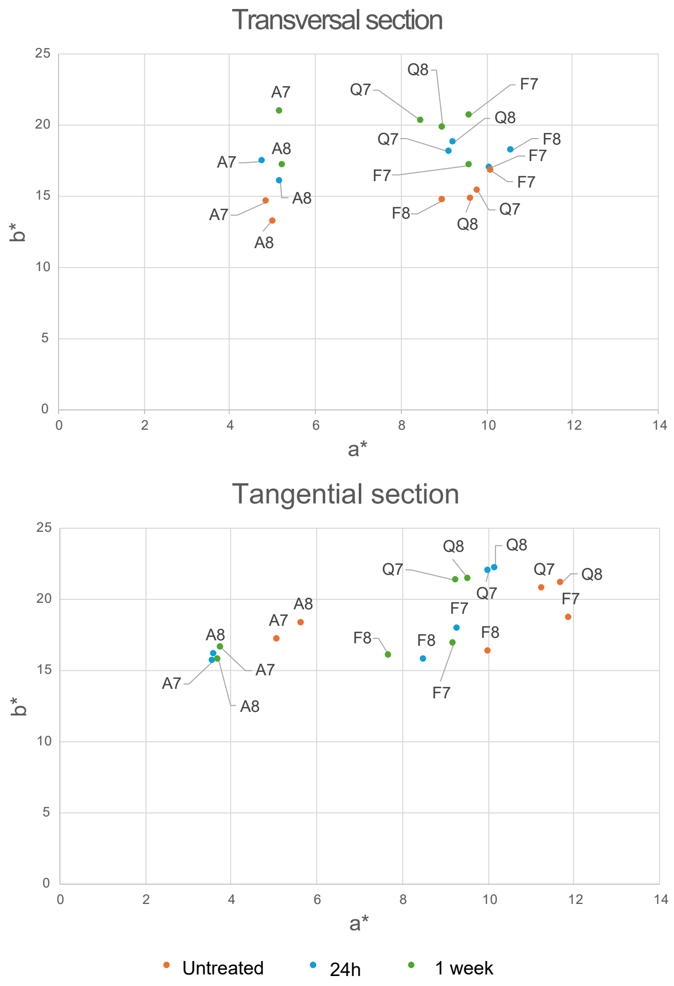

Figure 2. a* and b* coordinates from mediated surface values of all samples are plotted. Differences can be noted between the two examined surfaces: on transversal section yellower hues are detected while on tangential section values shift to greener hues. A= Silver fir; F= Beech; Q= Oak.

Upon concluding the colonization period, the samples were returned to the laboratory. Even without the aid of any instrument, a discernible darkening of the surface was observed in all buried samples, notably with the transversal surfaces displaying a significantly darker shade than the tangential surfaces. Among the wood types, beech exhibited heightened sensitivity to biological colonization, evident from the presence of fungi, a feature absent in other wood varieties. Furthermore, the samples housed in the underground chamber displayed a variation in surface colour. While this phenomenon was less pronounced for silver fir, beech wood emerged as the most conducive to colonization even in this controlled environment. Intriguingly, pre-treated beech samples appeared to undergo a more pronounced biological attack compared to their untreated counterparts.

Colorimetric measures taken onto two silver fir samples, two beech samples, and two oak samples for both the buried and underground sets were plotted in the CIELab 1931 chromaticity diagram (Figure 3 and Figure 4). Each diagram encompasses parameters related to colorimetric values of:

- untreated samples before exposure to NYMPHA (namely Reference), utilized as a benchmark to evaluate the absence of attack;
- pre-treated samples (Pre-treated) post-biological attack;
- respectively buried and hypogeum samples without NYMPHA application (Buried ref. and Tomb ref.), serving as a reference to estimate biological attack in the absence of the product's influence;
- post-treated samples (24h/3 weeks after treatment) at 24 hours and 3 weeks post-application.

The comparative analysis of colorimetric values before and after the biological attack in all instances reveals the product's inability to protect or restore the surface to its initial state. Notably, the colour variation before and after the biological attack is more pronounced for silver fir wood, attributed to its lighter natural colour compared to other, darker, wood types resulting in a greater fluctuation in colorimetric parameters.

3.1.1. Buried Samples

- *Transversal sections*

In the instances involving silver fir, all the described scenarios deviate from the reference values. When comparing samples amongst each other, no discernible colour distinctions emerge between a proactive or subsequent application of the NYMPHA product in either of the two repetitions (Figure 3a, b).

Concerning beech, it is evident that the surface condition remains unaltered after biological attack and post-treatment, indicating that the treatment applied after burial was ineffective. Furthermore, it appears that even the preventive treatment failed to avoid a colour change (Figure 3c, d).

In the case of oak samples, there is a minimal chromatic variation between the reference values and various treatments and attacks. It seems that this type of wood exhibits a lower propensity for chromatic changes and, consequently, appears less susceptible to degradation phenomena (Figure 3e, f).

- *Tangential Sections*

For silver fir samples, two distinct scenarios come to light. In one instance (Figure 3g), burial results in a shift in surface colour towards more reddish tones, whereas in the second scenario (Figure 3h), the hues lean more towards blue/green compared to the reference. In the first case, it is notably evident that the pre-treated sample closely aligns with the initial conditions; however, in the second case, none of the treatments induce significant colour variations compared to the buried sample.

Regarding beech samples, there are no noticeable colorimetric changes, despite a shift in colour between the reference and samples subjected to various analysis phases (Figure 3i, j).

In the case of oak samples, similar to beech, the surface remains nearly unchanged compared to the buried reference, with a slight shift, in this instance towards red, for samples treated after 24 hours and 3 weeks (Figure 3k, l).

3.1.2. Hypogeum Samples

The NYMPHA product appears ineffective in safeguarding wooden surfaces, even for samples placed in the tomb.

- *Transversal sections*

Consistent with the observations made for buried samples, silver fir wood displays a more pronounced colour difference between pre- and post-exposure to degrading agents compared to other wood types. It is noteworthy that pre-treatment appears to be the most effective for surface protection in the case of silver fir wood (Figure 4a, b).

In the case of beech samples, there is no significant variation in surface chroma observed in any of the scenarios (Figure 4 c, d).

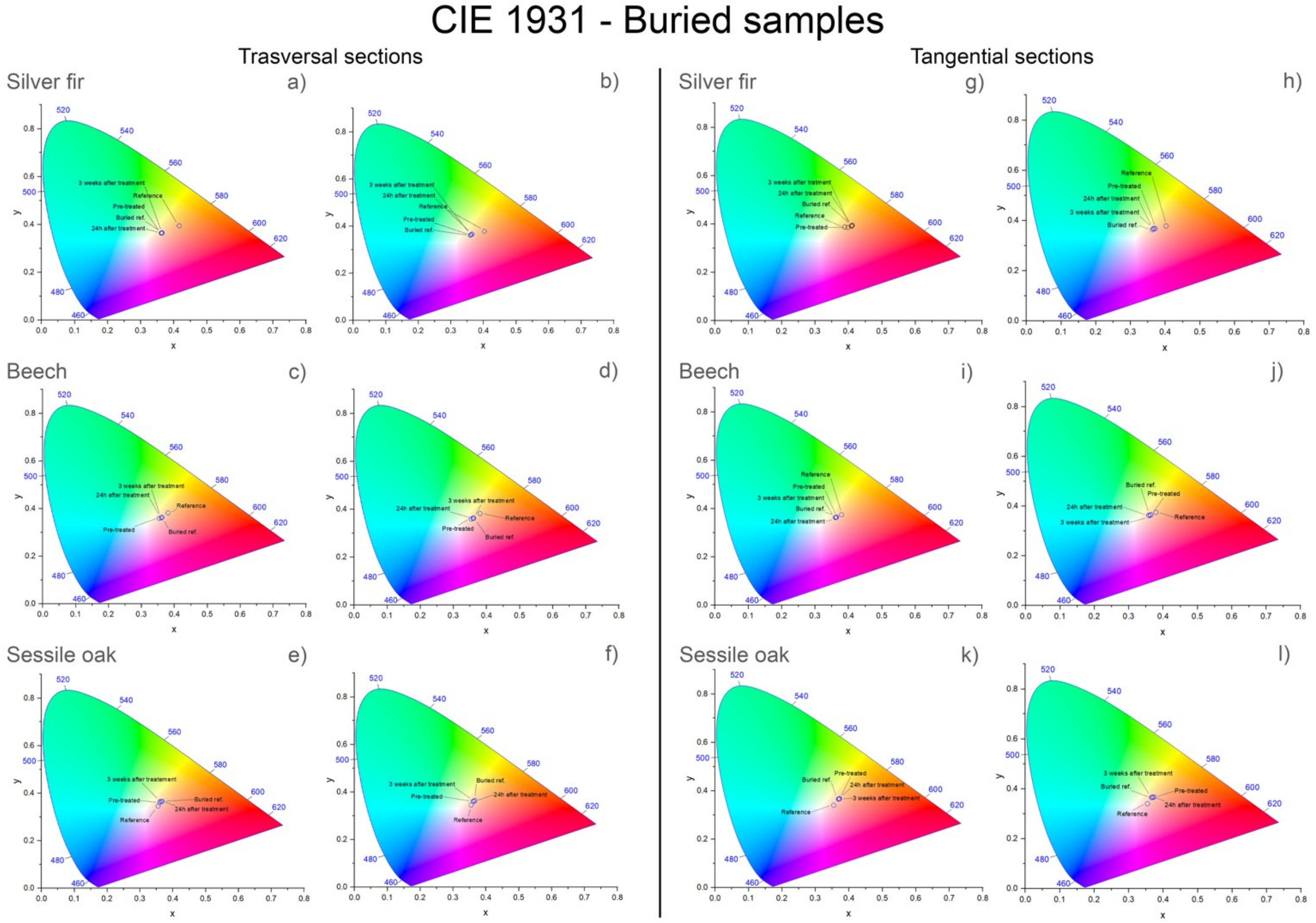

Figure 3. CIE 1931 diagram. The average of the six colorimetric values for transversal and tangential sections of buried silver fir (a, b, g, h), beech (c, d, i, j) and sessile oak (e, f, k, l) samples are reported for both the repetitions of the experiment. *Reference* = untreated, non-buried sample; *buried reference* = untreated buried sample; *pre-treated* = NYMPHA applied before burial; *24h and 3 weeks after treatment* = NYMPHA applied after burial and monitored 24h and 1 week from treatment.

As for oak samples, two distinct situations emerge. In the first scenario, post-treatment succeeds in approaching the initial conditions after 3 weeks (Figure 4e). However, in the second scenario (Figure 4f), there are no substantial colour variations compared to the tomb reference (Tomb ref.), except for a slight shift towards red observed 24 hours after treatment.

- *Tangential Sections*

In the case of silver fir samples, it is also evident on this surface that the pre-treated sample closely aligns with the reference sample, although there is a noticeable variation compared to the reference both after the application of the product and following the biological attack (Figure 4g, h).

Measurements on beech samples, akin to the transversal section, reveal no alterations in parameters compared to the hypogeal reference in any scenario (Figure 4i, j).

In both oak samples, the values from various treatments do not diverge significantly from the hypogeal reference (Tomb ref.) (Figure 4k, l).

4. CONCLUSIONS

In this initial investigation, we examined the aging behaviour of a novel, environmentally friendly product composed of algae polysaccharides, which holds potential to treat biological attacks on wood samples. Notably, the study highlights how the intricate surface structure of different woods pose challenges to the application of this product. The wooden material itself plays an important role in colorimetric measurements since the chromatic variation seems to depend also on different factors such as absorption rate, surface inhomogeneities and wood lightness stressing the need to take them into account for future studies of this kind. Nevertheless, the product appears to induce a subtle yellowing on some analysed surfaces.

Regarding the anticipated biocidal effect, despite previous studies suggesting potential biocidal properties of algae polysaccharides [22], [23], [24], our data did not confirm such effects.

However, it should be underlined that previous results published on potential biocidal and antioxidant properties were linked to the presence of sulphate groups in extracted polysaccharide mixtures [25], [26]. Preliminary analyses relating to the elemental chemical composition of the NYMPHA extracts revealed the absence of these functional groups (Antonacci et al., in preparation). The reason for this absence is probably due to the use of different strains of *C. reinhardtii*, as well as and the need to optimize the extraction and purification protocol in terms of yield and quality of the final product. For all these reasons, further research will be necessary always keeping in mind the complex nature of materials constituting cultural heritage.

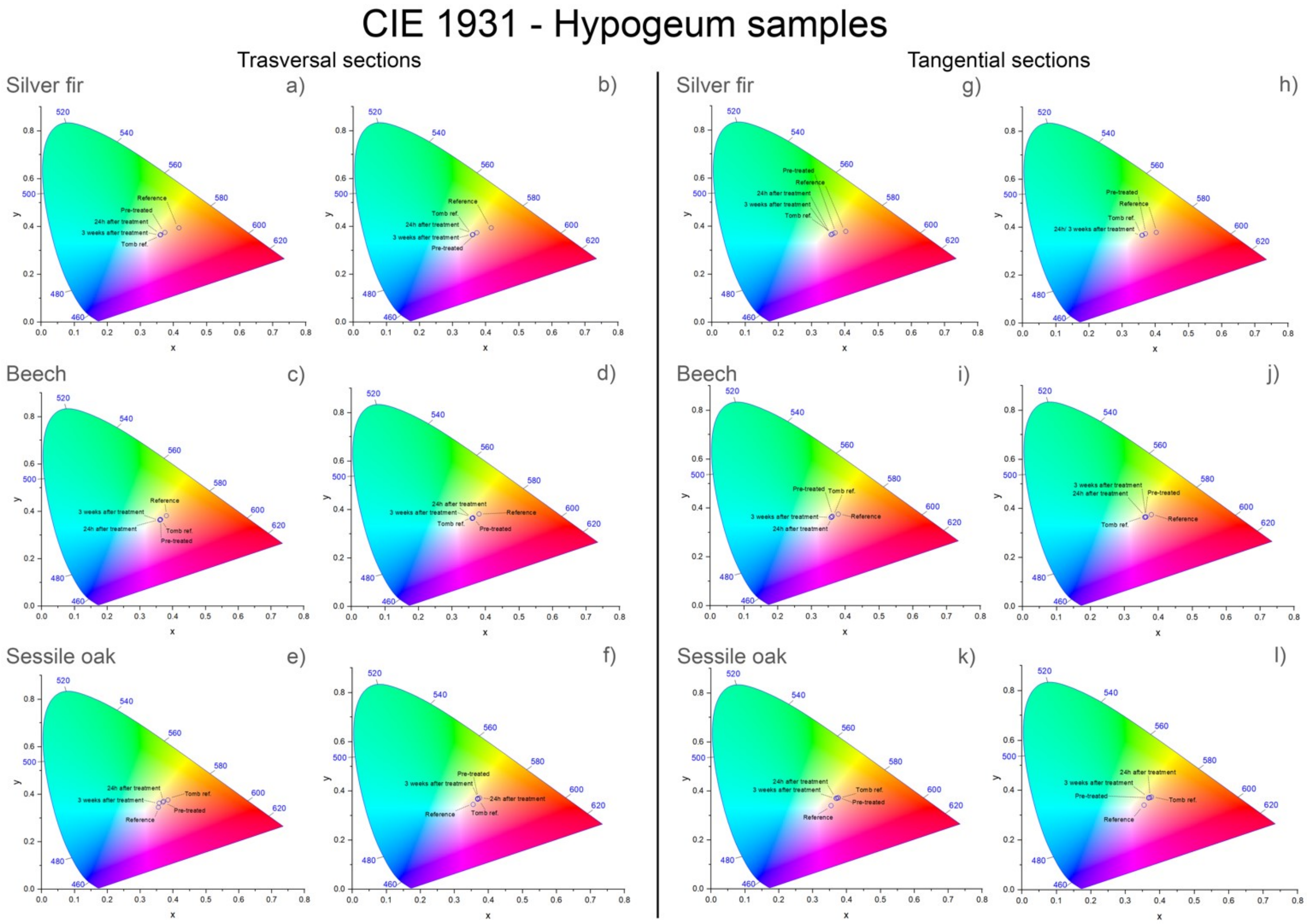

Figure 4. CIE 1931 diagram. The average of the six colorimetric values for transversal and tangential sections of the hypogeum silver fir (a, b, g, h), beech (c, d, i, j) and sessile oak (e, f, k, l) samples are reported for both the repetitions of the experiment. *Reference* = untreated, non-hypogeum sample; *tomb reference* = untreated hypogeum sample; *pre-treated* = NYMPHA applied before being put in hypogeum chamber; *24h and 3 weeks after treatment* = NYMPHA applied after being put in hypogeum chamber and monitored 24h and 1 week from treatment.

Particularly within the realm of cultural heritage, it is imperative to assess new solutions on naturally degraded materials, as was the focus of this study.

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REFERENCES

[1] H. J. Hueck, The biodeterioration of materials as a part of hylobiology, Material Und Organisms (1965), pp. 5-34.

[2] H. J. Hueck, The Biodeterioration of Materials - An appraisal, International Biodeterioration & Biodegradation 48(1–4) (2001), pp. 5-11, ISSN 0964-8305.
DOI: [10.1016/S0964-8305(01)00061-0](https://doi.org/10.1016/S0964-8305(01)00061-0)

[3] R. Arango, S. T. Lebow, J. A. Glaeser, Chapter 14: Biodeterioration of wood, in: Wood handbook wood as an engineering material. Forest Products Laboratory. General Technical Report FPL-GTR-282, Madison, WI: U.S. Department of Agriculture, Forest Service, Forest Products Laboratory, 2021, 543 pp.

[4] A. Unger, A. P. Schniewind, W. Unger, Conservation of Wood Artifacts - a handbook. Springer, Berlin, 2001, ISBN: 978-3-642-07480-6.

[5] D. Joshi, N. Adhikari, An overview on common organic Solvents and their toxicity, Journal of Pharmaceutical Research International 28(3) (2019), pp. 1-18.
DOI: [10.9734/jpri/2019/v28i330203](https://doi.org/10.9734/jpri/2019/v28i330203)

[6] M. R. Fidanza, G. Caneva, Natural biocides for the conservation of stone cultural heritage: A review, Journal of Cultural Heritage 38 (2019), pp. 271-286.
DOI: [10.1016/j.culher.2019.01.005](https://doi.org/10.1016/j.culher.2019.01.005)

[7] L. Campanella, F. Grimaldi, S. Plattner, A. Baiocco, Sustainable restoration: the contribution of algae, Natural Product Research 33(7) (2016), pp. 940-942.
DOI: [10.1080/14786419.2016.1163553](https://doi.org/10.1080/14786419.2016.1163553)

[8] C. Gattuso, L. Campanella, V. Giosafatto, L. Mariniello, V. Roviello, The consolidating and adhesive properties of funori: microscopy findings on common and ancient paper samples, Journal of Cultural Heritage 48 (2021), pp. 153-160.
DOI: [10.1016/j.culher.2020.11.013](https://doi.org/10.1016/j.culher.2020.11.013)

[9] H. Sheikh, A. El-Naggar, D. Al-Sobah, Evaluation of Antimycotic Activity of Extracts of Marine Algae Collected from Red Sea Coast, Jeddah, Saudi Arabia, Journal of Biosciences and Medicines 6(04) (2018), p. 51.
DOI: [10.4236/jbm.2018.64004](https://doi.org/10.4236/jbm.2018.64004)

[10] E. A. Cecilia Guedes, M. A. dos Santos Araújo, A. K. Pinheiro Souza, L. I. Oliveira de Souza, L. Dayse de Barros, F. C. de Albuquerque Maranhão, A. E. Antônio Euzébio Goulart Sant'Ana, Antifungal Activities of Different Extracts of Marine Macroalgae Against Dermatophytes and Candida Species, Mychopathologia 174 (2012), pp. 223-232.
DOI: 10.1007/s11046-012-9541-z

[11] E. A. Hassan, A. F. Hifney, E. F. Ali, A. M. Sayed, Fungicidal activities and molecular docking of the marine alga Ulva lactuca and Punica granatum peel extracts on Alternaria tomato spot disease, Environmental Science and Pollution Research 30(8) (2023), pp. 21872-21887.
DOI:10.1007/s11356-022-23733-y

[12] E. H. Harris, The genus Chlamydomonas in The Chlamydomonas Sourcebook: Introduction to Chlamydomonas and Its Laboratory Use: Volume 1, 2009, Academic press.
DOI: 10.1016/b978-0-12-370873-1.00001-0

[13] P. Kamble, S. Cheriyamundath, M. Lopus, V. L. Sirisha Chemical characteristics, antioxidant and anticancer potential of sulfated polysaccharides from Chlamydomonas reinhardtii, Journal of Applied Phycology 30(3) (2018), pp. 1641-1653.
DOI: 10.1007/s10811-018-1397-2

[14] J. Chen, J. Zhou, L. Zhang, Y. Nakamura, T. Norisuye, Chemical structure of the water-insoluble polysaccharide isolated from the fruiting body of Ganoderma lucidum, Polymer journal 30(10) (1998), pp. 838-842.
DOI: 10.1295/polymj.30.838

[15] B. Le, K. S. Golokhvast, S. H. Yang, S. Sun, Optimization of microwave-assisted extraction of polysaccharides from Ulva pertusa and evaluation of their antioxidant activity, Antioxidants 8(5) (2019), p. 129.
DOI: 10.3390/antiox8050129

[16] M. C. Tomassetti, A. Cirigliano, C. Arrighi, R. Negri, F. Mura, M. L. Maneschi, M. D. Gentili, C. Mazzoni, T. Rinaldi, A role for microbial selection in frescoes' deterioration in Tomba degli Scudi in Tarquinia, Italy, Scientific Reports 7 (2017), n. 6027.
DOI: 10.1038/s41598-017-06169-0

[17] J. Delgado Rodrigues, A. Grossi, Indicators and ratings for the compatibility assessment of conservation actions, Journal of Cultural Heritage 8(1) (2007), pp. 32-43.
DOI: 10.1016/j.culher.2006.04.007

[18] C. Genova, E. Fuentes, G. Favero, B. Prieto, Evaluation of the cleaning effect of natural-based biocides: application on different phototropic biofilms colonizing the same granite wall, Coatings 13(3) (2023), p. 520.
DOI: 10.3390/coatings13030520

[19] P. Sanmartín, E. Chorro, D. Vazquez-Nion, F. M. Martínez-Verdú, B. Prieto, Conversion of a digital camera into a non-contact colorimeter for use in stone cultural heritage: The application case to Spanish granites, Measurement 56 (2014), pp. 194-202.
DOI: 10.1016/j.measurement.2014.06.023

[20] P. Dietemann, C. Higgitt, M. Kälin, M. J. Edelmann, R. Knochenmuss, R. Zenobi, Aging and yellowing of triterpenoid resin varnishes–Influence of aging conditions and resin composition, Journal of Cultural Heritage 10(1) (2009), pp. 30-40.
DOI: 10.1016/j.culher.2008.04.007

[21] A. M. Conte, O. Pulci, A. Knapik, J. Bagniuk, R. Del Sole, J. Lojewska, M. Missori, Role of cellulose oxidation in the yellowing of ancient paper. Physical Review Letters, vol. 108, n. 15, 2012, p. 158301.
DOI: 10.1103/PhysRevLett.108.158301

[22] R. M. Abedin, H. M. Taha, Antibacterial and antifungal activity of cyanobacteria and green microalgae. Evaluation of medium components by Plackett-Burman design for antimicrobial activity of Spirulina platensis, Global Journal of Biotechnology and Biochemistry 3(1) (2008), pp. 22-31, ISSN 2078-466X. Online [Accessed 20 June 2024]
https://idosi.org/gjbb/gjbb3(1)08/4.pdf

[23] A. Bratchkova, A. D. Kroumov, Microalgae as producers of biologically active compounds with antibacterial, antiviral, antifungal, antialgal, antiprotozoal, antiparasitic and anticancer activity, Acta Microbiol. Bulg. 36(3) (2020), pp. 79-89. Online [Accessed 20 June 2024]
https://www.actamicrobio.bg/archive/issue-3-2020/amb-3-2020-article-2.pdf

[24] B. Schmid, L. Coelho, P. S. Schulze, H. Pereira, T. Santos, I. B. Maia, M. Reis, J. Varela, Antifungal properties of aqueous microalgal extracts, Bioresource Technology Reports 18 (2022), p. 101096.
DOI: 10.1016/j.biteb.2022.101096

[25] J. Vishwakarma, S. L. Vavilala, Evaluating the antibacterial and antibiofilm potential of sulphated polysaccharides extracted from green algae Chlamydomonas reinhardtii, Journal of Applied Microbiology 127(4) (2019), pp. 1004-1017.
DOI: 10.1111/jam.14364

[26] K. Palani, B. Balasubramanian, A. Malaisamy, V. Maluventhen, V. A. Arumugam, N. A. Al-Dhabi, M. V. Arasu, K. Pushparaj, (+2 more authors), Sulfated polysaccharides derived from Hypnea valentiae and their potential of antioxidant, antimicrobial, and anticoagulant activities with in silico docking, Evidence-Based Complementary and Alternative Medicine 2022 (2022).
DOI: 10.1155/2022/3715806